AWARD NUMBER: W81XWH-14-2-0129

TITLE: Restoring Bladder Function by Spinal Cord Neuromodulation in SCI

PRINCIPAL INVESTIGATOR: DANIEL C. LU M.D., PH.D. F.A.A.N.S., F.A.C.S.

CONTRACTING ORGANIZATION: UNIVERSITY OF CALIFORNIA, LOS ANGELES
Los Angeles, CA 90095

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Fort Detrick, Maryland 21702-5012

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Distribution Unlimited

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Restoring Bladder Function by Spinal Cord Neuromodulation in SCI

The central objective of this project is to use non-invasive neuromodulation that can produce improved bladder function by enabling the function of spared circuitry in the spinal cord. This normalization of the spinal cord function is accomplished through a process of functional neuroplasticity whereby neuromodulation (e.g. electromagnetic stimulation) activates spinal circuits associated with micturition. It also facilitates ascending projections for improved sensation and descending projections for volitional voiding. A subset of subjects appears to experience long-lasting improvements and can void in the absence of stimulation. A total of 18 male/female, age 18+, >1 year post (C2-T8, non-conus) injury with complete but stable severe motor paralysis (ASIA A, B) and catheterization dependent for urination will be enrolled.
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1. INTRODUCTION:
The central objective of this project is to use non-invasive neuromodulation that can produce improved bladder function by enabling the function of spared circuitry in the spinal cord. This normalization of the spinal cord function is accomplished through a process of functional neuroplasticity whereby neuromodulation (e.g. electromagnetic stimulation) activates spinal circuits associated with micturition. It also facilitates ascending projections for improved sensation and descending projections for volitional voiding. A subset of subjects appears to experience long-lasting improvements and can void in the absence of stimulation. A total of 18 male/female, age 18+, >1 year post (C2- T8, non-conus) injury with complete but stable severe motor paralysis (ASIA A, B) and catheterization dependent for urination will be enrolled.

2. KEYWORDS:

3. ACCOMPLISHMENTS:
In an allied area of sensorimotor rehabilitative research, we have discovered a method that is superior to transcutaneous electrical spinal cord stimulation (TESS) in the delivery of neuromodulatory stimulation to the spinal cord. We have identified magnetic stimulation, also known as transcranial magnetic stimulation (TMS) as a better 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 that of TESS. 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 TESS that requires a prolonged training period of 3-6 months.

b. Painless Stimulation. Our preliminary studies with TESS 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 6 months. This obviates the need to home use and a portable device. If patients experience a decline in function, they can return for therapy to improve function.

Because of the overwhelming superiority of the TMS device compared to TESS device, we elected to use the TMS for this study. With the TMS device, 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 6-month study. Results will be prepared for manuscript publication.
Percent Complete: 15%

Figure 1: Three magnetic stimulation sessions enable voluntary bladder function. Patient #001 (75yo male, C2 SCI, ASIA D, Injury 6/2014) underwent three stimulation sessions (indicated by the red arrows) with magnetic stimulation:
- Week 1: 1Hz @ 40% x 2 min; 5Hz @ 40% x 2 min; and 20Hz @ 40% x 2 min.
- Week 2: 1Hz @ 40% x 3 min; 5Hz @ 40% x 3 min.
- Week 3: 1Hz @ 40% x 3 min.
Our preliminary impressions are: 1) more than one stimulation session is required to activate spinal micturition-related circuitry; 2) the effect is modestly durable, lasting for 2-3 weeks after the last stimulation session; 3) the subject tolerated the intervention well.
Figure 2: Constructive urinary behaviors with magnetic stimulation. Patient #002 (41yo male, T4, ASIA A, Injury 1994) underwent six weeks of stimulation with magnetic stimulation.

- Week 1: 1Hz @ 40% x 2 min; 5Hz @ 40% x 2 min and 20Hz @ 40% x 2 min.
- Week 2: 1Hz @ 40% x 2 min; 5Hz @ 40% x 2 min and 20Hz @ 40% x 2 min.
- Week 3: 1Hz @ 40% x 2 min; 5Hz @ 40% x 2 min and 20Hz @ 40% x 2 min.
- Week 4: 1Hz @ 40% x 2 min; 5Hz @ 40% x 2 min and 20Hz @ 40% x 2 min.
- Week 5: 1Hz @ 40% x 2 min; 0.5Hz @ 40% x 2 min and 3Hz @ 40% x 2 min.
- Week 6: 1Hz @ 40% x 2 min; 0.7Hz @ 40% x 2 min and 1.3Hz @ 40% x 2 min.

Several observations have been noted: 1) The subject has reduced catheterization by over half (green line); 2) the bladder capacity has increased from 250mL to 400mL and appears less spastic (purple line); 3) voluntary urination increased from 0 to 250mL per day (red line); and 4) the responsiveness to stimulation increased from non-responsive to eliciting 100mL of voiding (blue line). We have noted that in this subject 5Hz stimulation is not useful, while 1Hz and even 0.7Hz is helpful. We have measured detrusor and urethral pressures and observed a reduction in dyssynergia with stimulation. The subject has had the following beneficial side effects: 1) improved bowel function; 2) improved erectile function; 3) decreased sensations of autonomic dysreflexia. Notably all of these are related to autonomic function. In contrast, the subject has had an increase in bilateral lower extremity spasms—a motor system under volitional control. This may indicate that: 1) the anatomic proximity of bladder and leg circuitries in the cord results in stimulation of both—even at lower frequencies (1-0.7Hz) that favor bladder behaviors; 2) leg motor training may be required to ‘contextualize’ the stimulation to a useful motor behavior at the expense of spasticity.
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 pre-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: 15%**

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: 15%**

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: 0%**

- **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). Specifically we have (at the time of this reporting):

  1. Revised and identified key personnel reflecting the change in research strategy.
  2. Obtained approval for USAMRAA for device and personnel change.
  3. Obtained budgetary approval of the change from USAMRAA.
  4. Acquired and purchased all necessary equipment for this study.
  5. Obtained UCLA IRB approval.
  6. Obtained final HRPO approval.
  7. Enrolled one subject.

We fully anticipate to accomplish the goals set forth within the same timeline described above. Because of the superiority and effectiveness of the device compared to original device in accessing the spinal cord circuitry, demonstrated in a separate study (Figure 1), the time required to decipher the optimal conditions of stimulation will be abbreviated. In the previous 2 months of
Task 1, we have begun to identify the optimal parameters for stimulation and proceeding onto the subsequent tasks as planned.

- **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 Tianyi Nui, MD, a Neurosurgery Fellow 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 is has been accepted at *Current Physical Medicine and Rehabilitation Reports*. This knowledge will help to guide the further design and interpretation of this study’s results.

- **How were the results disseminated to communities of interest?**
  William Alaynick, PhD, Project Scientist UCLA delivered a lecture, “Spinal Central Pattern Generating Circuitry: From Bench to Bedside” at the European Neuroscience Institute at the University of Göttingen in Germany on December 16th 2014.

- **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: Determine the optimal stimulation parameters to enable micturition in SCI subjects. We will use the TMS device to identify the optimal stimulation parameters to enable voluntary micturition. Having now achieved regulatory and administrative approvals, along with enrolling potential participants, and begun initial testing, we have begun to identify a stimulation parameter that enables micturition.

  Task 2: Determine the minimum training conditions to enable micturition in SCI subjects. The subjects will be motor trained to prime the lumbosacral circuitry. The minimum training conditions that will enable micturition will be assessed. We have begun have begun to decipher the training conditions, while finalizing it at year 3 of the project.

  Task 3: Application of machine learning strategy to determining the optimal stimulation and training parameters for micturition in SCI subjects. Machine learning will be utilized and applied throughout in Tasks 1 and 2 to obtain the optimal conditions to enable voluntary micturition.
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**
  In an allied area of sensorimotor rehabilitative research, we have discovered a method that is superior to transcutaneous electrical spinal cord stimulation (TESS) in the delivery of neuromodulatory stimulation to the spinal cord. We have identified magnetic stimulation, also known as transcranial magnetic stimulation (TMS) as a better 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 TESS.
  b. Painless Stimulation. Our preliminary studies with TESS 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 6 months. This obviates the need to home use and a portable device. If patients experience a decline in function, they can return for therapy to improve function.

- **Actual or anticipated problems or delays and actions or plans to resolve them**
  Nothing to Report
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 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.

   Status of publication: Accepted
   Acknowledgment of federal support: Yes
   Status of publication: Accepted
   Acknowledgment of federal support: Yes
   Status of publication: Accepted
   Acknowledgment of federal support: Yes
   Status of publication: Accepted
   Acknowledgment of federal support: Yes
Status of publication: Accepted
Acknowledgment of federal support: Yes

Status of publication: Accepted
Acknowledgment of federal support: Yes

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

Website(s) or other Internet site(s)
Nothing to Report

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 will be formally studying this discovery here and 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
2. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS
   o What individuals have worked on the project?

<table>
<thead>
<tr>
<th>Name</th>
<th>Daniel C. Lu, MD, PhD</th>
</tr>
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<tbody>
<tr>
<td>Project Role</td>
<td>Principle Investigator</td>
</tr>
<tr>
<td>Researcher Identifier (e.g. ORCID ID):</td>
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</tr>
<tr>
<td>Nearest person month worked:</td>
<td>2.52 person months per year. ~1 month this period</td>
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<tr>
<td>Contribution to Project:</td>
<td>Dr. Lu oversaw all aspect of research and administration of this program</td>
</tr>
<tr>
<td>Funding Support:</td>
<td>NIH U01</td>
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<tr>
<td>Name</td>
<td>William Alaynick, PhD</td>
</tr>
<tr>
<td>Project Role</td>
<td>Project Scientist</td>
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<td>Researcher Identifier (e.g. ORCID ID):</td>
<td>1234567</td>
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<tr>
<td>Nearest person month worked:</td>
<td>2.88 person months per year. ~1 month this period</td>
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<tr>
<td>Contribution to Project:</td>
<td>Dr. Alaynick contributed to the IRB regulatory approval and continued intellectual development of the research plan</td>
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<td>Funding Support:</td>
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   o 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

   o What other organizations were involved as partners?  
   Nothing to Report

3. SPECIAL REPORTING REQUIREMENTS
   o COLLABORATIVE AWARDS: Not Applicable
QUAD CHARTS:
Improving Cortical Sensorimotor Function and Headache with Spinal Cord Neuromodulation
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.

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 (cont.)
☐ Apply machine learning strategy to define best parameters in pilot
☐ Complete pilot trial with naïve subjects
CY17 Goal – Machine Learning; Pilot trial (cont.)
☐ Develop best parameters for trial
CY18 Goal –Clinical trial of MagStim + motor rehab for bladder function
☐ Complete clinical trial
☐ Organize and analyze data for conferences and publications

Comments/Challenges/Issues/Concerns
• We have obtained UCLA IRB approval for the use of MagStim in bladder rehabilitation work proposed here.

Timeline and Cost

<table>
<thead>
<tr>
<th>Activities</th>
<th>CY 15</th>
<th>CY 16</th>
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<td>Aim1-2: Explore rehab parameters</td>
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<td>Aim 3: Define best parameters</td>
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<td>Aim 4: Apply best in Phase1/2 trial</td>
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Updated: Oct 1 2015
4. **APPENDICES:**
   2. Publication.
### 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

   - 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*

   - 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 at item 3.6.1 above.

     - 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.
   - WILLIAM ALAYNICK

5.0 List the key personnel and study staff below.
Note: All personnel listed below are required to complete CITI training courses. HIPAA training is also required if personnel will be accessing protected health information. Please make sure to have all key personnel update their webIRB profile, contact information. Instructions on how to update the webIRB profile: Click here.

<table>
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<tr>
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<th>Role</th>
<th>Other Role (if applicable)</th>
<th>Will Obtain Consent?</th>
<th>Manage device accountability?</th>
<th>Access to personally identifiable info?</th>
<th>Access to code key?</th>
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<tr>
<td>View PARIN</td>
<td>NEUROSURGERY</td>
<td>Research Assistant</td>
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<tr>
<td>View WILLIAM</td>
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<td>View PAYMON</td>
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<tr>
<td>View CAROL</td>
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<tr>
<td>View DANIEL</td>
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<td>Other</td>
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<tr>
<td>View MORTEZA</td>
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ID: IRB#14-000932  View: NEW 1.1a - Other Personnel

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

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, indicate their responsibilities, training and qualifications and complete Item 2.1.

https://webirb.research.ucla.edu/WEBIRB/Doc/0/6AJR79U394LKPCNU2NDHOP0LE4/fromString.html
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.

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.

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:

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

2.2

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

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

- [ ] Yes
- [x] No

3.1

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

---

**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](https://example.com) 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.

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

- 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.

ID: IRB#14-000932  View: NEW 1.2 - Conflict of Interest Information

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?

   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: [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?

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

   Document Name: [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):

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

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

ID: IRB#14-000932  View: NEW 1.3 - Study Locations

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:
- [ ] a. UCLA Sites or UCLA Health System Sites
- [x] 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  [x] 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.

ID: IRB#14-000932

View: NEW 1.4 - UCLA Sites or UCLA Health System Sites

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

UCLA Sites or UCLA Health System Sites

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:

- Clinical & Translational Research Center (CTRC)
- Inpatient Medical Facility
- Outpatient Treatment Facility/Private Office
- Public Area
- Research Laboratory
- 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
- Basic Life Support (BLS) certified personnel
- Advanced Cardiac Life Support (ACLS) certified personnel
- Code Blue Team (hospital emergency response team)
- Emergency crash cart
- Paramedic Emergency Response Team (911)
- Suicide Protocol
- Other
1. Type of Submission (Select one)

- Research Study
- Application for Approval of "Research Participant Pool" or recruitment database only

2. 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. 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. 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 IRB to RELY on another IRB.
  - This includes reliance using UC MOU, CTSI, NCI, RAND, and Western IRBs.

5. 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 and/or involves
gene therapy?

☐ 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 6.2 of the webIRB application.

6.0 **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

- 6.1 If yes, indicate the source of scientific or scholarly review for the study.

- Check all that apply.
  
  ☐ National Institutes of Health (NIH)
  
  ☐ The funding agency (other than NIH)
  
  ☑ Faculty Sponsor
  
  ☐ JCCC Internal Scientific Peer Review Committee (ISPRC)
  
  ☐ Clinical Translational Research Center (CTRC)
  
  ☐ UCLA Department
  
  ☐ Other
  
  - 6.1.1 If you checked "other", describe.

- 6.2 Attach a copy of the scientific or scholarly review, if applicable.

<table>
<thead>
<tr>
<th>Document Name</th>
<th>Document Version #</th>
</tr>
</thead>
<tbody>
<tr>
<td>Summary Statement DoD Bladder 2013.pdf</td>
<td>0.01</td>
</tr>
</tbody>
</table>

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

**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,....**
4.0 * Is this study regulated by the Food and Drug Administration (FDA)?
  - Yes
  - No

  - If yes, check all that apply:
    - Medical Devices
    - Biological Products
    - Food Additives
    - Color Additives
    - Other

  4.1.1 If Other, describe:

ID: IRB#14-000932

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

1.0 * Indicate all that apply to this study.
  - Audio, Visual or Digital Recordings
  - Behavioral Observations (only applicable if you selected Exempt Category 2 in section 5.3)
  - Certificate of Confidentiality
  - Clinical Trial of a Drug, Biologic, Device or a Behavioral Intervention
  - Community Based Research
  - Controlled Substances (Schedule I or II)
  - Deception or Partial Disclosure
  - 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
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), professional medical services, clinical treatment, diagnostics, labs, medical supplies, etc.)?"

Please direct any questions about this to the Clinical Trials Administration Office at clinicaltrials@mednet.ucla.edu.

Yes ☐ No ☐

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

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?"

- Investigator-initiated study
- Expanded Access (aka Compassionate Use or Treatment Use)
- Humanitarian use device study
- Chemo/radiation therapy study
- UCLA IRB to rely on another IRB for this study

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:

<table>
<thead>
<tr>
<th>Document Name</th>
<th>Document Version #</th>
</tr>
</thead>
</table>

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) will be billed for investigational products (i.e., investigational drugs and/or devices), attach any documentation to support these charges including any FDA letter(s) if available.

<table>
<thead>
<tr>
<th>Document Name</th>
<th>Document Version #</th>
</tr>
</thead>
</table>

There are no items to display

---

**Warning:** Save your work at least every 15 minutes by clicking ✽Save✵ or ✽Continue✵.
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).

<table>
<thead>
<tr>
<th>Funding Source</th>
<th>Funding Source Information</th>
</tr>
</thead>
<tbody>
<tr>
<td>View DA-ARMY MEDICAL RESEARCH ACQUISITION ACTIVITY</td>
<td>Name of the Funding Source: DA-ARMY MEDICAL RESEARCH ACQUISITION ACTIVITY</td>
</tr>
<tr>
<td>If other, specify</td>
<td>No Value Entered</td>
</tr>
<tr>
<td>UCLA PI named on the grant, contract, subcontract or gift:</td>
<td>DANIEL LU</td>
</tr>
<tr>
<td>Indicate the type of award:</td>
<td>Grant</td>
</tr>
<tr>
<td>Indicate the Grant Title:</td>
<td>Restoring Bladder Function by Spinal Cord Neuromodulation in SCI</td>
</tr>
<tr>
<td>Indicate the Award Number assigned by the funding source:</td>
<td>SCI130209</td>
</tr>
<tr>
<td>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:</td>
<td>Federal</td>
</tr>
<tr>
<td>If Other, specify</td>
<td>No Value Entered</td>
</tr>
</tbody>
</table>

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

---

ID: IRB#14-000932

View: NEW 8.1 - Study Design

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

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

- 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

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

<table>
<thead>
<tr>
<th>Brand name of device</th>
<th>Investigational Devices Information</th>
</tr>
</thead>
<tbody>
<tr>
<td>MagStim</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Trade (Brand) name of the device:</td>
</tr>
<tr>
<td></td>
<td>Common (Generic) name of the device:</td>
</tr>
<tr>
<td></td>
<td>Manufacturer of the device (if UCLA research lab, identify the lab):</td>
</tr>
<tr>
<td></td>
<td>Source of the device:</td>
</tr>
<tr>
<td></td>
<td>If &quot;Other&quot; source, specify:</td>
</tr>
<tr>
<td></td>
<td>FDA Regulatory Status of the Device</td>
</tr>
<tr>
<td></td>
<td>Investigational Use of an Unapproved Device:</td>
</tr>
<tr>
<td></td>
<td>Device is Exempt from FDA approval:</td>
</tr>
<tr>
<td></td>
<td>Humanitarian Use Device (HUD):</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Document Name</th>
<th>Document Version #</th>
</tr>
</thead>
<tbody>
<tr>
<td>X100_productsheet.pdf</td>
<td>0.01</td>
</tr>
</tbody>
</table>

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 [X]

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 [X]

ID: IRB#14-000932

Warning: Save your work at least every 15 minutes by clicking Save or Continue.
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
       - [ ] 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
         - [ ] 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
1.0 * Are the radiologic procedures standard of care?  
**Note:** Please review the guidance to the right before completing this question.

- Yes
- No

- If Yes, please provide the following information for EACH procedure:
  - Type of standard of care radiological procedure.
  - Maximum number of times a subject will undergo this procedure in one year.
  - 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.
- Urodynamic 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 radiologic 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
- No

**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.
- Radiation Therapy - Form B required. Click [HERE](#) to download form.
- Radioactive Materials - Form C required. Click [HERE](#) to
1.0 *Indicate all that apply to the study data.

- 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

☑ 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 locked room 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
Privacy and Confidentiality

Important Notes:

เตรียมความพร้อม: Privacy is about people. Privacy refers to a person's wish to control the access of others to themselves.

เตรียมความพร้อม: 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.

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.

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

  There are no items to display

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

- 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.

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

Warning: Save your work at least every 15 minutes by clicking Save or Continue.
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#14-000932

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:

- 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

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 specimens would be stored for future use by (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:
- [ ] 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#14-000932

View: NEW 10.1 - Study Summary - Research Study

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

Study Summary - Research Study

1.0 Study Materials: As applicable to this study, attach the following:

- [ ] Protocol, Dissertation Proposal or Study Plan
- [ ] Preliminary Data
- [ ] Surveys, Questionnaires or other instruments to be used with study participants
- [ ] References

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2.0 *Specific Aims: Indicate the purpose of the research, specifying the problems and/or hypotheses to be addressed.

Specific Aim 1: Determine the optimal stimulation parameters to enable micturition in SCI subjects. We hypothesize that optimal spinal cord stimulation parameters exist that are selective or specific for micturition. We will test combinations of spinal cord levels (T10-L4, coccyx levels) and stimulation frequency (1-30 Hz) administered twice weekly for 24 weeks. A machine learning algorithm will guide subsequent stimulation parameters (Aim 3). Subjects will be evaluated at each session for urine flow, volume, detrusor pressure, and self-assessments of quality of life and urinary function. Formal urodynamics will be tested monthly and at the conclusion of the 24-week study.

Specific Aim 2: Determine the minimum training conditions to enable micturition in SCI subjects. We hypothesize that the naive post-injury spinal cord requires some minimal stimulation quality and duration to re-awaken dormant micturition neural circuitry. Using the optimum stimulation parameters determined in Aim 1, we will test a pre-training regimen in naive SCI subjects. In addition to determining the minimum number of sessions, we will examine the types of locomotor training that will best enable micturition function, administered twice weekly for 24 weeks. Assessments will be as in Aim 1.

Specific Aim 3: Application of machine learning strategies for determining the optimal stimulation and training parameters to induce micturition in SCI subjects. We hypothesize that existing machine learning techniques for locomotion can be adapted to determine the optimal stimulation
parameters for micturition. Data from each session will be added to a machine learning algorithm database to determine the most effective parameters for the most recent session and guide stimulation parameters for subsequent sessions. At the conclusion of this Aim, the most effective conditions for urination will be determined and used in the clinical trial.

Specific Aim 4: Application of the optimal stimulation and training parameters for inducing micturition in SCI subjects in a pilot clinical trial.

This Aim will combine the optimal stimulation and training parameters from Aims 1-3. In this Aim we will test the hypothesis that an optimized stimulation paradigm can improve micturition function in naïve, SCI subjects. 12 subjects will be tested twice weekly for 24 weeks. Urodynamic and self-assessments will be as Aim 1.

3.0 Background and Significance: Provide a summary of the background for this study and explain how it will contribute to existing knowledge.

For greater than minimal risk biomedical studies, include preliminary data. If necessary, attach in Item 1.0 graphs or tables used to convey information. If there no preliminary data are available, briefly indicate why this proposed study is a reasonable starting point.

Little progress has been made in developing any intervention that will enhance bladder function after a SCI. However our team has performed a systematic progression of experiments in animals and patients showing that several forms of electrical spinal cord stimulation can detect and improve spared function (Table 1, Figure 1). We have implanted an epidural stimulation (EDS) electrode array over the lumbosacral spinal cord in 4 human subjects with a motor complete SCI. Each has gained some voluntary control of urinary voiding in the absence of EDS (1, 2). Furthermore, we have preliminary data showing that single-electrode TESS or EDS of the cervical 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 somatomotor 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 abilities 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 $13.5B 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 viscerosympathetic 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 were 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 vesicourethral reflux during the filling phase. Subject will then be asked to void/voluntarily 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.

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 the meaning, reliability, and applicability of the test results for health care decisions.

- 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.
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.

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| 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. **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](https://webirb.research.ucla.edu/WEBIRB/Doc/06AJR79U394LKPCNU2NDHOP0LE4/fromString.html)
   - 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](https://webirb.research.ucla.edu/WEBIRB/Doc/06AJR79U394LKPCNU2NDHOP0LE4/fromString.html)

2. **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
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

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

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 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 strain 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
compilation 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.

☑ All types of studies - Choose not to participate in the study

☑
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 will still have access to his/her physician for care of the bladder associated issues related to spinal cord injury.

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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

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

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 6.1/Item 3.0)
3. This study involves treatment in an emergency setting (Section 8.1/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

Desigee 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
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.
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
- [ ] University check
- [ ] Course Credit
- [ ] Cash
- [ ] Gift Cards/Bruiocard 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/Bruiocard 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
ID: IRB#14-000932

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:*

- [ ] All UC participants will sign a UC HIPAA Research Authorization for Release of Personal Health Information for Research.
- [ ] 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.
- [ ] 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

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

- [ ] 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 ✉ ✉
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.

☐ The PHI is needed to identify potential participants with a specific medical condition

☐ 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

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.

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<tr>
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#### 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.
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.

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 on 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
- Other

- 3.1
- If other, describe

4.0 Indicate why the research could not practically 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
- Other

- 4.1
- If other, specify

5.0 NON-UC INSTITUTION(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) institution(s)/agency(ies)' HIPAA policies and procedures.

Agree ✗ ✓
### Eligibility Screening - Plans

You indicated that eligibility screening will be conducted during the recruitment process (Section 19.1/item 1). Please provide the following information.

#### 1. Will private identifiable information be collected during the screening?
- [ ] Yes  [ ] No

- **1.1** 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

- **1.2** 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
  - [ ] The data will be destroyed

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

#### 2. Indicate your plans for obtaining informed consent and/or parental permission for the screening procedures.

Check all that apply.
- [ ] Oral consent will be obtained for the screening procedures. Participants will not be asked to sign a consent form (Waiver of written consent).
- [ ] A waiver of informed consent is requested for the screening procedures
- [ ] A waiver of Research Authorization for HIPAA is requested for the screening procedures.
- [ ] Signed consent will be obtained prior to performing any of the screening procedures

- **2.1** If you checked more than one plan above, list the study groups and the plan that you will use for each.

#### 3. 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.

- **3.1** Attach screening script(s), if applicable.

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Oral Consent – For Screening Procedures
You indicated that you are obtaining oral consent for the screening procedures (Section 19.2/Item 2). Please provide the following information.

1.0 *Indicate the reason that you are requesting to conduct an oral consent process and/or parental permission instead of obtaining signed consent.

- The research is minimal risk and does not involve any procedures for which written consent is normally required outside the research setting (e.g., in everyday life written consent is not needed for minimal risk surveys, non-invasive health measurements, etc.) (45 CFR 46.117 c2)
- The only record linking the participants and the research would be the consent document, and the main risk of research would be a breach of confidentiality (45 CFR 46.117 c1).
  - e.g., Participants could suffer from social stigma, embarrassment, or other harms if it became known that they participated in research that identified them as having issues including, but not limited to, risky sexual behaviors, HIV, or mental health problems.

If you indicated that the main risk is a breach of confidentiality, answer 1.1 if appropriate.

- 1.1
  - According to DHHS regulations at 45 CFR 46.117(c1) when the main risk of the research would be a breach of confidentiality and an oral consent process is used, each participant should be asked whether he/she wants documentation linking the subject with the research and the subject’s wishes will govern.
  - Check here if you want the IRB to consider allowing a waiver of this regulation so that you do not need to ask each subject if he/she wishes documentation.
  - ✔ Request to waive documentation linking the participant with the research

2.0 *Provide a description of the oral screening procedures for the study.
Patients will be referred to Dr. Denis or Niu who will conduct a phone screening for eligibility.

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. If you checked more than one plan above, list the study groups and the plan that you will use for each.
2. 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

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
- 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.

- 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.

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
- 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.

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<thead>
<tr>
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<tr>
<td>DoD Bladder Consent Form Clean 5-28-15.docx</td>
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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.
- 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.

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 speak 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 fluent. Translations into the most common languages in the greater Los Angeles area are available for download on the OHRPP website.

☐ Agree

☐ Not Applicable

If minors are involved in the study, this would also include the processes of obtaining parental permission and assent, as applicable.

ID: IRB#14-000932

Warning: Save your work at least every 15 minutes by clicking Save or Continue.
2.0 Will surveys or interviews be conducted with DOD personnel as part of this study?
   - Yes [ ] No [ ]
     - 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).
     -

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 4.2)
    - Attached
    - Pending

- 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.

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<td>Data Collection Forms DoD Bladder.pdf</td>
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- 4.5
  - *FDA Letter for IND or IDE
    (Attach the FDA letter for an IND at Section 9.7/Item 1(1.1.2.3), 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

ID: IRB#14-000932

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.

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

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

  - Check all that apply.

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<thead>
<tr>
<th>Privacy and Confidentiality section of the consent form(s) (required only if the Monitor will have access to individually identifiable data)</th>
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<tbody>
<tr>
<td>In Section 15 (Data &amp; Safety Monitoring) of this application</td>
</tr>
<tr>
<td>In the attached protocol for this study</td>
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</table>

- 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

☐ 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

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  • If you indicated "other," describe.
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1.0 Attach any other documents that have not been specifically requested in previous items, but are needed for IRB Review.

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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.

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Instructions for Study Submission

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1. Click the **Finish** button to return to exit the SmartForm and return to the study workspace.
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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**.
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Behavioral Observations (only applicable if you selected Exempt Category 2 in section 5.3)

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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. The project does not need to be funded by NIH to obtain a Certificate of Confidentiality. For additional information see [http://grants.nih.gov/grants/policy/coc/](http://grants.nih.gov/grants/policy/coc/)

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.

Community Based Research

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](http://ag.ca.gov/research/guide.php)  
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), methylenedioxymethamphetamine (MDMA), marijuana, and psilocybin. 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](http://www.deadiversion.usdoj.gov/schedules/index.html)

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).

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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/ohrt/irbs/irbreview.pdf

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- 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

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Genetic Analyses/Genotyping

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

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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

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Studies involving gene transfer and/or recombinant DNA require approval of the UCLA Institutional Biosafety Committee (IBC) and the NIH Recombinant DNA Advisory Committee (RAC). 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.

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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).

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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.

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


None of the above
Molecular and cellular development of spinal cord locomotor circuitry

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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

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;
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 Isll, 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
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 movement 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 motor neurons that innervate skeletal muscle in the medial and lateral motor columns (LMCs), gamma motor neurons that innervate intrafusal fibers of the muscle spindles, and preganglionic motor neurons of the autonomic nervous system. Generation 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 into 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 MMC (Tsuchida et al., 1994). Motor neuron (Hb9 promoter) dependent expression of Lhx3 results in conversion of MMC motor neurons to a MMC identity (Sharma et al., 2000).

The lateral motor column (LMC)

At larval 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 down-regulate Hb9 and maintain Isl1 expression. 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 LMCm, Lim1 and Hb9 are expressed and Isl1 is downregulated. In the LMCl, 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.
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 dorsal Interneuron, 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 homebox 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: V0Δ, V0β, V0α, and V0ε (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+ V0β 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 Dbx1LacZ 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 Lhx1+ Pax2+ d6-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 V0D and V0β subclasses, whereas loss of Evx1 results in a loss of only the V0β 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 V0D and V0β 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

V0γ

The primarily inhibitory V0γ 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+
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 glycineric/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 Ptx2 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 Ptx2 immunoreactivity which, unlike many embryonic markers, could be detected until postnatal day 30 (Zagoraiou et al., 2009). Neurotransmitter markers can subdivide the Ptx2+ 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 Ptx2− cholinergic C3 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 Ptx2 immunoreactivity in the intermediate cord. Because V0_C and V0_G Ptx2+ cells transiently express Evx1, they appear to be subsets of the V0_V

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).
defined by expression of \( \text{En1} \) and \( \text{Hox5} \) (Pillai et al., 2007).

V1 Birth and Early Development

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

V1 Interneuron Subtypes

V1 Renshaw

Renshaw cells use both glycine and GABA as neurotransmitters, transiently express \( \text{Gad65} \) early in embryonic development and have both motor neurons and Ia interneurons as targets (Sauvage 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 \( \text{En1}^+ \) progenitor pool and, although they are not lost in the absence of \( \text{En1} \), they do have fewer motor neuron recurrent inputs (Sapir et al., 2004). They are, however, lost in the absence of \( \text{Pax6} \) (Sapir et al., 2004). Recent study showed that selective activation of the \( \text{Onecut} \) 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 \( \text{Foxd3} \), 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
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 glyceric 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 contribute 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, OCN, OC2, OC3, Foxd3, En1, MafB, Foxp2, Foxd3, Foxp4, Pax2, Arx, Evx1, Nurr1, Blhllh5, 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 Gata2a+/+ V2b class is inhibitory and uses both glycine and GABA (Figure 4D; Al-Mosawie et al., 2007; Lundfald et al., 2007). The transcription factor, Blhllh5, marks the V2, as well as V1 and dl6 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 tetraters active (Sander et al., 2006; Lee et al., 2008). The progenitor pool of V2 neurons becomes post-mitotically segregated into V2a and V2b neurons.

Time-lapse imaging in zebralab 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 zebralab 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 zebralab 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 DI4-Notch and subsequent BMP/TGFβ signaling events required for neuronal diversity in the V2 domain (Okigawa et al., 2014). V2b fate is specified by activ 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 contraterally 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
altemating 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 (Lundsfald et al., 2007). In zebrafish, alx, a zebrafish homolog of Cbx10, 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 BlhlhB5, 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 (Lundsfald 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 BlhlhB5, 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 contraterally and caudally (Goulding, 2009). Genetic tracing, using a Sim1-eGFP or Sim1Cre and reporter lines, and viral tracing, using pseudorabies, shows that 80–85% of these cells project contraterally 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 Sim1Cre AlsrR192 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+/− Nkx2.9−/− 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, BlhlhB5, 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 Sim1TaulacZ knock-in reporter mouse or Sim1Cre and reporter lines (R2glostop−/−GAP43−GFP and R2glostop−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 dl2 and dl3 neurons into dl4 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).

V3p and V3y
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).
reported V3v, primarily located in lamina VIII, possessed a few branching processes and were capable of generating rapid tonic firing spikes and V3d 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 V3y express Olig3, Prox1, Bhlhb5, and Nurr1, and V3y can be defined by expression of OC1, OC2, and OC3 (Francius et al., 2013).

**VX 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 VX include: what is the progenitor domain that gives rise to the VX 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, d11–6 and diLA and diLB. Of these, the dorsal-most d11–3 progenitors are dependent on signals from the roof plate and termed Class A (Liem et al., 1997; Lee et al., 2000). The remaining d14–6 and diLA and diLB 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 d14–6 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 diLA and diLB 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 dI1Comm) neurons express the Lim-HD transcription factors Lhx2high and Lhx9low, while dI1B (also known as dI1pm) 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 propriospinal input from the periphery and form commissural projections of dorsal and ventral SCTs (Helms and Johnson, 1998; Bermingham et al., 2001). Using an Atoh1lacZ 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 Cbh2+ and Smarca2+ and projects to the SCT in the contralateral (Tagt+) 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 dpl 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.
**dl2 Birth and Early Development**

The roof-plate-dependent Class A dl2 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 specific number of the corresponding dorsal interneurons (dl2 and dl3; Quiroga et al., 2015).

**dl3 Interneuron Characteristics**

The dl3 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, dl3 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 dl3 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 dl3 neurons is dependent on Isl1, and expression of Isl1 in dl1 neurons conferred dl3-like axon choice points to dl1 neurons (Avraham et al., 2010). Tlx1 (also known as Hox11) and Tlx3 (also known as Rnx and Hox11/12) are markers of glutamatergic signaling. Tlx3 functions cell-autonomously to specify a glutamatergic neurotransmitter phenotype (Cheng et al., 2004).

**dl3 Birth and Early Development**

The roof-plate dependent Class A d3 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 dl3 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 dl3 and dl5 neurons at the expense of dl2 and dl4 (Muller et al., 2005), while loss of Mash1 causes a decrease in dl3 and dl5 populations while dl4 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 dl1, dl3, and dl5 (Le Dréau et al., 2012).

**dl4 Interneuron Characteristics**

The early born (E10.5–E11) dl4 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 dl4 and dl5 interneurons also express Gsh1 (Gsx1) and Gsh2 post-mitotically, while dl3 only express Gsh2 (Kriks et al., 2005; Muller et al., 2005; Mizuguchi et al., 2006). They are GABAergic, calbindin\(^+\) and express the nociceptive marker PLCy (Chen et al., 2001; Helms and Johnson, 2003). The dl4 fate is dependent on Ptf1a and loss of this gene results in loss of all GABAergic dorsal neurons and respecification to dl5 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).

**dl5 Birth and Early Development**

The roof-plate independent Class B d5 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 dl1 progenitor domain that produces dl1A and dl1B progenitors, although both have very similar transcription factor expression patterns (below). Olig3 over-expression can inhibit formation of dl4, and loss can result in expansion of this domain (Muller et al., 2005). While these cells express Mash1, loss of Mash1 does not block dl4 formation, yet it does disrupt dl3 and dl5 (Helms et al., 2005).

**dl5 Interneuron Characteristics**

The roof-plate independent Class B dl5 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 Phox2A (Ding et al., 2004). These interneurons were previously known as D4.

**dl5 Birth and Early Development**

These cells are born between E10.5–E11 and arise from a Mash1\(^+\) and Pax7\(^+\) d5 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 dl5 domain expresses Gsh1 and Gsh2, as does the adjacent dl4 domain (Kriks et al., 2005). As noted previously, loss of function experiments with BMP7 in chick and Bmp7 mutant mice results in loss of dl1, dl3, and dl5 (Le Dréau et al., 2012).

**dl6 Interneuron Characteristics**

The roof-plate independent Class B dl6 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 dl6 group
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, Viatat, 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 WTI (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+, Dwxs+, Ngn1+ and Ngn2+ pD6 progenitor domain. Post-mitotically they express Bhlhb5, Wti1, 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).

**dILB 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 (Chen et al., 2004; Mizuguchi et al., 2006). The dILB 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).

**dILB Birth and Early Development**

These neurons are born later than the dI–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 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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