AWARD NUMBER: W81XWH-14-2-0129

TITLE: Restoring Bladder Function by Spinal Cord Neuromodulation in SCI

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CONTRACTING ORGANIZATION: UNIVERSITY OF CALIFORNIA,
LOS ANGLELES

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

Enclosed is the annual progress report for our grant entitled, "Restoring Bladder Function by Spinal Cord Neuromodulation in SCI" (SC103209). Over the last year, we have integrated the information from the pilot study of five naïve subjects to determine the optimal magnetic stimulating parameters for restoring bladder function in spinally-injured patients (Tasks 1-3). We have used these established parameters to begin a Triple Blind Randomized Control Trial (Task 4). Eleven participants have completed the clinical trial and we currently have no active participants. Our goal is to enroll 12 total participants through our clinical trial to validate our established optimal parameters of magnetic stimulation to restore bladder function in people with spinal cord injury (SCI). We believe this study will yield necessary information to enable therapies which non-invasively restore bladder function in patients with SCI and will make a dramatic impact on the lives of those living with SCI.

15. SUBJECT TERMS

Spinal Cord Injury, Urinary Function, Magnetic Stimulation

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			19b. TELEPHONE NUMBER *(include area code)*
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1. INTRODUCTION:

The central objective of this project is to demonstrate that **non-invasive neuromodulation can improve bladder function by stimulating spared circuitry in the spinal cord**. Such normalization of spinal cord function is accomplished through a process of functional neuroplasticity whereby neuromodulation (e.g. electromagnetic stimulation) activates spinal circuits associated with micturition. Repeated stimulation sessions are thought to potentiate these circuits, facilitating ascending projections for improved sensation and descending projections for volitional voiding. To date, a subset of subjects has experienced durable, long-lasting improvements in bladder function and have been able to void in the absence of stimulation. A total of 18 male, age 18+, >1 year post (C2-T8, non-conus) injury with complete and stable severe motor paralysis (ASIA A, B) and bladder catheterization dependence will be enrolled.

2. KEYWORDS:

Corticospinal tract, Dorsal Root Ganglia, Epidural Spinal Cord Stimulation, Electromyography, Machine learning, Magnetic Stimulation, Gaussian Process Optimization, Neurogenic Detrusor Overactivity, Spinal Cord Evoked Potentials, Urinary Tract Infection

3. ACCOMPLISHMENTS:

1. Completion of a pilot study in five initial patients with chronic ASIA A/B Spinal Cord Injury (SCI) and verified detrusor sphincter dyssynergia. Study subjects underwent pre-intervention baseline assessment followed by a 16-week treatment period and a remote post-treatment assessment.

a. Demonstrated definitive functional improvement in the key therapeutic target of volitional micturition in all five study subjects. Each subject regained at least partial volitional micturition with stimulation. Persistent function up to five weeks after the cessation of stimulation was noted in the study subjects.

b. Demonstrated improved bladder capacity, reduced reliance on self-catheterization, improved sexual function, and improved quality of life as assessed by objective and standardized metrics. Further, neurophysiological assessment of the bulbocavernosus reflex in treated subjects demonstrated a reduced level of activity, consistent with a more normal level of function as compared to abnormal hyperactive baseline measurements which were typical of chronic SCI.

c. Demonstrated improved bowel function and subjectively improved posture, ease of transfer, and duration of standing in study subjects, potentially opening new avenues of investigation and rehabilitation for future studies/therapies. All study interventions were tolerated without significant adverse effects.

d. Submitted study results for publication in Nature Scientific Reports.

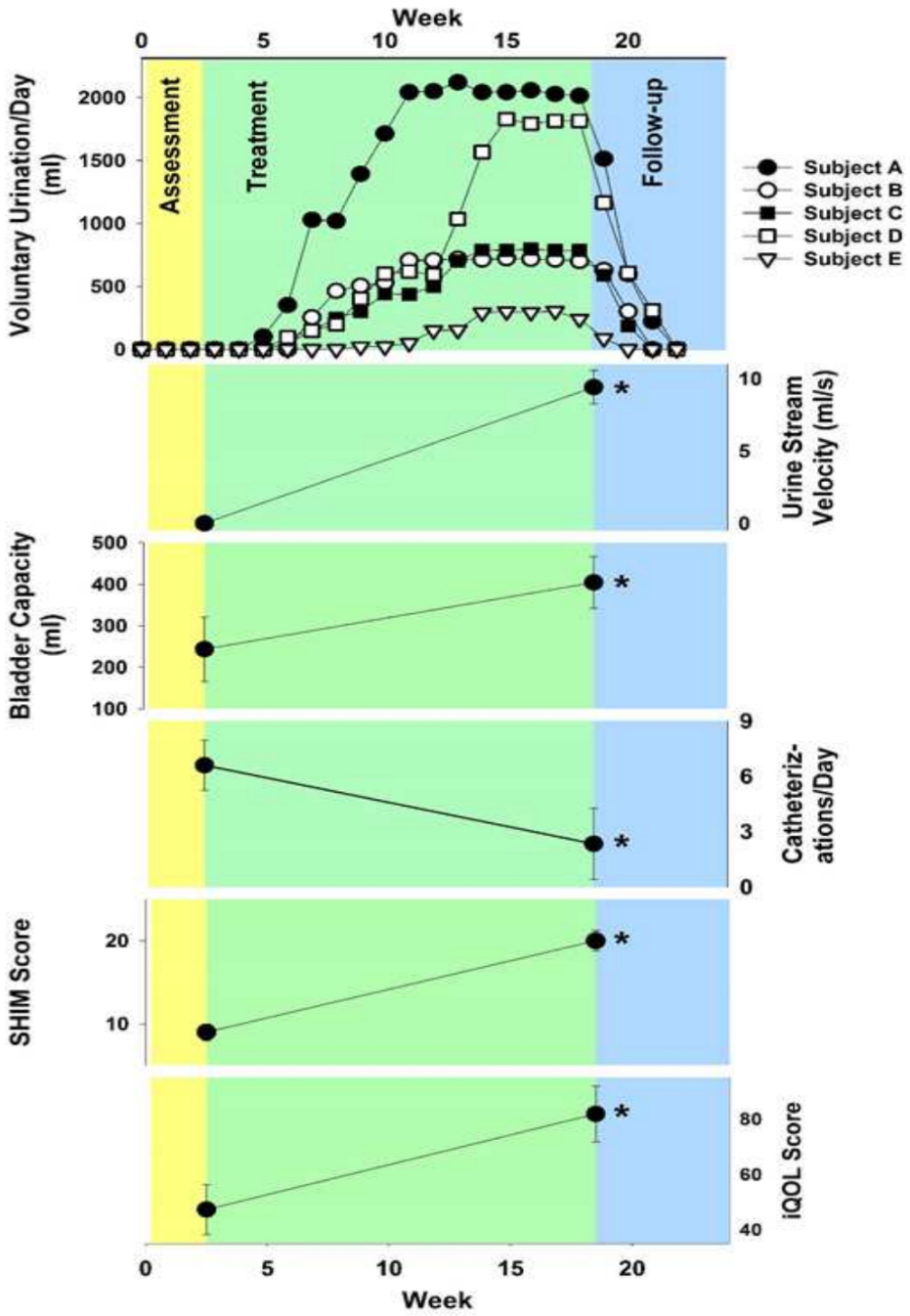


Figure 1: Summary of urological function and average daily volitional micturition volume for all subjects throughout study; all findings are statistically significant by paired t-test ($p < 0.05$). The top panel indicates timing of recovery and loss of voluntary control of micturition and the volume of urine produced each day over time. All subjects recovered the capacity to urinate voluntarily, and 2-3 weeks after termination of stimulation, the capacity to urinate voluntarily returned to the baseline (unable to void voluntarily). The remaining panels indicate the initial value of each variable before stimulation and after 16 weeks of stimulation. Urine stream velocity and the bladder capacity (both measured during urodynamic studies) increased significantly ($p < 0.05$) after 16 weeks of stimulation. The number of self-catheterizations diminished significantly ($p < 0.05$), and the SHIM Score and the iQOL, both quality of life measures, increased significantly ($p < 0.05$) after 16 weeks of stimulation.

2. In an allied area of sensorimotor rehabilitative research, we have discovered a method that is superior to transcutaneous electrical stimulation (TES) in the delivery of neuromodulatory stimulation to the spinal cord. We have identified transcutaneous magnetic stimulation (TMS) as a superior method for the following reasons.

a. Better energy delivery to deep structures. The magnitude of energy penetrating tissues and reaching the cord appears to be superior to that of TES. In our separate cohort of patients, TMS demonstrated superiority to activate the spinal cord interneurons to improve motor function, with an approximate 80% superiority in the generation of hand grip force. Additionally, this positive effect is observed immediately during the first session, unlike TES which requires a prolonged training period of 3-6 months.

b. Painless Stimulation. Our preliminary studies with TES were very promising; however, the consistently high levels of energy needed to reach the cord are prohibitive due to pain in about 40% of subjects with preserved sensation. It may be that reaching nerve roots with lower energies has provided some favorable preliminary results. We hypothesize that delivery of energy to the spinal cord is necessary to activate neuronal circuitry within the spinal cord that coordinates the activity of bladder function.

c. Access. Several manufacturers have marketed magnetic stimulation devices making these devices available to patients.

d. Durable Improvements. Our preliminary research in bladder and other regions of the spinal cord indicate that the improvements in function that occur after treatment can last for up to six months. This obviates the need of a portable device and home use. If patients experience a decline in function, they can return for therapy to restore function.

Due to the overwhelming superiority of magnetic stimulation compared to TES, we elected to use magnetic stimulation for this study. With magnetic stimulation we will likely accomplish the aims set forth in the project faster and more effectively.

- **What were the major goals of the project?**

Month 0-18. Task 1

Determine the optimal stimulation parameters to enable micturition in SCI subjects.

We will determine combinations of spinal cord stimulation level (vertebral body level T10-L4, +/- coccyx), stimulation frequency (1-30 Hz) twice weekly for 24 weeks. A machine learning algorithm will guide subsequent stimulation parameters (Task 3). Subjects will be evaluated at each session for urine flow and volume, and assessments of quality of life and urinary function. Formal urodynamics will be tested monthly and at the conclusion of the six-month study. Results will be prepared for manuscript publication.

Percent Complete: 100%

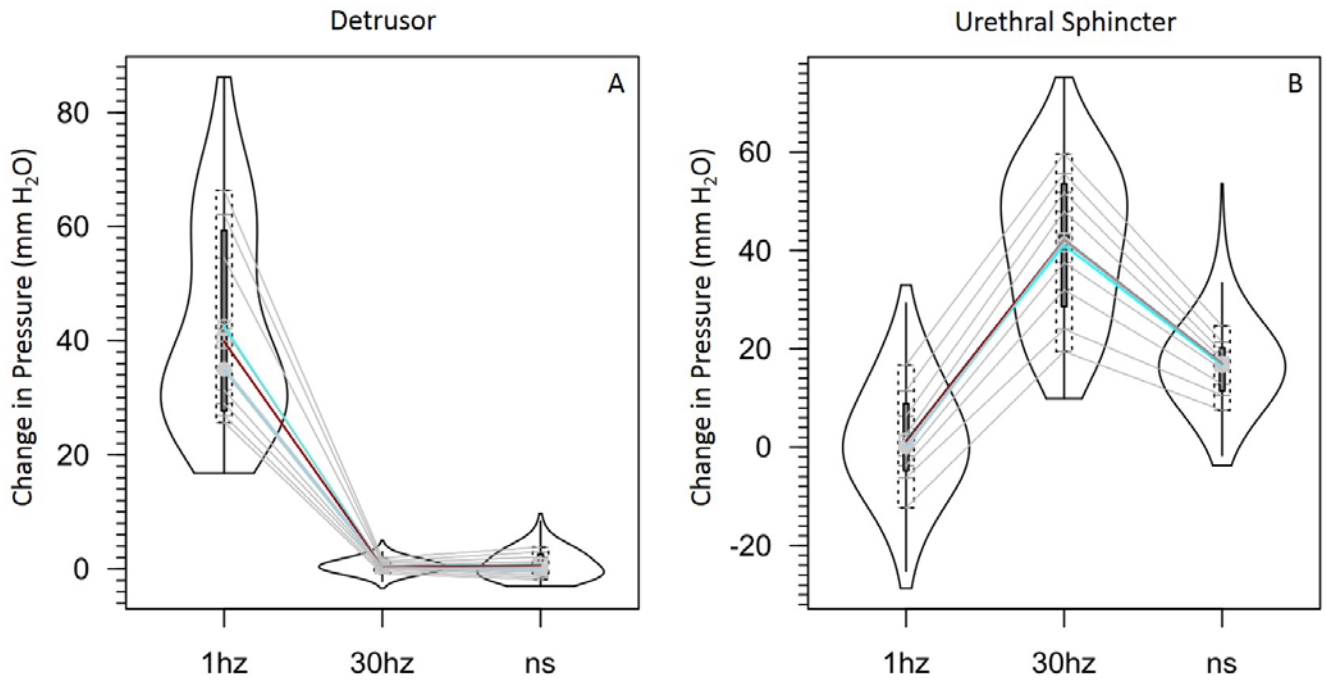


Figure 2: Violin plots of change in pressure, expressed as mm water, by stimulation condition for detrusor and urethral sphincters. Each violin shows mean and median (heavy horizontal lines) plus standard deviation (heavy vertical bars) and deciles (in light grey) using a kernal density estimator of the data distribution. For the measure labeled "Detrusor" (A) the 1hz condition resulted in a mean value of 42.47 (sd 17.27) while the no-stimulation and the 30hz conditions showed means of 0.47 (sd 1.06) and 0.75 (sd 2.38), respectively. For the measure labeled "Urethral sphincter" (B) the 1hz condition resulted in a mean of 1.44 (sd 10.94) while the no-stimulation and 30hz conditions showed means of 41.07 (sd 15.03) and 16.66 (sd 7.76), respectively. Analyses of variance (ANOVA) and Tukey HSD post-hoc testing were used to examine the differences between conditions in each of the two measures. In both instances, the results indicate that the 1Hz condition differed from both the no-stimulation condition and the 30Hz condition ($p < 0.0001$), but the latter two did not differ from one another.

We have several observations:

1) When there is no percutaneous magnetic stimulation, the urethral pressures increase more than the detrusor pressure increase during volitional attempt. This is characteristic of detrusor sphincter dyssynergia, a condition that is frequently recognized in SCI subjects.

2) With low frequency stimulation, subjects have minimal change in urethral pressure during volitional voiding while the detrusor pressure increases significantly; hence facilitate emptying.

3) With high frequency (30Hz) stimulation, subjects have significantly increased urethral pressure with minimal to no change in detrusor pressure; hence facilitating storage.

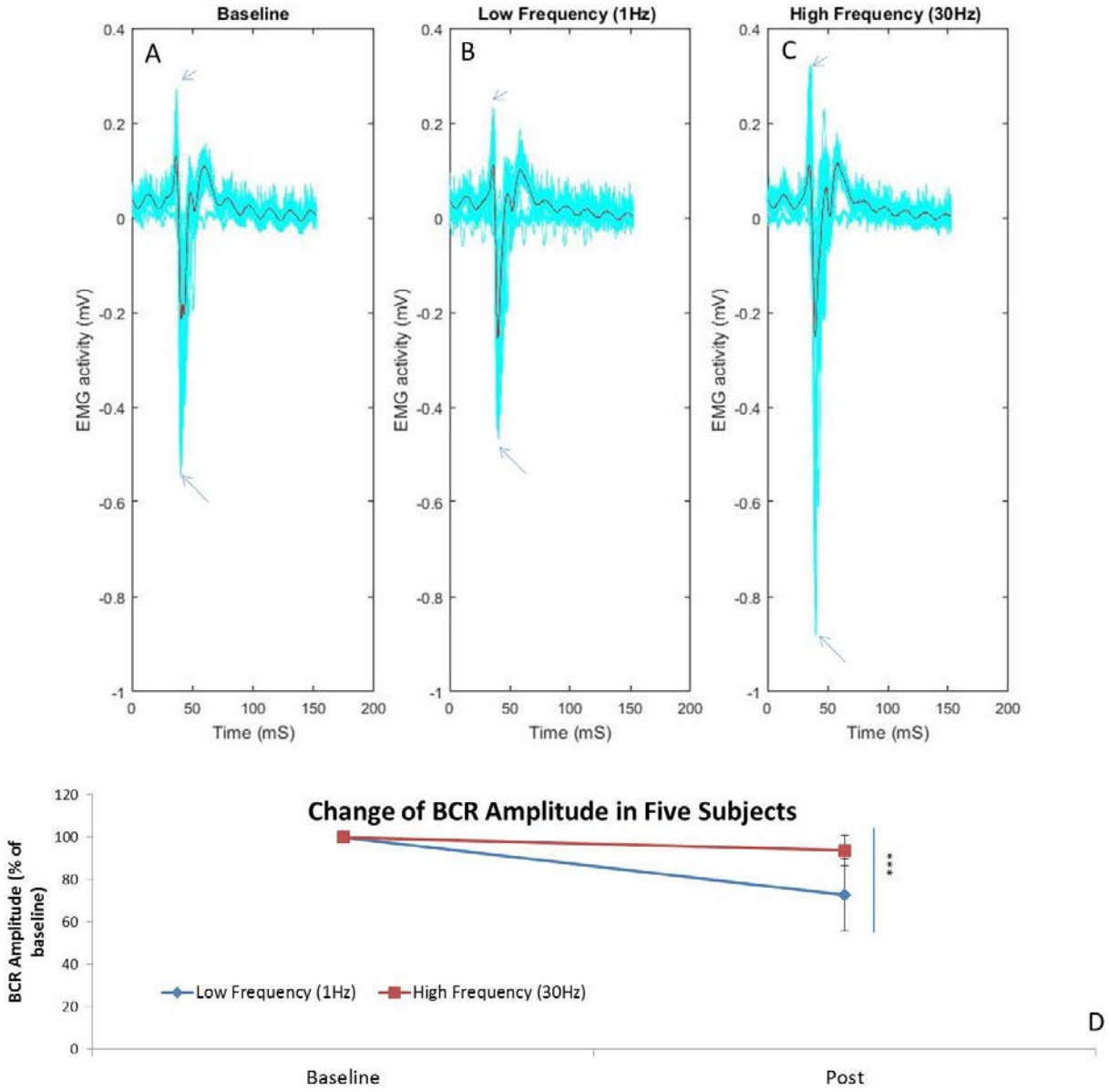


Figure 3: Change in bulbocavernosus reflex (BCR) amplitude, which is measured from the perineal muscle EMG, during low frequency (1 Hz) and high frequency (30 Hz) transcutaneous magnetic spinal cord stimulation. A, B & C. An example of measured the BCR from subject C at Baseline (A), after low frequency stimulation (B) and after high frequency stimulation (C) respectively; blue = individual electrical recordings; red = average. D = amplitude changes in all five subjects. Note a significantly higher degree of reduction of BCR amplitude after low frequency stimulation when compared to high frequency stimulation. Student's t-test: *** = $p < 0.0001$.

Several observations:

1) Low frequency TMS significantly reduced the BCR amplitude in all five subjects. In contrast, high frequency stimulation resulted in either further increased amplitude or no significant change (Figure 3). The average BCR latency was 35.2 ± 5.3 ms, which is similar to the latency of the BCR in normal individuals. The baseline amplitude, however, ranged from 490-3800 μV; amplitudes that are about 10-100 times greater than those of normal

individuals. During low frequency stimulation, the BCR amplitude was significantly decreased to between 440-3100 μV compared to the unstimulated baseline (an average reduction of 28%, $p < 0.0001$). High frequency stimulation did not alter the BCR amplitude from baseline, which ranged between 475-3700 μV ($p = 0.61$)

2) The modifications in the BCR also support the hypothesis that we accessed the micturition spinal circuitry rather than direct motor neuron stimulation as modifications of a polysynaptic reflex such as BCR require more than simple motor neuron stimulation. BCR amplitudes for our subjects at baseline were 10 to 100 times greater than those in normal individuals. This observation suggests that SCI subjects have decreased supraspinal inhibition of the BCR polysynaptic reflex. With low frequency stimulation, we observed a decrease in the BCR amplitudes and this implied an improved inhibition of the BCR polysynaptic reflex (likely via spinal micturition circuitry). However, high frequency stimulation did not decrease the BCR amplitude. (Figure 3) We hypothesize that this decrease in BCR amplitudes in chronic SCI with low frequency magnetic stimulation may signified a decreasing external sphincter motor pool hyperactivity or possibly modulating supraspinal input such that more supraspinal signal got through past the lesioned site; and hence enabling more volitional urinary functions that we observed in our subjects.

Month 19-36. Task 2

Determine the minimum training conditions to enable micturition in SCI subjects.

With the stimulation parameters from Task 1, we will determine a training regimen in naïve SCI subjects that are injury matched to those in Task 1. In addition to determining the minimum number sessions, we will examine the type of training sessions, twice weekly for 24 weeks. Assessments as in Task 1. Results will be prepared for manuscript publication.

Percent Complete: 100%

Month 1-48. Task 3

Application of machine learning strategy to determining the optimal stimulation and training parameters for micturition in SCI subjects.

Data from each session will be added to a machine learning algorithm database in order to determine the most effective parameters for the most recent session and guide stimulation parameters for subsequent sessions. At the conclusion of this Task, the optimal conditions for urination will be determined and used in the clinical trial. Results will be prepared for manuscript publication.

Percent Complete: 100%

Month 37-48 Task 4

Application of the optimal stimulation and training parameters for micturition in SCI subjects in a pilot clinical trial.

This Task will combine the optimal stimulation parameters from Tasks 1-3. We will determine the optimized stimulation paradigm that can improve micturition function in naïve, SCI subjects. In this Task, 12 subjects that are injury matched to Task 1-2 will be recruited. Each of 12 subjects will be tested twice weekly for 6 months. Urodynamics and self-assessments, as Task 1. Results will be prepared for manuscript publication.

Percent Complete: 91%

- **What was accomplished under these goals?**

Relevant to all above listed goals, we have completed the necessary approval to enroll subjects, recruited the necessary expertise and personnel to conduct the study with the revised device (TMS). Within the past year specifically, we have (at the time of this reporting):

1. Acquired and purchased all necessary equipment for this study.
2. Obtained UCLA IRB continuing review approval (3/13/2020) and UCLA Medical Radiation Safety Committee (MRSC) continuing review approval.
3. Completed the USAMRMC ORP HRPO continuing review
4. Submitted a one-year, no-cost extension to allow actively enrolled patients to complete the study and to allow us to reach the 12 planned subject total (11/2020).
5. Submitted an amendment to update key personnel to the study (12/02/2020).
6. Continued with the Triple Blind Randomized Control Trial, currently with no active participants (eleven completed).
7. Obtained approval for continued research activity at a reduced level during the ongoing COVI-19 pandemic (04/28/2020)

In Task 1, we identified the optimal parameters for stimulation and proceeded on to the subsequent tasks as planned. Figure 2 clearly demonstrated the different effects of stimulation when applied at low and high frequencies. We completed stimulation and follow-up in five subjects and used the information gathered from these subjects to establish optimal parameters for the ongoing Triple Blind Randomized Control Trial (RCT). With an additional year, we anticipate accomplishing the goals set forth in Task 4, as described in the above timeline.

- **What opportunities for training and professional development has the project provided?**
  This project has provided an opportunity for advancement in the study of molecular and cellular basis of spinal central pattern generator activity by Yevgeniy Freyvert, MD and Tianyi Niu, MD, Neurosurgery Residents with Dr. Lu; William Alaynick, PhD, a Project Scientist Step IV with Dr. Lu; and for the PI, Dr. Lu. A resulting manuscript has been accepted to Frontiers in Molecular Neuroscience and is attached in the Appendix. A second review manuscript with the same authors has been accepted at *Current Physical Medicine and Rehabilitation Reports* and is attached in the Appendix. A third manuscript on the results of the intervention on the first five patients was published in August 2018.

- **How were the results disseminated to communities of interest?**

  William Alaynick, PhD, Project Scientist at UCLA, delivered a lecture, "Spinal Central Pattern Generating Circuitry: From Bench to Bedside," at the European Neuroscience Institute at the University of Gottingen in Germany on December 16, 2014.

  Daniel Lu, MD PhD, Associate Professor of Neurosurgery at UCLA, presented on "Spinal modulation for rehabilitation" at the 2018 UCLA Symposium on Neurotechnology at the Norris Global Conference Room at UCLA on October 12, 2018.

  Daniel Lu, MD PhD, Associate Professor of Neurosurgery at UCLA, was featured in a segment, "Spinal Cord Stimulator Restores Bladder Control," on Ivanhoe Broadcast News (https://www.ivanhoe.com/medical-breakthroughs/spinal-cord-stimulator-restores-bladder-control/) on December 13, 2018 and later on ABC-7 News on December 27, 2018.

- **What do you plan to do during the next reporting period to accomplish the goals?**
  During the upcoming period, we plan to accomplish the following goals:

Task 1: We have established the optimal stimulation parameters to enable micturition in chronic SCI subjects, and have begun a Triple Blind Randomized Control Trial utilizing the established parameters. We plan to continue to enroll participants until we reach the goal of 12 to confirm consistent results of the established stimulation parameters.

Task 2: We have determined the minimum training conditions to improved lumbosacral circuitry to enable micturition in SCI subjects and have implemented these conditions to optimize the stimulation parameters from Task 1.

Task 3: We have applied a machine learning strategy to determine the optimal stimulation and training parameters for micturition in SCI subjects. Machine learning algorithms have been utilized and applied throughout in Tasks 1 and 2 to obtain the optimal conditions to enable voluntary micturition.

Task 4: We will continue with the Triple Blind Randomized Control Trial using optimal stimulation and training parameters established in Task 3. Eleven participants have completed the clinical trial. We currently have no active participants and are actively looking to enroll new individuals into our ongoing study. We are in communication with many potential participants.

4. **IMPACT:**

- **What was the impact on the development of the principal discipline(s) of the project?**
  Our hypothesis that non-invasive magnetic stimulation can improve urinary bladder function has been supported in a small number of initial experiments, as planned. This warrants continued investigation of this hypothesis and line of research.

- **What was the impact on other disciplines?**
  This research has led us to hypothesize that this type of sensorimotor rehabilitative intervention may be applicable to other indications, including central (e.g. cortical) injuries. This is only a hypothesis at this point and has not been disseminated by publication or lecture at this time.

- **What was the impact on technology transfer?**
  The PI has submitted an Invention Disclosure to the UCLA Technology Transfer Office related to the use of magnetic stimulation in rehabilitative bladder/urinary function therapy. The UCLA TTO will file a provisional patent application on behalf of the PI and UCLA.
- **What was the impact on society beyond science and technology?**
  The initial experiments are consistent with the hypotheses and objectives of this research plan and await further experimental results for confirmation.

5. **CHANGES/PROBLEMS:**

- **Changes in approach and reasons for change**
  (Recapitulating development described in the accomplishments section)

  In an allied area of sensorimotor rehabilitative research, we have discovered a method that is superior to TES in the delivery of neuromodulatory stimulation to the spinal cord. We identified TMS as a superior method as it allowed for better energy delivery to deep structures, painless stimulation, access, and durable improvements.

Related to access, several manufacturers have marketed magnetic stimulation devices, both for research and commercial use. Therefore, we conducted a small number of experiments to compare different TMS devices and coils available for research use to ensure we could replicate the settings/parameters established in our initial aims, using a new model.

The preliminary results of the effect of TMS on bladder function resulted in an influx of inquiries from females with SCI. Considering the potential impact on quality of life and function for this group, we are exploring methods to allow for comparable testing of female subjects.

- **Actual or anticipated problems or delays and actions or plans to resolve them**

- One subject discontinued participation due to ongoing health issues (not related to the current study) and associated time/travel constraints.

- The COVID-19 pandemic has also slowed our recruiting efforts, reducing our capacity to have subjects come in for scheduled visits. We continue to actively recruit individuals who are eligible to participate in this study.

- Several mission-critical research equipment items malfunctioned and required repair/replacement. At present, all relevant equipment is fully functioning.

- **Changes that had a significant impact on expenditures**
  Nothing to Report

  - **Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents**
    The UCLA IRB and DoD Humans Subjects approval were previously modified from the use of electrical stimulation to the use of magnetic stimulation.

  - **Significant changes in use or care of human subjects**
    The UCLA IRB and DoD Humans Subjects approval were modified from the use of electrical stimulation to the use of magnetic stimulation.

  - **Significant changes in use or care of vertebrate animals.**
    Not Applicable

  - **Significant changes in use of biohazards and/or select agents**
    Not Applicable

6. **PRODUCTS:**
   - **Publications, conference papers, and presentations**

     - **Journal publications.**

1. Niu, T, Bennett, CJ., Keller, TL, Leiter, JC, & **Lu, DC.** (2018). A proof-of-concept study of transcutaneous magnetic spinal cord stimulation for neurogenic bladder. *Scientific Reports, 8*, 1-12. DOI: 10.1038/s41598-018-30232-z
2. Gerasimenko Y, Gorodnichev R, Puhov A, Moshonkina T, Savochin A, Selionov V, Roy RR, **Lu DC**, Edgerton VR. (2015). Initiation and modulation of locomotor circuitry

output with multisite transcutaneous electrical stimulation of the spinal cord in noninjured humans. *J Neurophysiol, 113*(3). 834-42. doi: 10.1152/jn.00609.2014.
Status of publication: Accepted
Acknowledgment of federal support: Yes

3. Gad PN, Roy RR, Zhong H, **Lu DC**, Gerasimenko YP, Edgerton VR. Initiation of bladder voiding with epidural stimulation in paralyzed, step trained rats. *PLoS One*. 2014 Sep 29;9(9):e108184. doi: 10.1371/journal.pone.0108184. eCollection 2014.
Status of publication: Accepted
Acknowledgment of federal support: Yes
4. **Lu DC**, Niu T, Alaynick WA. Molecular and cellular development of spinal cord locomotor circuitry. Front Mol Neurosci. 2015 Jun 16;8:25.
Status of publication: Accepted
Acknowledgment of federal support: Yes
5. Niu T, Alaynick WA, **Lu DC**. Strategies and lessons in spinal cord injury rehabilitation. Current Physical Medicine and Rehabilitation Reports 2015 3;3, 206-213
Status of publication: Accepted
Acknowledgment of federal support: Yes
6. Hoffman H, Lee SI, Garst JH, Lu DS, Li CH, Nagasawa DT, Ghalehsari N, Jahanforouz N, Razaghy M, Espinal M, Ghavamrezaii A, Paak BH, Wu I, Sarrafzadeh M, **Lu DC**. Use of multivariate linear regression and support vector regression to predict functional outcome after surgery for cervical spondylotic myelopathy.mJ Clin Neurosci. 2015 Sep;22(9):1444-9.
Status of publication: Accepted
Acknowledgment of federal support: Yes

7. Gerasimenko YP, **Lu DC**, Modaber M, Zdunowski S, Gad P, Sayenko DG, Morikawa E, Haakana P, Ferguson AR, Roy RR, Edgerton VR. Noninvasive Reactivation of Motor Descending Control after Paralysis. J Neurotrauma. 2015 Aug 20.
Status of publication: Accepted
Acknowledgment of federal support: Yes
8. Lee S, Mortazavi B, Hoffman H, Lu D, Li C, Paak B, Garst J, Razaghy M, Espinal M, Park E, **Lu DC**, Sarrafzadeh M. A Prediction Model for Functional Outcomes in Spinal Cord DisorderPatients using Gaussian Process Regression. IEEE J Biomed Health Inform. 2014 Nov 20
Status of publication: Accepted
Acknowledgment of federal support: Yes

- **Books or other non-periodical, one-time publications.**
  Nothing to Report

- **Other publications, conference papers, and presentations.**

1. Alaynick. W. (2014, December). Spinal central pattern generating circuitry: From bench to bedside. Lecture presented at the European Neuroscience Institute at the University of Göttingen, Germany.

2. Lu, DC. (2018, October 12). Spinal modulation for rehabilitation. Lecture delivered at the 2018 UCLA Symposium on Neurotechnology, Los Angeles, CA.

- **Website(s) or other Internet site(s)**
  Daniel Lu, MD PhD, Associate Professor of Neurosurgery at UCLA, was interviewed in December 2018 by Ivanhoe Broadcast News. The article and video clip can be viewed at: https://www.ivanhoe.com/medical-breakthroughs/spinal-cord-stimulator-restores-bladder-control/

- **Technologies or techniques**

  In pursuit of allied research that is germane to this project, we found that magnetic stimulation is more effective than electrical stimulation. We are formally studying this discovery here and will report and disseminate these results for the rehabilitative bladder/urinary function therapy.

- **Inventions, patent applications, and/or licenses**

  The PI has submitted an Invention Disclosure to the UCLA Technology Transfer Office related to the use of magnetic stimulation in rehabilitative bladder/urinary function therapy. The UCLA TTO will file a provisional patent application on behalf of the PI and UCLA.

- **Other Products**
  Nothing to Report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name:	Daniel C. Lu, MD, PhD
Project Role:	Principal Investigator
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	2.52 person months per year. ~1 month this period
Contribution to Project:	Dr. Lu oversaw all aspects of research and administration of this program
Funding Support:	NIH U01
Name:	Yevgeniy Freyvert, MD
Project Role:	Research Resident
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	2 person months worked
Contribution to Project:	Dr. Freyvert continued intellectual development of the research plan, performed experimentation, and contributed to manuscript creation.
Funding Support:	
Name:	Aryeh Kashdan

Project Role:	Researcher
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	3 person months worked
Contribution to Project:	Aryeh Kashdan performs experimentation, assists with data collection, serves as a point of contact for the study participants, and contributes to ongoing development of the research protocol.
Funding Support:	
Name:	Kylee Tomasetti, PTA
Project Role:	Researcher
Researcher Identifier (e.g. ORCID ID):	1234567
Nearest person month worked:	1 person month worked
Contribution to Project:	Kylee Tomasetti assists with performing experimentation, data collection, and contributes to ongoing research protocol development
Funding Support:	

Personnel	**Role**	**Percent Effort**
Daniel Lu, MD, PHD	Principal investigator	17%
Yevgeniy Freyvert, MD	Research Resident	10%
Lorie Brinkman, OTD	Researcher	20%
Tina Keller, NP	Clinician	15%
David McArthur, PHD	Statistician	2%
Marc Nuwer, MD, PHD	Clinician	2%
Carol Bennett, MD	Clinician	3%
Aryeh Kashdan	Researcher	25%
Kylee Tomasetti	Researcher	6%

- **Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?**
  Nothing to Report

- **What other organizations were involved as partners?**
  Nothing to Report

8. **SPECIAL REPORTING REQUIREMENTS**

- **COLLABORATIVE AWARDS:** Not Applicable

- **QUAD CHARTS:**

Restoring Bladder Function by Spinal Cord Neuromodulation in SCI
Funding Opportunity: SC130209 (W81XWH-14-2-0129)
Clinical Trial Quarterly Progress Report

PI: Daniel C. Lu MD, PhD **Org:** UCLA, West LA Veterans Hospital **Award Amount:** $2,159,707

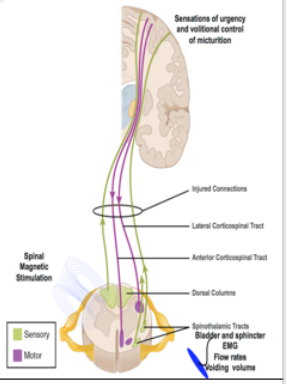
Study/Product Aim(s)

- Aim 1: Determine the optimal stimulation parameters to enable micturition in SCI subjects
- Aim 2: Determine the minimum training conditions to enable micturition in SCI subjects.
- Aim 3: Application of machine learning strategy to determining the optimal stimulation and training parameters for micturition in SCI subjects
- Aim 4: Application of the optimal stimulation and training parameters for micturition in SCI subjects in a pilot clinical trial

Approach

Aim 1 &2: Our established regimen of neuromodulation-facilitated sensorimotor rehabilitation will be applied with changes in bladder function as outcome. Aim 3: Machine leaning to determine best parameters for Aim 1-2. Aim 4: Phase 1/2 clinical trial to evaluate bladder function.

- We have shown that neuromodulation of the spinal cord in combination with conventional sensorimotor rehabilitation improves sensorimotor bladder function in SCI and TBI.
- We have established the optimal stimulation and training parameters to improve bladder function and micturition in SCI subjects
- We have started a Triple Blind Randomized Control Trial with Crossover
- Eight participants have completed the study and two subjects are active participants in the RCT.



Timeline and Cost

Activities CY	15	16	17	18	19-21
Aim1-2: Explore rehab parameters					
Aim 3: Define best parameters					
Aim 4: Apply best in Phase1/2 trial					
Estimated Budget ($K)	**$675**	**$504**	**$489**	**$329**	**$9**

Updated: (December 2020)

Goals/Milestones

CY15 Goal – Pilot trial of MagStim + motor rehab for bladder function
☑ Obtained UCLA and DoD IRB approvals for Magnetic Stimulation
☑ Explore MagStim and bladder rehab parameter space
CY16 Goals – Machine Learning; Pilot trial
☑ Apply machine learning strategy to define best parameters in pilot
☑ Complete pilot trial with naïve subjects
CY17 Goal – Machine Learning; Pilot trial
☑ Develop best parameters for trial
☑ Organize and plan for clinical trial
CY18-20 Goals –Clinical trial of MagStim + motor rehab for bladder function
☐ Complete clinical trial
☐ Organize and analyze data for conferences and publications

Comments/Challenges/Issues/Concerns

- Study enrollment and procedures that were paused due to the Covid-19 pandemic, have now resumed.
- Data analysis is in progress.

9. **APPENDICES:**
    1. University of California, Los Angeles (UCLA) Institutional Review Board Approval
    2. UCLA Continuing Review Approval
    3. Publications
        a. Lu, DC, Niu, T, & Alaynick, WA. (2015). Molecular and cellular development of spinal cord locomotor circuitry. *Frontiers in Molecular Neuroscience, 8*(25), 1-18.
        b. Niu, T, Alaynick, WA, & Lu, DC. (2015). Strategies and lessons in spinal cord injury rehabilitation. *Current Physical Medicine and rehabilitation Reports, 3*(3), 206-213.
        c. Niu, T, Bennett, CJ, Keller, TL, Leiter, JC, & Lu, DC. (2018). A proof-of-concept study of transcutaneous magnetic spinal cord stimulation for neurogenic bladder. *Scientific Reports, 8*: 12549.

Date: Monday, December 28, 2020 1:42:46 PM

Print Close

ID: IRB#14-000932 View: NEW 1.1 - Study Title and Key Personnel

This view has been locked by amendment(s)

Warning: Save your work at least every 15 minutes by clicking "Save" or "Continue."

Study Title and Key Personnel

All items marked with a red asterisk (*) are required. Items without an asterisk may or may not be required depending on whether the items are applicable to this study.

1.0 ***Full Title of the Submission:**
Restoring Bladder Function by Spinal Cord Neuromodulation in SCI

1.1 **Protocol Version Date and/or Number:**

2.0 ***Working or Lay Title:**
Restoring Bladder Function by Spinal Cord Neuromodulation in SCI

3.0 **Principal Investigator:**

3.1 ***Name:** DANIEL LU
Degree(s): If degrees are not shown here, please add them to the next section, Section 1.1a/Item 1.0, which will then update the Principal Investigator's webIRB account information.
MD, PhD.

3.2 **UCLA Title:** Associate Professor

3.3 ***Will the Principal Investigator conduct the informed consent process with potential study participants?**

- (●) **Yes**
- ( ) No
- ( ) Not Applicable

3.4 ***Is the Principal Investigator an undergraduate student, graduate student, post-doctoral fellow, or resident physician?**

( ) Yes (●) **No**

3.4.1 **If you answered "yes" to the above question, indicate the Faculty Sponsor for this study.**

3.5 **UCLA Policy 900 defines types of UCLA employees who may be eligible to serve as a Principal Investigator. Check the policy to see if the Principal Investigator for this study needs an exception to the eligibility requirements.**

If an exception is needed, either attach the letter of exception here, or indicate a Faculty Sponsor in the above item.

Document Name	Document Version #
There are no items to display	

4.0 **Study Contact Person: Indicate the person, in addition to the Principal Investigator, who should receive all of the study correspondence.**

LORIE BRINKMAN

5.0 List the key personnel and study staff below.

Note: All personnel listed below are required to complete CITI training courses (except for Fund Managers and Regulatory Coordinators). Please verify CITI training completion for all personnel prior to submitting a New Study application or Amendment application to add personnel. Verify using the Training Log tab in the application workspace (accessible by clicking the Exit button at the bottom of this page). HIPAA training is also required if personnel will be accessing protected health information.
Please make sure to have all personnel update their webIRB profile and contact information. Instructions on how to update the webIRB profile are available here.

	Name	Department	Role	Other Role (if applicable)	Will Obtain Consent?	Manage device accountability?	Access to personally identifiable info?	Access to code key?
View	CAROL BENNETT	UROLOGY	Co-Investigator		no	No	No	No
View	Marianne Bernardo	CLINICAL TRANSLATIONAL SCIENCE INSTITUTE	Study Coordinator		no	No	Yes	Yes
View	LORIE BRINKMAN	MEDCTR-REHABILITATION SERVICES	Study Coordinator		no	Yes	Yes	Yes
View	DANIEL DENIS	NEUROSURGERY	Co-Investigator		yes	Yes	Yes	Yes
View	YEVGENIY FREYVERT	NEUROSURGERY	Study Coordinator		yes	Yes	Yes	Yes
View	TALI HOMSEY	CLINICAL TRANSLATIONAL SCIENCE INSTITUTE	Research Assistant		no	Yes	No	No
View	DANIEL LU, MD, PhD	NEUROSURGERY	Other	PI	yes	No	No	No

ID: IRB#14-000932 View: NEW 1.1a - Other Personnel

This view has been locked by amendment(s)

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Other Personnel

All items marked with a red asterisk (*) are required. Items without an asterisk may or may not be required depending on whether the items are applicable to this study.

1.0 Principal Investigator

1.1 **Name:** DANIEL LU
***Please type the Degree(s):** MD, PhD.

1.2 **Principal Investigator's UCLA Department:** NEUROSURGERY

1.3 ***Protocol's UCLA Home Department:** NEUROSURGERY

This response defaults to the PI's payroll department. If you wish

to affiliate this protocol with another department, please select the department from the list above.

For tips on effective search, please see guidance to the right.

2.0 **If there will be other types of personnel working directly under the PI's supervision on aspects of the study, provide their name, title and institution, indicate their responsibilities, training and qualifications and complete Item 2.1.**

Please also indicate, if applicable, whether that person will obtain consent, manage device accountability, have access to personally identifiable information and/or have access to the code key.

Please use a new entry to add each individual unless describing a class of individuals who rotate through the study team (see guidance area to the right).

Note: If there will not be other types of personnel go to Item 3.0.

Name, title, institution	Study role(s): e.g., conduct interviews/surveys, recruit participants, obtain consent, review records, etc.

View

For existing protocols: Item 2.0 has been modified and this item cannot be edited. When submitting an amendment please use the information found in the text box below to complete Item 2.0 above.
Briefly describe the other study personnel.

2.1 **Indicate the human subjects research training these personnel have or will receive. If training is required in a language other than English or if research is occurring in a location where research personnel do not have access to the internet (e.g., rural community without internet capability), please describe how human subjects training requirements will be fulfilled.**

Check all that apply:

- [x] **CITI Training**
- [x] **UC HIPAA Training**
- [ ] Other

2.2 **If you indicated "Other" to item 2.1, describe:**

2.3 ***Will this study use the UCLA Health Sciences Volunteer Program to assist with the conduct of the research study?**

○ Yes ○ No

3.0 ***Will any of the study procedures or analyses be contracted to a consultant or an organization?**

○ Yes ◉ **No**

3.1 **If yes, specify the consultant(s) and/or organization(s) and the work that they will do for the study.**

Warning: Save your work at least every 15 minutes by clicking "Save" or "Continue."

Type of Study Review

1.0 ***Indicate the level of risk involved with this study.**

(if there are multiple groups or phases associated with this study, select the highest level of risk.)

- ○ Minimal risk or no known risks - Click here for the OHRPP tip sheet on minimal risk.
- ◉ **Greater than minimal risk**

2.0 ***Indicate the type of review that you are requesting for this study.**

- ◉ **IRB Review: Expedited or Full Board**
- ○ Certification of Exemption from IRB Review

2.1 **If you indicated "IRB Review: Expedited or Full Board" as the type of review in item 2.0, select the IRB that you think best matches your research.**

	Name	Description
○	Medical Institutional Review Board 1	MIRB1 reviews general and internal medicine, infectious diseases and ophthalmologic research.
○	Medical Institutional Review Board 2	MIRB2 reviews oncology and hematology research.
◉	**Medical Institutional Review Board 3**	MIRB3 reviews neuroscience, neurology, psychiatric, drug abuse and dental research.
○	North General Institutional Review Board	NGIRB reviews research from the College of Letters & Science and the Professional Schools.
○	South General Institutional Review Board	SGIRB reviews social-behavioral research from the Schools of Public Health, Nursing, and Medicine.

***Please note*: The above requests are for initial routing purposes only. The final decision as to committee assignment and type of review, rests with OHRPP and/or the IRBs.**

3.0 ***Is this a COVID-19 research proposal that falls under the following scope:**

a. Access to the suspected and confirmed UCLA Health COVID-19 patients.
b. Access to the electronic medical record chart or data of those patients.
c. Access to the remnant or research biospecimen collection of those patients.
d. Planning any clinical research interventional trial (drug/device) for those patients.
e. COVID Population-based studies that overlap the UCLA Health population or UCLA healthcare workers.

- ○ Yes
- ○ No

Warning: Save your work at least every 15 minutes by clicking "Save" or "Continue."

Conflict of Interest Information

1.0 * **Does the Principal Investigator, any of the key personnel, or their spouses, registered domestic partners, or dependent children, have a financial interest in the sponsor (profit, non-for-profit) of the research?**

○ Yes ◉ **No**

1.1 **If yes, attach a completed copy of the Financial Interests Form for each person who indicates a financial or related interest:**

Document Name	Document Version #
There are no items to display	

2.0 * **Does the Principal Investigator, any of the key personnel, or their spouses, registered domestic partners, or dependent children, have any financial interests related to the research sponsored by a government agency?**

○ Yes ◉ **No**

2.1 **If yes, attach a completed copy of the Financial Interests Form:**

Document Name	Document Version #
There are no items to display	

3.0 * **Indicate whether any of these financial interests have been submitted to or reviewed by the UCLA campus Conflict of Interest Review Committee (CIRC):**

◉ **Yes** ○ No

3.1 **If you have received a response from CIRC, attach it here:**

Document Name	Document Version #
Lu 2014-0038 follow up 3-13-2015.pdf	0.01
Lu 2014-0038 follow up 5-7-15 (1).pdf	0.01
Lu CIRC letter 3-19-14 DoD 14-000932.pdf	0.01

Warning: Save your work at least every 15 minutes by clicking "Save" or "Continue."

Study Locations

1.0 ***Indicate the locations where any research activities will be performed by the UCLA research team with participants and/or private information obtained.**

Check all that apply:

- [x] **a. UCLA Sites or UCLA Health System Sites (Does not include Harbor-UCLA Medical Center, Olive View-UCLA Medical Center, or Orthopaedic Institute for Children)**
- [ ] b. Off Campus (in California)
- [ ] c. Outside California (in the U.S.)
- [ ] d. Outside the United States ***See note at right**
- [ ] e. Internet

1.1

If you selected b, c or d above, please provide your assurance that documentation of each site's permission to conduct the research at the site(s) will be obtained and maintained by the UCLA PI as applicable:

Agree ☐

2.0 ***Is this a multi-institutional study (i.e., a collaborative project with other sites that have their own IRBs or principal investigators)?**
(Includes but not limited to UC MOU and CTSI MOU collaborations where UCLA IRB review is requested.)

○ Yes ◉ **No**

If no, please skip directly to the next page, do not complete the questions below.
If yes, please answer items 2.1-2.3:

2.1 **Will UCLA be responsible for the overall direction of the study at the other institutions?**

○ Yes ○ No

2.1.1 **Indicate the measures that will be taken to assure regulatory compliance at each site and that the following types of information will be communicated to the other sites: study procedures; modifications to the protocol and related documents; and safety updates, interim results and other information that may impact risks to study participants.**

Check all that apply:

- ☐ Conference calls or meetings with minutes distributed to each site
- ☐ Timely e-mail communications
- ☐ Postings on the study website
- ☐ Other

2.1.1.1 **If you chose "other", describe.**

2.1.2 **If you answered "yes" to item 2.1 above, please provide your assurance that the current IRB approval for each site(s) will be obtained and maintained by the UCLA PI as applicable:**

Agree ☐

2.2 **Will the UCLA principal investigator specified on this application be responsible for the data coordinating center?**

2.3 **Indicate the anticipated total number of study participants that will be enrolled across all of the institutions.**

*Please complete this section if you indicated that your study is greater than minimal risk **AND** that research activities will be performed at UCLA Sites or UCLA Health System Sites.*

1.0 ***Indicate where study procedures or data collection procedures - that are greater than minimal risk - will be conducted.**

Check all that apply:

- [x] **Clinical & Translational Research Center (CTRC)**
- [x] **Inpatient Medical Facility**
- [x] **Outpatient Treatment Facility/Private Office**
- [ ] Public Area
- [ ] Research Laboratory
- [x] **Other**

1.1 **If you indicated "other", specify.**
Semel institute, 760 Westwood Plaza, Room 18-265

2.0 ***Indicate the resources available to handle potential emergencies related to study procedures that are greater than minimal risk.**

Check all that apply:

- [ ] This item is not applicable to this study
- [x] **Basic Life Support (BLS) certified personnel**
- [x] **Advanced Cardiac Life Support (ACLS) certified personnel**
- [x] **Code Blue Team (hospital emergency response team)**
- [x] **Emergency crash cart**
- [x] **Paramedic Emergency Response Team (911)**
- [ ] Suicide Protocol
- [ ] Other

2.1 **If you indicated "other", specify.**

Warning: Save your work at least every 15 minutes by clicking "Save" or "Continue."

Project Identification Information

1.0 ***Type of Submission (Select one)**

- (●) **Research Study**
- ( ) Application for Approval of "Research Participant Pool" or recruitment database only

2.0 ***Type of Submission (Select one)**

For Amendments, do not undo the response below. Undoing the response may remove sections of the original application.

◉ **New Submission**

○ Transfer of Ongoing Research from Another Site from Investigator moving to UCLA. Please complete Item 2.1.

2.1 If you selected "Transfer of Ongoing Research" in Item 2.0 indicate the current status of the study and a brief summary of the work to date.

3.0 ***Who developed this study?**

Check all that apply:

- ☑ **UCLA investigator**
- ☐ Investigator from another institution
- ☐ Industry/Pharmaceutical Company
- ☐ Cooperative Group (e.g., Children's Oncology Group, AIDS Clinical Trial Group)
- ☐ Other

3.1 If other, specify.

4.0 Review For and Reliance Upon External IRBs.

***Indicate if one of the following applies to this study. (Select one)**

- ◉ **None of the options apply.**
- ○ UCLA IRB to serve as IRB of record for another institution.
- ○ UCLA to RELY on another IRB.
  This includes reliance using UC MOU, CTSI, NCI, RAND, and Western IRBs.

5.0 ***Is this study cancer related,** including the recruitment of individuals with cancer, collection of cancer human biological samples, specimens or data, or the recruitment of individuals because they are cancer survivors or at risk of developing cancer?

○ Yes ◉ **No**

Note: If you answered "Yes", you must submit an application to the Jonsson Comprehensive Cancer Center (JCCC) Internal Scientific Peer Review Committee (ISPRC). Click here for instructions for submitting to the ISPRC. The ISPRC approval notice or letter of exemption should be attached in Section 2.1/Item 7.2 of the webIRB application.

6.0 ***Nurse Involvement:** Does this study involve any nursing time, effort, and/or resources at UCLA Health System sites, including as subjects, investigators, clinical care providers or data or specimen collectors?

○ Yes ◉ **No**

Note: If you answer "Yes", please submit an application to the Nursing Practice Research Council (NPRC). For contact information or for more information about NPRC and how to apply, click here. ***IRB approval is not contingent on NPRC approval and you do not need to upload documentation of approval from the NPRC into webIRB.***

7.0 ***Federal regulations (45 CFR 46.111) require scientific review before an IRB approves a study. For the majority of studies being reviewed and approved by the UCLA IRB, the IRB performs this review.**
See http://ora.research.ucla.edu/OHRPP/Documents/Policy/4/Scientific_Review.pdf for additional details.

Do you want the IRB to consider external scientific or scholarly review?

(●) **Yes** ( ) No

7.1 **If yes, indicate the source of scientific or scholarly review for the study.**

Check all that apply.

- [ ] National Institutes of Health (NIH)
- [x] **The funding agency (other than NIH)**
- [ ] Faculty Sponsor
- [ ] JCCC – Internal Scientific Peer Review Committee (ISPRC)
- [ ] Clinical Translational Research Center (CTRC)
- [ ] UCLA Department
- [ ] Other

7.1.1 **If you checked "other", describe.**

7.2 **Attach a copy of the scientific or scholarly review, if applicable.**

Document Name	Document Version #
Summary Statement DoD Bladder 2013.pdf	0.01

ID: IRB#14-000932 View: NEW 2.2 - Lay Summary and Keywords

This view has been locked by amendment(s)

Warning: Save your work at least every 15 minutes by clicking "Save" or "Continue."

Lay Summary and Keywords

Please provide the following information about your study.

1.0 ***Provide a brief lay summary describing this study. (limit 500 words).**

We are performing a study on individuals with spinal cord injury that has resulted in reduced bladder function. We will administer a mild magnetic stimulation to the skin over the spinal cord to activate the part of the spinal cord that controls the bladder. The participants will undergo stimulation and training to move their legs for several sessions as it appears this helps activate the bladder-related parts of the spinal cord. Then subjects will be examined for bladder function using specialized equipment used for measuring urine flow and bladder pressure. 24 subjects will be enrolled in this study of 4 years. Each subject will participate in the study for 6 months where they will have twice-a-week visits and be followed for 1 year afterwards.

2.0 ***List three to five keywords describing this study (separate the words with commas). The keywords may be used for identifying certain types of studies.**

Spinal cord injury, bladder, stimulation

3.0 * **Is this study conducted or supported by HHS (e.g., the National Institutes of Health, Centers for Control and Prevention, etc.)?**

( ) Yes (●) **No**

4.0 * **Is this study regulated by the Food and Drug Administration (FDA)?**

(●) **Yes** ( ) No

4.1

If yes, check all that apply:

- [ ] Human Drugs
- [x] **Medical Devices**
- [ ] Biological Products
- [ ] Mobile Medical Applications
- [ ] Food Additives
- [ ] Color Additives
- [ ] Other

ID: IRB#14-000932 View: NEW 2.3 - Methods/Procedures - Descriptors

This view has been locked by amendment(s)

Warning: Save your work at least every 15 minutes by clicking "Save" or "Continue."

Methods/Procedures - Descriptors

Note: The items listed below are not an inclusive list of methods and procedures that may be used in research studies. The list only includes items that will trigger additional questions related to the research or are needed for the review process

1.0 ***Indicate all that apply to this study.**

- [x] **Audio, Visual or Digital Recordings**
- [ ] Certificate of Confidentiality for research not supported by NIH
- [ ] Clinical Trial of a Drug, Biologic, Device or a Behavioral Intervention
- [ ] Community Based Research
- [ ] Controlled Substances (Schedule I or II)
- [ ] Deception or Partial Disclosure
- [x] **Devices/Diagnostics (including Humanitarian Devices - HUD)**
- [ ] Drugs/Biologics/Dietary Supplements
- [ ] Expanded Access to Drug, Device or Biologic for Treatment Purposes (aka Compassionate Use, Treatment Use)
- [ ] Genetic Analyses/Genotyping
- [ ] Human Embryonic Stem Cells and/or Induced Pluripotent Stem Cells
- [ ] Human Gene Transfer/ Recombinant DNA
- [ ] Infectious Agents
- [ ] Non-FDA approved medical equipment used with UCLA hospital patients or research participants that operate under the UCLA Hospital License.
- [x] **Radiation (Standard of Care or Investigational Use of radioactive materials, radiation producing machines or ionizing radiation)**
- [ ] Substance Abuse Research (with Medication)
- [ ] Treatment in an Emergency Setting (with request to waive consent)
- [ ] **None of the above**

2.0 ***Will the study require services or resources owned/rented/operated or provided by the UCLA Health System (e.g. clinic and/or hospital visit(s), CTRC, professional medical services, clinical treatment, diagnostics, labs, medical supplies, etc.)?**

Please direct any questions about this to The Financial Coverage & Activation Team at coverageanalysis@mednet.ucla.edu.

(●) **Yes** ( ) No

ID: IRB#14-000932 View: NEW 2.4 - Coverage Analysis

This view has been locked by amendment(s)

Warning: Save your work at least every 15 minutes by clicking "Save" or "Continue."

Coverage Analysis

1.0 ***Will all protocol-required items and services that produce data for the study be funded by intramural or extramural funding/support?**

- (●) **Yes - we will not bill participants or their insurers for any protocol-required items or services**
- ( ) No - we will bill one or more protocol-required items or services to participants or their insurers
- ( ) Not Applicable – this is a non-interventional study (e.g., observational/registry/retrospective study without active treatment) that does not require additional visits, labs, items or services performed solely due to study participation

Note:

*If "**Yes**" is selected to the question above, then the corresponding "Research Only" cost language in the guidance to the right should be included in the ICF, and an abbreviated coverage analysis review is indicated.*

*If "**No**" is selected to the question above, then the "Mixed Cost" language in the guidance to the right should be included in the ICF, and a full coverage analysis review is indicated.*

*If "**Not Applicable**" is selected to the question above, then coverage analysis may not be applicable, and the corresponding "All Standard of Care" cost language in the guidance on the right should be included in the ICF.*

2.0 ***Is your study any of the following?**

- **Treatment Use (Expanded Access, Compassionate Use, or a Humanitarian Use Device)**
- **Hematology-Oncology Clinical Research Unit Study**
- **UCLA reliance on an external IRB review**

( ) Yes (●) **No**

Note: *If you have selected yes, then continue with question 3.0 below.*

3.0 **Please upload a copy of your study protocol below:**

Document Name	Document Version #
There are no items to display	

The following item pertains to investigational drugs and devices only.

4.0 **If the study participant or a third party payor (i.e., medical insurance/Medicare/Medicaid) may be billed for investigational products (i.e., investigational drugs and/or devices), attach relevant documentation to support charges including but not limited to FDA and/or CMS approval letter(s).**

Document Name	Document Version #
There are no items to display	

0… 1

ID: IRB#14-000932 View: NEW 6.1 - Funding and Other Study Characteristics

This view has been locked by amendment(s)

Warning: Save your work at least every 15 minutes by clicking "Save" or "Continue."

Funding and Other Study Characteristics

1.0 *Indicate the funding status for this study.

- (●) **Funded**
- ( ) Application for funding is pending
- ( ) Departmental funding / Self funding / No funding

2.0 *Check all that apply:

- [x] **The research will be conducted through the UCLA Clinical and Translational Research Center (CTRC)**
- [x] **The study will be supported by or conducted in collaboration with the U.S. Department of Defense (DOD)**
- [ ] The study will be supported by or conducted in collaboration with the U.S. Department of Energy (DOE)
- [ ] The study will be supported by or conducted in collaboration with the U.S. Department of Justice (DOJ)
- [ ] The study will be supported by or conducted in collaboration with the U.S. Department of Education (ED)
- [ ] The study will be supported by or conducted in collaboration with the U.S. Department of Protection Agency (EPA)
- [ ] **None of the above**

2.1 **If you selected DOD, DOE, DOJ, ED, and/or EPA support/collaboration, please provide your assurances that you will review the additional requirements for research supported by the relevant federal agency.**

Agree ☑

Note: Please refer to the Federally-Supported Research section of the OHRPP guidance document: Funding Considerations for Federally-Funded and Industry-Sponsored Human Research.

ID: IRB#14-000932 View: NEW 6.2 - Funding - Description

Warning: Save your work at least every 15 minutes by clicking "Save" or "Continue."

Funding - Description

Based on the response to section 6.1/item1, this study is or will be funded. Please provide the following information.

The Office of Contract and Grant Administration (OCGA) provides the list of funding sources used by webIRB in this section. Please check your OCGA paperwork to find the correct name of the funding source(s) for this study. Identifying the right funding source is important because:

- webIRB will auto-populate the designated funding source name on the approval letter for the study. Many funding sources require an accurate identification of their name on the IRB approval letter before they will release funding;
- The Office of Research Administration uses data from webIRB to generate funding reports.

Click here for tips on how to find the funding source name in webIRB.

1.0 **Identify the funding source(s).**
If a specific funding source has ended, do not delete it, instead please click Update next to the funding entry and **revise item 1.9**.

	Funding Source	Funding Source Information
View	DA-ARMY MEDICAL RESEARCH ACQUISITION ACTIVITY	

Name of the Funding Source	DA-ARMY MEDICAL RESEARCH ACQUISITION ACTIVITY
If other, specify	*No Value Entered*
UCLA PI named on the grant, contract, subcontract or gift:	DANIEL LU
Indicate the type of award:	Grant
If other award, specify	*No Value Entered*
Indicate the Grant Title:	Restoring Bladder Function by Spinal Cord Neuromodulation in SCI
Indicate the Award Number assigned by the funding source:	SCI130209
Indicate the description that applies to the source of funding named in the above item. If this is a subcontract, indicate the original source of funding:	Federal
If Other, specify	*No Value Entered*
Attach a copy of the funding proposal, subcontract, or scope of work.	**Document Name**: DOD-Lu-10-2013.pdf **Document Version #**: 0.01
Does the content of this IRB application differ from the activities described in the attached funding proposal, subcontract, or scope of work?	No
If yes, describe:	*No Value Entered*
Check this box to indicate that this specific funding has ended	*No Value Entered*

Warning: Save your work at least every 15 minutes by clicking "Save" or "Continue."

Study Design

1.0 ***Check all that apply to the study design.**

- [x] **Direct subject contact ONLY – The research activities involve direct contact with study participants (e.g., collection of data or specimens in person or via internet, phone, mail, etc.)**
- [ ] **No direct subject contact** – None of the research activities involve direct contact with study participants and include only analyses of data, records and/or human biological specimens (e.g., medical record or other record review, study of specimens left over from clinical procedures).
- [ ] **BOTH Direct subject contact AND No direct subject contact** – Some of the research activities involve direct contact with study participants and some of the research activities involve analyses of data, records and/or human specimens obtained without contact with participants.

ID: IRB#14-000932 View: NEW 8.5 - Devices/Diagnostics and/or Humanitarian Devices

This view has been locked by amendment(s)

Warning: Save your work at least every 15 minutes by clicking "Save" or "Continue."

Devices/Diagnostics and/or Humanitarian Devices

You indicated that this study includes devices/diagnostics and/or a Humanitarian Device (section 2.3/item 1.0). Please provide the following information.

1.0 For this study, list all Approved or Cleared (e.g., 510(k) or Premarket Notification (PMN); Premarket Application (PMA) devices that will be used within their approved labeling.
None

2.0 Complete only if one of the following apply:

- **The research involves *investigational use of an unapproved device*. The device is not approved by the FDA for marketing**
- **The research involves *investigational use of a marketed device*. The device will be used off label for an indication not in the approved labeling.**
- **The research involves use of a *device exempt from IDE regulations per 21 CFR 812(c).* Note: These exemptions apply in rare circumstances.**
- **The research involves a *humanitarian device.***

For additional information please refer to the OHRPP guidance documents on experimental drugs and devices.

	Brand name of device	Investigational Devices Information
View	MagStim	

Trade (Brand) name of the device:	MagStim
Common (Generic) name of the device:	Magnetic Stimulator, Transcranial Magnetic Stimulator , TMS
Manufacturer of the device (if UCLA research lab, identify the lab):	MagVenture
Source of the device:	Manufacturer
If "Other" source, specify:	*No Value Entered*
FDA Reguatory Status of the Device	**Investigational Use of a Marketed Device:** The device will be used off-label for an indication and not in the approved labeling
Investigational Use of an Unapproved Device:	
Device is Exempt from FDA approval	
Humanitarian Use Device (HUD):	

3.0 Attach a copy of the Device Brochure for each device listed above, including a picture, if available. (if applicable)

Document Name	Document Version #

Document Name	Document Version #
X100_productsheet.pdf	0.01

4.0 ***Is the investigational Device(s) controlled by the PI?**

◉ **Yes** ○ No

4.1 **If no, indicate by whom:**

5.0 ***By checking this box, I provide my assurance that all the person(s) who are authorized to manage the dispensation and accountability of the device have been identified in section 1.1/item 5.0.**

Agree ☑

6.0 ***Describe the specific location where the device(s) will be stored and how the device(s) will be secured.**

The device will be stored in a locked office.

7.0 ***Provide assurance that the device(s) will only be used:**
(1) By an authorized investigator
(2) With study participants who have consented to participate in the research *OR* with an IRB approved waiver of informed consent (in vitro device studies).

Agree ☑

ID: IRB#14-000932 View: NEW 8.8 - Audio, Visual or Digital Recordings

Warning: Save your work at least every 15 minutes by clicking "Save" or "Continue."

Audio, Visual or Digital Recordings

You indicated that this study includes recordings (audio or visual) (section 2.3/item 1.0). Please provide the following information.

1.0 ***Who will transcribe the research tapes/recordings?**

Check as many as apply:

- ☑ **Members of the research team**
- ☐ Persons outside the research team

2.0 ***Is the use of recordings an optional part of the research?**

○ Yes ◉ **No**

3.0 ***Will individual study participants be able to review, edit, and erase the tapes/recordings of their research participation?**

◉ **Yes** ○ No

3.1 **If no, provide an ethical and scientific justification for NOT allowing study participants to review, edit, and erase the tapes of their research participation.**

4.0 **Transcription of Research Tapes/Recordings**

4.1 ***Type of media (Check as many as apply):**

- ☐ CD ROM

- [ ] DVD
- [x] **Digital Files**
- [ ] VHS tape
- [ ] Cassette or microcassette
- [ ] Handwritten files
- [ ] Other

4.2 * **Method of transmission (Check as many as apply):**

- [ ] Courier or mail with delivery confirmation
- [ ] Posted to a secure website
- [x] **Email**
- [ ] Other
- [ ] **Not Applicable**

4.3 * **Transcription Service (Check as many as apply):**

- [ ] Transcription service secures tapes in a secure locked area
- [ ] Transcription(s) sign confidentiality agreements
- [ ] Transmission of voice files and text files is encrypted and password protected
- [ ] Other
- [x] **Not Applicable**

4.3.1 **If you selected "other" for any/all of the above items, describe.**

ID: IRB#14-000932 View: NEW 8.11 - Radiation

This view has been locked by amendment(s)

Warning: Save your work at least every 15 minutes by clicking "Save" or "Continue."

Radiation (read-only historical information)

NOTE: *This section includes read-only* ***historical information*** *for studies that included radiation procedures prior to the September 2018 transition from webIRB to SafetyNet for describing radiological procedures. The contents of this section are superseded by any related SafetyNet application and should* ***not*** *be considered part of the approved application.*

1.0 **Are the radiological procedures standard of care?**
Note: *Please review the guidance to the right before completing this question.*
No

1.1 **If Yes, please provide the following information for EACH procedure:**

a. Type of standard of care radiological procedure.
b. Maximum number of times a subject will undergo this procedure in one year.
c. Building and room number where this procedure will be performed.

The MRSC review process cannot begin until all of the above-referenced information has been provided in the field below.

NOTE: If procedures include a radiopharmaceutical then an Investigational New Drug (IND) or Abbreviated New Drug Application (ANDA) must be described in Section 8.6.

Urodynamics under fluoroscopy of the upper pelvic region to visualize bladder. 7 procedures per year of less than 2 minutes of beam-on time per procedure.

Air kerma values for
1:fluoroscopy-guided procedures: 1.38 mGy
2. Peak skin dose: 2.18 mGy
3. Effective dose: 0.07 mGy
4. Maximum expected air kerma value for the urinary bladder imaging session: 4.0 mGy
Procedure will be performed at: 200 Medical Plaza, Suites 140, Peter Morton Medical Building, Los Angeles, CA 90095

7 total procedures at 1 per month for 6 months (0,1,2,3,4,5 and 6 months)

2.0 **Will this study involve radiological procedures beyond the standard of care?**
Note: *If you have questions about what "beyond standard of care" means or questions about the forms to use in 2.1 below, or need help or additional information, please click* ***here****.*
Yes

Important Note: If your study involves beyond standard of care radiological procedures that have not changed since previous approval through the MRSC/RDRC CARE system, upload the previously completed eight-page CARE Application in 2.2 instead of Forms A, B and/or C.

2.1 **If Yes and this is an *initial submission* or *an amendment involving changes to radiological procedures*, check all applicable administrations of radiation.**

Radiation Producing Machines - *Form A required. Click* ***HERE*** *to download form.*

2.2 **Upload Forms A, B AND/OR C and other supporting documents.**

Document Name	Document Version #
Form A Rad Pro Mach - WebIRB.pdf	0.01

Warning: Save your work at least every 15 minutes by clicking "Save" or "Continue."

Information about Study Data

This information is needed to determine how you will best protect the confidentiality of data.

1.0 ***Indicate all that apply to the study data.**

Check all that apply:

- [ ] Obtained from a medical or clinical record
- [ ] Created or collected as part of health or mental health care
- [ ] Used to make healthcare or mental healthcare decisions and/or provided to other healthcare professionals
- [x] **Research data will be entered into the participants' medical or clinical record**
- [ ] **None of the above**

2.0 ***Is it reasonably foreseeable that the study will collect information that State or Federal law requires to be reported to other officials (e.g., child or elder abuse), ethically requires action (e.g., suicidal ideation), or**

is a reportable disease?

○ Yes ◉ **No**

2.1 **If yes, explain below and include a discussion of the reporting requirements in the consent document:**

3.0 ***Indicate if any of the following are being obtained and used without any direct contact with study participants.**

- ☐ Records (Not medical)
- ☐ Human biological specimens
- ☑ **None of the Above**

4.0 *Indicate all identifiers that may be **accessed or included in the research records** for the study:

- ☑ **Names**
- ☑ **Dates**
- ☐ Age (if over 89 years)
- ☑ **Postal Address**
- ☑ **Phone Numbers**
- ☐ Fax Numbers
- ☐ E-Mail Address
- ☑ **Social Security Number**
- ☑ **Medical Record Number**
- ☐ Health Plan Numbers
- ☐ Account Numbers
- ☐ License/Certificate Numbers
- ☐ Vehicle ID Numbers
- ☐ Device Identifiers/Serial Numbers
- ☐ Web URLS
- ☐ IP Address Numbers
- ☐ Biometric Identifiers (including finger and voice prints)
- ☐ Facial Photos/Images
- ☐ Any Other Unique Identifier (this does not include the code assigned by the investigator to identify the data)
- ☐ **None of the above**

4.1 **If social security numbers will be collected explain why they are necessary, how they will be used, how they will be protected and how long they will be retained.**
Social Security Numbers are used for reimbursement. They will be protected by being kept in a locked file cabinet in a lockedroom and will be destroyed at the end of the study.

5.0 ***Select all that apply:**

- ☑ **The data and/or specimens will be directly labeled with personal identifying information when acquired by the investigator for this research**
- ☐ The data and/or specimens will be labeled with a code that the research team can link to personal identifying information when acquired by the investigator for this research
- ☐ The data and/or specimens will not be labeled with any personal identifying information, nor with a code that the research team can link to personal identifying information when acquired by the investigator for this research

☐ The data are restricted use data (A term used in Social-Behavioral research. See guidance on the right.)

5.1 Indicate how the data will be used when this study is completed.

Check all that apply:

- ☑ **Use for this study**
- ☑ **Use for possible future research**
- ☐ Use to create a bank or repository at UCLA
- ☐ Add to existing repository
- ☐ Other

5.1.1 If Other, specify:

ID: IRB#14-000932 View: NEW 9.2a - Privacy and Confidentiality

This view has been locked by amendment(s)

Warning: Save your work at least every 15 minutes by clicking "Save" or "Continue."

Privacy and Confidentiality

Important Notes:

- **Privacy is about people.** Privacy refers to a person's wish to control the access of others to themselves.
- **Confidentiality is about data.** Confidentiality refers to the researcher's plan to handle, manage, and disseminate the participant's identifiable private information.

See OHRPP Quick Guide: Protecting Privacy and Maintaining Confidentiality

1.0 *Privacy: How will the investigator maintain privacy in the research setting(s)? (e.g., interviewing participant in a room or area where conversations cannot be overheard by others, or conducting medical procedures in an examination room, or behind a curtain in an emergency room).

Patient will be consented in the clinic in a private room, behind closed doors. Subsequent testing will be conducted in a clinical research laboratory space in UCLA CTRC.

During telehealth remote visits, we will either make phone calls to patients and confirm no other individuals are present. If using zoom, we will video call the patient using a specific meeting number only accessible to the patient, and ensure no one else is present during the call.

2.0 *Confidentiality: If the protocol will collect and maintain identifiable data, explain how the planned safeguards to maintain confidentiality of identifiable data and data security are appropriate to the degree of risk from disclosure.

Note: Other sections of the application (e.g., Sections 9.3, 9.3a, 9.4, 9.5, and 15.3) will request specifications such as identification of persons who will have access to code keys or measures to comply with HIPAA requirements.

All data collected will be placed on password protected files on an encoded hard drive. The data will be de-identified and coded key placed in a password protected file and on a separate encoded hard drive. The hard drives will be placed in a locked cabinet in the office of the PI in CHS, the door to the office is locked with only access from the PI, CHS is located behind security access points during off hours.

ID: IRB#14-000932 View: NEW 9.3 - Data Security

Warning: Save your work at least every 15 minutes by clicking "Save" or "Continue."

Data Security

You indicated that the study team will have access to personally identifiable or coded information (Section 9.2/item 5). Please complete the following items.

1.0 ***Do you agree to follow the OHRPP Data Security in Research guidance and procedures?**

- (●) **Yes**
- ( ) I have an alternate equally effective plan (*Note: The plan must be attached to item #2.1*)

2.0 ***Do you have a data security plan for this study?** *(Note: a plan is not required for all studies; it may be recommended in some instance).*

( ) Yes (●) **No**

2.1 **If yes, attach it here:**

Document Name	Document Version #
There are no items to display	

3.0 ***Indicate all that apply to personally identifiable information or codes during conduct of the study:**

- [x] **The data and/or specimens will be coded**
- [ ] The personal identifying information will be removed and destroyed
- [ ] Personally identifying information will be maintained with the data and/or specimens

3.1 **If you indicated that the personal identifying information will be removed or destroyed or that the data/specimens will be coded, provide the following information:**

- **The process for removing and destroying the personal identifying information or for coding the information, and**
- **Indicate who will perform the task**

Personally identifying information will be coded. Coding will be performed by a random number generator and code assigned to study participants. The coded key will be kept on a printout in a locked file cabinet behind a locked office door in CHS.

4.0 ***Will coded or personally identifiable data be collected, transmitted or stored via the internet?**

( ) Yes (●) **No**

4.1 **If yes, indicate all that apply:**

- [ ] A mechanism such as Survey Monkey, Zoomerang, or an e-mail anonymizing service will be used to strip off the IP addresses for data submitted via e-mail.
- [ ] The data will be encrypted.
- [ ] A firewall will be used to protect the research computer from unauthorized access.
- [ ] Controlled access privileges will be used on the hardware storing the data.
- [ ] Other.

4.1.1 If you indicated "Other", describe:

5.0 ***Provide your assurances that if there is a data security breach for this study, the PI will notify the IRB and your department's IT Compliance Coordinator.**

Agree ☑

ID: IRB#14-000932 View: NEW 9.4 - Data Security Plan - During the Study

This view has been locked by amendment(s)

Warning: Save your work at least every 15 minutes by clicking "Save" or "Continue."

Data Security Plan - During the Study

You indicated that data and/or specimens for this study will be coded (Section 9.3/item 3). Please complete the following information.

1.0 During the study indicate **how data will be stored and secured** including paper records, electronic files, audio/video tapes, specimens. Specify how the **code key** will be securely maintained, as applicable.

Check all that apply:

1.1 *Electronic Data

- ☑ **Encryption or password protection software will be used**
- ☐ Secure network server will be used to store data
- ☐ Stand alone desktop computer will be used to store data (not connected to server/internet)
- ☐ A contracted outside vendor will store the code key. The vendor will have a business associate agreement with UCLA.
- ☐ Other
- ☐ **Not Applicable**

1.2 *Hardcopy Data, Recordings and Specimens

- ☑ **Locked file cabinet or locked room with limited access by authorized personnel**
- ☐ Locked lab/refrigerator/freezer with limited access by authorized personnel
- ☐ The code key will be kept in a locked file in a locked room
- ☐ The coded data and/or specimens will be maintained in a different room
- ☐ Other
- ☐ **Not Applicable**

1.3 If you indicated "Other" in item 1.1 or 1.2 above, describe here.

2.0 ***By checking this box, I provide my assurance that all the person(s) who will have access to the code key have been identified in section 1.1 or section 1.1a.**

Agree ☑

ID: IRB#24-00093 View: NEW 9.5 - Data Security Plan

Warning: Save your work at least every 15 minutes by clicking "Save" or "Continue."

Data Security Plan

You indicated that the study will have access to personally identifiable or coded information (Section 9.2/item 5). Please complete the following items:

1.0 ***After the study is completed**, indicate how the data codes and/or personal identifying information will be handled.

Check all that apply:

- [x] **All data files will be stripped of personal identifiers and/or the key to the code destroyed.**
- [ ] All specimens will be stripped of personal identifiers and/or the key to the code destroyed.
- [ ] Personal identifiers and/or codes linking the data and/or specimens to personal identifiers will be maintained for future research.
- [ ] Audio or Video recordings will be transcribed and then destroyed or modified to eliminate the possibility that study participants could be identified.
- [ ] Photos or Images will be modified to eliminate the possibility that study participants could be identified.
- [ ] Restricted use data will be destroyed or returned to the source.

1.1 **If you indicated that personal identifiers will be maintained for future research, provide the following information:**
a) How the information will be securely handled and stored
b) assure confidentiality, and
c) who will have access to the identifiers and/or codes.

2.0 **Describe any additional steps, if any, to be taken to assure that the subjects' identities and any personal identifying information are kept confidential.**

ID: IRB#14-000932 View: NEW 9.8 - Data and/or Specimens for Possible Future Use

This view has been locked by amendment(s)

Warning: Save your work at least every 15 minutes by clicking "Save" or "Continue."

Data and/or Specimens for Possible Future Use

You indicated that prospectively collected data and/or specimens would be stored for future use (Section 9.2/item 5.1). Please provide the following information.

1.0 ***Specify what information directly or indirectly linked to the subject will be provided with data and/or specimens to other investigators.**

Check all that apply:

- [x] **No subject identifiers (The data/specimens are anonymous; no one including the investigator could identify the person from whom the materials were gathered.)**
- [ ] The data will be coded (A code links the data/specimens to the study participants. A key to the code exists.)
- [ ] Personal Identifying Information
- [ ] **Not applicable, the data will not be shared outside the study team.**

2.0 **Distribution Rules: Describe the criteria used to determine the adequacy of requests to obtain data and/or specimens (e.g., the type of researchers that will be eligible to receive data):**

ID: IRB#14-00093 View: NEW 10.1 - Study Summary - Research Study

Warning: Save your work at least every 15 minutes by clicking "Save" or "Continue."

y Summary - Research Study

spinal cord can improve fine motor function of the upper limb in human subjects with incomplete quadriplegia. The critical question here is whether TESS can be used to enable spared function of sacral neuromotor networks, i.e., neural networks related to bladder function in animals and humans (3-6). The potential impact of these therapeutic interventions on the lives of individuals with urinary incontinence cannot be overestimated (7, 8). Development of magnetic stimulation to activate spared, but silent, spinal cord pathways related to bladder function in humans could represent the beginning of a paradigm shift in the rehabilitative approach to bladder incontinence as a result of SCI and potentially other neurologic injuries or stroke (9-12). It could also provide new pathways toward more advanced technologies to further enable more potential success in improving bladder function.
As we have observed with studies of the lumbosacral spinal cord in completely paralyzed SCI subjects, future development of technical capabilities to use neuromodulation of the lumbosacral spinal cord for evaluation and enabling of spared function will undoubtedly enhance our ability to improve therapies for bladder function after paralysis. By discovering spared function and revealing the potential for treatments, the cost-savings from the proposed translational studies could include reduced assistive daily care costs, increased employment, and improved quality of life—especially for incomplete SCI patients who account for the majority of these SCI patients. The proposed studies could provide a partial solution to the US$40 billion/year care and $5.5 billion/year lost productivity costs (10, 46). Of the 10 million people in the US living with paralysis, 15,000 are the result of a SCI each year. The first year of care can range from $322,000-$986,000, with lifetime costs of $1.4-4M for someone injured at 25 years of age. In addition to potentially devastating sensorimotor disturbances, there is a huge financial cost of SCI, estimated to be $13.55B in medical care, therapy, and lost productivity nationwide. This proposal may positively impact patients with chronic or new SCI and other forms of nervous system damage or disease (stroke, multiple sclerosis).

4.0 *Research Design and Methods: Describe in detail the design and methodology of the study.

1. A description of each phase of the study. Details in Section 2.
Aim 1: Define stimulation parameters that improve bladder function over 24 weeks
Aim 2: Minimal number of exercise training session to improve bladder function over 24 weeks
Aim 3: Machine learning to define best parameters in Aims 1&2
Aim 4: Use best methods of Aims 1-3 in a cohort of 12 subjects for 24 weeks
2. Sequence and timing of study procedures to be performed.
Aim 1: Urodynamic testing: Start, monthly, conclusion: 7 sessions
Self-assessment: Start, monthly, conclusion: 7 sessions
Bladder diary: Daily for 6-months
Magnetic stimulation and bladder testing: twice weekly: 48 sessions
ASIA Score: Weekly

Aim 2: Urodynamic testing: Start, monthly, conclusion: 7 sessions
Self-assessment: Start, monthly, conclusion: 7 sessions
Bladder diary: Daily for 6-months
Magnetic stimulation and bladder testing: twice weekly: 48 sessions
Motor training: twice weekly: 48 sessions
ASIA Score: Weekly

Aim 4: Urodynamic testing: Start, monthly, conclusion: 7 sessions
Self-assessment: Start, monthly, conclusion: 7 sessions
Bladder diary: Daily for 6-months
Magnetic stimulation and bladder testing: twice weekly: 48 sessions
Motor training: twice weekly: 48 sessions
ASIA Score: Weekly

• Urodynamic testing (a standard procedure for research purposes to determine bladder function):
Subject will be placed in supine position. Bladder will be emptied of existing urine by inserting a Foley catheter using aseptic technique and sterile equipment. Pre-warmed 37°C saline will be instilled at a rate of <30 mL/Min, to avoid any viscerosymapthetic reflex, until subject reports an urge to void, or 400 mL, whichever happens first. To avoid autonomic dysreflexia brought on by bladder filling (viscerosympathetic reflex), arterial blood pressure will be monitored and fluid infusion will be stopped after observing >10 mmHg increase in systolic and/or diastolic blood pressure and/or flushing. Intermittent ultrasound will be applied to assess any reflux and measure bladder distention. Rapid bladder emptying with Foley catheter will be performed if any of these changes was observed; if the symptoms do not improve nifedipine and nitrates (e.g., nitroglycerine paste or sublingual nitroglycerine) will be the next step of management. If there are any poor responses to treatment, the patient will be transferred to Ronald Reagan hospital for further management. Fluoroscopic imaging will be performed to avoid any

vescicourethral reflux during the filling phase. Subject will then be asked to volitionally void without the use of any intervention for 5 minutes. Afterwards, the subject will be asked to void with the presence of magnetic stimulation applied to the skin overlying the subject's lower thoracic or lumbosacral spine for 5 minutes. Voided volume will be collected in a 1000 mL graduated plastic urinal. For the duration of the urodynamics testing, fluoroscopic imaging of the bladder and urethral sphincters will be performed intermittently, and rectal pressure, intravesicular pressure, external urethral sphincter and detrusor muscle pressures will be monitored and recorded. Upon completion of the test, the remaining saline instilled in the bladder will be emptied and recorded as residual volume. Duration: 30-60 minutes

• Magnetic Stimulation (experimental procedure to attempt to activate sacral parasympathetic nucleus (SPN) that contain parasympathetic preganglionic neurons bladder-related circuits in the spinal cord): application of electrodes or stimulation coil to skin over the lower thoracic or lumbosacral cord spinal column. Application of 1-30 Hz of stimulation. Stimulation amplitude will start at 1% Power (Magnetic stimulation) and will be increased in increments of 1% Power (Magnetic stimulation) until the subject reports an urge to void. Maximum amplitude will not exceed 100% Power (Magnetic stimulation). Stimulation will be administered for 1-5 minute intervals at up to 6 times per session. Duration: 30-60 minutes. Some subjects find the sensation of magnetic stimulation may be used.

• Bladder Testing (a standard procedure for research purposes to determine bladder function):
Bladder testing involves monitoring of rectal pressure, intravesicular pressure, external urethral sphincter and detrusor muscle pressures while subject receives stimulation therapy. Bladder testing follows the same protocol as Urodynamics. Testing (above), the only difference being that it does not involve fluoroscopic imaging. Instilled volume, voided volume, and residual volume will be recorded for each session. Duration 30-60 minutes

• Motor Training (experimental procedure to attempt to co-activate bladder-related circuits and motor-related circuits in the spinal cord): Subject will be placed left or right recumbent and legs positioned to extend beyond the edge of a cushioned bench. The legs will be supported at the knees and ankles with swings that allow the legs to move parallel to the floor in a gravity-neutral manner. Magnetic stimulation will be administered to the skin overlying the lower thoracic and lumbosacral cord to elicit movement of the legs. Experiments will be video recorded to measure the movement of the legs. The subject's face will not be recorded. Subjects cannot review or delete the recordings. This is not an optional procedure. Electrodes may be attached to record electromyographic (EMG) signals from the muscles and goniometers attached at the hip knee and ankle to record joint angles. Duration: 45-75 minutes
• Self-Assessment (standard procedures for research purposes): Functional Independence Measure (FIM), Incontinence Quality of Life (I-QOL), Spinal Cord Independence Measure (SCIM) weekly. Self-assessments will be performed at home and/or in the clinic. Duration: 1-10 minutes
• Bladder Diary (standard procedure for research purposes): Subject will be asked to record voiding by catheterization or without catheterization. Number of accidents and bladder infections will be recorded, as well. Bladder diary will be performed at home and/or in the clinic. Duration 1-2 minutes
3. How we will protect the privacy of the subjects during data collected.
Patient will be consented in the clinic in a private room, behind closed doors. Subsequent testing will be conducted in a clinical research laboratory space in UCLA CTRC. All data collected will be placed on password protected files on an encoded hard drive. The data will be de-identified and coded key placed in a password protected file and on a separate encoded hard drive. The hard drives will be placed in a locked cabinet in the office of the PI in CHS, the door to the office is locked with only access from the PI, CHS is located behind security access points during off hours. Personally identifying information will be coded. Coding will be performed by a random number generator and code assigned to study participants. The coded key will be kept on a printout in a locked file cabinet behind a locked office door in CHS.

• The approximate duration, intervals of administration and overall length of participation.
1 hour for recruitment, 3 hours for enrollment. The study will take place over 24 weeks with twice weekly testing and each session will take about 2 hours. 100 hours total.

The temporary modifications to the study due to COVID-19 is that we are pausing all enrollment and on-site interventions and procedures until further notice. (If the IRB reviewers need clarification, then that means when lock down restrictions are being lifted and we have established a system to allow patients to be seen while limiting exposure to other individuals.)

4.1 * **Will you be providing results of any experimental tests that are performed for the study?**

- ○ Yes - Complete Items 4.1.1 and 4.1.2
- ◉ **No**

◯ Not Applicable

4.1.1 **You indicated in Item 4.1 that the research involves experimental tests. Please describe the tests, provide a rationale for providing participants with the experimental test results and explain what, how and by whom participants and their health care provider will be told about the meaning, reliability, and applicability of the test results for health care decisions.**

4.1.2 **Will tests be performed by a Clinical Laboratory Improvement Amendments (CLIA) approved lab?**

◯ Yes ◯ No

5.0 ***Indicate how much time will be required of the subjects, per visit or contact, and in total for the study.**

Enrollment: 1 hour
Initial Inclusion/Exclusion Evaluation: 3 hours
Testing: 2 hours per session, twice a week X 24 weeks: 96 hours

Total: Approximately 100 hours per subject

6.0 ***Statistics and Data Analysis: Describe the proposed statistical procedures or descriptive analyses for the study. If applicable, indicate how the sample size was determined.**

In Aims 1, 2 and 4, subjects are measured at regularly scheduled sessions over a 27 or 24 week period. Since the stimulation (Aim 1) or training (Aim 2) parameters are being adjusted toward the optimum combination in each subject over time, the amount of improvement in the primary urine volume and urine flow outcomes from “off” stimulation to “on” stimulation should also improve with time. Therefore we will fit a repeated measures (mixed) analysis of variance model with urine flow change (“off” to “on”) or urine volume change versus session (time) and determine if there is a time trend and make comparisons to baseline. We will also consider parametric models over time such as a log or logistic curve or other parametric form that allows for a floor or ceiling effect since the trend over time is likely not linear but may be monotone. If such a parametric model can be fit, with separate regression estimates for each patient, we will report statistics that measure the rate of improvement per unit time. For example if there is a constant percent change in urine flow over time in each subject, the percent change can be computed for each patient and the mean percent change per week and its standard deviation over all patients can be reported. We will carry out similar trend analyses for the secondary outcomes such as FIM bladder score, ASIA sensory, and I-QOL score. We will carry out a Poisson or other count mixed model for the number of urinary tract infections over time.

ID: IRB#14-000932 View: NEW 11.1 - Characteristics of the Study Population

This view has been locked by amendment(s)

Warning: Save your work at least every 15 minutes by clicking "Save" or "Continue."

Characteristics of the Study Population

1.0 ***Is this an observational or ethnographic study for which the number of participants observed or interviewed cannot be determined in advance.**

○ Yes ◉ **No**

2.0 **If you answered "no" to item 1.0, indicate the maximum number of study participants you hope to enroll:**
24

3.0 **How many participants do you expect you will need to recruit, consent and/or screen to meet the target number above?**
48

4.0 ***Indicate the specific inclusion criteria for enrollment of each of the groups of research participants in this study.**
If there are any inclusion criteria based on *gender, pregnancy/childbearing potential, race, ethnicity or language spoken*, explain the nature of and scientific rationale for the inclusions.
1. Male 18-75 years; This is required to have one urethral anatomy. Secondarily, more males have spinal cord injury, especially in veteran populations.
2. At least 1 year post-injury;
3. Non-progressive SCI at C2-T8 (non-conus injury);
4. Motor Complete ASIA (A, B, C or D);
5. Neurogenic bladder requiring clean intermittent straight catheterization;
6. Able to attend twice weekly testing sessions for 6 months.
7. Have intact lower extremity anatomy and able to use lower extremity for assistive standing and stepping. This is required to assess the quality of motor-function-activating spinal cord stimulation.

5.0 ***Indicate the specific exclusion criteria for each of the groups of research participants in this study.**
If there are any exclusion criteria based on *gender, pregnancy/childbearing potential, race, ethnicity or language spoken*, explain the nature of and scientific rationale for the exclusions.
1. History of autonomic dysreflexia;
2. Ventilator dependency;
3. Musculoskeletal dysfunction, unhealed fracture, pressure ulcer, active infection;
4. Clinically significant depression or ongoing drug abuse;
5. Received botox injection, or bladder surgery (suprapubic access, Brindley procedure, etc.); 6. Prostatic hypertrophy or bladder outlet disorder;
7. Cardiopulmonary disease that precludes lower extremity training or rehabilitation.

6.0 ***How (chart review, additional tests/exams for study purposes, etc.), when and by whom will eligibility be determined?**
We will recruit subjects who have sustained a cervical SCI at least one year prior to enrollment to participate in the proposed experiments; specifically, individual subjects with non-progressive SCI at C2-T8 (non-conus injury), classified as motor complete (A or B) or incomplete (C or D) on the ASIA SCI scale; specifically SCI subjects with neurogenic bladder who are performing urethral catheterization procedures for bladder care will be recruited. With these criteria, we are screening for subjects with hypertonic or hyperreflexive neurogenic bladder and are excluding subjects with areflexive or hypotonic bladder (conus lesions). The reason for this is that our strategy depends on an intact spinal cord-bladder circuitry.

ID: IRB#14-000932 View: NEW 11.2 - Characteristics of Study Population

Warning: Save your work at least every 15 minutes by clicking "Save" or "Continue."

Characteristics of Study Population

1.0 ***Indicate the age range of the study participants.**

Check all that apply:

- [ ] 0 to 6 years
- [ ] 7 to 11 years
- [ ] 12 to 17 years
- [ ] 17 or younger **in California** who can consent for themselves - see note below
- [ ] 17 or younger **outside California** who can consent for themselves - see note below
- [x] **18 years or older**

NOTE:

- For additional information on minors **in California** who are permitted to consent for themselves please refer to the section "Legal Exceptions Permitting Certain Minors to Consent" in the OHRPP Guidance document, Child Assent and Permission by Parents or Guardians
- For additional information on minors **outside of California** who are permitted to consent for themselves please refer to the section "Exceptions Outside of California" in the OHRPP Guidance document, Child Assent and Permission by Parents or Guardians

2.0 ***Indicate if any of the following populations/specimens will be specifically recruited/obtained for the study.**

- [x] **Adults who are competent to give informed consent**
- [ ] Adults unable to give informed consent
- [ ] Adults with diminished capacity to consent
- [ ] Fetal Tissue
- [ ] Neonates
- [ ] Participants Unable to Read, Speak, or understand English
- [ ] Pregnant Women/Fetuses
- [ ] Prisoners
- [ ] UCLA Faculty/Staff
- [ ] UCLA Students
- [ ] Wards
- [ ] **Unknown/Not Applicable**

3.0 ***Is it possible that there may be non-English speakers enrolled in this study or children whose parents are non-English speaking?**

(●) **Yes** ( ) No

ID: IRB#14-000932 View: NEW 14.1 - Risks & Benefits

This view has been locked by amendment(s)

Warning: Save your work at least every 15 minutes by clicking "Save" or "Continue."

Risks & Benefits

Benefits

1.0 ***Are there any potential direct benefits (physical, psychological, social or other) to study participants?**

○ Yes ◉ **No**

1.1 **If yes, describe.**

Potential Benefits of the Proposed Research to the Subjects and Others: There may be no benefit. Exercise and rehabilitation has been considered beneficial for people with SCI who are confined to a wheelchair, as immobilization can contribute to secondary pathologies such as muscle contractures, decreased cardiovascular health, pressure sores, and muscle atrophy. Because individuals respond differently, it cannot be predetermined if this research will be beneficial to a specific type

of subject. Potential benefits may include: increase in cardiovascular fitness, decrease in spasticity, and/or an improved ability to utilize lower extremity function.

2.0 ***Describe the potential benefits to society including the importance of the knowledge to be gained.**

Importance of the Knowledge to Be Gained: The proposed experiments will demonstrate whether a new strategy of neuromodulation via non-invasive spinal cord stimulation can be used to improve bladder function. Positive demonstration of the proof-of-principle of the neuromodulatory strategy would almost certainly result in significant improvements in the quality of life after a spinal cord injury and could significantly reduce the cost of healthcare for these individuals by making them more independent. The knowledge gained also will demonstrate whether a medical stimulation device that is presently approved for other neuromotor dysfunctions can be used to improve bladder function. In addition we will learn whether a newly developed technology, transcutaneous stimulation, can be used to neuromodulate the spinal cord to improve bladder function. To date, there has been virtually no progress in improving bladder function after spinal cord injuries. The potential of the proposed studies are extremely positive. Given the magnitude of the scientific evidence from which the neuromodulatory strategy has evolved combined with our preliminary evidence in humans and rats, the potential gain that can be realized by so many impaired individuals given the modest total cost to be incurred in this grant cannot be denied.

Risks

3.0 ***Indicate the potential risks/discomforts, if any, associated with each intervention or research procedure.**

Additionally discuss any measures that will be taken to minimize risks. If data are available, estimate (a) the probability that a given harm may occur, (b) its severity, and (c) its potential reversibility. The information provided should be reflected in risks section of the informed consent documents.

If this is an exempt study and there are no risks, indicate N/A. Otherwise, please see the help text.

Risk from Transcutaneous Stimulation: The transcutaneous stimulation device is noninvasive and procedure has been approved by the UCLA Institutional Review Board (IRB#11-001720). There is a minor risk of discomfort during the stimulation procedure that stops after stimulation. There is also a minor risk of skin irritation with the adhesive electrode during stimulation. If this occurs, another site can be used, or testing postponed until skin heals.

Risk from Interventions and Experimental Procedures: Because subjects must meet the criteria listed above, we expect all subjects to be in good health. The studies described may involve the following physical risks and/or discomforts: 1) increased respiration or shortness of breath; 2) increased heart rate; 3) muscle and joint soreness; 4) lowering or elevation of blood pressure; 5) dizziness; 6) skin irritation from recording electrodes, or hand placements of trainers; 7) skin abrasion from hand placements of trainers; and 8) muscle strain or joint sprain from movement, or from the force exerted by the trainers.

Most subjects will have increased respiration and heart rate due to an increase in activity. However, we do not expect the increase in respiration and heart rate to be greater than what is normally experienced during regular exercise. Many SCI subjects will likely sustain skin irritation from the recording electrodes, or hand placements of the trainers. These conditions are considered to be minimal risks and are reversible. There is some chance that subjects may sustain muscle and joint soreness, lowering or elevation of blood pressure, dizziness, or skin abrasion from hand placements of the trainers. If these events occur the experiment would cease immediately. These conditions are considered to be minimal risks and are reversible.

It is highly unlikely that a subject would feel chest pain or high blood pressure would occur that did not resolve within several minutes. These events have not occurred in our past experience. Blood pressure will be monitored throughout the testing session at 1-5 minute intervals by arm blood pressure cuff. However, if this did occur the individual would be immediately transported to the University of California, Los Angeles Emergency Unit and Drs. Lu, and/or Denis, and/or Niu notified. It is also highly unlikely that a subject would suffer a muscle strain, joint sprain, or fracture from upper extremity physical therapy. These conditions are considered to be moderate risks but rarely occur. However if these events should occur, the subject would immediately stop training and would be immediately evaluated by Dr. Lu. Standard medical procedures will be provided. The subject's primary physician would be notified as needed. These conditions are considered to be moderate risks and are reversible.

There may be a risk of autonomic dysreflexia that may cause hypertension and headaches due to increase in bladder volume during urodynamic testing. This usually resolves after immediate evacuation of bladder fluid. If blood pressure is persistently high, topical nitroglycerine or nifedipine may be administered. The NRRC is

equipped with pharmacy, nursing, and code staff to provide immediate support. The likelihood of this occurring is very low if patient has no previous history of autonomic dysreflexia, which is an exclusionary criterion for this study.
There is a risk of urodynamic study during testing due to fluoroscopy use. There is a small risk of development of cancer from x-ray used during the monthly formal video urodynamic testing, however this risk is small. The aggregate dose of exposure is approximately 1 chest x-ray during each session. This dose is low and below limits and is not expected to be harmful.
There is a risk of urinary tract infection due to the catheterization during the urodynamic procedure. However all efforts are used to minimize this risk, such as use of sterile technique. Additionally, this risk of urinary tract infection with the urodynamic testing should not exceed the infection risk of daily catheterization and bladder care.
There are no identifiable psychological, sociological, economical, or legal risks to the subjects. Furthermore, there are no alternative treatments and procedures to this upper extremity training.

Risk/Benefit Analysis

4.0 ***RISKS/BENEFIT ANALYSIS: Indicate how the *risks to the participants are reasonable in relation to anticipated benefits*, if any, to participants and the importance of the knowledge that may reasonably be expected to result from the study:**
Protection against Risk: No subject will be allowed to participate in the study without being examined by Dr. Lu. In addition, all eligible subjects will be encouraged to discuss the study with their primary physician. Furthermore, to minimize the risks, certain measures will be implemented to protect our subjects. For example, the subjects will be monitored closely to assess for evidence of complication from experimental protocol. Dr. Lu will closely monitor the subjects in regards to the procedures and will be available for consultation.
During training and testing, every subject will be acclimated slowly to EMG, gravity neutral, and stance device to make him/her feel comfortable. Each subject will be closely monitored (blood pressure, oxygen saturation, and heart rate) through each experiment and training session. The experiment will immediately come to a halt if these values become abnormal or the subject feels tired or winded or has chest pain. If these conditions persist, Dr. Lu will be immediately contacted to assess the subject and will notify the person's primary care provider when necessary.
Before and after every experiment and training session, a nurse will examine the subject's skin for irritations and abrasions. If skin irritations or abrasions are caused by the recording electrodes or hand placements of trainers, electrode and hand placement will be modified appropriately. Furthermore, the physical therapist will constantly monitor the subject's skin and muscle for signs of muscle strain, joint sprain, and skin irritation (e.g., temperature and redness).
Dr. Lu and the nurse will continually assess the appropriate condition of back and lower extremities and continuously monitor manual assistance by trainers to avoid joint sprain. Furthermore, continuous monitoring of the subject will be conducted by the staff for potential injuries. For example, signs of skin redness, swelling of joints, or spasticity can be indicators of injury when subjects have impaired sensation. The physical therapist or trained staff member will stretch the muscles of the subjects before and after each training session to prevent injury.
If any signs of risks or discomfort are noted, the experiment or training session will be immediately discontinued. If any complications arise, training will immediately stop and Dr. Lu will be informed immediately. Dr. Lu, or a designated associate, will be available on campus during all experimental sessions involving SCI subjects. In addition, the subject's primary care provider will be notified as necessary.
To protect confidentiality, each subject will be assigned a coded identification number with no association to the identity of the subject. This number will be used to distinguish all evaluations and analyses. Data will be stored on computer media and videotape and will be secured in a locked storage area of the laboratory. Only members of the research team including research assistants, post-doctoral students, and graduate students will have access to the data for analyses. Only Dr. Lu, the PI, will have access to the coding of the identification number to the subjects.
Potential Benefits of the Proposed Research to the Subjects and Others: Exercise and rehabilitation has been considered beneficial for people with SCI who are confined to a wheelchair, as immobilization can contribute to secondary pathologies such as muscle contractures, decreased cardiovascular health, pressure sores, and muscle atrophy. Because individuals respond differently, it cannot be predetermined if this research will be beneficial to a specific type of subject. Potential benefits may include: increase in cardiovascular fitness, decrease in spasticity, and/or an improved ability to utilize upper extremity function. Furthermore, if this strategy can improve bladder function, the complications of bladder management can be minimize and independence to the SCI subject restored.

Alternatives

5.0 ***Indicate the alternatives to participating in this study.**

Check all that apply.

- [x] **All types of studies - Choose not to participate in the study**
- [x] **Clinical/Intervention Studies - Receive standard of care instead of participating in the study**
- [ ] Clinical/Intervention Studies - Medication, device, or other treatment is available off study
- [ ] Item is Not Applicable (e.g., study of existing data)
- [ ] Other

5.1 **If "other" was selected, specify.**

5.2 **If this is a clinical/intervention study:**

Describe the standard of care or activities at UCLA (or study site) that are available to prospective participants who do not enroll in this study. If not applicable to your study, state not applicable (N/A).
Interventions of stimulation for bladder function will not be performed. Patient wills still have access to his/her physician for care of the bladder associated issues related to spinal cord injury.

ID: IRB#14-000932 View: NEW 15.1 - Data & Safety Monitoring Plan

Warning: Save your work at least every 15 minutes by clicking "Save" or "Continue."

Data & Safety Monitoring Plan

1.0 ***Is a Data and Safety Monitoring Plan (DSMP) required by the funding agency or other entity?**

○ Yes ◉ **No**

ID: IRB#14-000932 View: NEW 15.2 - Data & Safety Monitoring Plan (continued)

This view has been locked by amendment(s)

Warning: Save your work at least every 15 minutes by clicking "Save" or "Continue."

Data & Safety Monitoring Plan (continued)

Important Note:

All interventional studies involving more than minimal risk must include a Data and Safety Monitoring Plan (DSMP). A DSMP is a plan established to assure that each research study has a mechanism for appropriate oversight and monitoring of the conduct of the study to ensure the safety of participants and the validity and integrity of the data. The DSMP should indicate specifically whether or not there will be a formal Data Safety Monitoring Board (DSMB) or Data Monitoring Committee (DMC).

Most, but not all studies (i.e., non-interventional studies) undergoing full board review will require a DSMP. You will need a DSMP if any of the following apply:

1. This is a Phase I, II or III clinical trial
2. This is an investigator initiated trial (Section 2.1/item 3.0)
3. This study involves treatment in an emergency setting (Section 2.3/item 1.0)
4. A Data/Safety Monitoring Plan is required by the funding agency (Section 15.1/item 1.0)
5. This study is greater than minimal risk (Section 1.1b/item 1.0)

1.0 ***Indicate who will be responsible for overseeing the study safety. Check all that apply.**

☐ The Principal Investigator

- [x] **Designee of the Principal Investigator**
- [ ] The DSMP includes at least one person who is not associated with the study
- [ ] A formally constituted Data and Safety Monitoring Board (DSMB)
- [ ] Medical monitor designated by the sponsor
- [ ] Other

1.1 **If you indicated that a designee would be responsible for overseeing the study safety, or that the DSMP would include at least one person not associated with the study, provide the name(s) of this individual (s). Also, provide a brief explanation of why this person(s) would be appropriate in this role(s).**

Dr. Daniel Denis and Niu will be responsible for overseeing the study safety, along with the External Research Monitor, Victor Chang, MD.

The Research Monitor, Victor Chang, MD (Director, Spine Research, Department of Neurosurgery, Henry Ford West Bloomfield Hospital, West Bloomfield, Michigan) is responsible to oversee the safety of the research and report observations/findings to the IRB of Record or a designated official. The Research Monitor will review all unanticipated problems involving risk to volunteers or others associated with the protocol and provide an unbiased written report of the event to the IRB of Record. The Research Monitor may discuss the research protocol with the investigators, interview human subjects, and consult with others outside of the study about the research. The Research Monitor shall have authority to stop the research protocol in progress, remove individual human subjects from the study, and take whatever steps are necessary to protect the safety and well-being of human subjects until the IRB can assess the monitor's report. The Research Monitor is responsible for promptly reporting their observations and findings to the IRB.

1.2 **If you indicated "other," describe or indicate where the information can be found in the attached protocol.**

2.0 ***Provide your assurance that information about serious, unanticipated problems related to the study (e.g., adverse events, incidents and violations) will be reported to the IRB within the time frames specified by the Summary Sheet of Reporting Requirements.**

Agree ☑

Provide the following information as appropriate to the study:

3.0 ***Are there plans to perform an interim safety analysis?**

○ Yes ◉ **No**

3.1 **If yes, describe or indicate where the information can be found in the attached protocol.**

4.0 ***Have stopping rules been established for the study?**

○ Yes ◉ **No**

4.1 **If yes, describe or indicate where the information can be found in the attached protocol.**

5.0 ***Are there defined rules for withdrawing participants from study interventions?**

○ Yes ◉ **No**

5.1 **If yes, describe or indicate where the information can be found in the attached protocol.**

ID: IRB#14-000932 View: NEW 16.1 - Payment, Costs, and Injury

Warning: Save your work at least every 15 minutes by clicking "Save" or "Continue."

Payment, Costs, and Injury

1.0 ***Indicate what the participants will receive for their participation in the study.**

Check all that apply.

- [ ] No payment will be provided
- [x] **University check**
- [ ] Course Credit
- [ ] Cash
- [ ] Gift Cards/Bruincard Deposit
- [ ] Non-Monetary Gifts or Services
- [ ] Other (including vouchers for parking)

1.1 **If you selected Non-Monetary Gifts or Services or Other, describe:**

1.2 **If you selected *Cash* and/or *Gift Cards/Bruincard Deposit* please specify the estimated total amount of money you will require to pay all participants during the length of the entire study. This information is required by UCLA Business and Finance Services (BFS), the office that will provide the cash/gift cards for payment.**

2.0 **If study participants will receive financial or other payment for their participation in the study, please provide the following information:**

- If applicable, the amount each participant will receive and the payment schedule to be followed including whether partial payment will be provided when the participant does not complete the study.
- If there are different plans for different populations or sub-studies, specify the groups and describe the plans.
- If families or children will be involved in the research, clarify how the payments, items or services will be apportioned.

Subjects will receive $35 per visit, two visits per week, for either 24 or 27 weeks.

Partial payment will be provided when the participant does not complete the study.

3.0 ***Will subjects** incur **any financial obligations from participation in the study?**

○ Yes ◉ **No**

3.1 If yes, describe:

4.0 ***Indicate below that you are familiar with UCLA policy related to treatment and compensation for injury and that you will use in the consent form for this study the appropriate UC required statement describing "Treatment and Compensation for Injury." Click here to access the UCLA policy: Treatment and Compensation for Research Related Injury.**

Note: Select **Not Applicable** if study is minimal risk.

◉ **Agree**

○ Not Applicable

ID: IRB#14-000932 View: NEW 17.1 - HIPAA Authorization

This view has been locked by amendment(s)

Warning: Save your work at least every 15 minutes by clicking "Save" or "Continue."

HIPAA Authorization

According to your responses to section 9.2/item 1.0, this study uses protected health information. Please provide the following information.

1.0 ***Indicate all that apply to use of or disclosure of PHI in this study:**

- [x] **All UC participants will sign a UC HIPAA Research Authorization for Release of Personal Health Information for Research.**
- [x] **Another Institutions' Healthcare Authorization for Release of Health Information will be used or a waiver for release of health information will be granted from another Institution.**
- [x] **A Waiver of HIPAA Research Authorization is requested for screening using UC medical records. I assure that the PHI collected for this study will not be reused or disclosed, except as indicated in this application.**
- [ ] **A Total Waiver of HIPAA Research Authorization** is requested for the entire study. I assure that the PHI collected for this study from UC records will not be reused or disclosed, except as indicated in this application.
- [ ] **Limited Data Set with a Data Use Agreement** will be obtained from UC medical records. I assure that I will follow the data security plan outlined in this application to protect the identifiers from improper use or disclosure.
- [ ] **None of the above. This study will be conducted outside the United States**

If you will access information from UCLA Health records, additional permissions are required. These permissions are described at: https://ctsi.ucla.edu/researcher-resources/pages/datarequests

1.1 Please identify the persons who will access UC Health records:

- ○ CTSI Informatics Program Staff (https://ctsi.ucla.edu/researcher-resources/pages/datarequests)
- ○ Key personnel listed in Section 1.1/5.0

2.0 ***Indicate to whom or where you will grant access to personal identifying information (including PHI) as part of the study process:**

- [x] **There is no plan to share identifiers outside the study team**
- [ ] The study sponsor; on site only (if there is more than one study sponsor, specify below).
- [ ] A foreign country or countries
- [ ] Other

2.1 **If you checked "other", "a foreign country or countries", or if "there is more than one sponsor", specify.**

3.0 ***The investigator's agreement is needed to the following:**

- The protected health information requested is the minimum necessary to meet the research objectives

- The protected health information that is obtained as part of this study will not be used or disclosed to any other person other than study personnel or to the parties listed in item Section 17.1/item 2, except as required by law.

- Study Sponsors will **not** be provided with personal identifying information (including PHI) to take from the study site at any time, including the end of the study.

- Data and specimens shared with outside entities, such as study sponsors, will be coded or de-identified.

Agree ☑

ID: IRB#14-000932 View: NEW 17.2 - HIPAA - Waiver of Authorization

This view has been locked by amendment(s)

Warning: Save your work at least every 15 minutes by clicking "Save" or "Continue."

HIPAA - Waiver of Authorization

According to your responses to Section 17.1/item 1, a waiver of authorization is requested. Please provide the following information.

In addition to the information that will be requested later in this application for a waiver of informed consent, HIPAA requires the following information for a waiver of authorization:

1.0 ***Indicate why the research could not be practicably conducted without access to and use of the protected health information.**

Check all that apply.

- [x] **The PHI is needed to identify potential participants with a specific medical condition**
- [x] **It would not be feasible to individually contact the large numbers of potential subjects in the study**
- [ ] It would not be possible to locate many of the individuals whose records would be used for the study
- [ ] Many of the individuals, whose records would be used for the study, are now deceased
- [ ] Other

1.1 **If you checked "other", specify.**

ID: IRB#14-000932 View: NEW 18.1 - Identification/Recruitment Methods

This view has been locked by amendment(s)

Warning: Save your work at least every 15 minutes by clicking "Save" or "Continue."

Identification/Recruitment Methods

1.0 ***How will you identify and/or recruit participants for this study.**

Check all that apply:

- [ ] Advertisements/Flyers/Information Sheet/Internet Postings
- [x] **Direct recruitment of potential study participants (e.g., physicians talking with their own or clinic patients about the study, contact between the study team and potential subjects in person, on the phone or on the internet, etc.)**
- [ ] Random or Other Probability Sampling
- [ ] Recruitment Letters/Emails
- [x] **Referrals (e.g., referrals from non-investigator healthcare providers, snowball sampling, participants referring other participants, etc.)**
- [x] **Review of medical records to identify potential research participants**
- [ ] Review of publicly available records
- [ ] Review of other records
- [ ] Participant pool for which potential research participants have given permission for future contact
- [ ] Potential Study Participants are identified from another IRB approved study or IRB approved screening protocol
- [ ] Other

ID: IRB#14-000932 View: NEW 18.2 - Recruitment Methods

Warning: Save your work at least every 15 minutes by clicking "Save" or "Continue."

Recruitment Methods

1.0 **Please upload copies of your recruitment materials below. This includes advertisements, flyers, internet postings, recruitment scripts and letters/emails.**

Document Name	Document Version #
There are no items to display	

Ads/Flyers/Info Sheets/Internet Postings

2.0 **If you have indicated that study participants will be recruited with advertisements/flyers (Section 18.1/Item 1.0), please indicate the type of media that will be used (e.g., newspaper, radio, internet, etc.) and/or where information will be posted or distributed.**

Direct Recruitment

3.0 **If you have indicated that participants will be recruited through direct contact (Section 18.1/Item 1.0), please provide the following information:**

- A description of how, when, and where initial contact would be made (e.g. in a public setting, in a waiting room, via a phone call, via a letter, via the internet, etc.)
- If applicable to the study, indicate how the potential research participant's privacy will be maintained.
- Who will make the contact (e.g. the investigator, a patient's physician, etc.)

Recruitment of individuals with SCI will be from VA Greater Los Angeles, VA Long Beach, and UCLA Health Care System. Additional referrals will be generated from treating physiatrists, urologist, neurologists, and other clinicians at referral sites.

Initial contact will be made in person by a potential participant's physiatrist, urologist, neurologists or other clinician during an office visit. The potential participant will be provided with contact information, e.g. phone number, email address, of the PI, Dr. Lu. Once Dr. Lu has been contacted, he and his staff will schedule a visit by the potential subject for potential consent and enrollment.

3.1 If you will be directly recruiting potential participants who are your patients, students, laboratory workers or any others with whom you have a relationship of authority or unequal power, describe what measures you will put in place to avoid those approached from feeling pressured or unduly influenced to participate in the study.

Recruitment Letters/Emails

4.0 If you have indicated that recruitment letters will be distributed to participants (Section 18.1/item 1.0), please indicate who will send out the recruitment letter (i.e. will it be the investigator or other persons who have authorized access to the information), how inquiries will be handled, and if there will be follow-up contacts.

Referrals

5.0 If you have indicated that study participants will be identified from referrals (Section 18.1/item 1.0), please indicate the source of the referral (e.g., friends, other participants, healthcare providers) and how the referral will be elicited.

The SCI patients with neurogenic bladder with the above inclusion/exclusion criteria will be identified by their treating physician at any referral site, or by the SCI database at UCLA. Recruitment of subjects will be generated from database of SCI subjects in UCLA Health Care System. Additional referrals will be generated from treating physiatrists, urologist, neurologists, and other clinicians at referral sites. Subjects who may qualify will be informed about the study by their treating physicians and given contact information for the PI, to call if they are interested. If they meet chart-review based inclusion and exclusion criteria they will be invited to attend an appointment for informed consent before further procedures are conducted.
Once the patients have been identified, they will be given the opportunity to meet with the principal investigator in order to discuss the purpose and the procedures involved in the trial. Dr. Lu will complete a chart review along with medical history and neurological examination to determine the medical eligibility for each SCI subject. Additionally, Dr. Lu will determine study eligibility based on the inclusion and exclusion criteria. Experimental testing and training interventions will be initiated after the subject has been evaluated and determined to be in compliance with the selection criteria. The subjects will not be concurrently enrolled in any other experimental studies. All subjects will sign an informed consent that has been approved by the UCLA Institutional Review Board (IRB) prior to entering the study. Each subject will be assigned a subject identification number to designate all evaluations.

Research Participant Pools/Recruitment Databases

6.0 If you have indicated that subjects will be identified and recruited from a subject pool(s) or recruitment database, (Section 18.1/item 1.0), please indicate the name of the Pool or Recruitment Database and UCLA Department. If the Pool or Recruitment Database is not at UCLA, identify the location.

Warning: Save your work at least every 15 minutes by clicking "Save" or "Continue."

Review of Medical Records

1.0 *You have indicated that potential research participants will be identified from medical records (Section 18.1/item 1). Indicate the specific records to be reviewed and the

information that will be obtained to identify potential participants for this study.
Clinic records of UCLA spine surgeons will be assessed for patients with SCI. After identification of the subject with SCI, the records will be assessed for satisfaction of enrollment criteria. If the enrollment criteria are satisfied, the patient may be contacted for enrollment.

1.1 If you have a data sheet summarizing the information that will be obtained from the records, you can upload it here instead of listing the information above.

Document Name	Document Version #
There are no items to display	

Federal and State Regulations require that the IRB review the information below to determine if a waiver of consent and authorization is appropriate for use of medical record information for recruitment purposes.

2.0 *Do you assure the following?

- The information that will be reviewed is the minimal necessary to identify potential research participants for this research.
- The information that will be obtained for identification of participants will not be reused or disclosed outside the research team, except as required by law.
- All study personnel will comply with HIPAA regulations.
- Review of the medical records will not result in greater than minimal risk by taking appropriate precautions to protect the confidentiality of the information.

Agree ☑

3.0 *Indicate why the potential study participants' rights and welfare would not be adversely affected by waiving consent to review their medical records.

Check all that apply.

- ☑ **Precautions will be taken to protect the confidentiality of the research participants**
- ☑ **The information from the medical records will not be used in any way other than to identify potential research participants**
- ☐ Other

3.1 If other, describe

4.0 *Indicate why the research could not practicably be carried out without a waiver of consent.

Check all that apply.

- ☑ **The identities of the potential study participants who would meet the criteria for this study would not be known without access to their medical records**
- ☐ Other

4.1 If other, specify

5.0 NON-UC INSTUTITION(S) / AGENCY(IES) HIPAA POLICIES AND PROCEDURES

If your research will involve access, use, or disclosure of PHI held by a non-UC institution/agency, please provide your assurances that you will comply with that (those) instutition(s)/agency(ies)' HIPAA policies and procedures.

Agree ☑

Warning: Save your work at least every 15 minutes by clicking "Save" or "Continue."

Eligibility Screening

1.0 ***Will you be conducting a preliminary assessment with potential research participants to determine study eligibility during the recruitment process?**

(●) **Yes** ( ) No

You indicated that eligibility screening will be conducted during the recruitment process (Section 19.1/item 1.0). Please provide the following information.

2.0 ***Will private identifiable information be collected during the screening?**

(●) **Yes** ( ) No

2.1 **If private identifiable information is collected during screening, are there plans to retain data from participants found to be ineligible for the study?**

( ) Yes (●) **No**

2.2 **If private identifiable data will be collected during the screening, indicate your plans for retaining the data.**

- [ ] The data will be retained with identifiers
- [ ] The data will be retained without identifiers
- [x] **The data will be destroyed**

2.2.1 **If you chose more than one response above, explain.**

3.0 **Describe how screening will be performed.**

The subject will be reached by phone or will visit the offices of Drs. Lu and/or Denis and/or Niu to be interviewed about potentially participating in the study. If by phone, the potential subject will be asked to provide Dr. Lu and/or Denis and/or Niu with access to their chart. A thorough history and physical and chart review will be used to determine eligibility/suitability for study participation.

4.0 **Attach screening script(s), if applicable.**

Document Name	Document Version #
Phone_Screening_Script_clean_07312015.doc	0.01
Phone_Screening_Script_track_07312015.doc	0.01

ID: IRB#14-000932 View: NEW 20.1 - Informed Consent Process

Warning: Save your work at least every 15 minutes by clicking "Save" or "Continue."

Informed Consent Process

You indicated that adults (and/or minors who are permitted to consent for themselves) are participating in the study (Section 11.2/item 1.0 or Section 12.2/item 1.0).

For additional information on minors who are permitted to consent for themselves please refer to the section "Legal Exceptions Permitting Certain Minors to Consent" in the OHRPP Guidance document, Child Assent and Permission by Parents or Guardians.

1.0

***Indicate your plans for obtaining informed consent for this study.**

Check all that apply:

☑ **Signed consent will be obtained from the research participant or Legally Authorized Representative.**

- **Signed consent means research participants will be asked to sign and date a written consent form.**

☐ **A waiver of signed consent is requested for the entire study.** One of the following procedures will be conducted:

- **A written information sheet will** be used. Signed consent will not be obtained from research participants.
- **Oral consent** will be obtained from the research participant or Legally Authorized Representative (LAR)
- This option should be selected if the study involves consenting participants via the internet.

☐ A **waiver of consent** is being requested.

- Research participants will **not** be asked to sign a consent form or give oral consent

☐ Consent will be obtained by a collaborating institution.

1.1 **- If you checked more than one plan above, list the study groups and the plan that you will use for each.**
- If you checked "Consent will be obtained by a collaborating institution", explain the consent process and upload a copy of the most recent approved consent document in item 1.2.

1.2 **If applicable, attach the consent document(s) from collaborating institution(s).**

Document Name	Document Version #
There are no items to display	

ID: IRB#14-000932 View: NEW 20.3 - Description of the Consent Process

This view has been locked by amendment(s)

Warning: Save your work at least every 15 minutes by clicking "Save" or "Continue."

Description of the Consent Process

1.0 ***Indicate the type of setting(s) in which the consent process will be conducted.**

Check all that apply.

- [ ] In a private home
- [x] **In a private room**
- [ ] In a waiting room
- [ ] In a public setting
- [ ] In a group setting
- [ ] On the internet
- [ ] Over the telephone
- [ ] Other

1.1

If you checked more than one response, or indicated other, describe.

1.2 **If the setting is not private, describe the measures to protect confidentiality or indicate "not applicable."**

2.0 ***Indicate the measures that will be taken to provide prospective research participants with sufficient opportunity to consider whether or not to participate in the study.**

Check all that apply.

- [x] **Member(s) of the study staff will meet with the prospective participants/families to review the consent document(s) and/or provide an oral explanation of the study. Individuals will be given a chance to ask questions before making a considered decision about whether or not to participate in the study.**
- [ ] Prospective participants/families will have the opportunity to take the consent form(s) home and may discuss the documents with others prior to deciding whether or not to participate in the study.
- [ ] Prospective participants will self-administer the consent and send it back if they decide to participate in the study.
- [ ] Other

2.1 **If you indicated other, describe.**

3.0 ***Indicate the length of time subjects are given to decide whether they wish to participate in the study.**
48 hours

4.0 ***How will you assess whether subjects understand the information conveyed during the consent process?**

Check all that apply.

- [ ] Use the Subject Comprehension Tool form for research
- [x] **Investigator or study team member will evaluate during the consent process**
- [ ] Other
- [ ] Not Applicable

4.1 **If you indicated other, describe.**

5.0 ***Attach copies of the informed consent documents, information sheets, consent scripts as applicable to this study. Include copies of translated forms, if applicable.**

Document Name	Document Version #
DoD Bladder Consent Form Clean 5-28-15.docx	0.02
DoD Bladder Consent Form TrackChanges 5-28-15.docx	0.02

Warning: Save your work at least every 15 minutes by clicking "Save" or "Continue."

Cultural Considerations

The following items are designed to acquaint the IRB with cultural features of the population that you are studying that may require procedures to ensure truly informed consent.

1.0 ***Check all that apply to the population(s) with which this study will be conducted.**

- [ ] Participants may be illiterate or insufficiently literate to be able to comprehend a conventional written informed consent form.
- [ ] The participants may be reluctant or unwilling to sign a written informed consent form.
- [ ] The husbands make decisions for their wives.
- [ ] Elders make decisions for younger adult family members.
- [ ] Elders make decisions for their community.
- [ ] It is considered impolite to refuse a request.
- [ ] People are fearful of refusing requests that they regard as coming from authorities.
- [x] **None of the above are applicable to this study.**

1.1 **If any of the above items are applicable to this study, indicate the steps that you will take to ensure voluntary participation after providing the study information, and if applicable, any planned involvement with the community regarding the consent process.**

Warning: Save your work at least every 15 minutes by clicking "Save" or "Continue."

Non-English Speaking Study Participants

You indicated that you would involve non-English speaking participants in the study (Section 11.2/Item 2.0) and/or that there is a possibility that non-English speaking participants may be enrolled in the study (Section 11.2/Item 3.0). Please provide the following information.

1.0 *Indicate the method that you use to conduct the consent process[1] with participants who do not speak English.

Check all that apply.

- ☐ The consent form and other study documents will be available in the participants' primary language. Study personnel (or qualified translators) able to discuss the participation in the patients' language will be present for the consent process.
- ☑ **Study staff or qualified translators will discuss the study in the participants' language.**
- ☐ An oral consent process will be used. Study personnel (or qualified translators) able to discuss the participation in the participants' language will be present for the consent process.
- ☐ The short form or another method will be used to conduct the consent process.

Important Note: The short form may be used in very limited circumstances. For additional information please refer to the " 'Short Form' Method" section of the OHRPP guidance document, Research Involving Non-English Speaking Research Participants.

1.1 **If you checked "short form or another method", provide additional details.**

2.0 ***How will you maintain the ability to communicate with non-English speakers throughout their participation in the study?**
Indicate "N/A" if not applicable to your study.
Members of the research staff a fluent in several languages.

3.0 ***If you are conducting research for which there is a real or foreseeable risk of biomedical harm in the state of California, indicate your agreement that you will provide the participants who do not read, speak, or understand English a copy of the Research Participants Bill of Rights in a language in which they are f uent. rans ations into the most common anguages in the greater os Ange es area a downlo d on the OHRPP website.**

- ◉ **Agr ee**
- ○ Not Appli cable

[1] *If minors are involved in the study, this would also include the processe s of obtaining paren assent, as applicable.*

Warning: Save your work at least every 15 minutes by clicking "Save" or "Continue."

Department of Defense

You indicated that this study is being supported and or conducted in collaboration with the Department of Defense (Section 6.1/item 2.0). Please provide the following information.

1.0 ***How is your project linked to the Department of Defense (DOD)?**

Check all that apply.

- [x] **The project is funded by the DOD**
- [ ] The project involves cooperation or collaboration with DOD
- [ ] The project uses DOD property, facilities, or assets
- [ ] DOD personnel (military or civilian) will be research participants

2.0 ***Will surveys or interviews be conducted with DOD personnel as part of this study?**

○ Yes ◉ **No**

2.1 **If yes, consult with your program officer to identify the survey requirements of the applicable branch of the DOD.**

- [ ] Survey approval is not required
- [ ] Documentation of Survey approval is attached below
- [ ] UCLA IRB approval is required prior to approval from DOD
- [ ] Other

2.1.1 **If you indicated "Other," specify.**

2.2 **Attach documentation of DOD survey approval (if applicable).**

Document Name	Document Version #
There are no items to display	

3.0 **Prohibitions or limitations related to DOD research**

Review and indicate your assurance that you will comply with the following limitations.

3.1 ***Captured or Detained Persons**

SECNAVINST 3900.39D (Section 6(a)(8)) prohibits research involving "any person captured, detained, held, or otherwise under the control of DoD personnel (military or civilian, or

contractor employee)" except DoD personnel held for law enforcement purposes.

Agree ☑

3.2 ***Payment to Active Duty Personnel**

Based on 24 USC 30, the military limits research payments for Active Duty personnel. Unless on leave status during participation, such personnel may not receive payment for participation except for blood donation. Payment for blood donation may not exceed $50 per blood draw.

Agree ☑

3.3 ***Classified (or Sensitive but Unclassified) Research**

Because classified research involves restriction of the dissemination of results, UCLA institutional practice is to not accept such research. This prohibition includes the designation of "sensitive but not classified."

Agree ☑

4.0 DOD Documentation Requirements

The DOD requires that the IRB receive and maintain the following documentation, as applicable for each study. Indicate if the following are attached elsewhere in the application, are pending, or not applicable to this study.

4.1 ***Attach documentation of completion of the DOD education requirements (required for PIs and if applicable, faculty sponsors)**

- ○ Attached
- ◉ **Pending**

4.2 ***PI, Co-PI and Co-investigator Curriculum Vitae (attach in Section 24.0/item 1.0)**

- ◉ **Attached**
- ○ Pending

4.3 ***Scientific Review (attach at Section 2.1/item 7.2)**

- ◉ **Attached**
- ○ Pending
- ○ Not Applicable

4.4 ***Data Collection Forms/Case Report Forms (attach below)**

- ◉ **Attached**
- ○ Pending
- ○ Not Applicable

4.4.1 **Attach the data collection forms/case report forms, as applicable.**

Document Name	Document Version #
Data Collection Forms DoD Bladder.pdf	0.01

4.5 ***FDA etter for IND or IDE**

(Attach the FDA letter for an IND at Section 9.7/Item 1(1.11.2.3), and attach the FDA letter for an IDE at Section 9.8/Item 1 (1.5.1.2), as applicable.)

- ○ Attached
- ○ Pending
- ◉ **Not Applicable**

4.6 ***FDA Form 1571(attach at Section 9.8/Item 1 (1.11.2.3))**

- ○ Attached
- ○ Pending
- ◉ **Not Applicable**

4.7 ***FDA Form 1572 (attach at Section 9.8/Item 1 (1.11.2.3))**

- ○ Attached
- ○ Pending
- ◉ **Not Applicable**

Warning: Save your work at least every 15 minutes by clicking "Save" or "Continue."

DOD - Study Greater than Minimal Risk

You have indicated that this study is greater than minimal risk (Section 5.1/item 1.0).The following information is required by the DOD.

1.0 **Research Monitor.** The following information is required regarding designation of a research monitor for this study.

1.1 ***Attach a copy of the Research Monitor's *curriculum vitae.***

Document Name	Document Version #
CV - Victor Chang.doc	0.01

1.2 ***Attach a copy of the letter from the Research Monitor accepting the role.**

Document Name	Document Version #
20140930083527367.pdf	0.01

1.3 ***Indicate where the Research Monitor is named and his/her role is described.**

Check all that apply.

- [ ] Privacy and Confidentiality section of the consent form(s) (required only if the Monitor will have access to individually identifiable data)
- [x] **In Section 15 (Data & Safety Monitoring) of this application**
- [ ] In the attached protocol for this study

1.3.1 **If you indicated that the Research Monitor's role is described in the attached protocol, indicate the page number and/or section where the information can be found.**

2.0 ***Protections for Military Personnel. Check the assurance(s) applicable to your recruitment plan**

- [ ] Not Applicable - Department of Defense personnel (military or civilian) are not a target population
- [x] **I will ascertain that an individual's decision about participation has not been influenced by unit officers or senior noncommissioned officers (NCOs)**
- [ ] I will exclude unit officers and senior NCOs from recruitment/consent sessions for units under their command
- [ ] I will offer separate recruitment/consent sessions for officers and NCOs excluded from sessions held for their units
- [ ] An ombudsperson not connected to the research or to the unit shall be present to monitor group recruitment briefings
- [ ] Other - I am implementing the following protections not specified above

2.1 **If you indicated "other," describe.**

Warning: Save your work at least every 15 minutes by clicking "Save" or "Continue."

Additional Information and/or Attachments

1.0 **Attach any other documents that have not been specifically requested in previous items, but are needed for IRB Review.**

Document Name	Document Version #
Alaynick_CV_Long_2014.pdf	0.01
CV_Morteza Modaber.pdf	0.01
CV_Paymon_Rezaii.pdf	0.01
DCL Blosketch.pdf	0.01
PersonnelCITIPackages.pdf	0.01

2.0 **If there is any additional information that you want to communicate about this study, include it in the area provided. Note: this section should not be used instead of the standard application items.**

ID: IRB#14-000932 View: NEW 100.0 - Instructions for Study Submission

Instructions for Study Submission

You have completed your application, **but it has *not yet been submitted*.**

FOLLOW THESE STEPS TO SUBMIT THE APPLICATION TO THE IRB FOR REVIEW:

1. Click the **Finish** button to return to exit the SmartForm and return to the study workspace.
2. Use the **View SmartForm Progress** function to make sure that the application is complete.
3. If you are the PI or PI Proxy, click **Submit Study** under **My Activities**. If you are a member of the study team, you can let the PI know that the study is ready to submit by clicking **Send Ready Notification.**
4. Once the study is submitted, the state indicator at the top of the page will no longer display **Pre-Submission.**
5. After submission of the study, the **PI Assurances** activity will immediately become available under **My Activities.** The PI should provide his/her assurances at that time. If the PI is not available, the study can be submitted by a PI Proxy and the assurances provided at a later time. The study will be reviewed by the IRB while the **PI Assurances** are pending; however, it will not be approved until the **PI assurances** are completed.
6. ***If there is a Faculty Sponsor for the study***: The study can not be submitted to the IRB until the Faculty Sponsor provides his/her assurances through **FS Assurances** activity.

ID: IRB#14-000932 View: Display - Method Description

Audio, Visual or Digital Recordings

Click "OK" below to return to the SmartForm page where you can select the appropriate response.

ID: IRB#14-000932 View: Display - Method Description

Certificate of Confidentiality for research not supported by NIH

The Certificate of Confidentiality button in this section is only if your study is NOT supported or conducted by NIH but you will obtain a Certificate of Confidentiality (for example, for studies collecting information about illegal drug use).

If you previously checked this box for an NIH-supported study before the policy change, you do not need to change your response here.

Certificates of Confidentiality are issued by the National Institutes of Health (NIH) to protect the privacy of research subjects by protecting investigators and institutions from being compelled to release information that could be used to identify subjects with a research project. Certificates of Confidentiality are issued to institutions or universities where the research is conducted. They allow the investigator and others who have access to research records to refuse to disclose identifying information in any civil, criminal, administrative, legislative, or other proceeding, whether at the federal, state, or local level.

Effective October 1, 2017, NIH has updated its policy for issuing Certificates of Confidentiality for NIH-funded and conducted research. For information about the policy change or about obtaining Certificates for research supported by other agencies, please see https://humansubjects.nih.gov/coc/index.

Click "OK" below to return to the SmartForm page where you can select the appropriate response.

ID: IRB#14-000932 View: Display - Method Description

Clinical Trial of a Drug, Biologic, Device or a Behavioral Intervention

A clinical trial is a research study designed to answer specific questions about medical or behavioral treatments. The trial may be interventional or observational. Interventional studies are those in which the research participants are assigned by the investigator to a treatment or other intervention, and the outcomes measured. Observational studies are those in which individuals are observed and the outcomes are measured by the investigators.

Click "OK" below to return to the SmartForm page where you can select the appropriate response.

ID: IRB#14-000932 View: Display - Method Description

Community Based Research

Click "OK" below to return to the SmartForm page where you can select the appropriate response.

ID: IRB#14-000932 View: Display - Method Description

Controlled Substances (Schedule I or II)

Check here only if you are using a Schedule I or II Controlled substance in this study. Research using Schedule I or Schedule II controlled Substances must be submitted to the Research Advisory Panel of California for review and approval prior to initiation. Research using Schedule III, IV, or V Controlled Substances as a study drug do not require review by the Research Advisory Panel. For further information see: http://ag.ca.gov/research/guide.php o Schedule I Controlled Substances are drugs or substances with a high potential for abuse, that have no currently accepted medical use in treatment in the United States. Examples of Schedule I Controlled Substances are: heroin, lysergic acid diethylamide (LSD), methylenedioxy-methamphetamine (MDMA), marijuana, and psilocybin. o Schedule II Controlled Substances are drugs or substances with a high potential for abuse, that have a currently accepted medical use in treatment in the United States, or a currently accepted medical use with severe restrictions. Examples of Schedule II Controlled Substances are: fentanyl, methadone, methylphenidate, morphine, and oxycodone. For further information see: http://www.deadiversion.usdoj.gov/schedules/index.html

Click "OK" below to return to the SmartForm page where you can select the appropriate response.

ID: IRB#14-000932 View: Display - Method Description

Deception or Partial Disclosure

Deception includes withholding information about the real purpose of the study or purposely giving subjects false information about some aspect of the research to prevent bias. Some professions, such as the American Psychological Association (APA) have ethical codes regarding the use of deception in research. (See sections 8.07 and 8.08 at http://www.apa.org/ethics/code/index.aspx#807) If deception is included in the study, you must also apply for approval of a waiver of the informed consent process (Section 20.1) in addition to selecting the other consent procedures planned for the study (e.g., written or oral consent).

Click "OK" below to return to the SmartForm page where you can select the appropriate response.

ID: IRB#14-000932 View: Display - Method Description

Click "OK" below to return to the SmartForm page where you can select the appropriate response.

ID: IRB#14-000932 View: Display - Method Description

Devices/Diagnostics (including Humanitarian Devices - HUD)

A medical device is defined, in part, as any health care product that does not achieve its primary intended purposes by chemical action or by being metabolized. Medical devices include, among other things, surgical lasers, wheelchairs, sutures, pacemakers, vascular grafts, intraocular lenses, and orthopedic pins. Medical devices also include diagnostic aids such as reagents and test kits for in vitro diagnosis (IVD) of disease and other medical conditions such as pregnancy. For further information see:
http://www.fda.gov/oc/ohrt/irbs/irbreview.pdf
Drugs/Biologics/Dietary Supplements

- Drug: The term "drug" means: articles recognized in the official United States Pharmacopoeia, official Homoeopathic Pharmacopoeia of the United States, or official National Formulary, or any supplement to any of them; and articles intended for use in the diagnosis, cure, mitigation, treatment, or prevention of disease in man or other animals; and articles (other than food) intended to affect the structure or any function of the body of man or other animals.
- Biologics vs. Drugs: Most drugs consist of pure chemical substances and their structures are known. Most biologics, however, are complex mixtures that are not easily identified or characterized. Biological products differ from conventional drugs in that they tend to be heat-sensitive and susceptible to microbial contamination. This requires sterile processes to be applied from initial manufacturing steps. For more information see: http://www.fda.gov/consumer/updates/biologics062608.html#drugs
- Dietary Supplements are products that are intended to supplement the diet and have one of the following ingredients:
  - ☐ A vitamin
  - ☐ A mineral
  - ☐ An herb or other botanical
  - ☐ An amino acid
  - ☐ A dietary substance for use by man to supplement the diet by increasing the total daily intake
  - ☐ A concentrate, metabolite, constituents, or an extract of combinations of these ingredients.

For additional information see: http://www.foodsafety.gov/~dms/supplmnt.html

Click "OK" below to return to the SmartForm page where you can select the appropriate response.

ID: IRB#14-000932 View: Display - Method Description

Expanded Access to Drug, Device or Biologic for Treatment Purposes (aka Compassionate Use, Treatment Use)

Click "OK" below to return to the SmartForm page where you can select the appropriate response.

ID: IRB#14-000932 View: Display - Method Description

Genetic Analyses/Genotyping

Genetic analyses/genotyping include, but are not limited to, studies of inheritable conditions or traits, gene markers or mutations, and pedigrees.

Click "OK" below to return to the SmartForm page where you can select the appropriate response.

ID: IRB#14-000932 View: Display - Method Description

Human Embryonic Stem Cells and/or Induced Pluripotent Stem Cells

Research with human embryonic stem cells (hESC) and related lines requires IRB review under the following conditions: o Clinical research in which human subjects are given hESCs or related products. o When the UCLA research team will have a research related direct interaction or intervention with the cell donors, including donation of blastocysts or gametes for the purpose of creating hESCs,. o Cells provided to the UCLA research team that have identifiers or codes that can be linked back to the donor. Research involving hESC requires review and approval by the ESCRO Committee. For further information see: http://www.stemcell.ucla.edu/research

Click "OK" below to return to the SmartForm page where you can select the appropriate response.

ID: IRB#14-000932 View: Display - Method Description

Human Gene Transfer/ Recombinant DNA

Studies involving gene transfer and/or recombinant DNA require approval of the UCLA Institutional Biosafety Committee (IBC). Human gene transfer is an investigational method for correcting defective genes responsible for disease development through one of the following techniques: o A normal gene may be inserted into a nonspecific location within the genome to replace a nonfunctional gene. o An abnormal gene could be swapped for a normal gene. o The abnormal gene could be repaired through selective reverse mutation, which returns the gene to its normal function. o The regulation of a particular gene could be altered. Recombinant DNA molecules, according to the NIH Guidelines, are defined as either: (i) molecules that are constructed outside living cells by joining natural or synthetic DNA segments to DNA molecules that can replicate in a living cell, or (ii) molecules that result from the replication of those described in (i) above.

Click "OK" below to return to the SmartForm page where you can select the appropriate response.

ID: IRB#14-000932 View: Display - Method Description

Infectious Agents

Studies involving the use of Risk Group 2 or 3 infectious agents (such as bacteria, fungi, parasites, prions, rickettsia, viruses, etc.) require approval of the UCLA Institutional Biosafety Committee (IBC).

Click "OK" below to return to the SmartForm page where you can select the appropriate response.

ID: IRB#14-000932 View: Display - Method Description

Non-FDA approved medical equipment used with UCLA hospital patients or research participants that operate under the UCLA Hospital License.

Clinical Engineering is responsible for completing incoming inspections on investigational devices that are used to diagnose, treat or monitor a patient and that are used in the patient care area on site at UCLA, but *not* in other hospitals such as Cedars Sinai, CHLA, or Drew. If a device is FDA and/or testing - laboratory approved for the purpose it was designed, then evaluation is not required of the device. If you have a copy of an inspection report from Clinical Engineering, please attach here. As appropriate, please contact Clinical Engineering at 310-267-9000 to arrange an inspection.

Click "OK" below to return to the SmartForm page where you can select the appropriate response.

ID: IRB#14-000932 View: Display - Method Description

Radiation (Standard of Care or Investigational Use of radioactive materials, radiation producing machines or ionizing radiation)

Note: This includes CT-guided biopsy, fluoroscopy use, etc.; MRI is not included. The radiological procedures included in this study must be described in the SafetyNet system. Please create a new SafetyNet application after submitting this webIRB application to the IRB for review.

Click "OK" below to return to the SmartForm page where you can select the appropriate response.

ID: IRB#14-000932 View: Display - Method Description

Substance Abuse Research (with Medication)

Research for the treatment of controlled substance addiction or abuse that uses any drug (scheduled or not) as treatment, requires the review and approval of the Research Advisory Panel of California prior to initiation. For further information see: For further information see: http://ag.ca.gov/research/guide.php

Click "OK" below to return to the SmartForm page where you can select the appropriate response.

ID: IRB#14-000932 View: Display - Method Description

Treatment in an Emergency Setting (with request to waive consent)

Federal regulations allow certain research activities to be conducted in emergency settings with waiver of informed consent - in the interest of facilitating potentially life-saving and life-enhancing research with protecting the rights and welfare of participants. For further information see: o OHRP Guidance: http://www.hhs.gov/ohrp/humansubjects/guidance/hsdc97-01.htm o FDA Guidance: http://www.fda.gov/oc/ohrt/irbs/except.html

Click "OK" below to return to the SmartForm page where you can select the appropriate response.

ID: IRB#14-000932 View: Display - Method Description

None of the above

Click "OK" below to return to the SmartForm page where you can select the appropriate response.

University of California Los Angeles
10889 Wilshire Blvd, Suite 830
Los Angeles, CA 90095-1406

http://ora.research.ucla.edu/ohrpp
General Campus IRB: (310) 825-7122
Medical IRB: (310) 825-5344

APPROVAL NOTICE

DATE:	4/25/2019
TO:	DANIEL LU, MD PhD NEUROSURGERY
FROM:	JAMES MC GOUGH, MD Chair, MIRB3
RE:	IRB#14-000932-CR-00005 2019 Review for IRB#14-000932 Restoring Bladder Function by Spinal Cord Neuromodulation in SCI

The UCLA Institutional Review Board (UCLA IRB) has approved the above-referenced study. UCLA's Federalwide Assurance (FWA) with Department of Health and Human Services is FWA00004642.

Submission and Review Information

Type of Submission	Continuing Review
Type of Review	Full Board Review
Approval Date	4/25/2019
Expiration Date of the Study	4/24/2020
Funding Source(s)	1) DA-ARMY MEDICAL RESEARCH ACQUISITION ACTIVITY *Grant PI*: DANIEL LU *Grant Title*: Restoring Bladder Function by Spinal Cord Neuromodulation in SCI *Grant Number*: SCI130209
Initial IRB Approval Type & Date for this Study	Full Board 11/3/2014

Specific Conditions for Approval

-- **Translations Needed** - Please submit translated copies of your consent and

screening documents as an amendment(s) before recruiting or consenting any subjects for whom these translations are required. [If medical study being conducted in CA--Be sure and provide subjects with the appropriately translated Research Participant's Bill of Rights. Numerous translations are available for download on the HRPP website at http://ora.research.ucla.edu/OHRPP/Pages/BillofRights.aspx.]

-- **Research Participants Bill of Rights** - By California law, a copy of the Research Participants Bill of Rights in a language in which the participant is fluent must be given to all research participants in this study as there is a real or foreseeable risk of biomedical harm. Numerous translations are available for download on the HRPP website at http://ora.research.ucla.edu/OHRPP/Pages/BillofRights.aspx .

Regulatory Determinations

-- **Waiver of Signed Informed Consent** - The UCLA IRB waived the requirement for signed informed consent for telephone screening under 45 CFR 46.117(c)(2) / 21 CFR 56.109(c)(1). However, appropriate signed HIPAA authorization will be obtained for any access to medical records during the screening process.

-- **Medical Device - Nonsignificant Risk** - The UCLA IRB determined that the medical device being used in this study is a non-significant risk device and the abbreviated IDE requirements must be followed [21CFR 812.2(b)].

Documents Reviewed included, but were not limited to:

Document Name	Document Version #
14-000932_DoD Bladder Consent Form Clean Copy_2017-06-19.pdf.pdf	0.01
14-000932_Phone_Screening_Script_clean_07312015.pdf.pdf	0.01

Important Note: Approval by the Institutional Review Board does not, in and of itself, constitute approval for the implementation of this research. Other UCLA clearances and approvals or other external agency or collaborating institutional approvals may be required before study activities are initiated. Research undertaken in conjunction with outside entities, such as drug or device companies, are typically contractual in nature and require an agreement between the University and the entity.

General Conditions of Approval
As indicated in the PI Assurances as part of the IRB requirements for approval, the PI has ultimate responsibility for the conduct of the study, the ethical performance of the project, the protection of the rights and welfare of human subjects, and strict adherence to any stipulations imposed by the IRB.

The PI and study team will comply with all UCLA policies and procedures, as well as with all applicable Federal, State, and local laws regarding the protection of human subjects in research, including, but not limited to, the following:

- Ensuring that the personnel performing the project are qualified, appropriately trained, and will adhere to the provisions of the approved protocol,
- Implementing no changes in the approved protocol or consent process or documents without prior IRB approval (except in an emergency, if necessary to safeguard the well-being of human subjects and then notifying the IRB as soon as possible afterwards),
- Obtaining the legally effective informed consent from human subjects of their legally responsible representative, and using only the currently approved consent process and stamped consent documents, as appropriate, with human subjects,
- Reporting serious or unexpected adverse events as well as protocol violations or other incidents related to the protocol to the IRB according to the OHRPP reporting requirements,

- Assuring that adequate resources to protect research participants (i.e., personnel, funding, time, equipment and space) are in place before implementing the research project, and that the research will stop if adequate resources become unavailable,
- Arranging for a co-investigator to assume direct responsibility of the study if the PI will be unavailable to direct this research personally, for example, when on sabbatical leave or vacation or other absences. Either this person is named as co-investigator in this application, or advising IRB via webIRB in advance of such arrangements.

frontiers in Molecular Neuroscience

REVIEW
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Molecular and cellular development of spinal cord locomotor circuitry

Daniel C. Lu, Tianyi Niu and William A. Alaynick*

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The spinal cord of vertebrate animals is comprised of intrinsic circuits that are capable of sensing the environment and generating complex motor behaviors. There are two major perspectives for understanding the biology of this complicated structure. The first approaches the spinal cord from the point of view of function and is based on classic and ongoing research in electrophysiology, adult behavior, and spinal cord injury. The second view considers the spinal cord from a developmental perspective and is founded mostly on gene expression and gain-of-function and loss-of-function genetic experiments. Together these studies have uncovered functional classes of neurons and their lineage relationships. In this review, we summarize our knowledge of developmental classes, with an eye toward understanding the functional roles of each group.

Keywords: interneuron, motor neuron, transcription factor, locomotion, sensory, circuit

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Introduction

More than 20 distinct embryonic classes of neurons have been described in the spinal cord, and the developmental sources of their diversity have been elucidated over the past decade (**Figure 1**). This cellular diversity has been organized into a schema that defines major groups of neurons based on their expression of embryonic transcription factors. The major characteristics of these classes their generation, transcription factors, subsets, positions, neurotransmitters, connections, and functions are summarized here.

Spinal cord development is subject to phylogenetically ancient organizing principles such as those that guide segmentation from the invertebrates, such as arthropods, to the vertebrates, such as mammals. Cellular identities in vertebrate spinal cord are specified during development along the three basic spatial axes of the embryonic body plan – rostral–caudal, dorsal–ventral, and medial–lateral. In addition, there is a temporal influence of development on these spatial coordinates such that distinct cell fates emerge at different times during development. This yields a four dimensional system for establishing spinal neuron cell fate that has been reviewed extensively (Jessell, 2000; Jankowska, 2001; Lee and Pfaff, 2001; Muroyama et al., 2002; Helms and Johnson, 2003; Goulding and Pfaff, 2005; Kiehn, 2006; Ladle et al., 2007; Stepien and Arber, 2008; Dasen and Jessell, 2009; Goulding, 2009; Grillner and Jessell, 2009; Hegarty et al., 2013).

To summarize briefly, the rostral–caudal positional identities are coordinated by opposing gradients of fibroblast growth factor (Fgf, caudalizing) and retinoic acid (RA, rostralizing; **Figure 2**; Muhr et al., 1999; Liu et al., 2001; Dasen et al., 2008). The dorsal–ventral axis is governed by ventralizing Sonic hedgehog (Shh) produced by the floorplate, and dorsalizing signals from the roof plate such as bone morphogenetic proteins (BMPs) and Wnts (which are members of the Wingless + MMTV integrants, *Int* family). These diffusible morphogens form gradients that activate specific transcriptional responses at defined points in the gradient (Roelink et al., 1994; Liem et al., 1995;

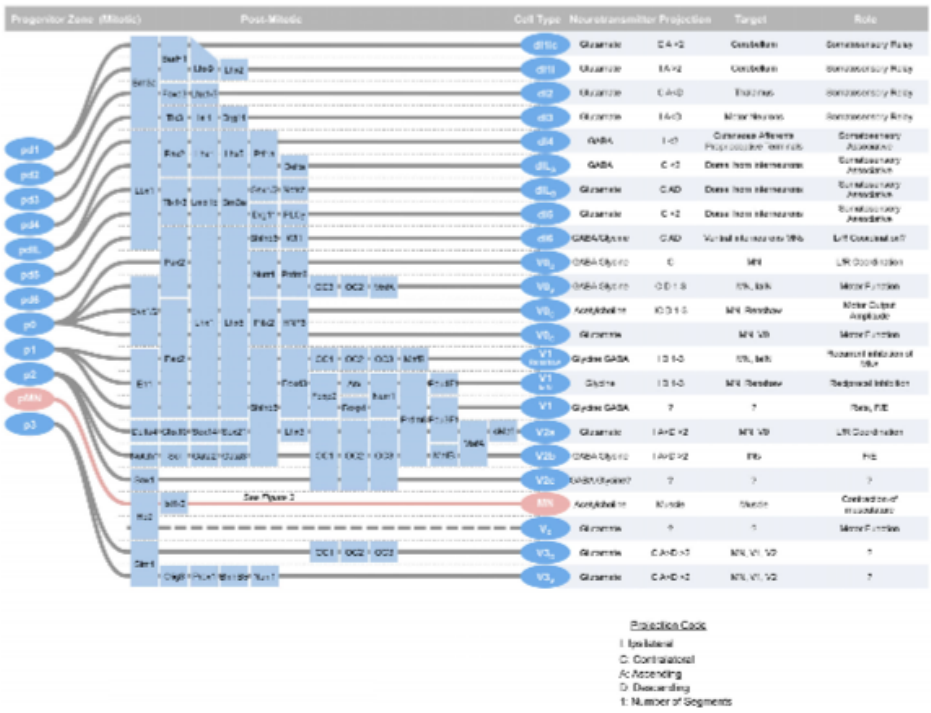


FIGURE 1 | At around mid-gestation, progenitors exit the cell cycle and begin to take up characteristic setting positions, extend axons, and express transcription factors and neurotransmitter biosynthetic enzymes. Over the last week of development, 23 classes of neurons can be defined by transcription factor expression. Adapted from Alaynick et al. (2011).

Ericson et al., 1996; Lee et al., 1998; Megason and McMahon, 2002; Muroyama et al., 2002; Timmer et al., 2002). These transcriptional programs first specify and reinforce the identities of progenitor cells, and second, act to oppose adjacent transcriptional programs and sharpen boundaries between progenitor zones. In the ventral cord, these transcription factors are grouped into two classes, those that are inhibited by Shh (Class I) and those that are activated by Shh (Class II; Briscoe et al., 2000). Spinal cord development is also organized along a medial–lateral axis dividing progenitor cells that are located adjacent to the lumen of the neural tube, medially, whereas differentiating progeny migrate laterally. Over time, a given progenitor domain defined by these spatial coordinates may sequentially produce distinct cellular classes.

Within an idealized spinal cord segment, this system establishes thirteen progenitor pools along the dorsal–ventral axis (**Figure 2**). There are eight dorsal interneuron progenitor divisions, pd1–6 and the late-born pdILA and pdILB, four ventral interneuron progenitor divisions, p0–3, and one motor neuron progenitor domain, pMN (Alaynick et al., 2011). The identities of these domains are predominantly defined by basic-helix-loop-helix (bHLH) domain transcription factors, such as Ngn, Olig2, and Math (Bermingham et al., 2001; Gowan et al., 2001; Novitch et al., 2001; Scardigli et al., 2001; and homeodomain proteins, such as Pax3, Dbx1, and Nkx6.1 (Briscoe et al., 2000; Vallstedt et al., 2001; Subsequently, additional transcription factors, predominantly of the LIM-homeodomain family, such as Lhx1 and Isl1, are expressed in sub-groups of these domains, further refining cell fate into at least 23 distinct classes (Tsuchida et al., 1994; Gross et al., 2002; Muller et al., 2002; Thaler et al., 2002; Cheng et al., 2004).

Ventral Compartment of Distinct Progenitor Cells

pMN Fate

The pMN cell domain gives rise to: (1) 100s of genetically distinct groups of cholinergic alpha motor neurons clustered into motor pools that innervate specific skeletal muscles; (2) gamma motor neurons that innervate intrafusal fibers of specific skeletal

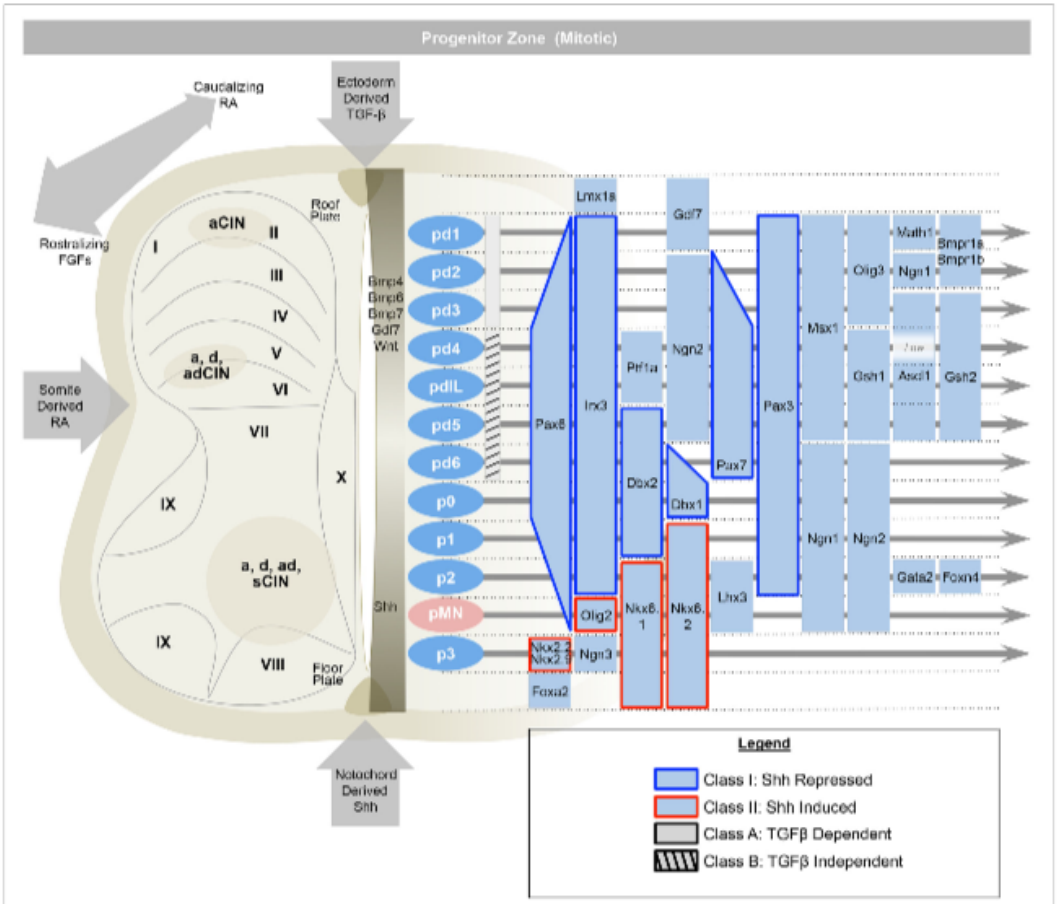


FIGURE 2 | The early spinal cord (e9.5–e11) is influenced by Sonic-hedgehog (Shh) ventrally, ectoderm-derived TGF-beta family members dorsally, and retinoic acid from the somite, laterally. This establishes 13 progenitor domains (including the late born pdlLA and pdlLB) that express transcription factors that help to define progenitor identities and refine boundaries between progenitor domains. Ventrally, Class I transcription factors are repressed by Shh (e.g., *Irx3*), while Class II are induced (e.g., *Olig2*). Similarly, the dorsal-most domains, pd1–pd3, are dependent on TGF-beta and the pd4–pd6 and pdlL domains are independent of TGF-beta signaling. Adapted from Alaynick et al. (2011).

muscles for proprioception; (3) the predominantly thoracic (T1–12, and to L1 and L2 **in some species) cholinergic preganglionic** sympathetic neurons; **(4) the cholinergic parasympathetic motor** neurons in the sacral **(S2–4) cord; and (5) oligodendrocytes found** throughout the spinal **cord (Figure 3). The motor neurons that** effect muscle move**ment are primarily alpha, with fewer beta,** motor neurons.

pMN Birth and Early Development

The recognition that a supernumerary notochord could induce the generation of additional motor neurons led to the identification of the diffusible morphogen, Shh, that induces **neural precursors to a MN fate (**Watterson, 1965**; Roelink et al., 1994). An early marker of motor neuron development, the LIM-HD transcription factor, *Isl1*, indicated that motor neuron precursors are born between HH Stages 15 and 17 in chick and beginning at E9.5 (~25 somites) in mouse (Figure 4A;** Ericson et al., 1992**; Roelink et al., 1994; Pfaff et al., 1996; Gould et al., 2006). These** progenitors **will** give rise to somatic alpha mo**tor neurons that** innervate skeletal muscle in the medial and **lateral motor** columns (LMCs), gamma motor neurons that **innervate intra**fusal fibers of the muscle spindles, and pre**ganglionic motor** neurons of the autonomic nervous system. Ge**neration of each** of these classes and their organization

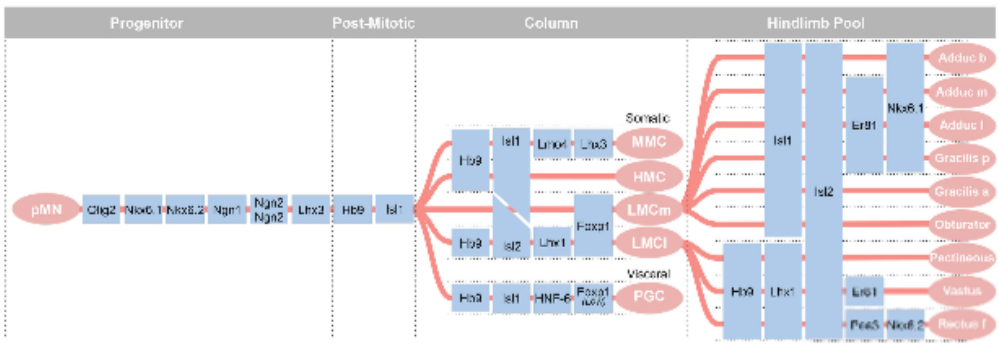


FIGURE 3 | The most diverse spinal cord neuron class belongs to the motor neurons. These arise from a uniform progenitor domain before differentiating in to classes that can be grouped by columns and by motor pools. Motor pools are clusters of motor neurons that innervate a single muscle. A transcription factor code is emerging to define each of the over 200 motor pools that innervate distinct muscles. Adapted from Alaynick et al. (2011).

into motor columns and motor pools requires the subsequent expression of additional transcription factors (Lin et al., 1998; Dasen et al., 2005, 2008). These factors then drive the unique characteristics of that motor pool, such as guidance to the target and establishment of proper connectivity with sensory neurons and interneurons. Interestingly, this transcriptionally defined program is complemented by activity-dependent processes that control cellular connectivity and function (Hanson and Landmesser, 2004; Myers et al., 2005). In the case of gamma motor neurons, the nuclear receptor *Errγ* is expressed in these motor neurons and their survival is dependent on GDNF signaling (Gould et al., 2008; Friese et al., 2009; Shneider et al., 2009; Ashrafi et al., 2012).

The motor neuron progenitor domain is ventral to the *Irx3* expressing p2 domain that delimits *Olig2* expression and is dorsal to the p3 domain that expresses *Nkx2.2* and *Nkx2.9* to delimit *Pax6* expression. The expression of *Nkx6.1* and *Nkx6.2* acts to limit transcription factor expression to *Olig2*, that in turn drives the expression of MN transcription factors *Hb9 (Mnr2 in chick)*, and *Ngn2* (Briscoe et al., 2000; Sander et al., 2000; Vallstedt et al., 2001; Shirasaki and Pfaff, 2002). *Hb9*, expressed during the final cell division of pMNs, is sufficient to drive the expression of *Isl1*, *Isl2*, *Lhx3*, and *ChAT*—as well as its own expression—establishing pMN independence from Shh (Tanabe et al., 1998). Like *Mnr2*, the HD transcription factor, *Hb9*, can induce the formation of motor neurons when ectopically expressed. Loss of *Hb9* in mouse, however, results in ectopic upregulation of a V2 IN marker gene, *Chx10*, but does not result in complete loss of motor neurons or of fictive locomotion (Arber et al., 1999; Thaler et al., 1999; Alaynick, Pfaff unpublished observations).

Motor Neuron Subtypes

The Medial and Hypaxial Motor Columns (MMC and HMC)

The medial sub-group of motor neurons innervates axial musculature and is found the length of the spinal cord. There are two divisions of this group, the medial motor column (MMC) and the hypaxial motor column (HMC or MMCl). Both express *Isl1* and *Isl2*, although the ratio of expression varies, with greater *Isl1* expression in the HMC than MMC at E11.5 and greater *Isl2* in the MMC than HMC by E13.5 in mouse (Tsuchida et al., 1994; Thaler et al., 2004). The MMC innervates dorsal or epaxial musculature, while HMC innervates ventral or hypaxial musculature. Initially all motor neuron progenitors express the LIM homeodomain transcription factor, *Lhx3*. *Lhx3* expression is maintained in the MMC while *Lhx3* expression is downregulated in the HMC and LMC (Tsuchida et al., 1994). Motor neuron (*Hb9* promoter) dependent expression of *Lhx3* results in conversion of LMC motor neurons to a MMC identity (Sharma et al., 2000).

The Lateral Motor Column (LMC)

At limb levels, the 50 or so muscles of the limb are innervated by motor neurons occupying a lateral motor column (Landmesser, 1978). Neurons of the lateral portion of the LMC (LMCl) are later born than the MMC motor neurons, and like the cortex, migrate in an inside-out arrangement such that LMC neurons are born in the proliferative ventricular zone of the pMN domain and then migrate through the MMC to form the LMC. While initially expressing *Lhx3*, a hallmark of MMC identity, these motor neurons down-regulate *Lhx3* by an unknown mechanism and begin to express transcription factors not found in MMC that are definitive for LMC identity. The factors include *Foxp1*, *Lim1*, and the enzyme *Raldh2* (Sharma et al., 1998, 2000; Sockanathan and Jessell, 1998). The lateral motor column has lateral (LMCl) and medial (LMCm) divisions that innervate the dorsal and ventral portions of the limb, respectively, and these cell fates are partially regulated by RA signaling (Sockanathan et al., 2003; Ji et al., 2006). In the LMCl, *Lim1* and *Hb9* are expressed while *Isl1* is downregulated. In the LMCm, there is low *Hb9* and maintained *Isl1* expression. The LMCm and LMCl both express *Isl2*, which is downregulated in the MMC and HMC (Misra et al., 2009). The LMCm and LMCl are further subdivided into motor pools, each

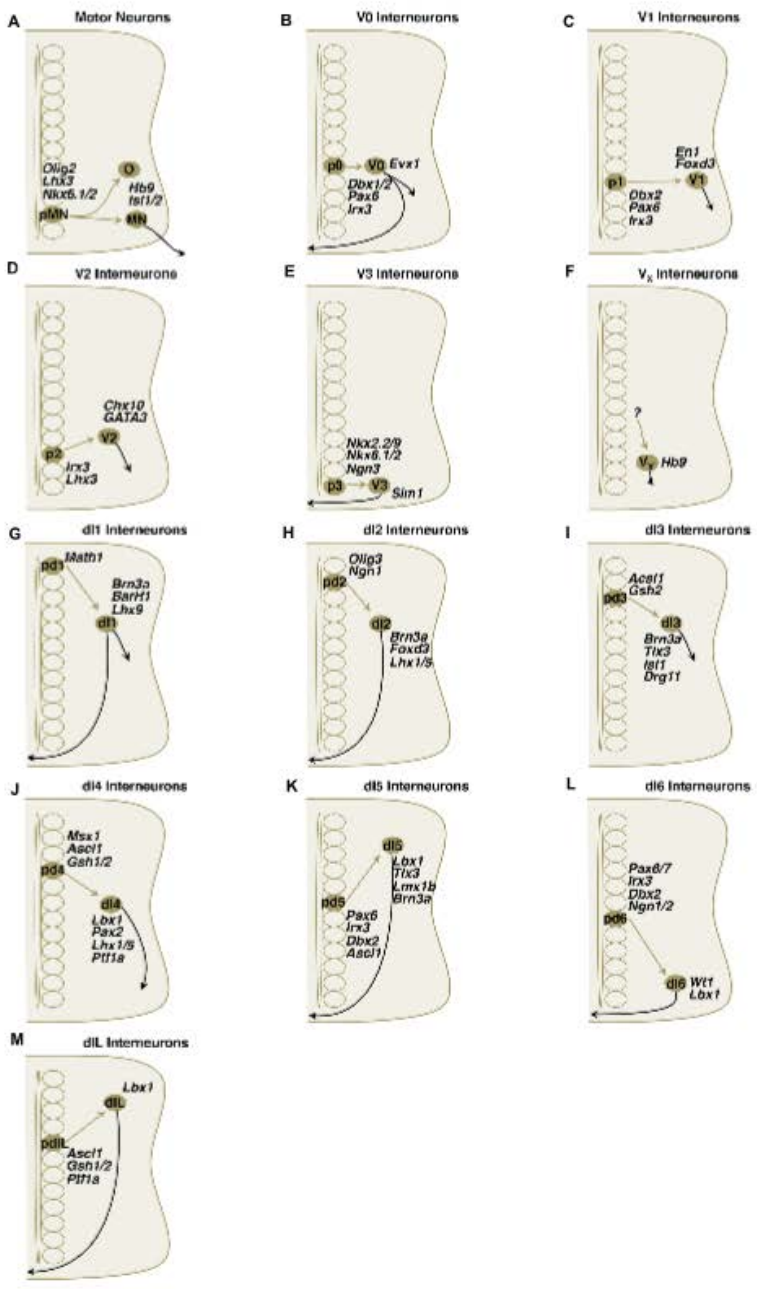


FIGURE 4 | (A–M) Simplified schematic illustrations of development of MNs and ventral/dorsal subclass interneurons with important transcriptional factors.

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innervating a specific muscle of the limb. These individual motor pools are defined by their expression of *Ets* and *Nkx* transcription factors that constitute a more refined transcriptional code (De Marco Garcia and Jessell, 2008).

The rostro-caudal regions of the LMC appear to be determined in part by homeobox (Hox) genes. *Hox6* is characteristic of brachial level, *Hox9* of thoracic and *Hox10* of lumbar. Disruption of the Hox genes in mouse or chick has shown that these boundaries can be profoundly altered to create an expansion of lateral motor columns into thoracic regions (Jung et al., 2010). More strikingly, loss of the *Hox* co-factor *Foxp1* disrupts the ability of motor neurons to incorporate the homeobox code for spatial information, and results in a loss of defined motor pools in the LMC (Dasen et al., 2008).

Preganglionic Motor Neurons (PGC)

Preganglionic motor neurons of the sympathetic nervous system are the most dorsal motor neurons and can be identified by their expression of *ChAT*, *NADPH Diaphorase*, and some members of the one-cut transcription factor class (Francius and Clotman, 2010). Preganglionic motor neurons are also dependent on the downregulation of *Lhx3* and are lost with continued, *Hb9*-dependent expression of *Lhx3* in all motor neurons, or loss of *Foxp1 or Isl2* (Sharma et al., 2000; Thaler et al., 2004; Dasen et al., 2005, 2008).

Spinal Interneurons

A great deal has been learned about the development of discrete classes of interneurons by describing them by electrophysiology, behavioral output, and by expression of proteins involved in transcription, neurotransmitter signaling, and intracellular signaling. Currently, this schema has defined over 20 interneuron types in the spinal cord. While one can argue that every neuron has a unique molecular/genetic expression profile, dendritic arborization and axonal projection pattern, this grouping schema has been useful in organizing interneurons into functionally related groups.

Historically, two broad groups have been defined: the "V" interneurons with progenitors that are found in the ventral cord and are grossly associated with motor function, and a *d*orsal *I*nterneuron, dI class, associated predominantly with sensory processing. Most studies have examined development within a single or a few segments. A recent study examined rostro-caudal differences at one time point, e12.5 (Francius et al., 2013). This showed that subclasses of ventral interneurons (V0, V1, V2, and V3) exhibit distinct organizational patterns at brachial, thoracic and lumbar levels of the developing spinal cord. Furthermore, each cardinal "V"' class of ventral interneurons can be subdivided into several subsets according to further combinatorial expression of transcription factors (Francius et al., 2013). Given these caveats that likely apply to other interneuron classes, the V and dI interneuron classifications are a simplification with exceptions, some of which are listed below. Despite these limitations, the V and dI schema is a useful approach to the subject.

V0 Interneuron Characteristics

Local projecting V0 neurons are a population of primarily contralateral, with some ipsilateral projecting neurons with inhibitory or excitatory identity that send axons 2–4 spinal segments rostrally (Moran-Rivard et al., 2001; Pierani et al., 2001). They receive inputs from ipsilaterally projecting Chx10$^+$ glutamatergic V2a interneurons (Crone et al., 2008; **Figure 5**). They are the dorsal-most ventral progenitor pool and are characterized by their expression of the *Dbx* (developing brain homeobox) homeodomain transcription factor, *Evx1/2* (even-skipped homeobox 1; **Figure 4B**). *Dbx1* and *Dbx2* are expressed in dividing cells, although *Dbx1* may be briefly expressed in post-mitotic cells (see V1 discussion Pierani et al., 1999). Four V0 interneuron subclasses have been described to date: V0$_V$, V0$_D$, V0$_C$, and V0$_G$ (Pierani et al., 1999, 2001; Moran-Rivard et al., 2001; Lanuza et al., 2004; Zagoraiou et al., 2009). Early studies addressed the V0 class by eliminating *Dbx1* and showing that the *Evx1*$^+$ V0$_V$ subclass was lost because these neurons become fated to an *En1*$^+$ V1-like subclass and astrocytes (Pierani et al., 2001; Lanuza et al., 2004). Because *Dbx1* is transiently expressed, a *Dbx1*^{LacZ} knock-in allele was used to show that with loss of *Dbx1*, E18.5 embryos retained 40% of the βgal$^+$ cells and resulted in a 25% expansion in the number of *Lbx1*$^+$ *Pax2*$^+$ dI6-like commissural neurons (Lanuza et al., 2004). By perinatal time points, genetic strategies to track *Dbx1*$^+$ cells using βgal find that most of these cells are neural by expression of *NeuN* and are found in lamina VIII where commissural interneurons reside. Lineage labeling of Dbx1-derived cell reveals a large abundance of glia (Lanuza et al., 2004). Moreover, the Dbx1 lineage includes many dorsal horn neurons as this transcription factor is also expressed in dorsal domains. Loss of *Dbx1* results in loss of V0$_D$ and V0$_V$ subclasses, whereas loss of *Evx1* results in a loss of only the V0$_V$ subclass (Moran-Rivard et al., 2001; Pierani et al., 2001; Lanuza et al., 2004). V0 and V1 classes both express *Lhx1* and *Lhx5*, markers of inhibitory spinal interneurons (Pillai et al., 2007).

V0 Birth and Early Development

In mouse, the majority of *Dbx1*$^+$ progenitors appear between E10 and E13 and give rise to V0$_D$ and V0$_V$ commissural interneurons (Moran-Rivard et al., 2001; Pierani et al., 2001; Lanuza et al., 2004). *Dbx1/2* expression is found in the rostral CNS at stage 13 in chick and more caudally by stage 15 (Pierani et al., 1999). *Evx1/2* positive V0 cells are generated at stages 17 and 18 and appear in the ventral domain of *Dbx1* and *Dbx2* expression (Pierani et al., 1999). Ventral *Evx1/2* expressing V0 neurons appear at stages 17–18 within the ventral expression domain of *Dbx1* and *Dbx2*, and then migrate ventrally (Pierani et al., 1999). The V0 class appears from a *Pax6*$^+$, *Dbx1/2*$^+$, *Pax3/7*$^-$ domain that is the dorsal-most ventral progenitor domain (Pierani et al., 1999).

V0 Interneuron Subtypes

V0$_V$

The primarily inhibitory V0$_V$ class is distinguished by transient expression of the homeodomain transcription factor, *Evx1*. These cells arise from the ventral portion of the *Dbx1*$^+$ progenitor domain, and like all post-mitotic cells arising from *Dbx1*$^+$

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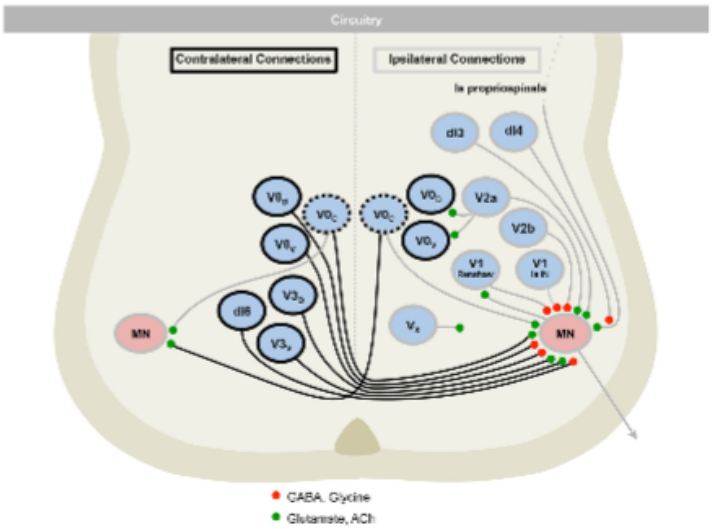


FIGURE 5 | The motor circuitry is shown in diagrammatic form in the lower panel. Here, neurons can be divided by projection patterns, that are ipsilateral, contralateral, or both. Three classes of neurotransmitter are found in the cord: excitatory glutamatergic (e.g., V2a), inhibitory GABAergic/glycinergic (e.g., V2b), and excitatory cholinergic neurons (e.g., motor neurons). Roles for neurons in defining rate (e.g., V1), left–right alternation (e.g., V0) and rhythmicity (e.g., V3), are emerging. Adapted from Alaynick et al. (2011).

progenitors, they share a similar post-mitotic migration and commissural axon pattern (Moran-Rivard et al., 2001; Pierani et al., 2001). The $V0_V$ interneurons are implicated in locomotion as indicated by increased *c-fos* immunoreactivity following fictive locomotion (Lanuza et al., 2004). However, *Evx1* knockout mice have grossly normal locomotion patterns despite a ~70% reduction in the $V0_V$ interneurons and loss of appropriate contralateral intersegmental axonal projections in the remaining ~30% of interneurons (Moran-Rivard et al., 2001). A subset of the $V0_V$ class has been reported to be excitatory in an unpublished observation (Zhang et al., 2008).

$V0_D$

Unlike the $V0_V$ subclass, the more dorsal *Dbx1*$^+$ progenitors of the glycinergic/GABAergic $V0_D$ class do not express *Evx1* (Pierani et al., 2001; Lanuza et al., 2004). And while both $V0_D$ and $V0_V$ classes have similar axon guidance and cell body position, the loss of the $V0_D$ class, in conjunction with $V0_V$ class, does appear to alter locomotor behavior. When *Dbx1* is knocked out, eliminating all V0 progenitors, a disruption of left–right coordination is observed at lumbar levels L2 and L5. These periods of left–right synchrony are intermittent and periods of normal left–right alternation are observed amidst episodes of synchrony (Lanuza et al., 2004). No disruption of flexor-extensor behavior, as indicated by alternating phasic activity of the L2 and L5 segments, was observed in a drug-induced isolated cord fictive locomotion assay (Lanuza et al., 2004). Recently, studies have showed that a cluster of $V0_D$ cells lateral to the central canal receive substantial input from primary afferents and preferentially project axons toward contralateral motoneurons via an oligosynaptic pathway, and are active during fictive locomotion. This suggests that this subset of V0 interneurons may be primarily responsible for coordination of left–right alternation during locomotion (Griener et al., 2015).

$V0_C$ and $V0_G$

The $V0_C$ and $V0_G$ subclass represent ~5% of V0 progenitors and are identified by expression of *Pitx2* and occupy a medial position dorsal to the central canal (Zagoraiou et al., 2009). These cells were first observed in lumbar levels at E11.5–12.0 by Pitx2 immunoreactivity which, unlike many embryonic markers, could be detected until postnatal day 30 (Zagoraiou et al., 2009). Neurotransmitter markers can subdivide the *Pitx2*$^+$ cells into cholinergic (*vAChT*$^+$ and *ChAT*$^+$) and glutamatergic (*vGluT2*$^+$) types that are distinct (Zagoraiou et al., 2009). While these are found at cervical and lumbar levels, within the lumbar cord, these two types are distributed in a gradient such that a greater number of cholinergic interneurons are found at more rostral levels and a greater number of glutamatergic interneurons at more caudal levels (Zagoraiou et al., 2009). The cholinergic cells are distinct from *Pitx2*$^-$ cholinergic C_3 propriospinal interneurons (Zagoraiou et al., 2009). By genetic tracing, ~80% of these neurons were determined to be from a *Dbx1*$^+$ progenitor domain at E12.5 and loss of *Dbx1* eliminated the Pitx2 immunoreactivity in the intermediate cord. Because $V0_C$ and $V0_G$ *Pitx2*$^+$ cells transiently express *Evx1*, they appear to be subsets of the $V0_V$

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class. This relatively small ipsilaterally and bilaterally projecting class, however, is responsible for perhaps all c-boutons on motor neurons found in P8 to P25 mice (Zagoraiou et al., 2009; Stepien et al., 2010). These interneurons provide relatively weak innervation to *Sox14::eGFP*$^+$ V2a and calbindin$^+$ V1 Renshaw cells. These cells appear to be involved in local circuitry as corticospinal and sensory *vGluT1*$^+$ glutamatergic boutons were not found, whereas serotonergic and *GAD67*$^+$ GABAergic boutons were observed (Zagoraiou et al., 2009). While previous experiments did not find a gross locomotor behavioral defect with loss of the $V0_V$ subclass in *Evx1* mutant animals, *Pitx2* mutant animals were found to have defects in locomotion revealed by EMG recordings during swimming (Zagoraiou et al., 2009). This deficit was argued to represent an abnormal integration of sensory inputs. It may, alternatively or in addition, represent a deficit in C-terminal modulation of motor neuron excitability. A survey in E12.5 mice showed that several V0 subclasses can be defined by expression of *Pax2, Pax6, Evx1, Ptx2, Nurr1, HNF-6, BhlhB5,* and *Prdm8* (Francius et al., 2013).

V1 Interneuron Characteristics

As a population, this group appears to control burst durations and is comprised of cells physiologists defined as Ia inhibitory and Renshaw cells. Mice models without V1 and V2b showed significant difficulty with limb articulation in flexion and extension (Zhang et al., 2014). The pV1 progenitor domain gives rise to important inhibitory subclasses of neurons that were previously described electrophysiologically: the Ia inhibitory interneurons that mediate reciprocal inhibition and the Renshaw cells that mediate inhibitory feedback to integrate limb and muscle length information into spinal circuitry. Renshaw cells and Ia inhibitory interneurons are V1 derived, but differ in morphology, location, calcium-binding protein expression, synaptic connectivity, and function. These differences are already present in neonates and their differentiation starts in the early embryo (Benito-Gonzalez and Alvarez, 2012). In addition, 75% of V1 interneurons are non-Ia, non-Renshaw subclasses that await characterization (Sapir et al., 2004; Alvarez et al., 2005). Short-range ipsilaterally and rostrally projecting glycinergic/GABAergic V1 neurons are characterized by transient expression of homeodomain transcription factor *En1* (**Figure 4C**; Burrill et al., 1997; Matise and Joyner, 1997; Pierani et al., 1999, 2001; Saueressig et al., 1999). Studies in embryonic chick indicate that these neurons project for only 1–2 segments and have been shown to make inhibitory contacts onto motor neurons and other interneurons, although this may not be the case in the mature mouse (Wenner et al., 1998, 2000). Loss of *Pax6* or *En1*-dependent DTA ablation eliminates the recurrent inhibition by Renshaw cells on motor neurons (Sapir et al., 2004; Gosgnach et al., 2006). Elimination of V1 interneurons results in a marked slowing on the drug-induced fictive locomotion period that is seen in conventional knockouts, targeted ablation, and acute inhibition with allatostatin (Gosgnach et al., 2006; Goulding, 2009). The mechanism by which elimination of an inhibitory class would prolong the locomotor cycle remains unknown and may result from the loss of inhibitory neurons to terminate MN firing. The V1 class, like V0, expresses the inhibitory spinal interneuron markers *Lhx1* and *Lhx5* (Pillai et al., 2007).

V1 Birth and Early Development

Unlike cells in the dorsal-most p0 domain that expresses *Dbx1* and *Dbx2*, the adjacent p1 domain only expresses *Dbx2* (Pierani et al., 1999). The V1 class appears from a *Pax6*$^+$, *Dbx2*$^+$, *Nkx6.2*$^+$, *Dbx1*$^-$ domain that is ventral to the *Dbx1/2*$^+$ V0 domain (Matise and Joyner, 1997; Pierani et al., 1999). In chick, *En1*$^+$, *Lim1/2*$^+$ V1 neurons appear at stage 17, and most appear ventral to the domain of *Dbx1* expression, within the ventral domain of these *Dbx2*$^+$, *Dbx1*$^-$ progenitors (Pierani et al., 1999). *Dbx* expression does not overlap with *En1*, perhaps due to the relatively late expression of *En1* (Pierani et al., 1999). Genetic tracing studies using *Dbx1*^{nlsLacZ} mice between ages E10 and E16.5 found that ~5–10% of *En1*$^+$ cells did express a low level of βgal, perhaps a reflection of transient *Dbx1* expression and more enduring βgal protein. The V1 class is marked by expression of *Foxd3*, found in the dI2 domain, as well (Ramos et al., 2010). The transcription factor, *Bhlhb5*, which marks the V1, V2 and dI6 domains, is required at least partially for V1 identity assessed by *En1* expression (Ramos et al., 2010; Skaggs et al., 2011). Expression of *Bhlhb5* in conjunction with *Ngn2* facilitates V1 identity ectopically (Skaggs et al., 2011).

V1 Interneuron Subtypes

V1 Renshaw

Renshaw cells use both glycine and GABA as neurotransmitters, transiently express *Gad65* early in embryonic development and have both motor neurons and Ia interneurons as targets (Saueressig et al., 1999; Sapir et al., 2004). They also express calbindin D28K embryonically and continue to express this marker into adulthood (Alvarez et al., 1999; Geiman et al., 2000). They receive input from motor neuron collaterals that release acetylcholine, glutamate, and aspartate (Mentis et al., 2005; Richards et al., 2014). Renshaw cells modulate proprioceptive sensory input and motor neuron output. Genetic tracing studies showed that Renshaw cells are derived from an *En1*$^+$ progenitor pool and, although they are not lost in the absence of *En1*, they do have fewer motor neuron recurrent inputs (Sapir et al., 2004). They are, however, lost in the absence of *Pax6* (Sapir et al., 2004). Recent study showed that selective activation of the Onecut transcription factors Oc1 and Oc2 during the first wave of V1 interneuron neurogenesis is a key step in the Renshaw cell differentiation; furthermore Renshaw cell development is dependent on the forkhead transcription factor Foxd3, which is more broadly expressed in post-mitotic V1 interneurons (Stam et al., 2012).

V1 Ia Interneuron

Although Ia interneurons have been rediscovered as a V1 subclass, like Renshaw cells, the Ia INs were functionally described before the advent of molecular genetic dissection of interneuron development (Eccles et al., 1954; Hultborn et al., 1971; Hultborn and Udo, 1972) These inhibitory glycinergic cells receive input from muscle spindle Ia proprioceptive afferents carrying muscle length information and provide inhibitory

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input onto motor neurons innervating antagonist muscles. Like motor neurons, Ia receive inhibitory inputs from Renshaw cells (Hultborn et al., 1971). In neonatal mice, disynaptic glycinergic reciprocal inhibition is mediated by Ia interneurons, although this activity is preserved in the absence of *Pax6*, indicating that cells of more than one origin contributes to this functional class (Wang et al., 2008). Only when V1 and V2b are both ablated is reciprocal inhibition profoundly altered. Renshaw cells constitute 8–19% of V1 interneurons and the $Foxp2^{+}$ (by immunohistochemistry) population accounts for around 33% of these neurons at P0 and 50% at E13 (Morikawa et al., 2009). Because there are no universal markers of Ia interneurons, all Ia interneurons cannot be accounted for, leaving the physiologic properties and connectivity patterns of V1 interneurons unaccounted for (Alvarez et al., 2005). Of note, some interneurons with synaptic organization like Ia interneurons have been found that arise from the V1 population and are Foxp2 positive (Morikawa et al., 2009). A survey in E12.5 mice showed that several V1 subclasses can be defined by expression of *Calbindin, OC1, OC2, OC3, Foxd3, En1, MafB, FoxP2, Foxd3, Foxp4, Pax2, Arx, Evx1, Nurr1, BhlhB5, Pou4F1, Pou3F1,* and *Prdm8* (Francius et al., 2013).

V2 Interneuron Characteristics

V2 interneurons become divided into V2a and V2b classes of ipsilaterally projecting interneurons that extend axons caudally across several segments (Goulding, 2009). The excitatory V2a class is glutamatergic and expresses *Chx10*, while the $Gata2/3^{+}$ V2b class is inhibitory and uses both glycine and GABA (**Figure 4D**; Al-Mosawie et al., 2007; Lundfald et al., 2007). The transcription factor, *Bhlhb5*, marks the V2, as well as V1 and dI6 domains (Ramos et al., 2010).

V2 Birth and Early Development (Notch-Delta)

V2 interneurons arise from a progenitor pool just dorsal to the pMN domain and share expression of *Lhx3* with the pMN domain. In addition, both domains share expression of NLI that forms homodimers. This NLI homodimer nucleates the formation of a higher-order tetramer with Lhx3 in the V2 progenitor domain, and in the case of pMNs this V2-defining tetramer (Lhx3-NLI-NLI-Lhx3) is disrupted by the insertion of Isl1 to form a hexamer (Lhx3-Isl1-NLI-NLI-Isl1-Lhx3). Transcriptional response elements that are active in V2 cells can bind both the motor neuron hexamers and the V2 associated tetramers, while response elements active in motor neurons are only responsive to the hexamers (Lee et al., 2008). Later the V2 domain expresses *Chx10* that acts as a repressor of motor neuron associated hexamers in V2 progenitors, leaving only the LIM tetramers active (Sander et al., 2000; Lee et al., 2008). The progenitor pool of V2 neurons becomes post-mitotically segregated into V2a and V2b neurons.

Time-lapse imaging in zebrafish showed that the majority of V2 progenitors give rise to a pair of V2a and V2b cells (Kimura et al., 2008), indicating that V2a and V2b arise from the same progenitor. This segregation into V2a and V2b is mediated by Notch/delta signaling in zebrafish and mouse models (Yang et al., 2006; Del Barrio et al., 2007; Peng et al., 2007). In mouse, Delta4, but not Delta 1, activates this signaling cascade and is downstream of *Foxn4*, which also induces expression of *Mash1/Ascl1* (Del Barrio et al., 2007; Peng et al., 2007). Mind bomb-1 (Mib1) is an E3 ubiquitin ligase that ubiquitinates and promotes the endocytosis of Notch ligands. In mice model, Mib1 plays an important role in Notch activity and specific differentiation, neurogenesis and gliogenesis of V2 interneurons. Mice models with abnormal Mib1 resulted in unclear spinal progenitors, premature or unbalanced differentiation or loss of astrocytes and oligodendrocytes (Kang et al., 2013). In zebrafish embryos two ligands, DeltaA and DeltaD, and three receptors, Notch1a, Notch1b, and Notch3 redundantly contribute to p2 progenitor maintenance; on the other hand, DeltaA, DeltaC, and Notch1a mainly contribute to the V2a/V2b cell fate determination (Okigawa et al., 2014). Misra et al. (2009) showed Foxn4 and proneural factors may serve as the trigger to initiate asymmetric Dll4-Notch and subsequent BMP/TGFβ signaling events required for neuronal diversity in the V2 domain (Okigawa et al., 2014). V2b fate is specified by active *Notch1, Foxn4, Mash1,* and *Scl* Notch-binding protein MAML is also required for this specification (Peng et al., 2007). Lack of active Notch1 results in V2a fate, shown in an increase of V2a interneurons at the expense of V2b in *Psn1* KO mice or *Notch1* KO mice (Del Barrio et al., 2007; Peng et al., 2007). Transcription factor Gata2 is necessary in the normal development of V2a and V2b neurons and Gata2 promotes the selective activation of V2b at the expense of V2a fate (Francius et al., 2014). Progenitors that express the notch ligand, Delta-like 4 generate almost all V2a and V2c neurons while producing only a small fraction of neurons of other subtypes along the dorsoventral axis (Zou et al., 2015).

V2 Interneuron Subtypes

V2a Sox14/Chx10

The V2a class of ipsilaterally projecting interneurons expresses the transcription factors Chx10 and Sox14 and is glutamatergic. These interneurons are composed of cells with diverse firing properties and morphologies with local as well as long-range ipsilateral projection patterns (Dougherty and Kiehn, 2010a,b; Zhong et al., 2010). This class of interneurons has been shown to contact motor neurons (Al-Mosawie et al., 2007; Stepien et al., 2010) and contralaterally projecting V0 interneurons (Crone et al., 2008). Loss of these cells has been shown to disturb locomotor function in a state-dependent manner (Crone et al., 2008, 2009; Dougherty and Kiehn, 2010a,b; Zhong et al., 2010). In a series of *in vitro* experiments it was found that *Chx10-DTA* V2a-ablated mice displayed more variable amplitude and period than wild-type controls during drug-induced fictive locomotion. Further, these mutant animals had incoherent left–right alternation during drug-induced fictive locomotion. Surprisingly, these animals failed to display coordinated brainstem stimulated or dorsal root stimulated fictive locomotion, suggesting that $Chx10^{+}$ cells mediate descending and sensory activation of locomotor activity (Crone et al., 2008).

A subsequent study, using a different strain of mice that avoided the neonatal lethality seen in previous work, showed that during treadmill running, *Chx10-DTA* mice can transition from

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alternating locomotion to synchronous hindlimb locomotion at higher speeds. High-speed synchronous left–right activity, or galloping, is not normally seen in mice, although it has been described in studies of *Eph* and *ephrin* signaling molecule mutant mice (Dottori et al., 1998; Kullander et al., 2001; Yokoyama et al., 2001). The *Eph/ephrin* mutant mice, however, have synchronous activity at both slow and fast speeds. Some V2a interneurons express *EphA4*, but a compelling correlation has yet to be discovered (Lundfald et al., 2007). In zebrafish, *alx*, a zebrafish homolog of *Chx10*, is expressed in an ipsilateral descending excitatory interneuron population named CiD (circumferential descending) neurons that monosynaptically contact motor neurons (Kimura et al., 2006; McLean et al., 2008). This population has been shown to be active during high-frequency swimming in larval zebrafish (McLean et al., 2008). Within this interneuron class, dorsally located cells are recruited at a high swimming frequency. As the frequency decreases, more ventral cells are recruited, accompanied by silencing of previously active dorsal cells (McLean et al., 2008). A survey in E12.5 mice showed that V2a subclasses can be defined by expression of *BhlhB5, Pou3F1, OC1, OC2, OC3, Prdm8, MafA*, and *cMaf* (Francius et al., 2013).

V2b Gata2/3

Ipsilaterally projecting V2b interneurons express *Gata2/3*, are inhibitory GABAergic neurons, and appear to make direct connections onto motor neurons (Lundfald et al., 2007; Peng et al., 2007). Observations by the Goulding lab indicate they project caudally (Zhang et al., 2014). These cells may underlie the retained reciprocal inhibitory pathways seen in V1 knockout mice (Wang et al., 2008). A survey in E12.5 mice showed that V2b subclasses can be defined by expression of *BhlhB5, Pou3F1, OC1, OC2, OC3, Prdm8, MafA*, and *MafB* (Francius et al., 2013). As pointed out earlier, V1- and V2b-derived neurons function as the core interneuronal components of the limb central pattern generator (CPG) that coordinate flexor-extensor motor activity (Zhang et al., 2014).

V2c Sox1

The V2 interneuron class has recently been shown to further diverge to a Sox1-expressing Gata3-negative population named V2c interneurons, function of which is still yet to be elucidated (Li et al., 2010; Panayi et al., 2010). A survey in E12.5 mice showed that V2c subclasses can be defined by expression of *Sox1, OC1, OC2, and OC3* (Francius et al., 2013).

V3 Interneuron Characteristics

The $Sim1^{+}$ $VGluT2^{+}$ glutamatergic V3 interneurons send projections predominantly contralaterally and caudally (Goulding, 2009). Genetic tracing, using a *Sim1-eGFP* or $Sim1^{Cre}$ and reporter lines, and viral tracing, using pseudorabies, shows that 80–85% of these cells project contralaterally and a minor proportion remain ipsilateral or project both contra- and ipsilaterally (Zhang et al., 2008). As a population, $Sim1^{+}$ V3 interneurons form 24% of glutamatergic connections on V1 Ia, 27% on Renshaw subclasses, 22% of glutamatergic synapses on lateral motor column motor neurons, as well as connections on $Lhx3^{+}$ V2 interneurons, and lamina VIII commissural interneurons (Zhang et al., 2008). Behaviorally, loss of V3 neuronal activity by genetic attenuation with tetanus toxin or allatostatin signaling resulted in a loss of CPG robustness. In isolated cord fictive locomotion, both dorsal root stimulation and drug-induced methods produced weak CPG activity in only some of the cords examined. The outputs were less consistent and had greater coefficients of variance. Although both right and left sides of the cord produced irregular outputs, the fidelity of left–right coordination was preserved suggesting that V3 interneurons do not regulate the coordination of left–right activity. In adult $Sim1^{Cre}$ *AlstR192* animals, application of allatostatin to the cord produced locomotor disturbances in gait, as well (Zhang et al., 2008). In Sim1 mutant mice, V3 interneurons are produced normally and maintain in the similar position and organizations as wild-type; however, there is significant reduction of interneurons in dorsal subgroup and there is significant reduction in the contralateral axonal projection. Therefore, Sim1 appears to be critical in migration and axonal projection of V3 interneuronal development (Blacklaws et al., 2015). Mice that are mutant for *Nkx2.2* and *Nkx2.9* lose V3 interneurons and $Nkx2.2^{+/-}$ $Nkx^{-/-}$ mice display intermittent or permanent hopping gait (Holz et al., 2010). Holz et al. (2010) indicate that this mutation affects floor plate, and therefore likely affects commissural interneuron projections that mediate left–right coordination. A survey in E12.5 mice showed that V2c subclasses can be defined by expression of *Olig3, Prox1, BhlhB5*, and *Nurr1* (Francius et al., 2013).

V3 Birth and Early Development

These V3 interneurons arise from the ventral-most p3 progenitor domain defined by homeobox transcription factors *Nkx2.2* and *Nkx2.9* and the PAS-bHLH transcription factor *Sim1* (simple-minded homolog 1; **Figure 4E**; Briscoe et al., 1999; Goulding et al., 2002). Genetic tracing techniques using a $Sim1^{TauLacZ}$ knock-in reporter mouse or $Sim1^{Cre}$ and reporter lines ($R26^{floxstop-GAP43-GFP}$ and $R26^{floxstop-lacZ}$) have shown similar expression at E11.5 to *in situ* hybridization data for *Sim1* expression that appeared just lateral to the *Nkx2.2* progenitors (Marion et al., 2005; Zhang et al., 2008). *Nkx2.2* also regulates the expression of *Olig3* in V3 neurons. While *Olig3* plays a key role in respecification of dI2 and dI3 neurons into dI4 interneurons in dorsal spinal cord (see below), it does not appear to affect the generation and migration of the ventral neurons (Liu et al., 2014).

$V3_D$ and $V3_V$

Each class of interneurons can likely be further subdivided. The existence of V3 subtype heterogeneity defined by cell body positions was first reported in a review of locomotor circuitry by the Goulding group (Goulding, 2009). This group recently examined both electrophysiological and morphological properties of mature V3 interneurons in adult mouse and were able to identify two V3 subpopulations with distinct intrinsic properties and distributions (ventral and dorsal), as well as an important intermediate subgroup (Borowska et al., 2013). They

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reported $V3_V$, primarily located in lamina VIII, possessed a few branching processes and were capable of generating rapid tonic firing spikes and $V3_D$ had a more complex morphology with relatively slow average spike frequency with strong adaptation (Borowska et al., 2013). A survey in E12.5 mice showed that $V3_V$ express *Olig3*, *Prox1*, *BhlhB5*, and *Nurr1*, and $V3_D$ can be defined by expression of *OC1*, *OC2*, and *OC3* (Francius et al., 2013).

V_X Hb9

A group of glutamatergic, rhythmically active interneurons with possible connections to motor neurons can be found along either side of the ventral midline in thoracic and upper lumbar segments (Thaler et al., 1999; Wichterle et al., 2002; Hinckley et al., 2005; Wilson et al., 2005). These $Hb9^+$ and $VGluT2^+$ interneurons are found in lamina VIII, although the developmental origin of these cells is unknown (**Figure 4F**). These cells have oscillatory behavior, make potential contacts with motor neurons, and are associated with motor rhythms (Hinckley et al., 2005; Wilson et al., 2005; Hinckley and Ziskind-Conhaim, 2006). These interneurons were the first to show oscillatory properties and efforts have been made to discover a relationship to rhythm generation or a pacemaker property for the CPG (Kwan et al., 2009). No cell class, however, has been found to act as a pacemaker for CPG activity. Remaining questions for the V_X include: what is the progenitor domain that gives rise to the V_X domain; and why are they not found below the L2 segment at E18.5.

Dorsal Interneuron Progenitors

There are eight canonical classes of dorsal progenitors, dI1–6 and dIL_A and dIL_B. Of these, the dorsal-most dI1–3 progenitors are dependent on signals from the roof plate and termed Class A (Liem et al., 1997; Lee et al., 2000). The remaining dI4–6 and dIL_A and dIL_B are independent of roof plate signals and termed Class B (Gross et al., 2002; Muller et al., 2002). The dorsal-most progenitors, pd1–pd3, are born between days E9.5 and 10.5 and become post-mitotic and begin to migrate ventrally between E10.5 and E11.5 (Helms and Johnson, 1998; Bermingham et al., 2001; Gross et al., 2002; Muller et al., 2002). These cells will eventually form the deeper layers of the dorsal horn. The more ventral Class B dI4–6 cells are born between E10 and 12.5 and then post-mitotically express *Lbx1* and migrate either dorsally to form the more superficial layers of the dorsal horn or migrate ventrally to the deep dorsal horn and the ventral spinal cord (Gross et al., 2002; Muller et al., 2002). The later born dIL_A and dIL_B classes are born between E11 and E13 and are intermixed with each other. They then migrate dorsally and constitute a significant portion of the cells in the superficial dorsal horn, including the substantia gelatinosa (Nornes and Carry, 1978; Gross et al., 2002; Muller et al., 2002; Mizuguchi et al., 2006). As with the ventral interneuron classes, each of these classes, or their subgroups, has characteristic features. For instance, each interneuron subclass appears to have a unique axonal projection that produces a tight fascicle within white matter tracts (Avraham et al., 2009, 2010).

dI1 Interneuron Characteristics

The dorsal-most progenitor domain pd1 expresses the bHLH transcription factor $Math1^+$ (Mouse atonal homolog 1, also known as *Atoh1*) and gives rise to at least two $VGluT2^+$ glutamatergic subclasses: dI1A and dI1B, characterized by Lim-HD expression and their spinocerebellar tract (SCT) contributions (**Figure 4G**). Recent study shows that *Msx1* and *Msx2*, two homeodomain transcription factors that are induced earlier than bHLH transcription factors, likely play a role as transcriptional activators of *Math1*/*Atoh1* in spinal cord development (Duval et al., 2014). The dI1A (also known as $dI1_{comm}$) neurons express the Lim-HD transcription factors $Lhx2_{high}$ and $Lhx9_{low}$, while dI1B (also known as $dI1_{ipsi}$) express the Lim-HD TF *Lhx9* (Helms and Johnson, 1998; Lee et al., 1998; Bermingham et al., 2001; Gowan et al., 2001; Wilson et al., 2008; Avraham et al., 2009). The dI1 interneurons migrate to the deep dorsal horn and intermediate gray where they receive proprioceptive input from the periphery and form commissural projections of dorsal and ventral SCTs (Helms and Johnson, 1998; Bermingham et al., 2001). Using an $Atoh1^{LacZ}$ allele to trace the fate of pd1 progenitors in developing mouse, at least two subsets of the dI1 class have been identified: (1) a medial cluster of vertically oriented neurons that are $Cbln2^+$ and $Smarca2^+$ and projects to the SCT in the contralateral ($Tag1^+$) lateral funiculus; (2) a more lateral $Sox6^+$, cluster of horizontally oriented neurons that contributes to the SCT in the ipsilateral lateral funiculus (Miesegaes et al., 2009). In chick, data with an enhancer that labels these cells suggests that both fascicles coalesce in the lateral funiculus ventral to the fascicle formed by the dI2 projections (Avraham et al., 2009).

dI1 Birth and Early Development

The roof-plate-dependent Class A dp1 progenitors of the dI1 class express the bHLH transcription factors *Olig3* and *Math1* (Muller et al., 2005; Gowan et al., 2001). The dI1 neurons in mouse are born between E10 and E12.5 and express *Lhx2/9*, *Barhl1* (bar homeobox like 1) and *Brn3a* (*Pou4f1*, a class IV POU domain-containing transcription factor; Helms and Johnson, 1998). Loss of function experiments with BMP7 in chick and *Bmp7* mutant mice results in loss of dI1, dI3, and dI5 (Le Dréau et al., 2012).

dI2 Interneuron Characteristics

dI2 interneurons are ascending, contralaterally projecting, relay interneurons that migrate to the intermediate spinal cord and ventral horn (Gowan et al., 2001; Gross et al., 2002). These interneurons have been suggested to convey sensory information via the spinothalamic tract to the thalamus, based on their location (**Figure 4E**; Brown, 1981; Tracey, 1985; Gross et al., 2002). The projections likely occupy the lateral funiculus and are dorsal to the dI1 fascicle, as analyzed by enhancer expression in chick (Avraham et al., 2009). Arising from bHLH transcription factor *Ngn1* (neurogenin 1) and *Ngn2* expressing progenitors, these neurons express LIM-HD transcription factors *Lhx1*, *Lhx5* and winged-helix domain *Foxd3* (forkhead homeobox D3) post-mitotically (Bermingham et al., 2001; Gowan et al., 2001; Gross et al., 2002). These interneurons were previously known as D3A interneurons.

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dI2 Birth and Early Development

The roof-plate-dependent Class A dI2 progenitor domain, pd2, is characterized by the expression of the bHLH transcription factors, *Olig3*, *Ngn1*, and *Ngn2* and are born between E10- and E12.5 (**Figure 4H**; Gowan et al., 2001; Muller et al., 2005). Two SoxD transcription factors, Sox5 and Sox6, are expressed in restricted domains of dorsal progenitors. Sox5 controls cell fate specification of dp2 and dp3 progenitors and, as a result, controls the correct number of the corresponding dorsal interneurons (dI2 and dI3; Quiroga et al., 2015).

dI3 Interneuron Characteristics

The dI3 neurons are excitatory interneurons in the deep dorsal horn and intermediate spinal cord (Liem et al., 1997; Gowan et al., 2001; Cheng et al., 2004). These cells target motor neurons monosynaptically, as revealed by recent rabies tracing experiments (Stepien et al., 2010). They have axons that project rostrally, ipsilaterally, and longitudinally in two fascicles. A ventral fascicle enters the ventral lateral funiculus (VLF) and the dorsal fascicle enters the dorsal funiculus (DF; Avraham et al., 2010). The dorsal projecting axons re-enter the cord when they encounter axons sensory axons at the dorsal root entry zone (DREZ; Avraham et al., 2010). Similarly, the ventrally projecting neurons re-enter the cord at ventral root exit points (Avraham et al., 2010). In mice model, dI3 appears to convey input from low threshold cutaneous afferents to the motor neurons that is critical in hand/forelimb grip (Bui et al., 2013). The dI3 pool also expresses *Tlx3* (T-cell leukemia homeobox 3) and LIM-HD transcription factor *Isl1*-expressing cells (**Figure 4I**; Gross et al., 2002). The turning behavior of dI3 neurons is dependent on *Isl1*, and expression of *Isl1* in dI1 neurons conferred dI3-like axon choice points to dI1 neurons (Avraham et al., 2010). *Tlx1* (also known as *Hox11*) and *Tlx3* (also known as *Rnx* and *Hox11L2*) are markers of glutamatergic signaling. *Tlx3* functions cell-autonomously to specify a glutamatergic neurotransmitter phenotype (Cheng et al., 2004).

dI3 Birth and Early Development

The roof-plate dependent Class A dP3 progenitors express the basic helix-loop-helix (bHLH) transcription factor *Mash1* (*Ascl1*, Mouse Achaete-scute complex-like 1), as do adjacent pd4 and pd5 domains (Gowan et al., 2001; Helms et al., 2005). They also express *Olig3*, *Pax7* and *Ngn2* and *Gsh2* (Muller et al., 2005). In chick spinal cord electroporation experiments it has been shown that over-expression of *Olig3* increases dI3 interneurons at the expense of other Classes A and B neuron classes and this effect is enhanced by *Mash1* (Muller et al., 2005). Over-expression of *Mash1* results in more dI3 and dI5 neurons at the expense of dI2 and dI4 (Muller et al., 2005), while loss of Mash1 causes a decrease in dI3 and dI5 populations while dI4 is maintained (Helms et al., 2005). As mentioned above, loss of function experiments with BMP7 in chick and *Bmp7* mutant mice results in loss of dI1, dI3, and dI5 (Le Dréau et al., 2012).

dI4 Interneuron Characteristics

The early born (E10.5–E11) dI4 interneurons become *Pax2*$^+$, *Lhx1*$^+$, and *Lhx5*$^+$ GABAergic ipsilaterally projecting somatosensory associative neurons that migrate laterally to the deep dorsal horn (**Figure 4J**; Gross et al., 2002; Muller et al., 2002; Pillai et al., 2007). In addition, both dI4 and dI5 interneurons also express *Gsh1* (*Gsx1*) and *Gsh2* post-mitotically, while dI3 only express *Gsh2* (Kriks et al., 2005; Muller et al., 2005; Mizuguchi et al., 2006). They are GABAergic, calbindin$^+$ and express the nociceptive marker *PLCγ* (Chen et al., 2001; Helms and Johnson, 2003). The dI4 fate is dependent on *Ptf1a* and loss of this gene results in loss of all GABAergic dorsal neurons and respecification to dI5 fate (Henke et al., 2009; Meredith et al., 2009). Loss of *Lhx1* and *Lhx5* results in a loss of *Pax2*, *Viaat*, and *Gad1* (Pillai et al., 2007). In addition, *Pax2* is required for the maintained expression of *Lhx1*, *Lhx5*, *Pax5*, and *Pax8* (Pillai et al., 2007).

dI4 Birth and Early Development

The roof-plate independent Class B dP4 domain expresses *Lbx1*, *Mash1* and higher levels of *Pax7* than the dorsally adjacent pD3 domain (Gross et al., 2002; Muller et al., 2002). These progenitors are born between E10.5 and E11 and this domain is distinct from the dIL progenitor domain that produces dIL_A and dIL_B progenitors, although both have very similar transcription factor expression patterns (below). *Olig3* over-expression can inhibit formation of dI4, and loss can result in expansion of this domain (Muller et al., 2005). While these cells express *Mash1*, loss of *Mash1* does not block dI4 formation, yet it does disrupt dI3 and dI5 (Helms et al., 2005).

dI5 Interneuron Characteristics

The roof-plate independent Class B dI5 neurons become contralaterally projecting glutamatergic somatosensory interneurons of the deep dorsal horn (nucleus proprius) and ventral horn that express the homeodomain transcription factors *Lbx1*, *Brn3a*, *Tlx1*, *Tlx3*, and *Lmx1b* (**Figure 4K**; Gross et al., 2002; Muller et al., 2002; Qian et al., 2002; Ding et al., 2005; Glasgow et al., 2005). In addition, a subset expresses *PhoxA2* (Ding et al., 2004). These interneurons were previously known as D4.

dI5 Birth and Early Development

These cells are born between E10.5–E11 and arise from a *Mash1*$^+$ and *Pax7*$^+$ dP5 progenitor domain that express *Lbx1* post-mitotically to both reinforce Class B fate and oppose Class A fates (**Figure 4K**; Gross et al., 2002; Muller et al., 2002). The dI5 domain expresses *Gsh1* and *Gsh2*, as does the adjacent dI4 domain (Kriks et al., 2005). As noted previously, loss of function experiments with BMP7 in chick and *Bmp7* mutant mice results in loss of dI1, dI3, and dI5 (Le Dréau et al., 2012).

dI6 Interneuron Characteristics

The roof-plate independent Class B dI6 commissural inhibitory interneurons express *Lbx1*, *Lhx1*, *Lhx5*, and are *Pax2* positive, indicating a GABAergic fate (**Figure 4L**; Gross et al., 2002; Muller et al., 2002; Cheng et al., 2004; Glasgow et al., 2005; Pillai et al., 2007). These cells may also use glycine for neurotransmission (Goulding, 2009). Although arising from a dorsal progenitor pool, and not being part of the "V" interneurons, the dI6 group

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of interneurons gives rise to more than one subtype and appears to contribute to motor function (Gross et al., 2002; Muller et al., 2002; Lanuza et al., 2004). These inhibitory neurons are reported in unpublished observations to be commissural and may be involved in right–left alternation, as well (Goulding, 2009). Dmrt3, a novel marker in dI6 interneuron was traced to play a key role in locomotor circuitry and in development of commissural interneurons, and mutation in dmrt3 result in divergent in gait pattern in mice models (Andersson et al., 2012; Vallstedt and Kullander, 2013). Double knockout of *Lhx1* and *Lhx5* results in a loss of *Pax2*, *Viaat*, and *Gad1* expression (Pillai et al., 2007). Furthermore, *Pax2* is required for the maintained expression of *Lhx1*, *Lhx5*, *Pax5*, and *Pax8* (Pillai et al., 2007). These cells also express *WT1* (Wilms' tumor 1; Goulding, 2009). The transcription factor, *Bhlhb5*, marks the dI6, V1 and V2 domains (Ramos et al., 2010). Electrophysiologic characteristics of the dI6 interneurons around a central canal reveal two possible subtypes: one firing trains of action potentials that are loosely coupled to the ventral root output and expressing intrinsic rhythmic activity which suggests a role in locomotor rhythm generation. The other subtype fires action potentials that are tightly coupled to the ventral root output (Dyck et al., 2012).

dI6 Birth and Early Development

The dI6 neurons are born around E10.5–E11 and originate from a $Pax7^+$, $Dbx2^+$, $Ngn1^+$ and $Ngn2^+$ pD6 progenitor domain. Post-mitotically they express *Bhlhb5*, *Wt1*, *Lbx1*, *Lhx1*, *Lhx5*, and *Pax2* (references above).

Late Born Dorsal Interneurons

The dIL neurons represent a second wave of neurogenesis from the dIL progenitor domain that constitutes most of the interneurons in the superficial dorsal horn in Rexed laminae II–IV (Gross et al., 2002; Muller et al., 2002). These cells are formed from common progenitors, and their cell fates are controlled by *Ascl1/Mash1* (**Figure 4M**; Mizuguchi et al., 2006).

dIL_A Interneuron Characteristics

The roof-plate independent Class B IL_A interneurons are ipsilaterally projecting association neurons that occupy the superficial dorsal horn in Rexed laminae I–III (Gross et al., 2000). These inhibitory neurons are GABAergic and are calbindin+, and a subset express *Gbx1* (John et al., 2005; Mizuguchi et al., 2006).

dIL_A Birth and Early Development

The dIL_A interneurons are late born (E.13.5) and arise from the dIL progenitor domain that is $Lbx1^+$, $Lhx1/5^+$, and $Pax2^+$ (Pillai et al., 2007). The sensory relay interneuron maker *Foxd3* is downregulated in these cells (Gross et al., 2002). *Pax2* has been shown to be necessary for GABAergic differentiation and 98% of these cells also express *Gad1* (*GAD67*; Cheng et al., 2004). The dIL_A subclass is dependent on *Ptf1a* and loss of this gene results in trans-fate to the dIL^B identity (Glasgow et al., 2005).

dIL_B Interneuron Characteristics

The roof-plate independent Class B are $DRG11^+$ ipsilaterally projecting association interneurons that integrate input from cutaneous sensory neurons that detect noxious stimuli (Chen et al., 2001; Gross et al., 2002). This last-born subclass gives rise to glutamatergic neurons expressing *Tlx1* and *Tlx3* and *Lmx1b* (Cheng et al., 2004; Mizuguchi et al., 2006). The dIL_B neurons migrate dorsolaterally to settle in the superficial dorsal horn in Rexed laminae I–III (Gross et al., 2000; Muller et al., 2002). Over 96% of the dorsolateral population of these cells expresses both *Tlx3* and *VGluT2* (Cheng et al., 2004; Glasgow et al., 2005).

dIL_B Birth and Early Development

These neurons are born later than the dI1–6 class and express *Lbx1* post-mitotically (Gross et al., 2000; Muller et al., 2002). Mash1 controls the upregulation of Notch signaling to direct formation of dILB from common dIL progenitors (Mizuguchi et al., 2006).

Discussion

Combinatorial transcriptional control of cell fate is a mature perspective for understanding spinal cord development. This focus on transcription factors has been powerful in two major respects. First, it has allowed the identification of downstream factors that direct cell-specific characteristics, such as neurotransmitter status (Cheng et al., 2004). Second, it has permitted a powerful genetic analysis of spinal neurons, using transcription factors as class-specific tools to drive changes in cell fate and function (Lee et al., 2000; Zhang et al., 2008). Novel techniques are emerging for tracing neuronal circuitry and for the sophisticated manipulation of neuronal activity, including selective cellular ablation, optogenetic activation and silencing, and chemically induced activation and silencing (Boyden et al., 2005; Wickersham et al., 2007; Alexander et al., 2009). These techniques may reach their most exciting potential when coupled with the increasingly specific genetic control available in the spinal cord.

The further refinement of developmental neuronal classes is showing that subclasses may reflect functionally coherent groups of cells that can be mapped onto physiologically identified populations (**Figure 5**). Therefore, as the spinal cord development field grows to incorporate circuitry and behavior, it is merging with the rich field of adult spinal cord electrophysiology that has uncovered major mechanisms of spinal cord function. The combination of these disciplines will advance spinal cord biology to a state that fully encompasses both form and function.

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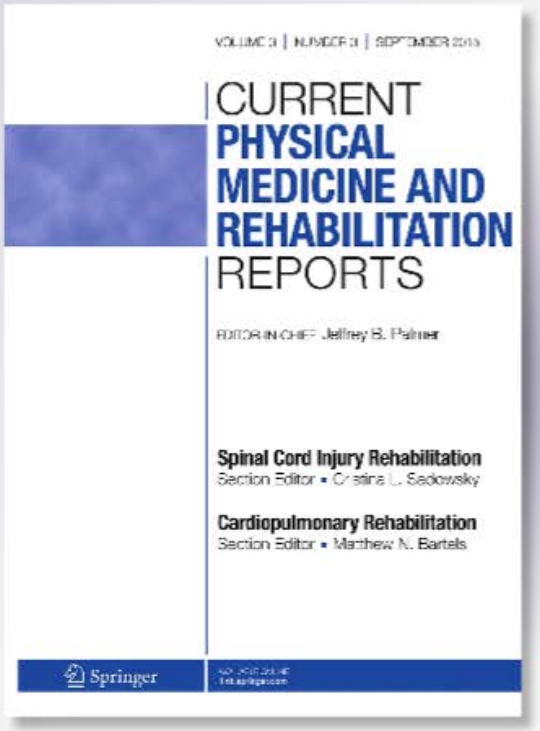
Strategies and lessons in spinal cord injury rehabilitation

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SPINAL CORD INJURY REHABILITATION (CL. SADOWSKY)

Strategies and lessons in spinal cord injury rehabilitation

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Abstract The spinal cord is often underappreciated as part of the central nervous system. Like the brain, the spinal cord can independently carry out relatively complex behaviors, such as left–right and flexor–extension alternation of the limbs, through activation of resident central pattern generator circuitry. Here the spinal cord integrates ascending or local proprioceptive information with descending sensory and volitional information. In the context of injury, portions of the isolated spinal cord may still be capable of carrying out sophisticated processing for sensorimotor function. Several modes of stimulation appear to activate the central pattern generating circuitry in SCI: treadmill stepping, magnetic stimulation, electrical stimulation, vibratory stimulation, and pharmacologic agents. Like the brain, the spinal cord is capable of classical and operant conditioning. This observation highlights the need for well-planned therapeutic interventions that work with the innate behavior of the cord and avoid maladaptive learning that can occur if noxious stimuli are present during rehabilitation. Patient-specific multimodal therapies that work with innate spinal cord behaviors are most likely to benefit patients with SCI.

Keywords Spinal cord injury · Rehabilitation · Electrical stimulation · Magnetic stimulation · Treadmill stepping · Operant conditioning

This article is part of the Topical Collection on *Spinal Cord Injury Rehabilitation*.

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Introduction

The central nervous system consists of the spinal cord, hindbrain, midbrain, and forebrain. The spinal cord contains 31 segments that mediate all motor and sensory functions (save those of the cranial nerves). The spinal cord does not simple relay these descending commands and ascending sensory signals. In addition to substantial fibers of passage that surround the cord with white matter, the central region of the cord contains gray matter. This gray matter contains dozens of distinct interneuron cell types, as well as the pools of **motor neurons** that are distinct for each muscle of the body **[1, 2•]**.

The majority of **spinal cord** injuries happen at one location affecting **one, or a few, spi**nal levels. As a result, a great deal of spinal **cord tissue is** unharmed—although it may be isolated from the brain—unable to convey ascending sensory signals or receive descending motor commands. The field of rehabilitation for spinal cord injury has developed, and demonstrated, the concept that spared spinal cord can be activated by several means to restore function. The combination of activation modalities, and in what context they are delivered, appears to be critical to the appropriate re-activation of spinal cord following injury. Below we review key concepts in the field of spinal cord injury rehabilitation.

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Basis of human locomotion

Coordination of the limbs during locomotion, or during stereotyped behaviors, is mediated by several interdependent regions of the central nervous system, including the spinal cord. The recognition that the spinal cord can semi-autonomously coordinate limb movement was proposed by Graham-Brown in 1911 with his half-center hypothesis to explain the observation that reduced preparations can produce coordinated movement [3]. This early model, developed in cats, did not account for descending or sensory information. Over 70 years later, work in isolated lamprey spinal cords resulted in the concept of Central Pattern Generators (CPGs) [4]. Within the lumbosacral cord of model mammals, cat, rat, and mouse, lesion and isolated cord experiments have demonstrated that left–right and flexor–extensor coordination of the hindlimbs can be induced (in actual or fictive locomotion) by pharmacologic or electrical stimulation, or by sensory stimulation [5]. With the advent of molecular genetic experiments in mice, several interneuron classes have been identified that contribute to aspects of left–right and flexor–extensor coordination, and rates of CPG activity [1, 6, 2•].

These reductionist experiments have been useful in solidifying the CPG model; however, rehabilitation requires that complex peripheral proprioceptive (such as walking over varied terrain), central unconscious sensory (vestibular, auditory, visual), and volitional (direction, speed) information to seamlessly modulate the intrinsic CPG circuitry [7]. It appears that CPGs are cyclically permissive in integrating or restrictive in gating these information streams depending on the behavioral state of the animal [8, 9]. While the CPG has robust self-regulation, it can incorporate descending tone [10] from primary and supplementary motor cortex during locomotion [11, 12] and in proportion to walking speed [13].

Activation of lumbosacral CPG circuitry, such as by electrically stimulating the tibial nerve, results in coordination of the upper/fore with the lower/hind limbs via propriospinal connections [14, 15]. As such, leg locomotion alters the reflex behavior in proximal arm muscles, revealing task-dependent coupling of these upper and lower limb spinal circuitries. This status-dependent interaction of distributed motor systems highlights the importance of designing rehabilitation schemes that work within the innate permissive "up states" or resistive "down states" to external modulation through ascending or descending inputs.

Plasticity with locomotor training

Learning and plasticity in the spinal cord is demonstrated by the observation that animals lacking motor input to the hind/lower limbs can learn to stand and walk on a treadmill without input from the brain [16–18], including human subjects [19]. Here the rehabilitative task is well matched to the innate behavior of the CPG: alternating flexion–extension and left–right alternation [20, 21]. This results in a useful and adaptive behavioral response by the spinal circuitry and CPG. Care must be taken by healthcare professionals to be assured that the rehabilitative strategy is appropriate to the natural behavior of the task being trained, as well and the receptivity status of the cord.

Spinal cord injuries in clinical settings may occur with additional trauma within dermatomes and myotomes innervated by spinal segments below the level of injury. Rehabilitative strategies that are performed in the presence of pain signaling to the isolated cord level(s) may result in adaptive or maladaptive spinal cord learning. For instance, a form of Pavlovian leaning, where a behavior (e.g., salivation) can be elicited with a conditioned stimulus (e.g., a bell) that has been paired with and unconditioned stimulus (e.g., food) that can be found within the spinal cord. In spinal animals, it has been shown that innocuous thigh stimulation can be paired with a noxious plantar stimulation. As a result, the conditioned thigh stimulus can produce a paw withdrawal without the unconditioned noxious stimulus [22, 23]. Here, as in the treadmill training above, the learned behavioral response is appropriate to the environmental conditions and the avoidance of pain. Care must be taken to avoid inadvertent, potentially maladaptive, Pavlovian conditioning of isolated spinal segments during rehabilitation.

In similar experiments in spinalized rats, animals can be trained to flex their foot to avoid a shock when the foot is lowered [24]. This context-appropriate behavior improves recovery from spinal cord injury. Importantly, if, however, the stimulus is delivered at random and without consideration of the limb position, a spinal learning deficit with tactile hyperreactivity and impaired recovery of function is observed [25]. It is therefore likely that rehabilitative strategies that elicit limb pain in a random manner or regardless of limb position, even in sensory complete individuals, could elicit maladaptive spinal learning.

Locomotor ability after SCI

Most SCIs result from trauma and majority of those injuries result in incomplete loss of motor and sensory function with some residual functions below the level of injury [26]. This indicates that there are some intact functional neuronal connections across the injury level. Those connections, if activated, may contribute to the plasticity of the nervous system and enhance the potential of functional recovery after SCI [27]. In cases of complete SCI where there is no function below the level of injury, the functional recovery rests heavily on the plasticity of resident spinal locomotor circuitry below the level of injury [28]. The

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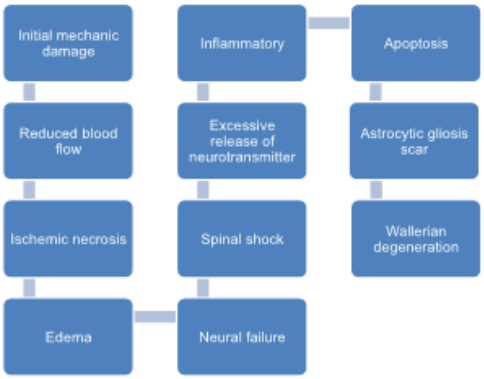


Fig. 1 Sequence of physiologic events following SCI. In SCI, there is the initial insult resulting often from mechanical trauma, such as the shearing force of displaced vertebral bodies. This initial trauma disrupts the vasculature or compresses tissue preventing normal blood flow. Tissue that has been deprived of blood flow for sufficient duration will undergo ischemic necrosis. This is followed by edema due to increased capillary permeability. Neurons in the affected regions that are not killed are exposed to milieu that is not conducive to normal function and undergo neural failure. As a result of these conditions, an acute period of spinal shock lasting for days or weeks can produce a transient loss of movement and sensation. During this period of spinal shock, excessive neurotransmitter may be released, perhaps in an attempt by the local neurons to re-establish homeostasis. Excessive neuronal activity and the necrosis of cells due to ischemia result in inflammation. Inflammation results in apoptotic death of cells in and within the penumbra of the affected region. Over several months, inappropriately a large number of glia are recruited to the region. As a result, an astrocytic glial scar can form that deposits excess myelin and other molecules that inhibit axon growth. Neurons with soma that are outside the focus of the injury or the penumbra may still have damaged axons within the injured region. Neurons with severed axons will slowly retract their axons and die in a characteristic manner called Wallerian degeneration

basis of any functional recovery after SCI is the idea that the central nervous system is a dynamic system that constantly changes, adapts, and reorganizes, particularly after an injury. Bareyre and Schwab summarized the order of events following SCI: from swelling to excess neurotransmitter release to gliosis to eventual scarring and Wallerian degeneration [29] (Fig. 1).

It has been well documented in both mammalian [30] and rodent models [31] that following severe SCI, a significant proportion of locomotor function is maintained by spinal central pattern generator circuitry that generates oscillatory patterns in response to stimulation, such as propriospinal feedback from the limbs. In fact, experimental neonatal rats with complete transection of the spinal cord are still capable of performing treadmill locomotion [32]. Corticospinal tracts, along with the proprioceptive afferent signals from the muscles and extremities, appear to play a more prominent role in modulating and fine tuning of the otherwise crude locomotion and therefore are much more important in skilled movements [33]. This is especially true in humans, unlike rodents, where corticospinal projections synapse directly on motor neurons.

Electrophysiology studies reveal that the amplitudes of locomotor EMG signals and polysynaptic spinal reflexes increase slowly over time as patients recover from SCI. These plateau after a few months [34] at an EMG activity level that is significantly lower than a healthy individual [35]. Clinically, the patient exhibits increased spinal reflexes, increased muscle tone, and spasticity. In SCI individuals, after some minutes of assisted locomotion, the EMG amplitude decreases to background noise level. This is termed "EMG exhaustion" by Hubli et al. [36••]. This characteristic change is unique to humans, as so far, there are no animal model conditions that can recreate this phenomenon. The phenomenon appears to be closely related to immobility rather than the quality of the injury, as it is evident that incomplete SCI patients who are wheelchair bound show same exhaustion as patients with complete SCI; while patients who undergo frequent locomotion activities show no such exhaustion [37]. The exact cause of the exhaustion is unknown at this time. One theory is that SCI results in reduced excitatory, or increased inhibitory, input from supraspinal sources and afferent input from peripheral sources, and thus an overall increase in inhibitory influence of the locomotor circuitry [37]. This theory, if true, offers a therapeutic opportunity to enhance functions through blocking of inhibitory signals (or improving excitatory signals).

Effect of locomotor training after SCI

As mentioned above, after SCI, with neuronal plasticity, there is a spontaneous re-organization of cortical sensory and motor representations, demonstrating substantial CNS plasticity, particularly in incomplete subjects. In addition to this, however, there is also re-organization and re-structuring of the spinal circuitry after locomotor training [38]. One of the most common forms of locomotor training is body weight supported treadmill training (BWSTT). During the training, the patient's body weight is supported by a harness or a robotic apparatus. The subject is then asked to step on a motorized treadmill. In complete SCI subjects, steps can also be assisted by therapists or assistive robotics. In both animal and human models, there are numerous studies that support BWSTT as a method to restore locomotion.

In mice model, BWSTT increases axonal sprouting proximal to the injury level [39] and the expression of neurotrophic factors in the spinal cord [40, 41]. In human subjects, functional MRI evidence suggests that BWSTT induces greater activation of bilateral cortical and cerebellar sensory, and motor areas [42]. That this may be a general phenomenon of recovery is reflected in the

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observation that similar changes are seen in post-stroke patients after BWSTT training [43]. Electrophysiological evidence shows that BWSTT increases MEP amplitude, reorganizes and re-establishes cortical control of spinal reflexes [44], and spinal interneuron afferents [45].

Despite the above promising findings, a recent Cochrane review by Mehrholz showed that there is no statistically significant effect of locomotor training on walking function in human subjects after SCI, when comparing BWSTT, with or without functional electrical stimulation, or robotic-assisted locomotor training. However, as pointed out by Mehrholz et al., the Cochrane review is limited in its utility because there are only four randomized control trials available for comparison [46••].

Importance of sensory/proprioceptive cues

Following an injury to the spinal cord that results in a reduction in spinal neural and motor activity [47], there is an attendant reduction in the amount of proprioceptive sensory information generated and fed into the spinal cord. Rehabilitative strategies have sought to replace this deficit in proprioceptive feedback onto spinal and CPG circuitries by using vibratory stimulation of the quadriceps and hamstring muscles [48], epidural stimulation of the dorsal cord [19], electrical stimulation of the sural or peroneal nerves [49], and TMS stimulation of the cord [50]. These approaches may create a condition where the CPG circuitry is modulated such that it is in a permissive state to allow incorporation of sensory input.

Work in cats and rodents have demonstrated that a multimodal method of modulation is most effective. Here rehabilitative strategies have used treadmill training, electrical stimulation of the spinal cord, and the use of serotonergic and noradrenergic agonists [51–54]. While limited evidence has been published regarding the use of pharmacology for the recovery of motor function in human subjects, other modalities are being attempted [55]. Other methods, such as transcutaneous spinal direct current stimulation (tsDCS) [56] and the use of paired spinal associative stimulations of H-reflexes and transcranial magnetic stimulation [57], are being applied in the hopes of producing a permissive spinal cord state and behaviors appropriate to the natural function of the spinal cord.

Novel treatments: modulation of spinal cord excitability

Experimental studies in spinalized animals confirm the importance of afferent information in modulating locomotion [51, 52]. The functional state of the spinal cord is highly dependent on afferent sensory information [58]. And it has been argued that after a severe SCI, due to loss of sensory input, the functional state of the spinal cord is depressed and this further negatively impacts locomotor recovery [36••]. Therefore, tools to activate the locomotor circuitry after SCI may be useful in restoration of locomotor function. Approaches include, body loading (by body weight support treadmill apparatus) [59•], vibratory stimuli of the muscles [60], electrical stimulation of the peripheral nerves [61], electrical [62] and magnetic [50] stimulation of the spinal cord, and pharmacological agents [51, 52] that activate the spinal locomotor circuitry.

Body weight support with manually or robotically assisted devices provides sensory feedback to the cord and is often used to activate the spinal locomotor circuitry [61]. During axial loading, load information from joint and muscles, and tactile sensory information from foot, are thought to be integrated and influence the locomotor central pattern generator to adapt to changing terrain. The effect of this strategy in activating the excitability of the spinal cord circuitry has been demonstrated by an associated increase in H-reflex and data suggesting that functional recovery can be realized in incomplete and complete SCI patients [59•].

Vibratory stimuli at the muscle and joint serve to activate predominantly Ia afferent fibers (muscle spindles) and to lesser extent Ib fibers (Golgi tendon organs) [60] and have the capacity of imparting kinesthesia—sense and positional movement of extremity—in a non-injured subject. Additionally, the vibrated muscle can generate activity that can induce step-like movement in normal and incomplete SCI subject [63•]. Electrical stimulation of peripheral nerves, which may recapitulate some aspects of vibratory stimuli, can modulate the functional state of the CPG. Specific stimulation of the sural of peroneal nerves (0.3 ms pulse width, 2–3 mA, 60 Hz), can induce air-stepping in a gravity-eliminating apparatus that can be enhanced with vibratory muscle stimuli, in healthy subjects [49].

Spinal cord modulation by electrical and magnetic energy is thought to enhance the activation state of the spinal cord such that residual supraspinal input is unmasked and voluntary function revealed (reviewed in [64] Fig. 2), hypothesized to re-enable lower extremity function after severe SCI [65]. Non-invasive means of delivering magnetic (via magnetic coil; [50]) and electrical (via surface electrodes; [66•]) stimulation have also been demonstrated to facilitate rhythmic locomotor-like activity in normal subjects. Whether these methods can impact locomotor function in SCI awaits further investigation.

Activation of the spinal cord locomotor circuitry in animals can be performed with pharmacological agents, particularly serotonergic and noradrenergic agonists. However, there is limited clinical evidence that pharmacological treatment can improve locomotor function after SCI [55].

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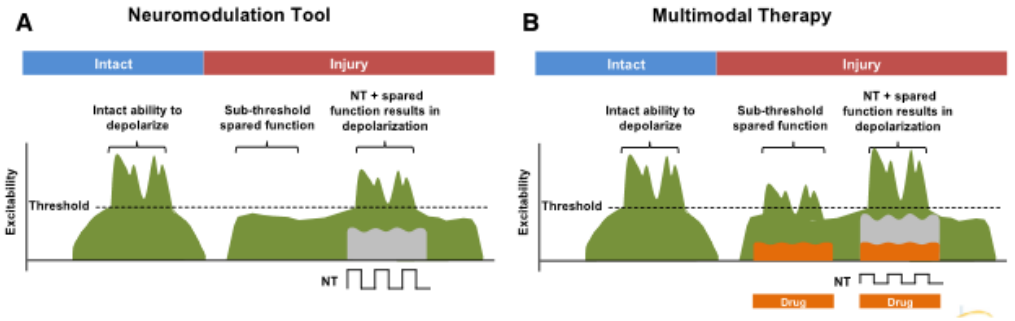


Fig. 2 **a** Neuromodulatory Interventions in SCI. In the intact cord, there is sufficient ascending, local, and descending input to effect depolarization of motor neurons to produce movement. In the context of injury, however, the reduction in inputs to the areas of the spinal cord below the injury results in a state that does not reach the threshold and cannot depolarize motor neurons resulting in paralysis. The addition of electrical or magnetic stimulation helps increase the state of the spinal cord such that previously subthreshold inputs can now depolarize some motor neurons to produce movement. **b** Rehabilitative strategies that employ interventions with mechanistically discreet actions might result in additive or synergistic improvements in function to recruit greater numbers of motor neurons

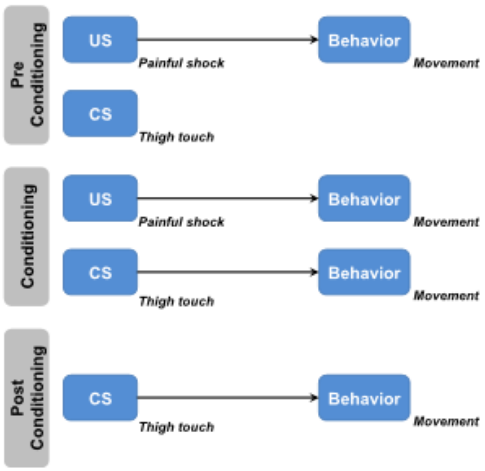


Fig. 3 The spinal cord is capable of learning. Preconditioning. In Pavlovian classical conditioning, the Unconditioned Stimulus, US, is an innate, unlearned behavior, such as retracting the limb from a painful shock. A stimulus for which there is no innate behavior, such as light touch on the thigh, does not result in any innate behavior, and the animal or in the case isolated spinal cord, can be conditioned to respond. Conditioning. Here the Conditioned Stimulus, CS, is paired (delivered at the same time) as the US. This is done over multiple learning sessions to establish the conditioning. Post Conditioning. Once conditioning has been established, the CS will evoke the same behavior as the US. In the context of spinal cord rehabilitation, care must be taken to avoid inadvertently painful stimulation even in sensory complete individuals. Maladaptive learning could occur with pairing of tasks or stimuli meant to improve movement if they co-occur with or produce pain, respectively

There are currently no published studies that assess the utility of pharmacological treatment in patients with complete spinal cord injury (ASIA Impairment Scale A) to determine whether **treatment can** unmask residual lower extremity function. **Such an approach** may be fruitful as interventions that **enhance spinal** cord activity state may serve to enable attenuated **supraspinal** volitional input distal to lesion of injury (**Fig. 3**). **Additionally**, there are currently no multimodal studies that combine electrical/magnetic forms of neuromodulation with pharmacological means to further activate the spinal cord, which may be useful.

Changes in other systems

While there may be limited functional recovery after locomotor training, numerous changes in other systems have been well documented. For example, locomotor **training has been** shown to increase **muscle and bone mass** [67, 68]. **It has also** been shown **to increase heart rate, blood pressure regulation**, and reduce **ventilatory need** [69–71]. **And perhaps**, most importantly, **many studies have shown significant** increased subjective **sense of independence in SCI patients** after locomotor **training** [72, 73].

Conclusion

Because the spinal cord can carry **our semi-independent** behaviors and is capable of learning, it is critical to design rehabilitative strategies that work with the innate behaviors

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of the cord. The innate behavior of integrating proprioceptive information from the limbs and body axis in order to modulate the central pattern generating circuitry is one that shows great promise. Several methods of increasing proprioceptive input (treadmill with body weight support and vibratory stimuli of muscle proprioceptors), or altering the tone of the cord (electric, magnetic, and pharmacologic stimulation) appear to that each method has great promise. Lessons from noxious stimulation experiments demonstrating the ability of the cord to learn operant and classical conditioning, especially to noxious stimuli, should caution caregivers in therapeutic design as some schedules of learning are maladaptive and could result in inadvertent reductions in function. Rehabilitation of the spinal cord, like other regions of the CNS, will have to choose a stimulus or the stimuli that best serve the behavior to be restored, and do so in a schedule that matches the ability of the spinal cord to constructively integrate these inputs.

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Compliance with Ethics Guidelines

Conflict of Interest Tianyi Niu, William A. Alaynick, and Daniel C. Lu declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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A Proof-of-Concept Study of Transcutaneous Magnetic Spinal Cord Stimulation for Neurogenic Bladder

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Patients with chronic spinal cord injury (SCI) cannot urinate at will and must empty the bladder by self-catheterization. We tested the hypothesis that non-invasive, transcutaneous magnetic spinal cord stimulation (TMSCS) would improve bladder function in individuals with SCI. Five individuals with American Spinal Injury Association Impairment Scale A/B, chronic SCI and detrusor sphincter dyssynergia enrolled in this prospective, interventional study. After a two-week assessment to determine effective stimulation characteristics, each patient received sixteen weekly TMSCS treatments and then received "sham" weekly stimulation for six weeks while bladder function was monitored. Bladder function improved in all five subjects, but only during and after repeated weekly sessions of 1 Hz TMSCS. All subjects achieved volitional urination. The volume of urine produced voluntarily increased from 0 cc/day to 1120 cc/day ($p = 0.03$); self-catheterization frequency decreased from 6.6/day to 2.4/day ($p = 0.04$); the capacity of the bladder increased from 244 ml to 404 ml ($p = 0.02$); and the average quality of life ranking increased significantly ($p = 0.007$). Volitional bladder function was re-enabled in five individuals with SCI following intermittent, non-invasive TMSCS. We conclude that neuromodulation of spinal micturition circuitry by TMSCS may be used to ameliorate bladder function.

Spinal cord injury (SCI) leads to long-term disabilities with significant social and economic consequences. After SCI, bladder dysfunction is common and improved bladder function consistently ranks as the top quality of life priority in individuals with SCI[1,2]. Patients with a neurogenic bladder following SCI often catheterize themselves to empty the bladder, and urinary tract infections and obstructive uropathies are common[3–5]. Direct muscle stimulation[6], stimulation of peripheral nerves[7], or rhizotomy[8] to restore bladder function all have limitations. Most of these interventions fail to restore the complex, orchestrated sequence of muscle contraction and relaxation that normal, voluntary micturition requires[9].

Recently, epidural spinal cord stimulation (SCS) was used to enhance motor function in individuals with chronic SCI[10–12]. It is our hypothesis that spinal networks have the capacity to execute a range of complicated movements requiring detailed coordination among motor pools within the spine with minimal or even no input from the brain[12], and electrical or magnetic stimulation of the spine restores or permits coordinated activation of these spinal circuits. We hypothesize that a similar mechanism of SCS to the restoration of reaching and grasping function may be at play with respect to bladder function whereby co-contraction of agonist-antagonist muscles is abolished and voluntary motor control of micturition may be restored[13]. Thus, SCS can be used to address detrusor-sphincter dyssnergia (DSD), where there is agonist/antagonist muscle co-contraction, and disinhibit or

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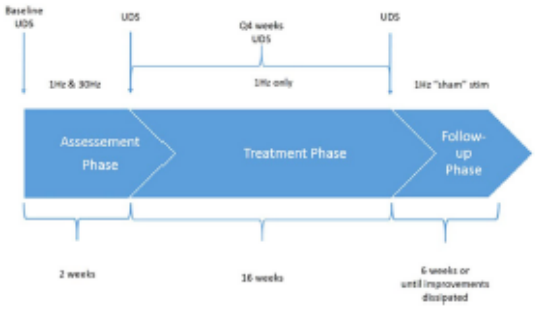


Figure 1. Overview of the study. There were three phases of the study: assessment, treatment and follow-up. The time frame for each is shown in the flow chart. During the assessment phase, each subject received stimulation with both 1 Hz and 30 Hz, each stimulation frequency delivered for one week, and underwent urodynamic testing (UDS) with video recording at the end of the assessment phase to determine the optimal frequency based on the changes in urethral and detrusor pressures during micturition attempts with either stimulating frequency. The 1 Hz stimulation frequency reduced urethral pressure and increased detrusor pressure in all subjects more effectively than 30 Hz stimulation. Therefore, each subject received 1 Hz stimulation during the treatment phase and received weekly stimulation treatment for 16 weeks. During the follow-up phase, the subject received "sham" stimulation at < 5% intensity in order to blind each subject to the change in stimulation treatment. The follow-up phase lasted 6 weeks or until each subject's urological improvements completely dissipated.

enable volitional control of the spinal micturition circuit that coordinates detrusor constriction with sphincter relaxation.

Epidural electrical stimulation can activate micturition in rodents[14], but epidural stimulation is invasive and costly. We were able to demonstrate recently that transcutaneous electrical stimulation of the spine can activate descending motor pathways non-invasively in paraplegic individuals, but such stimulation can be painful, and the spread of electrical current may activate other susceptible structures with adverse or painful consequences[15].

Magnetic stimulation can also be used to modulate neural circuits, and with figure-eight coils, the energy can be targeted to some extent. Moreover, transcutaneous magnetic stimulation is non-invasive and painless. Transcranial magnetic stimulation (TMS) has been used to modulate neuronal function in a variety of settings from migraine treatment[16] to depression[17] to restoration of motor function after ischemic stroke[18]. In the current study, we used transcutaneous magnetic spinal cord stimulation (TMSCS) to stimulate the lumbar spine to try to improve bladder function in five patients with SCI who were unable to urinate voluntarily. We hypothesized that neuromodulation of the spine using TMSCS would allow these patients to achieve voluntary micturition and reduce or eliminate the need for bladder self-catheterization.

Results

Subjects underwent 3 study phases (Fig. 1). Demographic information and indices of bladder function for all five subjects are shown in Table 1. The magnetic resonance images (MRI) indicating the level and extent of SCI for each subject are shown in Fig. 2. The average duration of SCI was 8.8 ± 7.5 years. None of the subjects had been able to void voluntarily since the time of injury as shown in at least three prior urodynamic studies in each subject.

Determining the optimal frequency: spinal function. The bulbocavernosus reflex (BCR) is disinhibited and pathologically hyperactive after SCI (Fig. 3). The BCR amplitude was significantly reduced during 1 Hz TMSCS in all five subjects ($p < 0.001$). In contrast, high frequency stimulation either increased the BCR amplitude or had no significant effect. The average BCR latency was 35.2 ± 5.3 ms during both 1 Hz and 30 Hz TMSCS, which is similar to the latency of the BCR in normal individuals[19].

During 1 Hz TMSCS, spinal cord evoked potentials could be elicited in selected lower extremity muscle groups (perineal, vastus lateralis and quadriceps femoris); whereas we were unable to detect any spinal evoked potentials at 30 Hz stimulation (Fig. 3).

Determining the optimal frequency: bladder function. During the assessment phase, the urethral (P urethra) and detrusor pressures (P detrusor) obtained during urodynamic testing during attempted volitional micturition were significantly different during high and low frequency TMSCS (Fig. 4). The urethral and detrusor pressures are shown in Tables 2 and 3, respectively. The average urethral pressure was significantly lower than the baseline, unstimulated value during 1 Hz stimulation ($p < 0.05$) and the average urethral pressure was greater than the unstimulated, baseline value during 30 Hz stimulation (though this was not statistically significant $P < 0.10$). On the other hand, the average detrusor pressure was significantly elevated during 1 Hz stimulation compared to both the baseline, unstimulated condition and 30 Hz stimulation ($P < 0.01$ for both comparisons), and the detrusor pressure was not different from baseline conditions during 30 Hz stimulation ($p = 0.5$). Thus, stimulation at low frequency allowed each subject to elevate the bladder pressure and reduce the urethral pressure

#	Sex	Age	Injury level	ASIA Grade	Disease duration (years)	Mechanism of Injury	Length of stimulation until volitional micturition (weeks)	Decay of the effect duration (weeks)	CIC/ day Pre	CIC/ day Post	Volitional Void (Y/N)	Stream Velocity (ml/s)	Bladder Capacity Pre (ml)	Bladder Capacity Post (ml)	Daily Voiding Volume Post (ml)
A	M	42	T4	A	13	MVA	4	4	9	0	Y	10	141	431	2000
B	M	43	T4	A	5	Wrestling	6	3	6	3	Y	10	238	462	700
C	M	22	C5	B	8	Football	5	3	6	3	Y	10	270	351	800
D	M	25	C6	B	8	MVA	5	4	6	1	Y	8	215	325	1800
E	M	23	C7	A	8	MVA	8	2	6	5	Y	8	354	452	300
Avg	—	—	—	—	—	—	5.6	3.2	6.6	2.4	—	9.3	244	404	1120
SD	—	—	—	—	—	—	1.5	0.8	1.3	1.9	—	1.1	78	62	740

Table 1. Demographic information, the origin and nature of the SCI and urinary indices. AIS = American Spinal Injury Association Impairment Scale. MVA = motor vehicle accident. CIC = clean intermittent catheterization.

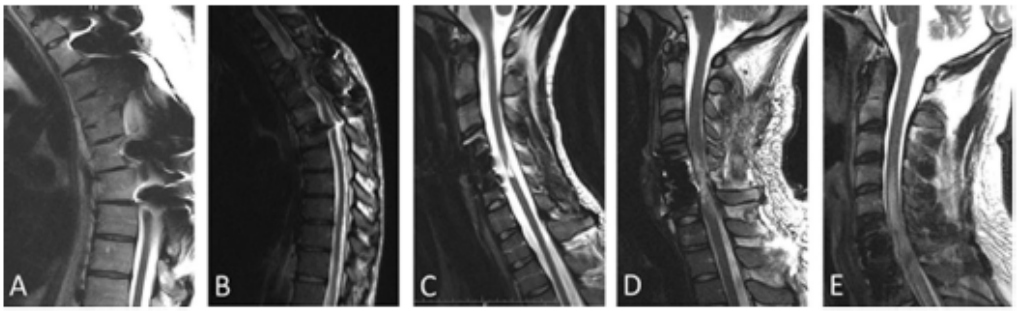


Figure 2. T2-weighted MRI imaging showing the degree of SCI in all five subjects enrolled in the study. The MRIs were obtained to ensure there was no spinal cord transection and to assess the anatomical level of injury (cervical/thoracic/lumbar). Authors (TN and DCL) reviewed all the MRIs prior to enrolling each subject in the study. A synopsis of the formal neuroradiology report was reviewed and included here for reference. (**A**) Prominent metallic artifact from fusion hardware in the superior to mid thoracic spine significantly obscures evaluation at these levels. The small segment in which the cord can be visualized at T4-T5 demonstrates prominent cord myelomalacia. Stable compression deformity of T5 without retropulsion. Scattered discogenic changes are seen in the thoracic spine from T8 through T12 without significant foraminal or canal stenosis. The cord is unremarkable at these levels. (**B**) Metallic artifact from instrumentation hardware in upper thoracic spine makes the evaluation of the spinal cord difficult at high thoracic spine levels. On axial images, significant myelomalacia is noted at T3-4 level. Below T5, the spinal cord appears to have normal caliber. No significant canal or foraminal stenosis. (**C**) Severe spinal cord myelomalacia at C5-C6. No evidence of spinal cord edema. Grossly stable anterior and posterior fusion from C4 to C6. Left vertebral artery occlusion, possibly related to chronic traumatic dissection. (**D**) Status post anterior fusion from C5 to C7 and posterior fusion. Metallic distortion artefact is noted through the fused C5 to C7 levels and significant myelomalacia or cord edema is noted at these levels. Visualized upper thoracic spinal cord appears to be in normal caliber with no compression. (**E**) Status post ACDF from C6 to T1 for repair of C7 burst fracture. Spinal cord edema and swelling spans from C4-T1.

(conditions conducive to urine flow), and 30 Hz stimulation had the opposite effect: urethral pressure increased significantly, but detrusor pressure was not modified by 30 Hz stimulation (Tables 2 and 3). Not surprisingly, increasing detrusor contraction and bladder pressure while simultaneously decreasing urethral pressure allowed voluntary micturition (Fig. 4).

Based on the BCR response, the evoked EMG activity and the responses of urethral and detrusor pressures, only 1 Hz TMSCS was used for weekly TMSCS during the treatment period.

Bladder function before, during and after TMSCS. All five subjects achieved at least some volitional urination following 16 weeks of bladder rehabilitation with TMSCS (Fig. 5). No subject achieved volitional urination until at least 4 weekly TMSCS treatments had been given, and the capacity to urinate voluntarily was restored in all 5 subjects on average 5.6 ± 1.5 weeks after TMSCS was begun. The capacity to urinate voluntarily was maintained throughout the 16-week treatment period.

Daily self-catheterization decreased from 6.6 times per day at baseline to 2.4 times per day at the conclusion of the 16-week bladder rehabilitation ($p = 0.04$). Based on urodynamic studies conducted at the end of the

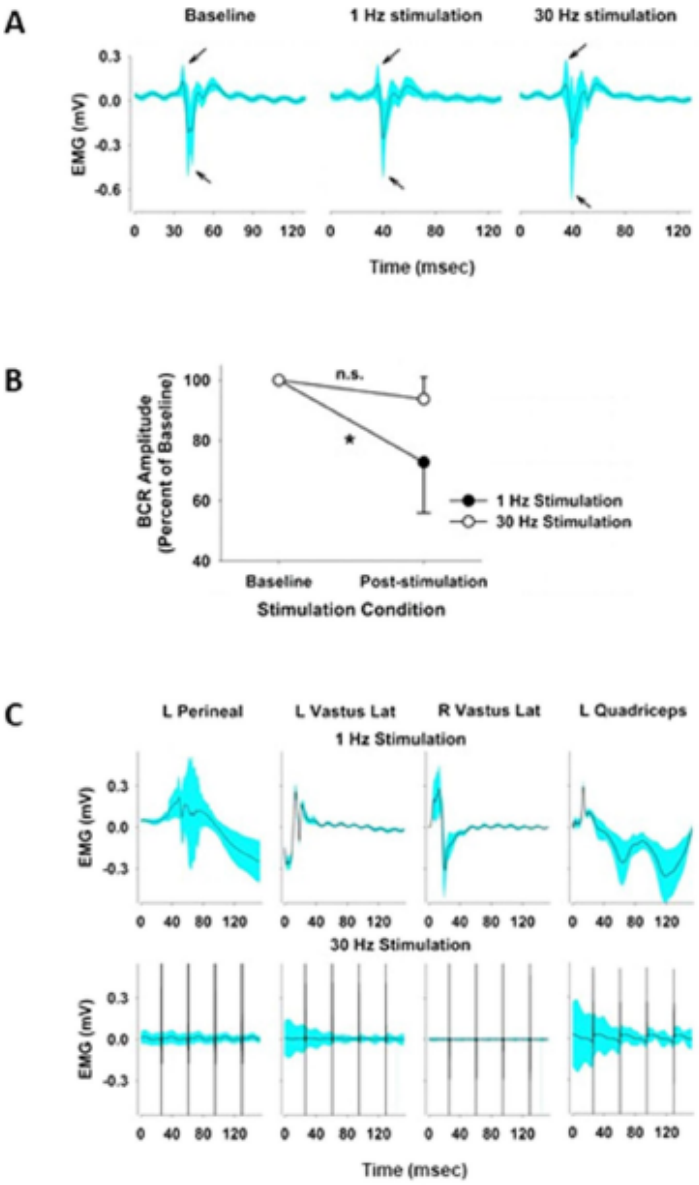


Figure 3. An example of the BCR amplitude (**A**), which is measured from the perineal muscle EMG activity, obtained from subject C at baseline and during low frequency (1 Hz) and high frequency (30 Hz) TCSMS of the lumbar spine at the end of the assessment phase of the study. The BCR was elicited serially >100 times, and the mean (solid black line) ± 2 times the SD (cyan shading) are shown for each stimulation condition. The average and standard deviation of BCR responses to 1 and 30 Hz TCSMS (**B**), expressed as a percent of the baseline value in each subject, are shown to illustrate that the BCR amplitude was significantly reduced during 1 Hz TCSMS compared to 30 Hz TCSMS. Student's t-test: *$p < 0.0001$, n.s. = non-significant, N = 100 BCR cycles. BCR = bulbocavernosus reflex. Examples of evoked EMG activity from a single subject in selected muscles are shown in C. Lumbar TCSMS at 1 Hz elicited significant EMG activity, but 30 Hz TCSMS did not alter EMG activity. Ensemble averages of EMG activity (solid black line) ± 2 times the SD (cyan shading) were derived from greater than 100 cycles of stimulation. The stimulus artifacts are shown in the 30 Hz stimulation sequences since stimulation occurred multiple times within the recording window (large black spikes). The left (L) perineum, left vastus lateralis, right (R) vastus lateralis and left quadriceps femoris muscles were recorded. The arrows in panel A represent the peak and the nadir of the BCR.

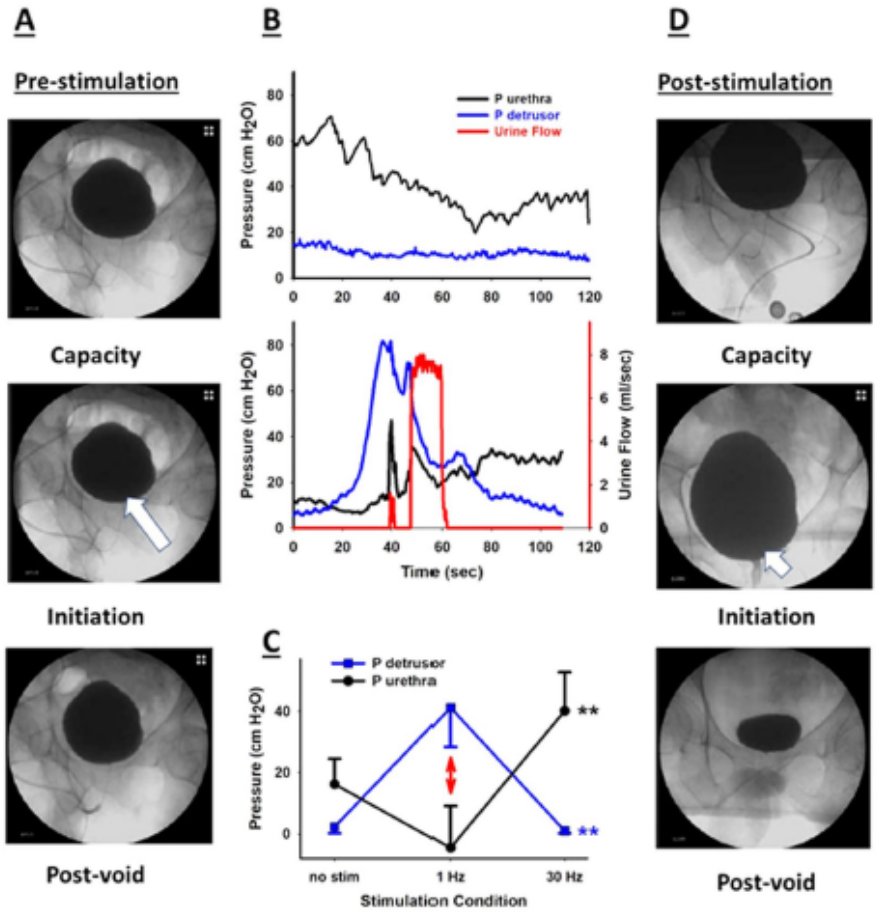


Figure 4. Examples of video urodynamics are shown from patient A (panels A – before the 16-week TMSCS treatment and D – after the 16 week TMSCS treatment). The first video images in each sequence show the pre-voiding bladder capacity, which increased after TMSCS. The second images show the initiation of volitional voiding and opening the bladder neck (white arrows), and the final images show the post-void residuals. In panel B, examples of urine flow (red line); urethral pressure (black line) and detrusor pressure (blue line) are shown before (upper graph) and after the 16-week TCSMS treatment (lower graph). Note that detrusor pressure remained below urethral pressure before TMSCS, and no urine flow was generated; whereas detrusor pressure exceeded urethral pressure and urine flow was generated after 16 weeks of TMSCS. The average urethral and detrusor pressures ± SD obtained during efforts to void at the end of the assessment phase are shown in panel C during baseline and 1 and 30 Hz TMSCS. The detrusor pressure rose significantly and the urethral pressure fell significantly only during 1 Hz TMSCS compared to the non-stimulated condition and the 30 Hz condition (**$p < 0.0001$), but the baseline, unstimulated state and the 30 Hz condition did not differ from each other based on an ANOVA and specific comparisons using Tukey's HSD.

TMSCS treatment, the average volume of urine generated voluntarily increased from 0 cc/day to 1120 cc/day ($p = 0.03$), and the subjects were able to generate significant urine stream velocities, which rose on average from 0 cc/sec to 9.3 cc/sec ($p < 0.001$). The bladder capacity increased from 244 ml to 404 ml ($p = 0.02$). Sexual function also improved from 9 to 20 as measured by Sexual Health Inventory for Men (SHIM) ($p = 0.0003$). The subjects enjoyed a much higher quality of life; the average i-QOL score rose from 47 to 82 ($p = 0.007$, Fig. 4). While all five subjects had improved bladder function and were able to achieve volitional micturition, their responses to TMSCS varied (the responsiveness order was A > D > B = C > E). This variation did not appear to be the result of differences in their AIS. (Table 1).

The average time that volitional micturition was maintained after the sham stimulation began was 3.2 ± 0.8 weeks. Follow-up diary entries confirmed that the ability to void voluntarily rapidly decayed in all subjects after

Δ P ure (mmHg)	No stimulation (NoS)	Low frequency (1 Hz)	High frequency (30 Hz)
Subject A	28.4 ± 5.0	−25.3 ± 2.7	36.2 ± 11.1
Subject B	2.5 ± 4.8	0.4 ± 5.2	50.3 ± 17.0
Subject C	19.8 ± 3.4	−8.3 ± 6.3	20.0 ± 10.0
Subject D	18.9 ± 1.5	5.6 ± 7.3	27.6 ± 4.1
Subject E	15.3 ± 5.2	−0.9 ± 9.6	46.6 ± 11.1
Average	17.0 ± 9.4	−5.7 ± 12.0 1 Hz vs NoS $p < 0.05$ 1 Hz vs 30 Hz $p < 0.001$	36.1 ± 12.7 30 Hz vs NoS $p < 0.10$

Table 2. At the end of the assessment phase, changes in urethral pressure in five subjects during micturition attempts compared to the pre-attempt baseline. Positive numbers indicate an increase in the pressure during the attempts while negative numbers indicate a decrease in the urethral pressure during the attempts. Notice that high frequency stimulation (30 Hz) resulted in increased/unchanged urethral pressure during attempted micturition when compared to the no-stimulation/baseline; low frequency stimulation (1 Hz), on the other hand, significant decreased urethral pressure compared to both unstimulated and 30 Hz stimulation conditions.

Δ P det (mmHg)	No stimulation	Low frequency (1 Hz)	High frequency (30 Hz)
Subject A	19.4 ± 2.2	38.8 ± 0.9	24.5 ± 4.5
Subject B	26.1 ± 1.5	28.2 ± 3.7	0.6 ± 1.3
Subject C	1.3 ± 0.6	30.4 ± 4.3	1.3 ± 0.9
Subject D	3.0 ± 1.5	49.4 ± 19.5	1.6 ± 0.8
Subject E	−1.1 ± 0.7	58.5 ± 9.4	−0.1 ± 0.7
Average	9.7 ± 12.2	41.1 ± 12.8 1 Hz vs NoS $p < 0.001$ 1 Hz vs 30 Hz $p < 0.001$	5.6 ± 10.6 30 Hz vs NoS $p = 0.50$

Table 3. At the end of the assessment phase, the change in detrusor pressure in five subjects during micturition attempts compared to the pre-attempt baseline. Positive numbers indicate an increase in the pressure during the attempts while negative numbers indicate a decrease in the pressure during the attempts. Notice that high frequency stimulation (30 Hz) did not change in detrusor pressure during attempted micturition when compared to the no-stimulation/baseline; low frequency stimulation (1 Hz), on the other hand, significantly increased detrusor pressure compared to the non-stimulated and 30 Hz conditions.

the cessation of effective TMSCS, and no subject maintained the capacity for voluntary micturition five weeks after the last effective stimulation.

Discussion

Voluntary micturition requires complex, orchestrated neuromuscular control of the urinary bladder by sensory, motor and autonomic systems. During voluntary micturition, sympathetic inhibition of bladder contraction is withdrawn, parasympathetic activation of the detrusor contraction emerges to increase vesicular pressure, and contraction of the urethral sphincter is inhibited to allow urine to flow out of the bladder. This control is achieved through fronto-pontine-spinal cord projections to parasympathetic ganglia in the abdomen and to sympathetic and somatic neurons in the caudal spine. In individuals with SCI, coordination among parasympathetic, sympathetic and somatic nerve activities is lost: bladder pressure is elevated, but the bladder cannot be completely emptied because contraction of the external sphincter is not inhibited. Patients with SCI must perform multiple bladder self-catheterizations each day to evacuate urine and to prevent kidney injury due to high pressure; which increase the risk and frequency of infection and traumatic injury to the urethra. Any decrease in catheterization frequency, which was achieved in all study subjects, represents a potential decrease in complications associated with catheterization.

Isolated regions of lumbosacral spinal cord contain circuits that are capable of carrying out complex motor activities[20,21]. Furthermore, spinal cord injury in most motor complete, AIS A and B SCI subjects is not anatomically complete, and many spinal circuits remain intact, especially those below the level of the spinal cord injury[22]. In both animal and human subjects with chronic paralysis from SCI, motor movements have improved after invasive, epidural, electrical stimulation[10–12]. In this study, we hypothesized that the spinal micturition circuit remains intact in subjects with SCI, and since this circuit is semiautonomous, we should be able to enhance activation of patterned muscle activities controlled by these circuits and activate or modulate them using TMSCS over the thoracolumbar spine. The mechanism of action appears to be similar to the use of stimulation to improve upper extremity function in which the threshold of motor circuit activation is diminished to enable volitional, coordinated agonist-antagonist muscle activity[13]. Voluntary bladder control was restored to some extent by TMSCS in all five individuals with chronic SCI. Four out of five subjects (80%) were able to decrease the frequency of self-catheterization by at least 50%. One subject was able to void normally without any self-catheterization while another subject only needed one catheterization each day (Table 1).

Other attempts to restore urination in SCI patients by stimulating multiple peripheral nerves, specifically the pudendal, pelvic, hypogastric and tibial nerves[23–25], did not consistently improve bladder function. Furthermore, sacral nerve modulation requires electrode implantation, which is invasive and risky[26,27]. TMSCS differs in that

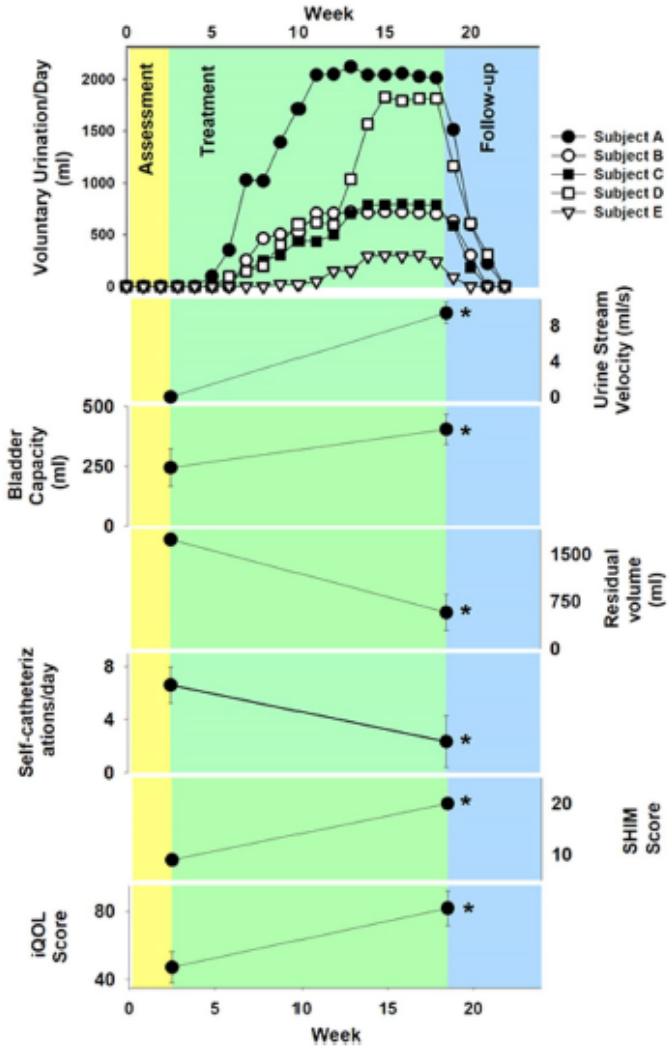


Figure 5. Summary of urological functions for all five subjects and average daily volitional micturition volume for all five subjects during follow-up phase; all changes were statistical significant when tested with paired t-tests ($p < 0.05$; see Results for details). The top panel shows the timing of recovery and loss of voluntary control of micturition and the volume of urine produced each day as a function of time. All five subjects recovered the capacity to urinate voluntarily, and about 2–3 weeks after the termination of TMSCS, the capacity to urinate voluntarily declined rapidly back to the baseline (unable to void voluntarily). The remaining panels indicate the initial value of each variable before the start of TMSCS and after 16 weeks of TMSCS. The urine stream velocity and the bladder capacity (both measured during urodynamic studies) increased significantly ($p < 0.05$) after 16 weeks of TMSCS. The residual volume and the number of self-catheterizations diminished significantly ($p < 0.05$, for both variables), and the SHIM Score and the iQOL, both quality of life measures, increased significantly ($p < 0.05$) after 16 weeks of TMSCS.

it is non-invasive and painless in patients with SCI. In addition, TMSCS provides more consistent and effective bladder emptying than existing epidural stimulation of selected peripheral nerves[6–8].

We believe that TMSCS allowed volitional activation of a coordinated pattern of parasympathetic withdrawal and sympathetic activation and somatic muscle inhibition as demonstrated in urodynamic studies. While the precise mechanism of TMSCS remains unknown, the coordinated activity of detrusor and sphincter muscles suggests that TMSCS works by activating or enhancing activation of central pattern generating circuits within the lumbosacral spinal cord and does not rely solely on activation of motor neurons or peripheral nerves. This

hypothesis receives further support from the divergent responses to TMSCS at 1 Hz and 30 Hz: 1 Hz TMSCS resulted in decreased urethral pressure, increased detrusor pressure and micturition, as opposed to 30 Hz TMSCS, which increased urethral pressure, decreased detrusor pressure and enhanced urine storage within the bladder. The different stimulation frequencies elicited different bladder behaviors as if different central pattern generators (CPGs) or different aspects of a micturition CPG were activated. These divergent responses suggest that TMSCS may be applicable to a broader range of conditions such as hyperactive bladder, which may benefit from higher frequency stimulation.

We selected patients with detrusor-sphincter dyssynergia specifically because this is the group of SCI patients most recalcitrant to treatment. A regular schedule of self-catheterization prevents ureteral reflux and the development of obstructive uropathy and chronic renal failure, but frequent catheterization has risks of its own: infection, creation of false passages, urethral stricture[28,29], a reduced quality of life, and a loss of independence. Improving quality of life is our ultimate goal using TMSCS, but this cannot be achieved if the risk of ureteral reflux and chronic renal failure increases. Therefore, any benefits of TMSCS, such as a more physiological voiding sequence with low storage pressures and increased bladder capacity and a better coordination of increased detrusor compliance and reduced external sphincter pressures that enable unobstructed voiding in a low pressure system, will be beneficial in the long term only if ureteral reflux is not increased. Video urodynamics performed at the initiation and termination of our study demonstrated no evidence of reflux. While this was a proof of concept, pilot study with patients followed for 16 weeks, additional studies are needed in an expanded cohort with extended follow-up to ensure that stable bladder and renal function are maintained when TMSCS is used to increase voluntary micturition and reduce the frequency of self-catheterization.

The BCR is a polysynaptic reflex, and BCR amplitudes in our subjects were 10 to 100 times larger at baseline than in normal individuals. Hyperactivity of the BCR may be analogous to the hyperactivity of tendon reflexes following SCI and suggests that subjects with SCI have decreased supraspinal inhibition of the BCR. During low frequency TMSCS, the amplitude of the BCR decreased, from which we infer that TMSCS induced greater inhibition of the BCR. Magnetic stimulation may achieve these effects by modulation of spinal interneurons via dorsal root ganglion or dorsal column stimulation, which is a putative mechanism of action for epidural spinal cord stimulation[30], or TMSCS may modulate responses within the sympathetic chain and sacral parasympathetic centers and facilitate the process of micturition.

Improvements in urinary function were not instantaneous; progressive improvement became apparent over the course of the study. Initially, simultaneous measurements of urethral and bladder pressures during volitional urination attempts revealed little (if any) sustained bladder contraction and persistently elevated urethral pressures, but after completion of at least 4 weeks of effective TMSCS, subjects became better able to generate sustained bladder contractions although detrusor-sphincter dyssynergia persisted (increased bladder pressures, but also increased urethral pressures, which prevented bladder emptying). At the end of the 16-week rehabilitation period, subjects were able to produce voluntary, coordinated bladder contractions with high detrusor pressures and reduced urethral pressures. Since bladder pressure exceed urethral pressure, urine flow velocity was increased and significantly higher urine volumes were achieved (Fig. 2).

Our subjects were able to urinate voluntarily in between treatment sessions when magnetic stimulation was not present. We believe that TMSCS persistently raised the activation state (or reduced inhibition) of the micturition circuit so that residual neural pathways between the supraspinal micturition centers and lumbosacral micturition central pattern generators were re-invigorated, which is consistent with previous findings using epidural stimulation to enhance recovery of motor function[12]. Restoration of voluntary micturition required repetitive TMSCS over at least 4 weeks. The benefits of epidural electric stimulations on motor function also required 3–5 sessions/weeks before improvements in motor functions were seen[12]. Once supraspinal to spinal communication had been restored or re-enabled by TMSCS, it remained enabled so long as the subject received some minimal amount of TMSCS during each weekly treatment session, but the benefits of TMSCS were not permanent. All subjects lost the ability to control micturition soon after the termination of effective TMSCS (Fig. 5). The temporal dynamics of the onset and offset of benefit of TMSCS are consistent with remodeling of the spinal circuitry in which some relatively slow neuronal or circuit remodeling is required to re-establish effective synaptic or supraspinal communication[31–33], and some aspect of TMSCS was necessary between periods of volitional bladder emptying to maintain the integrity of communication between supraspinal and lumbar micturition circuits. The once weekly treatment interval and stimulation protocol represent a surprisingly small recurrent input to maintain volitional micturition, but this schedule is feasible for patients, and TMSCS could be administered in weekly physical therapy sessions at low cost. In any event, neuronal plasticity or remodeling are well recognized in TMS studies, specifically with low frequency (1 Hz) stimulation[34,35]. These results and our study of hand function[12] provide two examples of the capacity of neuromodulation of spinal circuits to enable volitional control of motor functions below the level of SCI.

The responses to TMSCS varied among our five subjects. While we do not have a precise explanation for this, we know that the variation was not a result of differences among the AIS (Subject A, B, E were all category A, but subject A improved much more than the other two). The reasons for the variation are likely multifactorial, but perhaps most importantly, our subjects have variable amounts of residual spinal function. The current AIS is not sensitive to the subtleties of residual spinal functions among subjects.

The main limitations of our study are its small size and the lack of proof of the actual mechanism of action. As this is a pilot study, we plan to continue to expand the study and enroll additional subjects. Further studies will focus on the molecular and cellular processes that follow magnetic stimulation to investigate the precise mechanism of action of magnetic stimulation.

Methods

Subject selection. We conducted a pilot, prospective, interventional study in five subjects. All aspects of the study were approved by the UCLA IRB (IRB# 14-000932) and filed with ClinicalTrials.gov (registration number: NCT02331979, date of registration: 06/01/2015). All methods were performed in accordance with the relevant guidelines and regulations as stipulated by UCLA IRB. Informed consent was obtained prior to subject participation. The inclusion criteria for the study were male age 18–75, a stable American Spinal Injury Association Impairment Scale (AIS) A/B, motor complete spinal cord injury between spinal levels C2-T8 present for greater than 1 year, and a documented history of neurogenic bladder requiring intermittent catheterization. Each subject was required to have at least three prior urodynamic studies to confirm the diagnosis of neurogenic bladder with detrusor sphincter dyssynergia (DSD), which was diagnosed with urodynamic study in which a rise in detrusor pressure and concomitant needle EMG activity and rise in urethral pressure were demonstrated (see Tables 2 and 3). Patients with a history of autonomic dysreflexia were excluded from the study. Any patient who was ventilator dependent, abusing drugs, had musculoskeletal dysfunction (i.e., unstable fractures), cardiopulmonary diseases, active infections or ongoing depression requiring treatment, or had previous exposure to and use of spinal cord stimulation was excluded from the study. Patients with a history of bladder botox injection or bladder/sphincter surgeries were excluded. Five subjects were recruited and completed the study. There was no subject attrition.

Intervention. Each study subject underwent baseline urodynamic testing (UDS) at the beginning of the study to confirm the diagnosis of a neurogenic bladder with DSD and establish baseline bladder functions. The study was divided into three phases: an Assessment phase (2 weeks), a Treatment phase (16 weeks) and a Follow-up phase (6 weeks). During the Assessment phase, each subject underwent once/week transcutaneous magnetic spinal cord stimulation (TMSCS) at both 1 Hz (low) and 30 Hz (high) frequency (40–60% intensity) over the lumbar spine (described below). 1 Hz and 30 Hz were both administered during the assessment phase because the optimal stimulation frequency in human subjects was unknown to us prior to this study. The two frequencies were administered in random order. These frequencies was chosen based on previous results in animals in which low frequency stimulation promoted and high frequency stimulation inhibited micturition in animals with SCI[13]. At the conclusion of the Assessment phase, each subject underwent another UDS to determine the better stimulation frequency (the characteristics of optimal stimulation are defined below). Once the better frequency was established (and it turned out that 1 Hz was better than 30 Hz stimulation in all five subjects), each subject entered the treatment phase of the study and received weekly transcutaneous lumbar spinal cord magnetic stimulation for a total of 16 weeks (described below). This 16-week period of TMSCS constituted bladder rehabilitation. Each subject received non-video urodynamic testing once every four weeks during the treatment phase to monitor progress and insure that bladder function was not further impaired. After the initial four-week stimulation period, each subject was asked to attempt volitional urination for 5–10 minutes prior to bladder catheterization. The subjects were instructed to keep the environment quiet, relax and focus on voiding. Specifically, they were instructed to perform no straining/Valsalva maneuver, external compression by Crede maneuver, reflex triggering by tapping, anal stretch, or pushing. Attempts were limited to 10 minutes. Each subject was given a urine/stool specimen collection pan (Medline DYND36600H, Mundelein, IL) to collect any volitional urinary output. In order to prevent potential urinary retention, the subjects were asked to self-catheterize after the volitional attempt and to record the catheterization output. The urinary output and the volume of the residual urine in the bladder (collected after attempted void by each patient's routine bladder catheterization) were recorded in a diary after every attempt to urinate voluntarily. Each subject was also asked to record any other changes that he may have noticed in the diary throughout the study period. During the follow-up period, sham transcutaneous magnetic stimulation (sham) was employed at reduced intensity (5%), which replicated the auditory, partial sensory and mechanical cues of real stimulation. Each subject was instructed to continue to attempt to urinate voluntarily as he had during the treatment phase, and each subject continued to keep a detailed urological lifestyle diary until the end of the follow-up phase (Fig. 1).

Each subject was also given an incontinence quality of life (iQOL) questionnaire to complete prior to the start of the study and at the end of the 16-week treatment stimulation. iQOL has been validated in multiple urological quality of life studies in patients with SCI[36,37]. Sexual functions were assessed by Sexual Health Inventory for Men (SHIM) questionnaire[38] at the beginning of the study and at the end of 16-week treatment phase.

Blinding. The state of knowledge at the start of the study, in which we did not know the effective parameters of stimulation to effect micturition, precluded a randomized trial. Therefore, we conducted a single arm study in which each subject acted as his own control. Additionally, a sham phase was conducted at the end after stimulation because there was exposure to stimulation during initial Assessment Phase (we had no initial, baseline, non-stimulated collection period), and we were unsure of the wash-out period for this exposure due to the pilot nature of this study. However, subjects and experimenters were blinded throughout the process in the assessment, treatment and follow-up phases. Given that these spinal cord injury patients have diminished/no sensation due to their injuries, The subjects did not feel any sensations at 1 Hz or 30 Hz at the level of stimulation used during treatment as they have muted sensory capacity due to their spinal cord injury. We purposefully selected a relatively low intensity stimulus to avoid any painful sensations, and the stimulation level was below the sensory and motor threshold. The subjects did hear a "click" during each stimulation (especially at 1 Hz when the click was very predicable). This auditory cue was re-created during sham stimulation as well. Approximately from T11-L3 level. The coil dimensions are 172 × 92 × 51 mm in a figure-of-eight formation with two rings each 75 mm in diameter. The target (focality) is the center of the figure of eight, which in our case is T12-L1 area, which overlies the conus medullaris in humans. We used a research coil (with identical sham and treatment faces), which allowed blinding of both experimenter and subject; thereby double blinding the follow-up phase of the study. The staff member responsible for controlling of the stimulator and the dose of stimulation was not blinded as the

stimulation parameters were manipulated during the various phases of the study; however, this person did not interact with the subject (he sat behind a curtain), and each staff member was instructed to follow the same script when administering the various tasks regardless of the particular stimulation values used. To assess integrity of blinding, we asked subjects at the conclusion of the study what each study phases consisted of; their responses were no better than chance.

Urodynamic testing. We employed a commercially available urodynamic machine (Laborie Aquarius® XT, Laborie International, Mississauga, ON, Canada). Prior to the urodynamic testing, each subject emptied his bladder by direct catheterization. The volume of urine was recorded. The patient was then placed in a supine position and a triple lumen catheter (TLC-7M, Laborie International, Mississauga, ON, Canada) was inserted. Two needle recording electrodes (1512A-M, Laborie International, Mississauga, ON, Canada) were inserted bilaterally into the perineal muscles approximately halfway between the base of the scrotum and anus and 1 cm lateral to the midline. An EMG grounding pad was placed on the knee joint. A rectal catheter (RPC-9, Laborie International, Mississauga, ON, Canada) was inserted to record abdominal pressure. The subject was next placed in a left decubitus position. A condom catheter was used to collect any urine output, which was directed through a funnel into a graduated cylinder (DIS173, Laborie International, Mississauga, ON, Canada) on a scale (UROCAP IV, Laborie International, Mississauga, ON, Canada) to record the volume of urine produced and the stream velocity.

Transcutaneous magnetic stimulation. A MagVenture Magnetic Stimulator (MagPro R30, Atlanta, GA) with an active/placebo figure-8 research coil (Cool-B65 A/P Coil) was used for all transcutaneous magnetic stimulation sessions. The spinous processes of the lower vertebrae in each subject were palpated, and thoracic 11 to lumbar 4 vertebrae were marked. The coil was centered along the midline at the L1 vertebral level during the stimulation and oriented such that the magnetic field generated was parallel to the spinal cord (rostral-caudal). We used trains of biphasic, single pulse (duration 250 μs), continuous, magnetic stimulation. Each stimulation session consisted of three 4-min continuous stimulation periods with a 30 second break between each stimulation period for a total of 13 minutes (a total of 12 minutes of stimulation plus 1 minute of breaks). For the first two weeks, each subject underwent stimulation at 1 Hz and 30 Hz frequencies (week one: 1 Hz/30 Hz/1 Hz, and week two: 30 Hz/1 Hz/30 Hz) until the better frequency was determined for the patient at the first follow-up UDS after the 2nd week of stimulation. The frequency of 1 Hz and 30 Hz was selected based on our previous work in animals and humans[12,14]. Changes in urethral (directly measured) and detrusor (vesicular – abdominal) pressures during micturition attempts were measured during both low frequency stimulation (1 Hz) and high frequency stimulation (30 Hz). The stimulation frequency that resulted in the combination of increased detrusor pressure and decreased urethral pressure during attempted micturition (hence, promoting bladder emptying) was selected as optimal. The intensity of stimulation was set 20% below the intensity that elicited local paraspinal muscular contraction for each subject (since muscle contractions would have unmasked the double blinding). This stimulation intensity was usually around 40–50% of the maximal field strength of 2 Tesla. Once the optimal frequency was determined, all subjects received the optimal stimulation frequency only at a constant intensity for the remaining 16, weekly bladder rehabilitation sessions.

Electrophysiology. At the end of the assessment phase, the following electrophysiological data were obtained on each subject before, during and after low frequency (1 Hz) and high frequency (30 Hz) transcutaneous magnetic stimulation: bulbocavernosus reflex (BCR), electromyography (EMG) and spinal evoked potentials (SEP) bilaterally in the pelvic floor and in the vastus lateralis, gastrocnemius, gluteus and quadriceps femoris muscles.

Pelvic floor EMGs were obtained using needle electrodes (Laborie 1512A-M, Laborie International, Mississauga, ON, Canada). All other muscle EMGs were obtained with 1 inch surface pad electrodes (MultiBioSensors, El Paso, TX).

The BCR was obtained by using ring stimulating electrodes (Cadwell 302243-200, Cadwell Industries, Kennewick, WA) that were stimulated with a monophasic electric pulse at 1.5 Hz, pulse width 0.2 ms, and intensity at three times the sensory threshold (or 35 mA if the subject had no sensation). At least 100 pulses were given in each BCR test session.

Recording, amplification and digitization of all data were done using an RZ2 amplifier and a PZ5–32 TDT digitizer (Tucker Davis Technologies, Alachua, FL) with a 60 Hz notch filter and band pass filtering to exclude frequencies < 3 Hz and > 200 Hz.

Data Analysis. The primary outcome was voluntary urination volume per day. Pre-specified secondary outcomes included urine stream flow rate, bladder capacity, catheterizations per day, sexual health inventory for men (SHIM), and urinary incontinence quality of life scale (iQOL). All electrophysiological data from the TDT system (Tucker Davis Technologies, Alachua, FL) were exported to a computer and analyzed using MatLab (Matlab2015b, MathWorks, Natick, MA). The BCR amplitudes and latency were calculated for every single electrical pudendal stimulation. Spinal evoked potentials (if present) were identified in the continuous recording of lower extremity EMGs.

Urodynamic data were exported from the Laborie system and analyzed using Microsoft Excel (Excel2010, Microsoft, Redmond, WA). The changes in urethral pressure (P urethra) and detrusor pressure (P detrusor) were measured and compared during baseline and during attempted micturition.

Statistical significance was assessed with Analysis of Variance (ANOVA) and paired Student's T-tests and the Bonferroni correction for multiple preplanned comparisons, when appropriate, using R 3.25 (https://www.r-project.org) and Graphpad Prism (Graphpad Software, La Jolla, CA), respectively.

Data Availability. All data generated during and/or analyzed during the current study are available from the corresponding author on reasonable request. For further details of study protocol please see Supplementary Information.

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Author Contributions
D.C.L. conceived the study and designed the experiments. T.N. and T.K. performed the experiments. T.N. and J.C.L. performed data analysis. C.B. interpreted urodynamic studies. D.C.L. and J.C.L. critically reviewed and revised the manuscript. All authors reviewed and approved the manuscript.

Additional Information
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