

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

REPORT DATE: OCTOBER 2015

TYPE OF REPORT: ANNUAL

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

REPORT DOCUMENTATION PAGE				Form Approved OMB No. 0704-0188	
Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.					
1. REPORT DATE October 2015		2. REPORT TYPE Annual		3. DATES COVERED 30 Sep 2014 - 29 Sep 2015	
4. TITLE AND SUBTITLE Restoring Bladder Function by Spinal Cord Neuromodulation in SCI				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER W81XWH-14-2-0129	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) DANIEL C. LU Email: DCLu@mednet.ucla.edu				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) UNIVERSITY OF CALIFORNIA, LOS ANGELES Los Angeles, CA 90095				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012				10. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT 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.					
15. SUBJECT TERMS-					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT UU	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON USAMRMC
a. REPORT U	b. ABSTRACT U	c. THIS PAGE U			19b. TELEPHONE NUMBER (include area code)

Table of Contents

	<u>Page</u>
1. Introduction.....	3
2. Keywords.....	3
3. Accomplishments.....	3
4. Impact.....	8
5. Changes/Problems.....	8
6. Products.....	9
7. Participants & Other Collaborating Organizations.....	11
8. Special Reporting Requirements.....	11
9. Appendices.....	13

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:

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

3. ACCOMPLISHMENTS:

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%

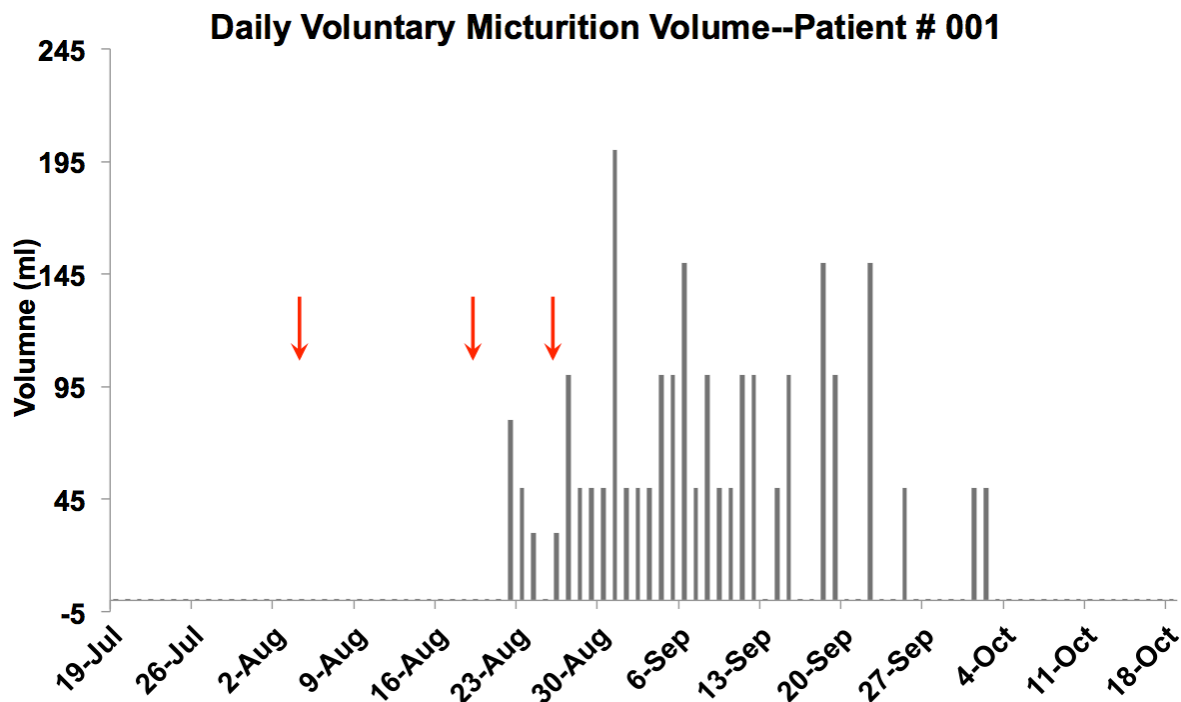


Figure 1: Three magnetic stimulation sessions enable voluntary bladder function. Patient #001 (75yo male, C2 SCI, ASIA D, Injury 6/2014) underwent three stimulation sessions (indicated by the red arrows) with magnetic stimulation:

- 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.

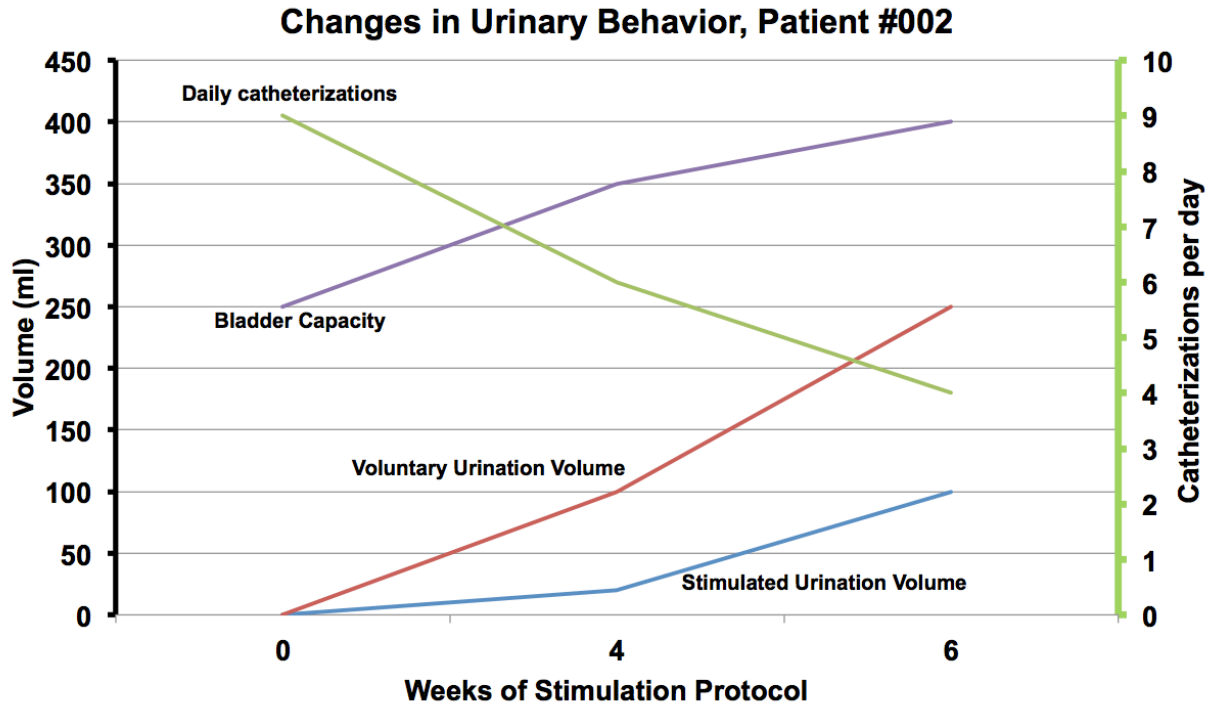


Figure 2: Constructive urinary behaviors with magnetic stimulation. Patient #002 (41yo male, T4, ASIA A, Injury 1994) underwent six weeks of stimulation with magnetic stimulation.

- 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 to 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 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.**
 1. Gerasimenko Y, Gorodnichev R, Puhov A, Moshonkina T, Savochin A, Selionov V, Roy RR, **Lu DC**, Edgerton VR. Initiation and modulation of locomotor circuitry output with multisite transcutaneous electrical stimulation of the spinal cord in noninjured humans. *J Neurophysiol*. 2015 Feb 1;113(3):834-42. doi: 10.1152/jn.00609.2014. Epub 2014 Nov 5
Status of publication: Accepted
Acknowledgment of federal support: Yes
 2. Gad PN, Roy RR, Zhong H, **Lu DC**, Gerasimenko YP, Edgerton VR. Initiation of bladder voiding with epidural stimulation in paralyzed, step trained rats. *PLoS One*. 2014 Sep 29;9(9):e108184. doi: 10.1371/journal.pone.0108184. eCollection 2014.
Status of publication: Accepted
Acknowledgment of federal support: Yes
 3. **Lu DC**, Niu T, Alaynick WA. Molecular and cellular development of spinal cord locomotor circuitry. *Front Mol Neurosci*. 2015 Jun 16;8:25.
Status of publication: Accepted
Acknowledgment of federal support: Yes
 4. Niu T, Alaynick WA, **Lu DC**. Strategies and lessons in spinal cord injury rehabilitation. *Current Physical Medicine and Rehabilitation Reports* 2015 3;3, 206-213
Status of publication: Accepted
Acknowledgment of federal support: Yes
 5. Hoffman H, Lee SI, Garst JH, Lu DS, Li CH, Nagasawa DT, Ghalehsari N, Jahanforouz N, Razaghy M, Espinal M, Ghavamrezaei A, Paak BH, Wu I, Sarrafzadeh M, **Lu DC**.

Use of multivariate linear regression and support vector regression to predict functional outcome after surgery for cervical spondylotic myelopathy. *mJ Clin Neurosci*. 2015 Sep;22(9):1444-9.

Status of publication: Accepted

Acknowledgment of federal support: Yes

6. Gerasimenko YP, **Lu DC**, Modaber M, Zdunowski S, Gad P, Sayenko DG, Morikawa E, Haakana P, Ferguson AR, Roy RR, Edgerton VR. Noninvasive Reactivation of Motor Descending Control after Paralysis. *J Neurotrauma*. 2015 Aug 20.

Status of publication: Accepted

Acknowledgment of federal support: Yes

7. Lee S, Mortazavi B, Hoffman H, Lu D, Li C, Paak B, Garst J, Razaghy M, Espinal M, Park E, **Lu DC**, Sarrafzadeh M. A Prediction Model for Functional Outcomes in Spinal Cord Disorder Patients using Gaussian Process Regression. *IEEE J Biomed Health Inform*. 2014 Nov 20

Status of publication: Accepted

Acknowledgment of federal support: Yes

- **Books or other non-periodical, one-time publications.**

Nothing to Report

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

1. 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**

Nothing to Report

2. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

- **What individuals have worked on the project?**

■

Name:	Daniel C. Lu, MD, PhD
Project Role:	Principle Investigator
Researcher Identifier (e.g. ORCID ID):	1234567
Nearest person month worked:	2.52 person months per year. ~1 month this period
Contribution to Project:	Dr. Lu oversaw all aspect of research and administration of this program
Funding Support:	NIH U01
Name:	William Alaynick, PhD
Project Role:	Project Scientist
Researcher Identifier (e.g. ORCID ID):	1234567
Nearest person month worked:	2.88 person months per year. ~1 month this period
Contribution to Project:	Dr. Alaynick contributed to the IRB regulatory approval and continued intellectual development of the research plan
Funding Support:	None

- **Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?**

Nothing to Report

- **What other organizations were involved as partners?**

Nothing to Report

3. SPECIAL REPORTING REQUIREMENTS

- **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

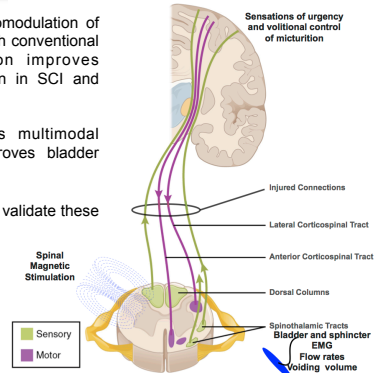
Study/Product Aim(s)

- 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 learning to determine best parameters for Aim 1-2. Aim 4: Phase 1/2 clinical trial to evaluate bladder function.

- We have shown that neuromodulation of spinal cord in combination with conventional sensorimotor rehabilitation improves sensorimotor bladder function in SCI and TBI.
- We discovered that this multimodal approach has reduced improves bladder function.
- We propose a clinical trial to validate these discoveries.



Timeline and Cost

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

Updated: (Oct 1 2015)

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.

4. **APPENDICES:**

1. University of California, Los Angeles Institutional Review Board Approval.
2. Publication.

Date Generated: Tuesday, August 4, 2015 9:41:16 AM**Current Date:** 10/28/2015, 10:59:29 AM

Date: Tuesday, August 4, 2015 9:41:17 AM

Print

Close

ID: IRB#14-000932

View: NEW 1.1 - Study Title and Key Personnel

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

Study Title and Key Personnel

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

1.0 *Full Title of the Submission:

Restoring Bladder Function by Spinal Cord Neuromodulation in SCI

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

2.0 *Working or Lay Title:

Restoring Bladder Function by Spinal Cord Neuromodulation in SCI

3.0 Principal Investigator:

- 3.1
 - ***Name:** DANIEL LU
 - ***Degree(s):** If degrees are not shown here, please add them to the next section, Section 1.1a/Item 1.0, which will then update the Principal Investigator's webIRB account information.
MD PhD
 - 3.2
 - **UCLA Title:** Associate Professor
 - 3.3
 - ***Will the Principal Investigator conduct the informed consent process with potential study participants?**

☐ Yes

☒ No

☐ 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](#).

Name	Department	Role	Other Role (if applicable)	Will Obtain Consent?	Manage device accountability?	Access to personally identifiable info?	Access to code key?
View PARIN YAZDANIFARD	NEUROSURGERY	Research Assistant		no	Yes	Yes	No
View ERIKA MORIKAWA	NEUROSURGERY	Research Assistant		no	No	Yes	Yes
View WILLIAM ALAYNICK	NEUROSURGERY	Co-Investigator		yes	Yes	Yes	Yes
View DANIEL DENIS	NEUROSURGERY	Co-Investigator		yes	Yes	Yes	Yes
View PAYMON REZAI	NEUROSURGERY	Co-Investigator		no	No	No	No
View CAROL BENNETT	UROLOGY	Co-Investigator		no	No	No	No
View BIT NA RAE OK	NEUROSURGERY	Research Assistant		no	Yes	Yes	No
View TIANYI NIU	NEUROSURGERY	Co-Investigator		yes	Yes	Yes	Yes
View DANIEL LU, MD, PhD	NEUROSURGERY	Other	PI	no	No	No	No
View MORTEZA MODABER	NEUROSURGERY	Co-Investigator		no	Yes	Yes	Yes

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.

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.

View

-
-
- For existing protocols: Item 2.0 has been modified and this item cannot be edited. When submitting an amendment please use the information found in the text box below to complete Item 2.0 above.
Briefly describe the other study personnel.
-
- 2.1
- Indicate the human subjects research training these personnel have or will receive. If training is required in a language other than English or if research is occurring in a location where research personnel do not have access to the internet (e.g., rural community without internet capability), please describe how human subjects training requirements will be fulfilled.
-
- Check all that apply:
 - ☒ 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 ☒ No

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

ID: IRB#14-000932

View: NEW 1.1b - Type of Study Review

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

Type of Study Review

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

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

☐ Minimal risk or no known risks - [Click here](#) for the OHRPP tip sheet on minimal risk.

☐

☐ Greater than minimal risk

☒

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

☐ IRB Review: Expedited or Full Board

☒

☐ Certification of Exemption from IRB Review

☐

- 2.1

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

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

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

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?**

☐ Yes ☒ No

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

Document Name

Document Version #

There are no items to display

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

☐ Yes ☒ No

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

Document Name

Document Version #

There are no items to display

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

☒ Yes ☐ No

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

Document Name

Document Version #

Lu 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
- ☒ b. Off Campus (in California)
- ☐ c. Outside California (in the U.S.)
- ☐ d. Outside the United States ***See note at right**
- ☐ e. Internet

- 1.1
- If you selected b, c or d above, please provide your assurance that documentation of each site's permission to conduct the research at the site(s) will be obtained and maintained by the UCLA PI as applicable:

Agree ☐ ☐**2.0 *Is this a multi-institutional study (i.e., a collaborative project with other sites that have their own IRBs or principal investigators)?**

(Includes but not limited to UC MOU and CTSI MOU collaborations where UCLA IRB review is requested.)

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

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

☐ Yes ☐ No

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

Check all that apply:

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

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

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

Agree ☐ ☐

- 2.2
- Will the UCLA principal investigator specified on this application be responsible for the data coordinating center?
- 2.3
- Indicate the anticipated total number of study participants that will be enrolled across all of the institutions.

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

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

ID: IRB#14-000932

View: NEW 2.1 - Project Identification Information

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

Project Identification Information

1.0 *Type of Submission (Select one)

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

2.0 *Type of Submission (Select one)

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

- ☐ New Submission
- ☒
- ☐ Transfer of Ongoing Research from Another Site from Investigator moving to UCLA. Please complete Item 2.1.
- ☐

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

3.0 *Who developed this study?

Check all that apply:

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

- 3.1
- If other, specify.

4.0 Review For and Reliance Upon External IRBs.

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

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

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

Document Name	Document Version #
<u>Summary Statement DoD Bladder 2013.pdf</u>	0.01

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,

etc.)?

☐ Yes ☒ No
4.0 * Is this study regulated by the Food and Drug Administration (FDA)?
☒ Yes ☐ No

- 4.1
- If yes, check all that apply:

- ☐ Human Drugs
- ☐
- ☒ Medical Devices
- ☒
- ☐ Biological Products
- ☐
- ☐ Food Additives
- ☐
- ☐ Color Additives
- ☐
- ☐ Other
- ☐

- 4.1.1
- If Other, describe:

ID: IRB#14-000932

View: NEW 2.3 - Methods/Procedures - Descriptors

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

Methods/Procedures - Descriptors

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

1.0 *Indicate all that apply to this study.

- ☒ 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

☐ Human Gene Transfer/ Recombinant DNA

☐ Infectious Agents

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

☒ Radiation (Standard of Care or Investigational use of radioactive materials or ionizing radiation)

☒ Substance Abuse Research (with Medication)

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

☐ None of the above

2.0 *Will the study require services or resources owned/rented/operated or provided by the UCLA Health System (e.g. clinic and/or hospital visit(s), 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:**Document Name****Document Version #**

There are no items to display

The following item pertains to investigational drugs and devices only.**4.0 If the study participant or a third party payor (i.e., medical insurance/Medicare) 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.****Document Name****Document Version #**

There are no items to display

ID: IRB#14-000932

View: NEW 6.1 - Funding and Other Study Characteristics

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

Funding and Other Study Characteristics

1.0 *Indicate the funding status for this study.☐ Funded☐ Application for funding is pending☐ Departmental funding / Self funding / No funding**2.0 *Check all that apply:**☒ The research will be conducted through the UCLA Clinical and Translational Research Center (CTRC)☒ The study will be supported by or conducted in collaboration with the U.S. Department of Defense (DOD)☐ The study will be supported by or conducted in collaboration with the U.S. Department of Energy (DOE)☐ The study will be supported by or conducted in collaboration with the U.S. Department of Justice (DOJ)☐ The study will be supported by or conducted in collaboration with the U.S. Department of Education (ED)☐ The study will be supported by or conducted in collaboration with the U.S. Department of Protection Agency (EPA)☐ None of the above

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

Note: Please refer to the Federally-Supported Research section of the OHRPP guidance document: [Funding Considerations for Federally-Funded and Industry-Sponsored Human Research](https://webirb.research.ucla.edu/WEBIRB/Doc/0/6AJR79U394LKPCNU2NDHOP0LE4/fromString.html).

ID: IRB#14-000932

View: NEW 6.2 - Funding - Description

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

Funding - Description

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

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

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

[Click here](#) for tips on how to find the funding source name in webIRB.

1.0 Identify the funding source(s).

Funding Source	Funding Source Information					
View DA-ARMY MEDICAL RESEARCH ACQUISITION ACTIVITY	Name of the Funding Source	DA-ARMY MEDICAL RESEARCH ACQUISITION ACTIVITY				
	If other, specify	No Value Entered				
	UCLA PI named on the grant, contract, subcontract or gift:	DANIEL LU				
	Indicate the type of award:	Grant				
	Indicate the Grant Title:	Restoring Bladder Function by Spinal Cord Neuromodulation in SCI				
	Indicate the Award Number assigned by the funding source:	SCI130209				
	Indicate the description that applies to the source of funding named in the above item. If this is a subcontract, indicate the original source of funding:	Federal				
	If Other, specify	No Value Entered				
	Attach a copy of the funding proposal, subcontract, or scope of work.	<table border="1"> <tr> <td>Document Name</td> <td>DOD-Lu-10-2013.pdf</td> </tr> <tr> <td>Document Version #</td> <td>0.01</td> </tr> </table>	Document Name	DOD-Lu-10-2013.pdf	Document Version #	0.01
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](#).

Study Design

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](#).

Devices/Diagnostics and/or Humanitarian Devices

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

1.0 For this study, list all Approved or Cleared (e.g., 510(k) or Premarket Notification (PMN); Premarket Application (PMA) devices that will be used within their approved labeling.

None

2.0 Complete only if one of the following apply:

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

For additional information please refer to the OHRPP guidance documents on [experimental drugs and devices](#).

Brand name Investigational Devices Information
of device

[View](#) MagStim

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

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

Document Name

Document Version #

X100_productsheet.pdf

0.01

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

☒ Yes ☐ No

• 4.1

• If no, indicate by whom:

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

Agree ☒ ☒

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

The device will be stored in a locked office.

7.0 *Provide assurance that the device(s) will only be used:

(1) By an authorized investigator

(2) With study participants who have consented to participate in the research OR with an IRB approved waiver of informed consent (in vitro device studies).

Agree ☒ ☒

ID: IRB#14-000932

View: NEW 8.8 - Audio, Visual or Digital Recordings

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

Audio, Visual or Digital Recordings

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

1.0 *Who will transcribe the research tapes/recordings?

Check as many as apply:

☒ Members of the research team



☐ Persons outside the research team



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

☐ Yes ☒ No

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

☒ Yes ☐ No

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

4.0 Transcription of Research Tapes/Recordings

- 4.1
- * **Type of media (Check as many as apply):**
 - ☐ CD ROM
 - ☐
 - ☐ DVD
 - ☐
 - ☒ 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
 - ☐

☒ Not Applicable


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

ID: IRB#14-000932

View: NEW 8.11 - Radiation

Warning: Save your work at least every 15 minutes by clicking [Save](#) or [Continue](#).

Radiation

Based on the response to section 2.3/item 1.0, you are seeking approval from MRSC committee. Please complete the following items.

1.0 * Are the radiologic procedures standard of care?

Note: Please review the guidance to the right before completing this question.

☐ Yes ☒ No

- 1.1
- If Yes, please provide the following information for EACH procedure:
 - a. Type of standard of care radiological procedure.
 - b. Maximum number of times a subject will undergo this procedure in one year.
 - c. Building and room number where this procedure will be performed.
- The MRSC review process cannot begin until all of the above-referenced information has been provided in the field below.
- NOTE: If procedures include a radiopharmaceutical then an Investigational New Drug (IND) or Abbreviated New Drug Application (ANDA) must be described in Section 8.6.
- Urodynamics under fluoroscopy of the upper pelvic region to visualize bladder. 7 procedures per year of less than 2 minutes of beam-on time per procedure.

Air kerma values for

1:fluoroscopy-guided procedures: 1.38 mGy

2. Peak skin dose: 2.18 mGy

3. Effective dose: 0.07 mGy

4. Maximum expected air kerma value for the urinary bladder imaging session: 4.0 mGy

Procedure will be performed at: 200 Medical Plaza, Suites 140, Peter Morton Medical Building, Los Angeles, CA 90095

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

2.0 * Will this study involve 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

☐ download form.



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

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

ID: IRB#14-000932

View: NEW 9.2 - Information about Study Data

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

Information about Study Data

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

1.0 *Indicate all that apply to the study data.

Check all that apply:

- ☐ Obtained from a medical or clinical record
- ☐
- ☐ Created or collected as part of health or mental health care
- ☐
- ☐ Used to make healthcare or mental healthcare decisions and/or provided to other healthcare professionals
- ☐
- ☒ 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 <u>directly labeled with personal identifying information</u> when acquired by the investigator for this research
☒	
☐	The data and/or specimens will be <u>labeled with a code that the research team can link to personal identifying information</u> when acquired by the investigator for this research
☐	
☐	The data and/or specimens <u>will not be labeled</u> with any personal identifying information, nor with a code that the research team can link to personal identifying information when acquired by the investigator for this research
☐	
☐	The data are restricted use data (A term used in Social-Behavioral research. See guidance on the right.)
☐	

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

Check all that apply:

☒	Use for this study
☒	
☒	Use for possible future research

☐ Use to create a bank or repository at UCLA☐ Add to existing repository☐ Other

o 5.1.1

o If Other, specify:

ID: IRB#14-000932

View: NEW 9.2a - Privacy and Confidentiality

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

Privacy and Confidentiality

Important Notes:**Privacy is about people.** Privacy refers to a person's wish to control the access of others to themselves.**Confidentiality is about data.** Confidentiality refers to the researcher's plan to handle, manage, and disseminate the participant's identifiable private information.See OHRPP Quick Guide: [Protecting Privacy and Maintaining Confidentiality](#)

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

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

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

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

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

ID: IRB#14-000932

View: NEW 9.3 - Data Security

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

Data Security

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

- 1.0 *Do you agree to follow the [OHRPP Data Security in Research](#) guidance and procedures?**



Yes



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



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



Yes



No

• 2.1

• If yes, attach it here:

•

Document Name

Document Version #

There are no items to display

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

- ☒ The data and/or specimens will be coded
- ☒
- ☐ The personal identifying information will be removed and destroyed
- ☐
- ☐ Personally identifying information will be maintained with the data and/or specimens
- ☐

- 3.1
- **If you indicated that the personal identifying information will be removed or destroyed or that the data/specimens will be coded, provide the following information:**
 - The process for removing and destroying the personal identifying information or for coding the information, and
 - Indicate who will perform the task
- Personally identifying information will be coded. Coding will be performed by a random number generator and code assigned to study participants. The coded key will be kept on a printout in a locked file cabinet behind a locked office door in CHS.

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

☐ Yes ☒ No

- 4.1
- **If yes, indicate all that apply:**
 - ☐ A mechanism such as Survey Monkey, Zoomerang, or an e-mail anonymizing service will be used to strip off the IP addresses for data submitted via e-mail.
 - ☐ The data will be encrypted.
 - ☐
 - ☐ A firewall will be used to protect the research computer from unauthorized access.
 - ☐
 - ☐ Controlled access privileges will be used on the hardware storing the data.
 - ☐
 - ☐ Other.
 - ☐
-
- - 4.1.1
 - If you indicated "Other", describe:

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

Agree ☒ ☒

ID: IRB#14-000932

View: NEW 9.4 - Data Security Plan - During the Study

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

Data Security Plan - During the Study

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

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

Check all that apply:

- 1.1
- ***Electronic Data**
- - ☒ Encryption or password protection software will be used
 - ☒
 - ☐ Secure network server will be used to store data

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

- 1.2

- *Hardcopy Data, Recordings and Specimens**

-

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

- 1.3

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

-

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

Agree ☒ ☒

ID: IRB#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**

Document Name	Document Version #
<u>Procedures_Clean.docx</u>	0.01
<u>References Cited DoD Bladder.docx</u>	0.01
<u>Procedures_Redlined.docx</u>	0.01

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 naïve post-injury spinal cord requires some minimal stimulation quality and duration to re-awaken dormant micturition neural circuitries. Using the optimum stimulation parameters determined in Aim 1, we will test a pre-training regimen in naïve 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. Urodynamics 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 neuromotor networks, i.e., neural networks related to bladder function in animals and humans (3-6). The potential impact of these therapeutic interventions on the lives of individuals with urinary incontinence cannot be overestimated (7, 8). Development of magnetic stimulation to activate spared, but silent, spinal cord pathways related to bladder function in humans could represent the beginning of a paradigm shift in the rehabilitative approach to bladder incontinence as a result of SCI and potentially other neurologic injuries or stroke (9-12). It could also provide new pathways toward more advanced technologies to further enable more potential success in improving bladder function.

As we have observed with studies of the lumbosacral spinal cord in completely paralyzed SCI subjects, future development of technical capabilities to use neuromodulation of the lumbosacral spinal cord for evaluation and enabling of spared function will undoubtedly enhance our ability to improve therapies for bladder function after paralysis. By discovering spared function and revealing the potential for treatments, the cost-savings from the proposed translational studies could include reduced assistive daily care costs, increased employment, and improved quality of life—especially for incomplete SCI patients who account for the majority of these SCI patients. The proposed studies could provide a partial solution to the US\$40 billion/year care and \$5.5 billion/year lost productivity costs (10, 46). Of the 10 million people in the US living with paralysis, 15,000 are the result of a SCI each year. The first year of care can range from \$322,000-\$986,000, with lifetime costs of \$1.4-4M for someone injured at 25 years of age. In addition to potentially devastating sensorimotor disturbances, there is a huge financial cost of SCI, estimated to be \$13.55B in medical care, therapy, and lost productivity nationwide. This proposal may positively impact patients with chronic or new SCI and other forms of nervous system damage or disease (stroke, multiple sclerosis).

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

1. A description of each phase of the study. Details in Section 2.

Aim 1: Define stimulation parameters that improve bladder function over 24 weeks

Aim 2: Minimal number of exercise training session to improve bladder function over 24 weeks

Aim 3: Machine learning to define best parameters in Aims 1&2

Aim 4: Use best methods of Aims 1-3 in a cohort of 12 subjects for 24 weeks

2. Sequence and timing of study procedures to be performed.

Aim 1: Urodynamic testing: Start, monthly, conclusion: 7 sessions

Self-assessment: Start, monthly, conclusion: 7 sessions

Bladder diary: Daily for 6-months

Magnetic stimulation and bladder testing: twice weekly: 48 sessions

ASIA Score: Weekly

Aim 2: Urodynamic testing: Start, monthly, conclusion: 7 sessions

Self-assessment: Start, monthly, conclusion: 7 sessions

Bladder diary: Daily for 6-months

Magnetic stimulation and bladder testing: twice weekly: 48 sessions

Motor training: twice weekly: 48 sessions

ASIA Score: Weekly

Aim 4: Urodynamic testing: Start, monthly, conclusion: 7 sessions

Self-assessment: Start, monthly, conclusion: 7 sessions

Bladder diary: Daily for 6-months

Magnetic stimulation and bladder testing: twice weekly: 48 sessions

Motor training: twice weekly: 48 sessions

ASIA Score: Weekly

◆ Urodynamic testing (a standard procedure for research purposes to determine bladder function):

Subject will be placed in supine position. Bladder will be emptied of existing urine by inserting a Foley catheter using aseptic technique and sterile equipment. Pre-warmed 37°C saline will be instilled at a rate of <30 mL/Min, to avoid any 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 was observed; if the symptoms do not improve nifedipine and nitrates (e.g., nitroglycerine paste or sublingual nitroglycerine) will be the next step of management. If there are any poor responses to treatment, the patient will be transferred to Ronald Reagan hospital for further management. Fluoroscopic imaging will be performed to avoid any vesicourethral reflux during the filling phase. Subject will then be asked to 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 about the meaning, reliability, and applicability of the test results for health care decisions.

- o
- o
- o 4.1.2
- o **Will tests be performed by a Clinical Laboratory Improvement Amendments (CLIA) approved lab?**
- o ☐ Yes ☐ No

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

Enrollment: 1 hour

Initial Inclusion/Exclusion Evaluation: 3 hours

Testing: 2 hours per session, twice a week X 24 weeks: 96 hours

Total: Approximately 100 hours per subject

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

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

ID: IRB#14-000932

View: NEW 11.1 - Characteristics of the Study Population

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

Characteristics of the Study Population

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

☐ Yes ☒ No

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

24

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

48

4.0 *Indicate the specific inclusion criteria for enrollment of each of the groups of research participants in this study.

If there are any inclusion criteria based on *gender, pregnancy/childbearing potential, race, ethnicity or language spoken*, explain the nature of and scientific rationale for the inclusions.

1. Male 18-75 years; This is required to have one urethral anatomy. Secondarily, more males have spinal cord injury, especially in veteran populations.
2. At least 1 year post-injury;
3. Non-progressive SCI at C2-T8 (non-conus injury);
4. Motor Complete ASIA (A, B, C or D);
5. Neurogenic bladder requiring clean intermittent straight catheterization;
6. Able to attend twice weekly testing sessions for 6 months.
7. Have intact lower extremity anatomy and able to use lower extremity for assistive standing and stepping. This is required to assess the quality of motor-function-activating spinal cord stimulation.

5.0 *Indicate the specific exclusion criteria for each of the groups of research participants in this study.

If there are any exclusion criteria based on *gender, pregnancy/childbearing potential, race, ethnicity or language spoken*, explain the nature of and scientific rationale for the exclusions.

1. History of autonomic dysreflexia;
2. Ventilator dependency;
3. Musculoskeletal dysfunction, unhealed fracture, pressure ulcer, active infection;
4. Clinically significant depression or ongoing drug abuse;
5. Received botox injection, or bladder surgery (suprapubic access, Brindley procedure, etc.);
6. Prostatic hypertrophy or bladder outlet disorder;

7. Cardiopulmonary disease that precludes lower extremity training or rehabilitation.

6.0 *How (chart review, additional tests/exams for study purposes, etc.), when and by whom will eligibility be determined?

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

ID: IRB#14-000932

View: NEW 11.2 - Characteristics of Study Population

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

Characteristics of Study Population

1.0 *Indicate the age range of the study participants.

Check all that apply:

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

NOTE:

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

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

- ☒ Adults who are competent to give informed consent
- ☐ Adults unable to give informed consent
- ☐ Adults with diminished capacity to consent
- ☐ Fetal Tissue
- ☐ Neonates
- ☐ Participants Unable to Read, Speak, or understand English
- ☐ Pregnant Women/Fetuses
- ☐ Prisoners
- ☐ UCLA Faculty/Staff

☐ UCLA Students☐☐ Wards☐☐ Unknown/Not Applicable☐

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

☐ Yes ☒ No

ID: IRB#14-000932

View: NEW 14.1 - Risks & Benefits

Warning: Save your work at least every 15 minutes by clicking [Save](#) or [Continue](#).

Risks & Benefits

Benefits

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

☐ Yes ☒ No

- 1.1
- If yes, describe.
- Potential Benefits of the Proposed Research to the Subjects and Others: There may be no benefit. Exercise and rehabilitation has been considered beneficial for people with SCI who are confined to a wheelchair, as immobilization can contribute to secondary pathologies such as muscle contractures, decreased cardiovascular health, pressure sores, and muscle atrophy. Because individuals respond differently, it cannot be predetermined if this research will be beneficial to a specific type of subject. Potential benefits may include: increase in cardiovascular fitness, decrease in spasticity, and/or an improved ability to utilize lower extremity function.

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

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

Risks

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

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

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

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

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

Most subjects will have increased respiration and heart rate due to an increase in activity. However, we do not expect the increase in respiration and heart rate to be greater than what is normally experienced during regular exercise. Many SCI subjects will likely sustain skin irritation from the recording electrodes, or hand placements of the trainers. These conditions are considered to be minimal risks and are reversible. There is some chance that subjects may sustain muscle

and joint soreness, lowering or elevation of blood pressure, dizziness, or skin abrasion from hand placements of the trainers. If these events occur the experiment would cease immediately. These conditions are considered to be minimal risks and are reversible.

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

There may be a risk of autonomic dysreflexia that may cause hypertension and headaches due to increase in bladder volume during urodynamic testing. This usually resolves after immediate evacuation of bladder fluid. If blood pressure is persistently high, topical nitroglycerine or nifedipine may be administered. The NRRC is equipped with pharmacy, nursing, and code staff to provide immediate support. The likelihood of this occurring is very low if patient has no previous history of autonomic dysreflexia, which is an exclusionary criterion for this study.

There is a risk of urodynamic study during testing due to fluoroscopy use. There is a small risk of development of cancer from x-ray used during the monthly formal video urodynamic testing, however this risk is small. The aggregate dose of exposure is approximately 1 chest x-ray during each session. This dose is low and below limits and is not expected to be harmful.

There is a risk of urinary tract infection due to the catheterization during the urodynamic procedure. However all efforts are used to minimize this risk, such as use of sterile technique. Additionally, this risk of urinary tract infection with the urodynamic testing should not exceed the infection risk of daily catheterization and bladder care.

There are no identifiable psychological, sociological, economical, or legal risks to the subjects. Furthermore, there are no alternative treatments and procedures to this upper extremity training.

Risk/Benefit Analysis

4.0 ***RISKS/BENEFIT ANALYSIS:** Indicate how the *risks to the participants are reasonable in relation to anticipated benefits*, if any, to participants and the importance of the knowledge that may reasonably be expected to result from the study:

Protection against Risk: No subject will be allowed to participate in the study without being examined by Dr. Lu. In addition, all eligible subjects will be encouraged to discuss the study with their primary physician. Furthermore, to minimize the risks, certain measures will be implemented to protect our subjects. For example, the subjects will be monitored closely to assess for evidence of complication from experimental protocol. Dr. Lu will closely monitor the subjects in regards to the procedures and will be available for consultation.

During training and testing, every subject will be acclimated slowly to EMG, gravity neutral, and stance device to make him/her feel comfortable. Each subject will be closely monitored (blood pressure, oxygen saturation, and heart rate) through each experiment and training session. The experiment will immediately come to a halt if these values become abnormal or the subject feels tired or winded or has chest pain. If these conditions persist, Dr. Lu will be immediately contacted to assess the subject and will notify the person's primary care provider when necessary.

Before and after every experiment and training session, a nurse will examine the subject's skin for irritations and abrasions. If skin irritations or abrasions are caused by the recording electrodes or hand placements of trainers, electrode and hand placement will be modified appropriately. Furthermore, the physical therapist will constantly monitor the subject's skin and muscle for signs of muscle strain, joint sprain, and skin irritation (e.g., temperature and redness).

Dr. Lu and the nurse will continually assess the appropriate condition of back and lower extremities and continuously monitor manual assistance by trainers to avoid joint sprain. Furthermore, continuous monitoring of the subject will be conducted by the staff for potential injuries. For example, signs of skin redness, swelling of joints, or spasticity can be indicators of injury when subjects have impaired sensation. The physical therapist or trained staff member will stretch the muscles of the subjects before and after each training session to prevent injury.

If any signs of risks or discomfort are noted, the experiment or training session will be immediately discontinued. If any complications arise, training will immediately stop and Dr. Lu will be informed immediately. Dr. Lu, or a designated associate, will be available on campus during all experimental sessions involving SCI subjects. In addition, the subject's primary care provider will be notified as necessary.

To protect confidentiality, each subject will be assigned a coded identification number with no association to the identity of the subject. This number will be used to distinguish all evaluations and analyses. Data will be stored on computer media and videotape and will be secured in a locked storage area of the laboratory. Only members of the research team including research assistants, post-doctoral students, and graduate students will have access to the data for analyses. Only Dr. Lu, the PI, will have access to the coding of the identification number to the subjects.

Potential Benefits of the Proposed Research to the Subjects and Others: Exercise and rehabilitation has been considered beneficial for people with SCI who are confined to a wheelchair, as immobilization can contribute to secondary pathologies such as muscle contractures, decreased cardiovascular health, pressure sores, and muscle atrophy. Because individuals respond differently, it cannot be predetermined if this research will be beneficial to a specific type of subject. Potential benefits may include: increase in cardiovascular fitness, decrease in spasticity, and/or an improved ability to utilize upper extremity function. Furthermore, if this strategy can improve bladder function, the complications of bladder management can be minimize and independence to the SCI subject restored.

Alternatives

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

Check all that apply.

- ☒ 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.

ID: IRB#14-000932

View: NEW 15.1 - Data & Safety Monitoring Plan

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

Data & Safety Monitoring Plan

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

☐ Yes ☒ No

ID: IRB#14-000932

View: NEW 15.2 - Data & Safety Monitoring Plan (continued)

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

Data & Safety Monitoring Plan (continued)

Important Note:

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

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

1. This is a Phase I, II or III clinical trial
2. This is an investigator initiated trial (Section 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
- ☐
- ☒ Designee of the Principal Investigator
- ☒
- ☐ The DSMP includes at least one person who is not associated with the study
- ☐
- ☐ A formally constituted Data and Safety Monitoring Board (DSMB)
- ☐
- ☐ Medical monitor designated by the sponsor
- ☐

☐ Other

☐

- 1.1
- **If you indicated that a designee would be responsible for overseeing the study safety, or that the DSMP would include at least one person not associated with the study, provide the name(s) of this individual (s). Also, provide a brief explanation of why this person(s) would be appropriate in this role(s).**
- Dr. Daniel Denis and Niu will be responsible for overseeing the study safety, along with the External Research Monitor, Victor Chang, MD.

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

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

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

Agree ☒ ☒

Provide the following information as appropriate to the study:

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

☐ Yes ☒ No

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

4.0 *Have stopping rules been established for the study?

☐ Yes ☒ No

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

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

☐ Yes ☒ No

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

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/Bruincard Deposit

☐

☐ Non-Monetary Gifts or Services

☐

☐ Other (including vouchers for parking)

☐

- 1.1
- If you selected **Non-Monetary Gifts or Services or Other**, describe:
 -
- 1.2
- If you selected **Cash and/or Gift Cards/Bruincard Deposit** please specify the estimated total amount of money you will require to pay all participants during the length of the entire study. This information is required by UCLA Business and Finance Services (BFS), the office that will provide the cash/gift cards for payment.
-

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

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

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

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

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

☐ Yes ☒ No

- 3.1
- If yes, describe:
 -

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

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

☐ Agree

☒

☐ Not Applicable


ID: IRB#14-000932

View: NEW 17.1 - HIPAA Authorization

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 ☒ ☒

ID: IRB#14-000932

View: NEW 17.2 - HIPAA - Waiver of Authorization

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

HIPAA - Waiver of Authorization

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

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

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

Check all that apply.

- ☒ 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.

Identification/Recruitment Methods

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

Check all that apply:

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

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

Recruitment Methods

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

Document Name

Document Version

There are no items to display

Ads/Flyers/Info Sheets/Internet Postings

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

Direct Recruitment

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

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

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

Initial contact will be made in person by a potential participant's psychiatrist, 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 psychiatrists, urologist, neurologists, and other clinicians at referral sites. Subjects who may qualify will be informed about the study by their treating physicians and given contact information for the PI, to call if they are interested. If they meet chart-review based inclusion and exclusion criteria they will be invited to attend an appointment for informed consent before further procedures are conducted.

Once the patients have been identified, they will be given the opportunity to meet with the principal investigator in order to discuss the purpose and the procedures involved in the trial. Dr. Lu will complete a chart review along with medical history and neurological examination to determine the medical eligibility for each SCI subject. Additionally, Dr. Lu will determine study eligibility based on the inclusion and exclusion criteria. Experimental testing and training interventions will be initiated after the subject has been evaluated and determined to be in compliance with the selection criteria. The subjects will not be concurrently enrolled in any other experimental studies. All subjects will sign an informed consent that has been approved by the UCLA Institutional Review Board (IRB) prior to entering the study. Each subject will be assigned a subject identification number to designate all evaluations.

Research Participant Pools/Recruitment Databases

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

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

Review of Medical Records

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

Clinic records of UCLA spine surgeons will be assessed for patients with SCI. After identification of the subject with SCI, the records will be assessed for satisfaction of enrollment criteria. If the enrollment criteria are satisfied, the patient may be contacted for enrollment.

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

Document Name

Document Version #

There are no items to display

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

2.0 *Do you assure the following?

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

Agree ☒ ☒

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

Check all that apply.

☒ Precautions will be taken 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



- 3.1
- If other, describe

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

Check all that apply.

☒ The identities of the potential study participants who would meet the criteria for this study would not be known without access to their medical records



☐ Other



- 4.1
- If other, specify

5.0 NON-UC 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 ☒ ☒

ID: IRB#14-000932

View: NEW 19.1 - Eligibility Screening

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

Eligibility Screening

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

☐ Yes ☒ No

ID: IRB#14-000932

View: NEW 19.2 - Eligibility Screening - Plans

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

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.

1.0 *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.
 -

2.0 *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.

•

3.0 Describe how screening will be performed.

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

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

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

View: NEW 19.3 - Oral Consent - For Screening Procedures

ID: IRB#14-000932

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

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.

ID: IRB#14-000932

View: NEW 20.1 - Informed Consent Process

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

Informed Consent Process

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

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

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

Check **all** that apply:

- ☒ **Signed consent** will be obtained from the research participant or Legally Authorized Representative.
- ☒ Signed consent means research participants will be asked to **sign and date** a written consent form.
- ☐ **A waiver of signed consent is requested for the entire study.** One of the following procedures will be conducted:
- ☐ **A written information sheet will be used.** Signed consent will not be obtained from research participants.
- ☐ **Oral consent** will be obtained from the research participant or Legally Authorized Representative (LAR)
- ☐ This option should be selected if the study involves consenting participants via the internet.
- ☐ **A waiver of consent** is being requested.
- ☐ Research participants will **not** be asked to sign a consent form or give oral consent

☐ Consent will be obtained by a collaborating institution.

☐

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

Document Name

Document Version #

There are no items to display

ID: IRB#14-000932

View: NEW 20.3 - Description of the Consent Process

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.

☐

☐ Other

☐

- 2.1
- If you indicated other, describe.

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

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

Check all that apply.

☐ Use the Subject Comprehension Tool form for research

☐

☒ Investigator or study team member will evaluate during the consent process

☒

☐ Other

☐

☐ Not Applicable

☐

- 4.1
- If you indicated other, describe.
-

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

Document Name	Document Version #
<u>DoD Bladder Consent Form Clean 5-28-15.docx</u>	0.01
<u>DoD Bladder Consent Form TrackChanges 5-28-15.docx</u>	0.01

ID: IRB#14-000932

View: NEW 22.1 - Cultural Considerations

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

Cultural Considerations

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

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

☐ Participants may be illiterate or insufficiently literate to be able to comprehend a conventional written informed consent form.

☐

☐ The participants may be reluctant or unwilling to sign a written informed consent form.

☐

☐ The husbands make decisions for their wives.

☐

☐ Elders make decisions for younger adult family members.

☐

☐ Elders make decisions for their community.

☐

☐ It is considered impolite to refuse a request.

☐

☐ People are fearful of refusing requests that they regard as coming from authorities.

☐

☒ **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.
-

ID: IRB#14-000932

View: NEW 22.2 - Non-English Speaking Study Participants

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

Non-English Speaking Study Participants

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

1.0 *Indicate the method that you use to conduct the consent process¹ with participants who do not speak English.

Check all that apply.

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

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

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

2.0 *How will you maintain the ability to communicate with non-English speakers throughout their participation in the study? Indicate "N/A" if not applicable to your study.

Members of the research staff a fluent in several languages.

3.0 *If you are conducting research for which there is a real or foreseeable risk of biomedical harm in the state of California, indicate your agreement that you will provide the participants who do not read, speak, or understand English a copy of the Research Participants Bill of Rights in a language in which they are fluent. Translations into the most common languages in the greater Los Angeles area are available for download on the [OHRPP website](#).

- ☒ Agree
- ☐ Not Applicable

¹ 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

View: NEW 23.1 - Department of Defense

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

Department of Defense

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

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

Check all that apply.

The project is funded by the DOD



The project involves cooperation or collaboration with DOD



The project uses DOD property, facilities, or assets



DOD personnel (military or civilian) will be research participants



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

☐ Yes ☒ No

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

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

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

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

Document Name

Document Version #

There are no items to display

3.0 Prohibitions or limitations related to DOD research

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

- 3.1
- ***Captured or Detained Persons**
SECNAVINST 3900.39D (Section 6(a)(8)) prohibits research involving "any person captured, detained, held, or otherwise under the control of DoD personnel (military or civilian, or contractor employee)" except DoD personnel held for law enforcement purposes.
- **Agree** ☒ ☒
- 3.2
- ***Payment to Active Duty Personnel**
Based on 24 USC 30, the military limits research payments for Active Duty personnel. Unless on leave status during participation, such personnel may not receive payment for participation except for blood donation. Payment for blood donation may not exceed \$50 per blood draw.
- **Agree** ☒ ☒
- 3.3
- ***Classified (or Sensitive but Unclassified) Research**
Because classified research involves restriction of the dissemination of results, UCLA institutional practice is to not accept such research. This prohibition includes the designation of "sensitive but not classified."
- **Agree** ☒ ☒

4.0 DOD Documentation Requirements

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

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

☐ Attached

☐

☒ Pending

☒

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

☐ Attached

☒

☐ Pending

☐

- 4.3
- ***Scientific Review (attach at Section 2.1/item 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.**
-

Document Name	Document Version #
<u>Data Collection Forms DoD Bladder.pdf</u>	0.01

- 4.5
- ***FDA Letter for IND or IDE**

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

☐ Attached

☐

☐ Pending

☐

☒ Not Applicable

☒

- 4.6
- ***FDA Form 1571(attach at Section 9.8/Item 1 (1.11.2.3))**
- | | |
|----------------------------------|----------------|
| ☐ | Attached |
| ☐ | |
| ☐ | Pending |
| ☐ | |
| ☒ | Not Applicable |
- 4.7
- ***FDA Form 1572 (attach at Section 9.8/Item 1 (1.11.2.3))**
- | | |
|----------------------------------|----------------|
| ☐ | Attached |
| ☐ | |
| ☐ | Pending |
| ☐ | |
| ☒ | Not Applicable |

ID: IRB#14-000932

View: NEW 23.2 - DOD - Study Greater than Minimal Risk

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

DOD - Study Greater than Minimal Risk

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

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

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

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

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

Document Name	Document Version #
20140930083527367.pdf	0.01

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

- **Check all that apply.**
-

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

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

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

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

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

ID: IRB#14-000932

View: NEW 24.0 - Additional Information and/or Attachments

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

Additional Information and/or Attachments

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

Document Name	Document Version #
PersonnelCITIPackages.pdf	0.01
Alaynick CV Long 2014.pdf	0.01
CV Morteza Modaber.pdf	0.01
CV Paymon Rezaii.pdf	0.01
DCL Blosketch.pdf	0.01

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

ID: IRB#14-000932

View: NEW 100.0 - Instructions for Study Submission

Instructions for Study Submission

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

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

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

ID: IRB#14-000932

View: Display - Method Description

Audio, Visual or Digital Recordings

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

ID: IRB#14-000932

View: Display - Method Description

Behavioral Observations (only applicable if you selected Exempt Category 2 in section 5.3)

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

ID: IRB#14-000932

View: Display - Method Description

Certificate of Confidentiality

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/>

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

ID: IRB#14-000932

View: Display - Method Description

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

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

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

ID: IRB#14-000932

View: Display - Method Description

Community Based Research

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

ID: IRB#14-000932

View: Display - Method Description

Controlled Substances (Schedule I or II)

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

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

ID: IRB#14-000932

View: Display - Method Description

Deception or Partial Disclosure

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

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

ID: IRB#14-000932

View: Display - Method Description

Devices/Diagnostics (including Humanitarian Devices - HUD)

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

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

ID: IRB#14-000932

View: Display - Method Description

Drugs/Biologics/Dietary Supplements

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

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

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

ID: IRB#14-000932

View: Display - Method Description

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

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

ID: IRB#14-000932

View: Display - Method Description

Genetic Analyses/Genotyping

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

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

ID: IRB#14-000932

View: Display - Method Description

Human Embryonic Stem Cells and/or Induced Pluripotent Stem Cells

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

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

ID: IRB#14-000932

View: Display - Method Description

Human Gene Transfer/ Recombinant DNA

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

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

ID: IRB#14-000932

View: Display - Method Description

Infectious Agents

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

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

ID: IRB#14-000932

View: Display - Method Description

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

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

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

ID: IRB#14-000932

View: Display - Method Description

Radiation (Standard of Care or Investigational use of radioactive materials or ionizing radiation)

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

ID: IRB#14-000932

View: Display - Method Description

Substance Abuse Research (with Medication)

Research for the treatment of 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>

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

ID: IRB#14-000932

View: Display - Method Description

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

Federal regulations allow certain research activities to be conducted in emergency settings with waiver of informed consent - in the interest of facilitating potentially life-saving and life-enhancing research with protecting the rights and welfare of participants. For further information see: OHRP Guidance: <http://www.hhs.gov/ohrp/humansubjects/guidance/hSDC97-01.htm> or FDA Guidance: <http://www.fda.gov/oc/ohrt/irbs/except.html>

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

ID: IRB#14-000932

View: Display - Method Description

None of the above

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

Molecular and cellular development of spinal cord locomotor circuitry

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

Department of Neurosurgery, David Geffen School of Medicine, University of California, Los Angeles, Los Angeles, CA, USA

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

OPEN ACCESS

Edited by:

Robert W. Burgess,
The Jackson Laboratory, USA

Reviewed by:

Bruce R. Johnson,
Cornell University, USA
Francisco Javier Alvarez,
Emory University, USA

*Correspondence:

William A. Alaynick,
Department of Neurosurgery, David
Geffen School of Medicine, University
of California, Los Angeles,
650 Charles East Young Drive,
Wasserman Building, Room 562,
Los Angeles, CA 90095, USA
walaynick@mednet.ucla.edu

Received: 02 April 2015

Accepted: 30 May 2015

Published: 16 June 2015

Citation:

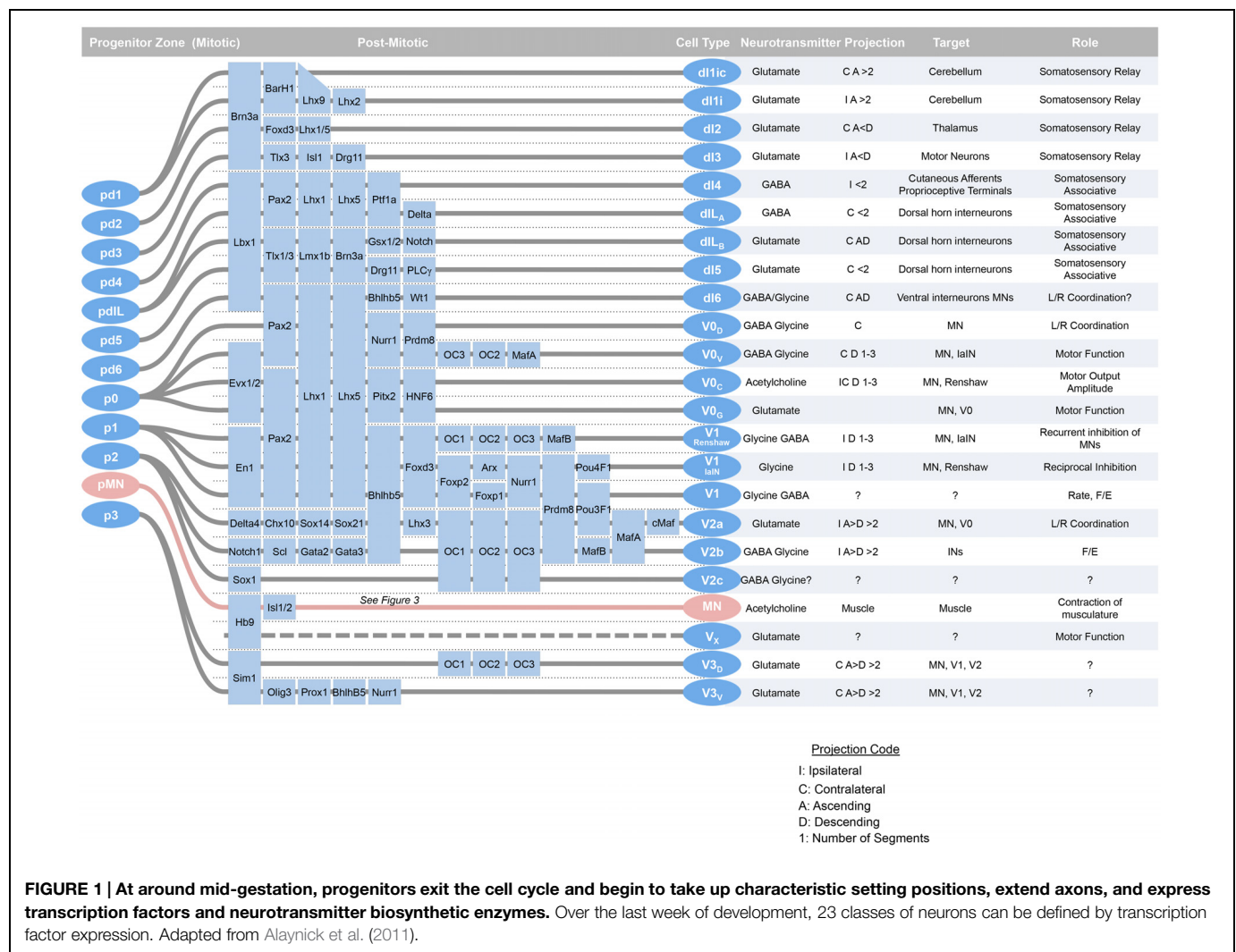
Lu DC, Niu T and Alaynick WA (2015)
Molecular and cellular development
of spinal cord locomotor circuitry.
Front. Mol. Neurosci. 8:25.
doi: 10.3389/fnmol.2015.00025

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;



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.

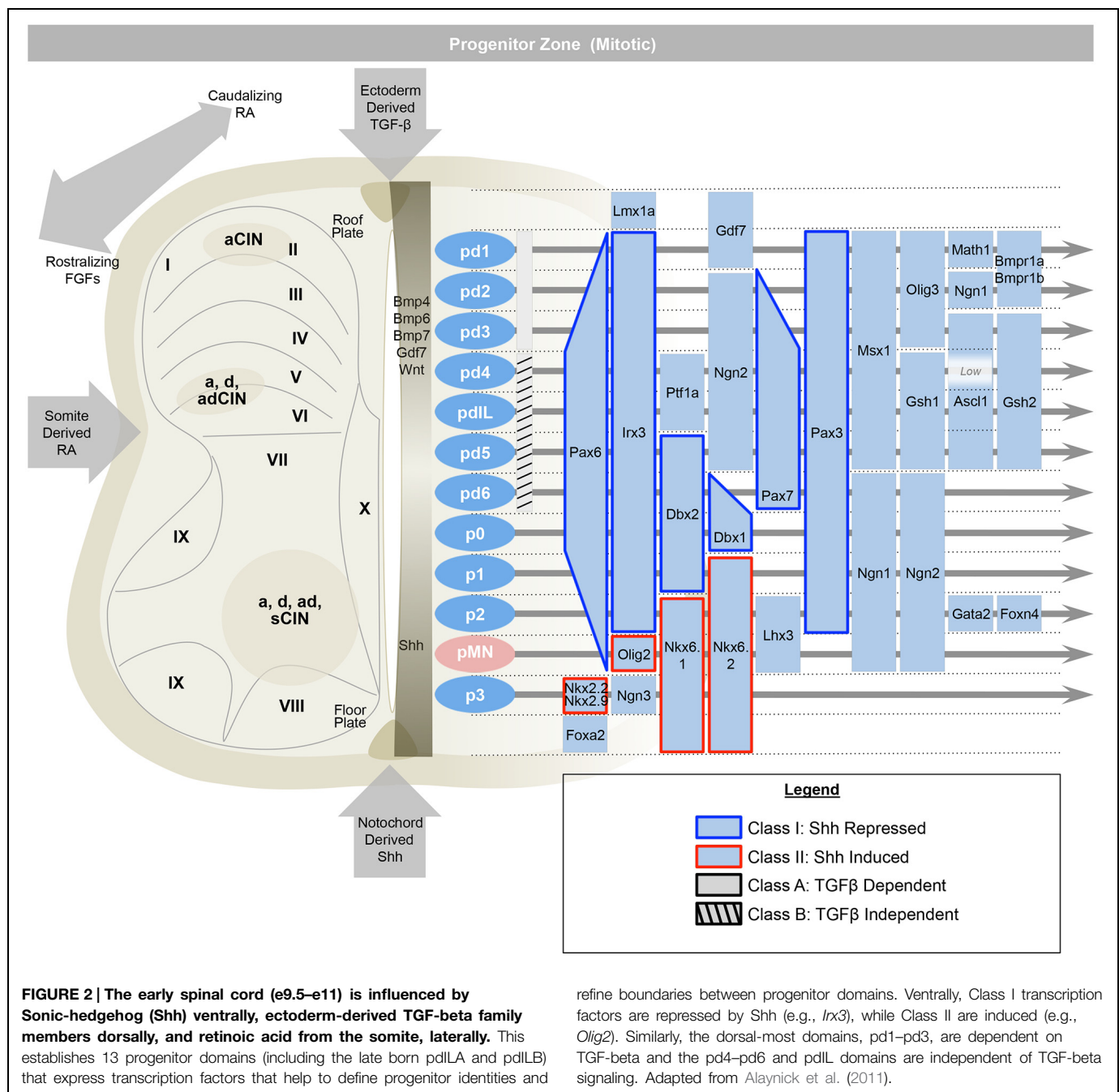
Within an idealized spinal cord segment, this system establishes thirteen progenitor pools along the dorsal–ventral axis (Figure 2). There are eight dorsal interneuron progenitor divisions, pd1–6 and the late-born pdILA and pdILB, four ventral interneuron progenitor divisions, p0–3, and one motor neuron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

Ventral Compartment of Distinct Progenitor Cells

pMN Fate

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

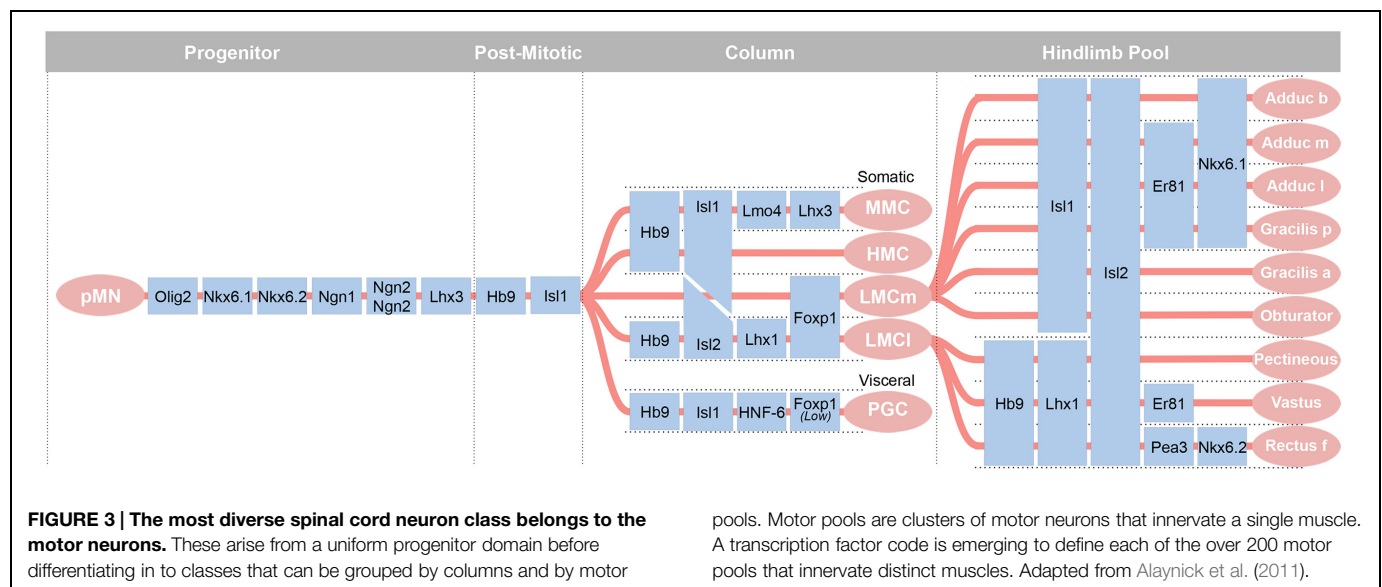


muscles for proprioception; (3) the predominantly thoracic (T1–12, and to L1 and L2 in some species) cholinergic preganglionic sympathetic neurons; (4) the cholinergic parasympathetic motor neurons in the sacral (S2–4) cord; and (5) oligodendrocytes found throughout the spinal cord (Figure 3). The motor 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



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

The Lateral Motor Column (LMC)

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

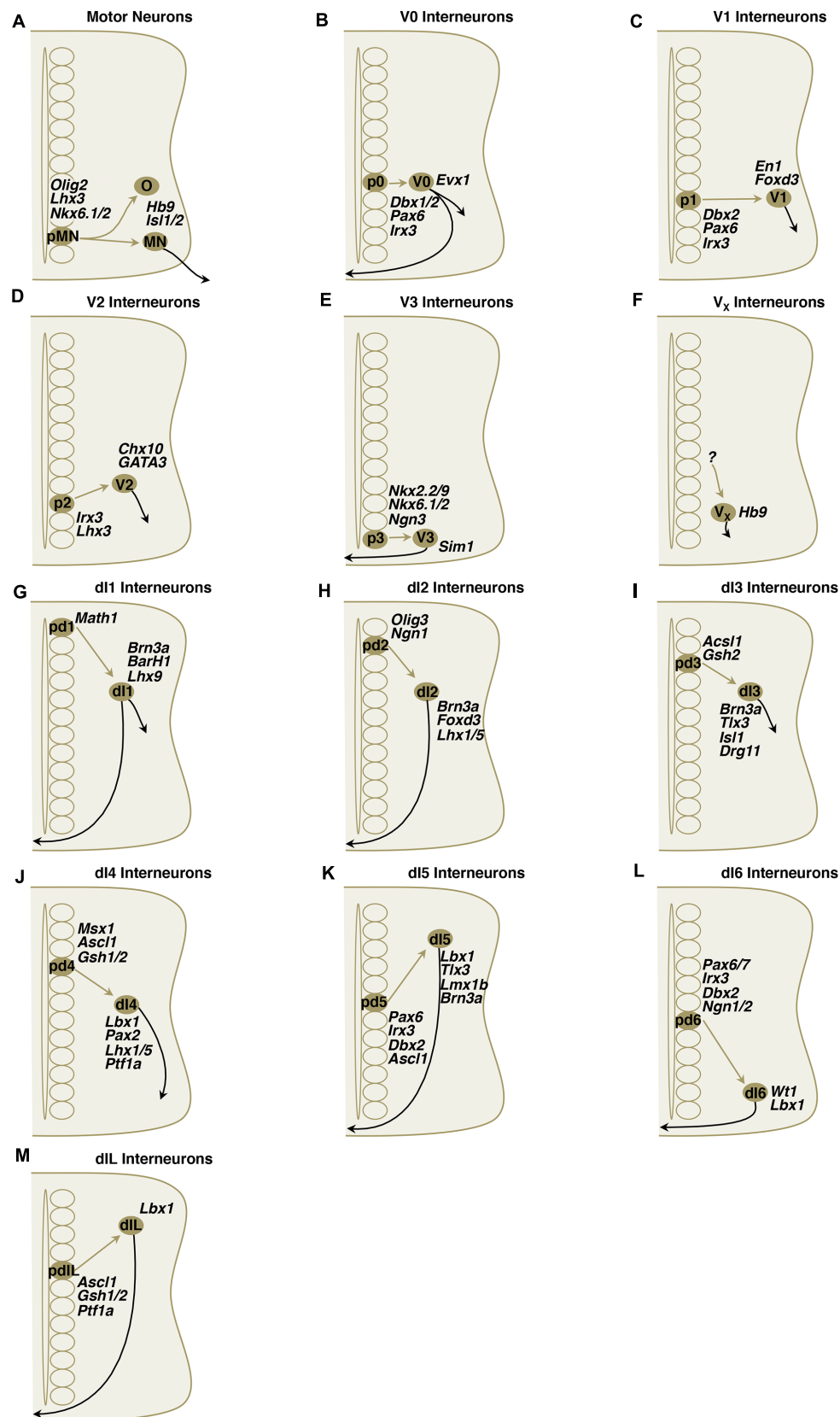


FIGURE 4 | (A–M) Simplified schematic illustrations of development of MNs and ventral/dorsal subclass interneurons with important transcriptional factors.

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

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

Preganglionic Motor Neurons (PGC)

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

Spinal Interneurons

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

Historically, two broad groups have been defined: the “V” interneurons with progenitors that are found in the ventral cord and are grossly associated with motor function, and a 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 homeobox 1; **Figure 4B**). *Dbx1* and *Dbx2* are expressed in dividing cells, although *Dbx1* may be briefly expressed in post-mitotic cells (see V1 discussion Pierani et al., 1999). Four V0 interneuron subclasses have been described to date: V0_v, V0_D, V0_C, and V0_G (Pierani et al., 1999, 2001; Moran-Rivard et al., 2001; Lanuza et al., 2004; Zagoraoui et al., 2009). Early studies addressed the V0 class by eliminating *Dbx1* and showing that the *Evx1*⁺ V0_v subclass was lost because these neurons become fated to an *En1*⁺ V1-like subclass and astrocytes (Pierani et al., 2001; Lanuza et al., 2004). Because *Dbx1* is transiently expressed, a *Dbx1*^{LacZ} knock-in allele was used to show that with loss of *Dbx1*, E18.5 embryos retained 40% of the βgal⁺ cells and resulted in a 25% expansion in the number of *Lbx1*⁺ *Pax2*⁺ dl6-like commissural neurons (Lanuza et al., 2004). By perinatal time points, genetic strategies to track *Dbx1*⁺ cells using βgal find that most of these cells are neural by expression of *NeuN* and are found in lamina VIII where commissural interneurons reside. Lineage labeling of *Dbx1*-derived cell reveals a large abundance of glia (Lanuza et al., 2004). Moreover, the *Dbx1* lineage includes many dorsal horn neurons as this transcription factor is also expressed in dorsal domains. Loss of *Dbx1* results in loss of V0_D and V0_v subclasses, whereas loss of *Evx1* results in a loss of only the V0_v subclass (Moran-Rivard et al., 2001; Pierani et al., 2001; Lanuza et al., 2004). V0 and V1 classes both express *Lhx1* and *Lhx5*, markers of inhibitory spinal interneurons (Pillai et al., 2007).

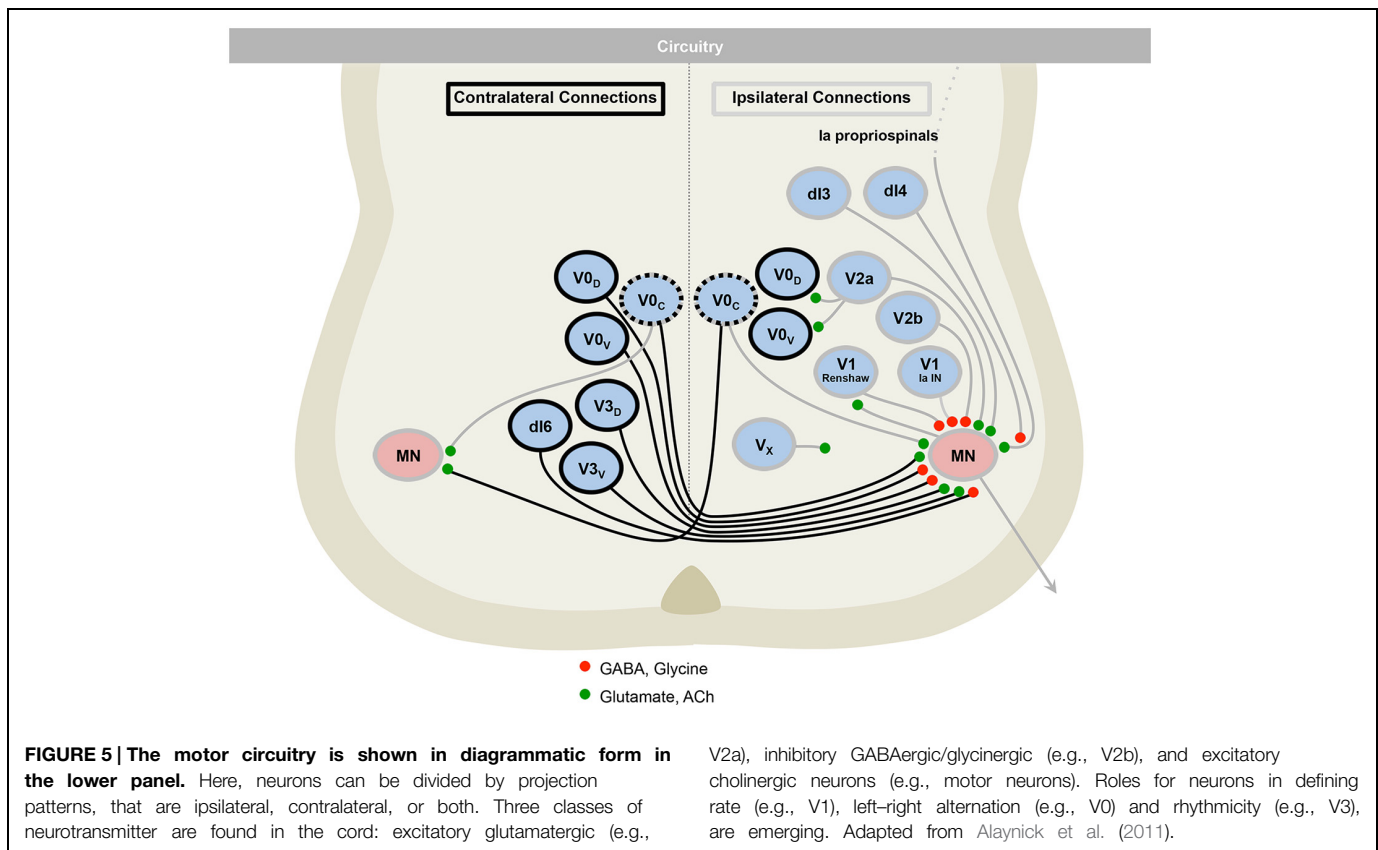
V0 Birth and Early Development

In mouse, the majority of *Dbx1*⁺ progenitors appear between E10 and E13 and give rise to V0_D and V0_v commissural interneurons (Moran-Rivard et al., 2001; Pierani et al., 2001; Lanuza et al., 2004). *Dbx1/2* expression is found in the rostral CNS at stage 13 in chick and more caudally by stage 15 (Pierani et al., 1999). *Evx1/2* positive V0 cells are generated at stages 17 and 18 and appear in the ventral domain of *Dbx1* and *Dbx2* expression (Pierani et al., 1999). Ventral *Evx1/2* expressing V0 neurons appear at stages 17–18 within the ventral expression domain of *Dbx1* and *Dbx2*, and then migrate ventrally (Pierani et al., 1999). The V0 class appears from a *Pax6*⁺, *Dbx1/2*⁺, *Pax3/7*[−] domain that is the dorsal-most ventral progenitor domain (Pierani et al., 1999).

V0 Interneuron Subtypes

V0_v

The primarily inhibitory V0_v class is distinguished by transient expression of the homeodomain transcription factor, *Evx1*. These cells arise from the ventral portion of the *Dbx1*⁺ progenitor domain, and like all post-mitotic cells arising from *Dbx1*⁺



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

$V0_D$

Unlike the $V0_V$ subclass, the more dorsal *Dbx1*⁺ progenitors of the glycinergic/GABAergic $V0_D$ class do not express *Evx1* (Pierani et al., 2001; Lanuza et al., 2004). And while both $V0_D$ and $V0_V$ classes have similar axon guidance and cell body position, the loss of the $V0_D$ class, in conjunction with $V0_V$ class, does appear to alter locomotor behavior. When *Dbx1* is knocked out, eliminating all $V0$ progenitors, a disruption of left-right coordination is observed at lumbar levels L2 and L5. These periods of left-right synchrony are intermittent and periods of normal left-right alternation are observed amidst episodes of synchrony (Lanuza et al., 2004). No disruption of flexor-extensor behavior, as indicated by alternating phasic activity of the L2 and L5 segments, was observed in a drug-induced isolated cord fictive locomotion assay (Lanuza et al., 2004). Recently, studies have showed that a cluster of $V0_D$ cells lateral to the

central canal receive substantial input from primary afferents and preferentially project axons toward contralateral motoneurons via an oligosynaptic pathway, and are active during fictive locomotion. This suggests that this subset of $V0$ interneurons may be primarily responsible for coordination of left-right alternation during locomotion (Griener et al., 2015).

$V0_C$ and $V0_G$

The $V0_C$ and $V0_G$ subclass represent ~5% of $V0$ progenitors and are identified by expression of *Pitx2* and occupy a medial position dorsal to the central canal (Zagoraoui et al., 2009). These cells were first observed in lumbar levels at E11.5–12.0 by *Pitx2* immunoreactivity which, unlike many embryonic markers, could be detected until postnatal day 30 (Zagoraoui et al., 2009). Neurotransmitter markers can subdivide the *Pitx2*⁺ cells into cholinergic (*vAChT*⁺ and *ChAT*⁺) and glutamatergic (*vGluT2*⁺) types that are distinct (Zagoraoui 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 (Zagoraoui et al., 2009). The cholinergic cells are distinct from *Pitx2*[−] cholinergic C₃ propriospinal interneurons (Zagoraoui et al., 2009). By genetic tracing, ~80% of these neurons were determined to be from a *Dbx1*⁺ progenitor domain at E12.5 and loss of *Dbx1* eliminated the *Pitx2* immunoreactivity in the intermediate cord. Because $V0_C$ and $V0_G$ *Pitx2*⁺ cells transiently express *Evx1*, they appear to be subsets of the $V0_V$

class. This relatively small ipsilaterally and bilaterally projecting class, however, is responsible for perhaps all c-boutons on motor neurons found in P8 to P25 mice (Zagoraïou et al., 2009; Stepien et al., 2010). These interneurons provide relatively weak innervation to *Sox14::eGFP⁺* V2a and calbindin⁺ V1 Renshaw cells. These cells appear to be involved in local circuitry as corticospinal and sensory *vGluT1⁺* glutamatergic boutons were not found, whereas serotonergic and *GAD67⁺* GABAergic boutons were observed (Zagoraïou et al., 2009). While previous experiments did not find a gross locomotor behavioral defect with loss of the V0v subclass in *Evx1* mutant animals, *Pitx2* mutant animals were found to have defects in locomotion revealed by EMG recordings during swimming (Zagoraïou et al., 2009). This deficit was argued to represent an abnormal integration of sensory inputs. It may, alternatively or in addition, represent a deficit in C-terminal modulation of motor neuron excitability. A survey in E12.5 mice showed that several V0 subclasses can be defined by expression of *Pax2*, *Pax6*, *Evx1*, *Ptx2*, *Nurr1*, *HNF-6*, *Bhlhb5*, and *Prdm8* (Francius et al., 2013).

V1 Interneuron Characteristics

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

inhibitory spinal interneuron markers *Lhx1* and *Lhx5* (Pillai et al., 2007).

V1 Birth and Early Development

Unlike cells in the dorsal-most p0 domain that expresses *Dbx1* and *Dbx2*, the adjacent p1 domain only expresses *Dbx2* (Pierani et al., 1999). The V1 class appears from a *Pax6⁺*, *Dbx2⁺*, *Nkx6.2⁺*, *Dbx1⁻* domain that is ventral to the *Dbx1/2⁺* V0 domain (Matisse and Joyner, 1997; Pierani et al., 1999). In chick, *En1⁺*, *Lim1/2⁺* V1 neurons appear at stage 17, and most appear ventral to the domain of *Dbx1* expression, within the ventral domain of these *Dbx2⁺*, *Dbx1⁻* progenitors (Pierani et al., 1999). *Dbx* expression does not overlap with *En1*, perhaps due to the relatively late expression of *En1* (Pierani et al., 1999). Genetic tracing studies using *Dbx1^{nlslacZ}* mice between ages E10 and E16.5 found that ~5–10% of *En1⁺* cells did express a low level of β gal, perhaps a reflection of transient *Dbx1* expression and more enduring β gal protein. The V1 class is marked by expression of *Foxd3*, found in the dI2 domain, as well (Ramos et al., 2010). The transcription factor, *Bhlhb5*, which marks the V1, V2 and dI6 domains, is required at least partially for V1 identity assessed by *En1* expression (Ramos et al., 2010; Skaggs et al., 2011). Expression of *Bhlhb5* in conjunction with *Ng2* facilitates V1 identity ectopically (Skaggs et al., 2011).

V1 Interneuron Subtypes

V1 Renshaw

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

V1 Ia Interneuron

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

input onto motor neurons innervating antagonist muscles. Like motor neurons, Ia receive inhibitory inputs from Renshaw cells (Hultborn et al., 1971). In neonatal mice, disynaptic glycinergic reciprocal inhibition is mediated by Ia interneurons, although this activity is preserved in the absence of *Pax6*, indicating that cells of more than one origin 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*, *OC1*, *OC2*, *OC3*, *Foxd3*, *En1*, *MafB*, *FoxP2*, *Foxd3*, *Foxp4*, *Pax2*, *Arx*, *Evx1*, *Nurr1*, *Bhlhb5*, *Pou4F1*, *Pou3F1*, and *Prdm8* (Francius et al., 2013).

V2 Interneuron Characteristics

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

V2 Birth and Early Development (Notch-Delta)

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

Time-lapse imaging in zebrafish showed that the majority of V2 progenitors give rise to a pair of V2a and V2b cells (Kimura et al., 2008), indicating that V2a and V2b arise from the same progenitor. This segregation into V2a and V2b is mediated by Notch/delta signaling in zebrafish and mouse models (Yang et al., 2006; Del Barrio et al., 2007; Peng et al., 2007). In

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

V2 Interneuron Subtypes

V2a Sox14/Chx10

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

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

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

V2b *Gata2/3*

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

V2c *Sox1*

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

V3 Interneuron Characteristics

The *Sim1*⁺ *VGluT2*⁺ glutamatergic V3 interneurons send projections predominantly contralaterally and caudally (Goulding, 2009). Genetic tracing, using a *Sim1-eGFP* or *Sim1*^{Cre} and reporter lines, and viral tracing, using pseudorabies, shows that 80–85% of these cells project contralaterally and a minor proportion remain ipsilateral or project both contra- and ipsilaterally (Zhang et al., 2008). As a population, *Sim1*⁺ V3 interneurons form 24% of glutamatergic connections on V1 Ia, 27% on Renshaw subclasses, 22% of glutamatergic synapses on lateral motor column motor neurons, as well as connections

on *Lhx3*⁺ V2 interneurons, and lamina VIII commissural interneurons (Zhang et al., 2008). Behaviorally, loss of V3 neuronal activity by genetic attenuation with tetanus toxin or allatostatin signaling resulted in a loss of CPG robustness. In isolated cord fictive locomotion, both dorsal root stimulation and drug-induced methods produced weak CPG activity in only some of the cords examined. The outputs were less consistent and had greater coefficients of variance. Although both right and left sides of the cord produced irregular outputs, the fidelity of left–right coordination was preserved suggesting that V3 interneurons do not regulate the coordination of left–right activity. In adult *Sim1*^{Cre} *AlstR192* animals, application of allatostatin to the cord produced locomotor disturbances in gait, as well (Zhang et al., 2008). In *Sim1* mutant mice, V3 interneurons are produced normally and maintain in the similar position and organizations as wild-type; however, there is significant reduction of interneurons in dorsal subgroup and there is significant reduction in the contralateral axonal projection. Therefore, *Sim1* appears to be critical in migration and axonal projection of V3 interneuronal development (Blacklaws et al., 2015). Mice that are mutant for *Nkx2.2* and *Nkx2.9* lose V3 interneurons and *Nkx2.2*^{+/-} *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*, *BhlhB5*, and *Nurr1* (Francius et al., 2013).

V3 Birth and Early Development

These V3 interneurons arise from the ventral-most p3 progenitor domain defined by homeobox transcription factors *Nkx2.2* and *Nkx2.9* and the PAS-bHLH transcription factor *Sim1* (simple-minded homolog 1; Figure 4E; Briscoe et al., 1999; Goulding et al., 2002). Genetic tracing techniques using a *Sim1*^{TauLacZ} knock-in reporter mouse or *Sim1*^{Cre} and reporter lines (*R26*^{floxstop-GAP43-GFP} and *R26*^{floxstop-lacZ}) have shown similar expression at E11.5 to *in situ* hybridization data for *Sim1* expression that appeared just lateral to the *Nkx2.2* progenitors (Marion et al., 2005; Zhang et al., 2008). *Nkx2.2* also regulates the expression of *Olig3* in V3 neurons. While *Olig3* plays a key role in respecification of 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).

V3_D and V3_V

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

reported V3_V, primarily located in lamina VIII, possessed a few branching processes and were capable of generating rapid tonic firing spikes and V3_D had a more complex morphology with relatively slow average spike frequency with strong adaptation (Borowska et al., 2013). A survey in E12.5 mice showed that V3_V express *Olig3*, *Prox1*, *Bhlhb5*, and *Nurr1*, and V3_D can be defined by expression of *OC1*, *OC2*, and *OC3* (Francius et al., 2013).

V_X Hb9

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

Dorsal Interneuron Progenitors

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

dI1 Interneuron Characteristics

The dorsal-most progenitor domain pd1 expresses the bHLH transcription factor *Math1*⁺ (Mouse atonal homolog 1, also known as *Atoh1*) and gives rise to at least two *VGluT2*⁺ glutamatergic subclasses: dI1A and dI1B, characterized by Lim-HD expression and their spinocerebellar tract (SCT) contributions (Figure 4G). Recent study shows that *Msx1* and *Msx2*, two homeodomain transcription factors that are induced earlier than bHLH transcription factors, likely play a role as transcriptional activators of *Math1/Atoh1* in spinal cord development (Duval et al., 2014). The dI1A (also known as dI1_{comm}) neurons express the Lim-HD transcription factors *Lhx2*_{high} and *Lhx9*_{low}, while dI1B (also known as dI1_{ipsi}) express the Lim-HD TF *Lhx9* (Helms and Johnson, 1998; Lee et al., 1998; Bermingham et al., 2001; Gowan et al., 2001; Wilson et al., 2008; Avraham et al., 2009). The dI1 interneurons migrate to the deep dorsal horn and intermediate gray where they receive proprioceptive input from the periphery and form commissural projections of dorsal and ventral SCTs (Helms and Johnson, 1998; Bermingham et al., 2001). Using an *Atoh1*^{LacZ} allele to trace the fate of pd1 progenitors in developing mouse, at least two subsets of the dI1 class have been identified: (1) a medial cluster of vertically oriented neurons that are *Cbln2*⁺ and *Smarca2*⁺ and projects to the SCT in the contralateral (*Tag1*⁺) lateral funiculus; (2) a more lateral *Sox6*⁺, cluster of horizontally oriented neurons that contributes to the SCT in the ipsilateral lateral funiculus (Miesegaes et al., 2009). In chick, data with an enhancer that labels these cells suggests that both fascicles coalesce in the lateral funiculus ventral to the fascicle formed by the dI2 projections (Avraham et al., 2009).

dI1 Birth and Early Development

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

dI2 Interneuron Characteristics

dI2 interneurons are ascending, contralaterally projecting, relay interneurons that migrate to the intermediate spinal cord and ventral horn (Gowan et al., 2001; Gross et al., 2002). These interneurons have been suggested to convey sensory information via the spinothalamic tract to the thalamus, based on their location (Figure 4E; Brown, 1981; Tracey, 1985; Gross et al., 2002). The projections likely occupy the lateral funiculus and are dorsal to the dI1 fascicle, as analyzed by enhancer expression in chick (Avraham et al., 2009). Arising from bHLH transcription factor *Ng1* (neurogenin 1) and *Ng2* 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 correct 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 *Hox11L2*) 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 dp3 progenitors express the basic helix-loop-helix (bHLH) transcription factor *Mash1* (*Ascl1*, Mouse Achaete-scute complex-like 1), as do adjacent pd4 and pd5 domains (Gowan et al., 2001; Helms et al., 2005). They also express *Olig3*, *Pax7* and *Ngn2* and *Gsh2* (Muller et al., 2005). In chick spinal cord electroporation experiments it has been shown that over-expression of *Olig3* increases 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*, *Viaa*, 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).

dl4 Birth and Early Development

The roof-plate independent Class B dp4 domain expresses *Lbx1*, *Mash1* and higher levels of *Pax7* than the dorsally adjacent pd3 domain (Gross et al., 2002; Muller et al., 2002). These progenitors are born between E10.5 and E11 and this domain is distinct from the dlL progenitor domain that produces dlL_A and dlL_B 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 *PhoxA2* (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*⁺ dp5 progenitor domain that express *Lbx1* post-mitotically to both reinforce Class B fate and oppose Class A fates (Figure 4K; Gross et al., 2002; Muller et al., 2002). The 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 dl6 interneuron was traced to play a key role in locomotor circuitry and in development of commissural interneurons, and mutation in *dmrt3* result in divergent in gait pattern in mice models (Andersson et al., 2012; Vallstedt and Kullander, 2013). Double knockout of *Lhx1* and *Lhx5* results in a loss of *Pax2*, *Viaat*, and *Gad1* expression (Pillai et al., 2007). Furthermore, *Pax2* is required for the maintained expression of *Lhx1*, *Lhx5*, *Pax5*, and *Pax8* (Pillai et al., 2007). These cells also express *WT1* (Wilms' tumor 1; Goulding, 2009). The transcription factor, *Bhlhb5*, marks the dl6, V1 and V2 domains (Ramos et al., 2010). Electrophysiologic characteristics of the dl6 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).

dl6 Birth and Early Development

The dl6 neurons are born around E10.5–E11 and originate from a *Pax7*⁺, *Dbx2*⁺, *Ngn1*⁺ and *Ngn2*⁺ pD6 progenitor domain. Post-mitotically they express *Bhlhb5*, *Wt1*, *Lbx1*, *Lhx1*, *Lhx5*, and *Pax2* (references above).

Late Born Dorsal Interneurons

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

dIL_A Interneuron Characteristics

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

dIL_A Birth and Early Development

The dIL_A interneurons are late born (E13.5) and arise from the dIL progenitor domain that is *Lbx1*⁺, *Lhx1/5*⁺, and *Pax2*⁺ (Pillai et al., 2007). The sensory relay interneuron marker *Foxd3* is downregulated in these cells (Gross et al., 2002). *Pax2* has been shown to be necessary for GABAergic differentiation and 98% of these cells also express *Gad1* (GAD67; Cheng et al., 2004). The dIL_A subclass is dependent on *Ptf1a* and loss of this gene results in trans-fate to the dIL^B identity (Glasgow et al., 2005).

dIL_B Interneuron Characteristics

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

dIL_B Birth and Early Development

These neurons are born later than the dl1–6 class and express *Lbx1* post-mitotically (Gross et al., 2000; Muller et al., 2002). *Mash1* controls the upregulation of Notch signaling to direct formation of dILB from common dIL progenitors (Mizuguchi et al., 2006).

Discussion

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

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

Acknowledgments

We apologize to the researchers whose work was not included due to space constraints. We thank Samuel Pfaff for support; and Ariel Levine, Marito Hayashi, and members of the Pfaff Laboratory for critical reading of this manuscript.

References

- Al-Mosawie, A., Wilson, J. M., and Brownstone, R. M. (2007). Heterogeneity of V2-derived interneurons in the adult mouse spinal cord. *Eur. J. Neurosci.* 26, 3003–3015. doi: 10.1111/j.1460-9568.2007.05907.x
- Alaynick, W. A., Jessell, T. M., and Pfaff, S. L. (2011). SnapShot: spinal cord development. *Cell* 146, 178–178. doi: 10.1016/j.cell.2011.06.038
- Alexander, G. M., Rogan, S. C., Abbas, A. I., Armbruster, B. N., Pei, Y., Allen, A. J., et al. (2009). Remote control of neuronal activity in transgenic mice expressing evolved G protein-coupled receptors. *Neuron* 63, 27–39. doi: 10.1016/j.neuron.2009.06.014
- Alvarez, F. J., Dewey, D. E., McMillin, P., and Fyffe, R. E. (1999). Distribution of cholinergic contacts on Renshaw cells in the rat spinal cord: a light microscopic study. *J. Physiol.* 515(Pt 3), 787–797. doi: 10.1111/j.1469-7793.1999.787ab.x
- Alvarez, F. J., Jonas, P. C., Sapir, T., Hartley, R., Berrocal, M. C., Geiman, E. J., et al. (2005). Postnatal phenotype and localization of spinal cord V1 derived interneurons. *J. Comp. Neurol.* 493, 177–92. doi: 10.1002/cne.20711
- Andersson, L. S., Larhammar, M., Memic, F., Wootz, H., Schwachow, D., Rubin, C.-J., et al. (2012). Mutations in DMRT3 affect locomotion in horses and spinal circuit function in mice. *Nature* 488, 642–646. doi: 10.1038/nature11399
- Arber, S., Han, B., Mendelsohn, M., Smith, M., Jessell, T. M., and Sockanathan, S. (1999). Requirement for the homeobox gene Hb9 in the consolidation of motor neuron identity. *Neuron* 23, 659–674. doi: 10.1016/S0896-6273(01)80026-X
- Ashrafi, S., Lalancette-Hebert, M., Friesse, A., Sigrist, M., Arber, S., Schneider, N. A., et al. (2015). Wnt7A identifies embryonic gamma-motor neurons and reveals early postnatal dependence of gamma-motor neurons on a muscle spindle-derived signal. *J. Neurosci.* 32, 8725–8731. doi: 10.1523/JNEUROSCI.1160-12.2012
- Avraham, O., Hadas, Y., Vald, L., Hong, S., Song, M. R., and Klar, A. (2010). Motor and dorsal root ganglion axons serve as choice points for the ipsilateral turning of d13 axons. *J. Neurosci.* 30, 15546–15557. doi: 10.1523/JNEUROSCI.2380-10.2010
- Avraham, O., Hadas, Y., Vald, L., Zisman, S., Schejter, A., Visel, A., et al. (2009). Transcriptional control of axonal guidance and sorting in dorsal interneurons by the Lim-HD proteins Lhx9 and Lhx1. *Neural Dev.* 4:21. doi: 10.1186/1749-8104-4-21
- Benito-Gonzalez, A., and Alvarez, F. J. (2012). Renshaw cells and Ia inhibitory interneurons are generated at different times from p1 progenitors and differentiate shortly after exiting the cell cycle. *J. Neurosci.* 32, 1156–1170. doi: 10.1523/JNEUROSCI.3630-12.2012
- Bermingham, N. A., Hassan, B. A., Wang, V. Y., Fernandez, M., Banfi, S., Bellen, H. J., et al. (2001). Proprioceptor pathway development is dependent on Math1. *Neuron* 30, 411–422. doi: 10.1016/S0896-6273(01)00305-1
- Blacklaws, J., Deska-Gauthier, D., Jones, C. T., Petracca, Y. L., Liu, M., Zhang, H., et al. (2015). Sim1 is required for the migration and axonal projections of V3 interneurons in the developing mouse spinal cord. *Dev. Neurobiol.* doi: 10.1002/dneu.22266 [Epub ahead of print].
- Borowska, J., Jones, C. T., Zhang, H., Blacklaws, J., Goulding, M., and Zhang, Y. (2013). Functional subpopulations of V3 interneurons in the mature mouse spinal cord. *J. Neurosci.* 33, 18553–18565. doi: 10.1523/JNEUROSCI.2005-13.2013
- Boyden, E. S., Zhang, F., Bamberg, E., Nagel, G., and Deisseroth, K. (2005). Millisecond-timescale, genetically targeted optical control of neural activity. *Nat. Neurosci.* 8, 1263–1268. doi: 10.1038/nn1525
- Briscoe, J., Pierani, A., Jessell, T. M., and Ericson, J. (2000). A homeodomain protein code specifies progenitor cell identity and neuronal fate in the ventral neural tube. *Cell* 101, 435–45. doi: 10.1016/S0092-8674(00)80853-3
- Briscoe, J., Sussel, L., Serup, P., Hartigan-O'Connor, D., Jessell, T. M., Rubenstein, J. L., et al. (1999). Homeobox gene Nkx2.2 and specification of neuronal identity by graded Sonic hedgehog signalling. *Nature* 398, 622–627. doi: 10.1038/19315
- Brown, A. G. (1981). *Organization of the Spinal Cord: The Anatomy and Physiology of Identified Neurones*. New York, NY: Springer-Verlag. 238. doi: 10.1007/978-1-4471-1305-8
- Bui, T. V., Akay, T., Loubani, O., Hnasko, T. S., Jessell, T. M., and Brownstone, R. M. (2013). Circuits for grasping: spinal d13 interneurons mediate cutaneous control of motor behavior. *Neuron* 78, 191–204. doi: 10.1016/j.neuron.2013.02.007
- Burrill, J. D., Moran, L., Goulding, M. D., and Saueressig, H. (1997). PAX2 is expressed in multiple spinal cord interneurons, including a population of EN1+ interneurons that require PAX6 for their development. *Development* 124, 4493–4503.
- Chen, Z. F., Rebelo, S., White, F., Malmberg, A. B., Baba, H., Lima, D., et al. (2001). The paired homeodomain protein DRG11 is required for the projection of cutaneous sensory afferent fibers to the dorsal spinal cord. *Neuron* 31, 59–73. doi: 10.1016/S0896-6273(01)00341-5
- Cheng, L., Arata, A., Mizuguchi, R., Qian, Y., Karunaratne, A., Gray, P. A., et al. (2004). Tlx3 and Tlx1 are post-mitotic selector genes determining glutamatergic over GABAergic cell fates. *Nat. Neurosci.* 7, 510–517. doi: 10.1038/nn1221
- Crone, S. A., Quinlan, K. A., Zagoraoui, L., Droho, S., Restrepo, C. E., Lundfald, L., et al. (2008). Genetic ablation of V2a ipsilateral interneurons disrupts left-right locomotor coordination in mammalian spinal cord. *Neuron* 60, 70–83. doi: 10.1016/j.neuron.2008.08.009
- Crone, S. A., Zhong, G., Harris-Warrick, R., and Sharma, K. (2009). In mice lacking V2a interneurons, gait depends on speed of locomotion. *J. Neurosci.* 29, 7098–7109. doi: 10.1523/JNEUROSCI.1206-09.2009
- Dasen, J. S., De Camilli, A., Wang, B., Tucker, P. W., and Jessell, T. M. (2008). Hox repertoires for motor neuron diversity and connectivity gated by a single accessory factor, FoxP1. *Cell* 134, 304–316. doi: 10.1016/j.cell.2008.06.019
- Dasen, J. S., and Jessell, T. M. (2009). Hox networks and the origins of motor neuron diversity. *Curr. Top. Dev. Biol.* 88, 169–200. doi: 10.1016/S0070-2153(09)88006-X
- Dasen, J. S., Tice, B. C., Brenner-Morton, S., and Jessell, T. M. (2005). A Hox regulatory network establishes motor neuron pool identity and target-muscle connectivity. *Cell* 123, 477–491. doi: 10.1016/j.cell.2005.09.009
- Del Barrio, M. G., Taveira-Marques, R., Muromiya, Y., Yuk, D. I., Li, S., Wines-Samuelson, M., et al. (2007). A regulatory network involving Foxn4, Mash1 and delta-like 4/Notch1 generates V2a and V2b spinal interneurons from a common progenitor pool. *Development* 134, 3427–3436. doi: 10.1242/dev.005868
- De Marco Garcia, N. V., and Jessell, T. M. (2008). Early motor neuron pool identity and muscle nerve trajectory defined by postmitotic restrictions in Nkx6.1 activity. *Neuron* 57, 217–231. doi: 10.1016/j.neuron.2007.11.033
- Ding, Y. Q., Kim, J. Y., Xu, Y. S., Rao, Y., and Chen, Z. F. (2005). Ventral migration of early-born neurons requires Dcc and is essential for the projections of primary afferents in the spinal cord. *Development* 132, 2047–2056. doi: 10.1242/dev.01798
- Ding, Y. Q., Yin, J., Kania, A., Zhao, Z. Q., Johnson, R. L., and Chen, Z. F. (2004). Lmx1b controls the differentiation and migration of the superficial dorsal horn neurons of the spinal cord. *Development* 131, 3693–3703. doi: 10.1242/dev.01250
- Dottori, M., Hartley, L., Galea, M., Paxinos, G., Polizzotto, M., Kilpatrick, T., et al. (1998). EphA4 (Sek1) receptor tyrosine kinase is required for the development of the corticospinal tract. *Proc. Natl. Acad. Sci. U.S.A.* 95, 13248–13253. doi: 10.1073/pnas.95.22.13248
- Dougherty, K. J., and Kiehn, O. (2010a). Firing and cellular properties of V2a interneurons in the rodent spinal cord. *J. Neurosci.* 30, 24–37. doi: 10.1523/JNEUROSCI.4821-09.2010
- Dougherty, K. J., and Kiehn, O. (2010b). Functional organization of V2a-related locomotor circuits in the rodent spinal cord. *Ann. N. Y. Acad. Sci.* 1198, 85–93. doi: 10.1111/j.1749-6632.2010.05502.x
- Duval, N., Daubas, P., Bourcier de Carbon, C., St Clément, C., Tinevez, J. Y., Lopes, M., et al. (2014). Msx1 and Msx2 act as essential activators of Atoh1 expression in the murine spinal cord. *Development* 141, 1726–1736. doi: 10.1242/dev.099002
- Dyck, J., Lanuza, G. M., and Gosgnach, S. (2012). Functional characterization of d16 interneurons in the neonatal mouse spinal cord. *J. Neurophysiol.* 107, 3256–3266. doi: 10.1152/jn.01132.2011
- Eccles, J. C., Fatt, P., and Koketsu, K. (1954). Cholinergic and inhibitory synapses in a pathway from motor-axon collaterals to motoneurons. *J. Physiol.* 126, 524–562. doi: 10.1113/jphysiol.1954.sp005226
- Ericson, J., Morton, S., Kawakami, A., Roelink, H., and Jessell, T. M. (1996). Two critical periods of Sonic Hedgehog signaling required for the specification of motor neuron identity. *Cell* 87, 661–673. doi: 10.1016/S0092-8674(00)81386-0
- Ericson, J., Thor, S., Edlund, T., Jessell, T. M., and Yamada, T. (1992). Early stages of motor neuron differentiation revealed by expression of homeobox gene Islet-1. *Science* 256, 1555–1560. doi: 10.1126/science.1350865

- Francius, C., and Clotman, F. (2010). Dynamic expression of the *Onecut* transcription factors HNF-6, OC-2 and OC-3 during spinal motor neuron development. *Neuroscience* 165, 116–129. doi: 10.1016/j.neuroscience.2009.09.076
- Francius, C., Harris, A., Rucchin, V., Hendricks, T. J., Stam, F. J., Barber, M., et al. (2013). Identification of multiple subsets of ventral interneurons and differential distribution along the rostrocaudal axis of the developing spinal cord. *PLoS ONE* 8:e70325. doi: 10.1371/journal.pone.0070325
- Francius, C., Ravassard, P., Hidalgo-Figueroa, M., Mallet, J., Clotman, F., and Nardelli, J. (2014). Genetic dissection of *Gata2* selective functions during specification of V2 interneurons in the developing spinal cord. *Dev. Neurobiol.* doi: 10.1002/dneu.22244 [Epub ahead of print].
- Friese, A., Kaltschmidt, J. A., Ladle, D. R., Sigrist, M., Jessell, T. M., and Arber, S. (2009). Gamma and alpha motor neurons distinguished by expression of transcription factor *Err3*. *Proc. Natl. Acad. Sci. U.S.A.* 106, 13588–13593. doi: 10.1073/pnas.0906809106
- Geiman, E. J., Knox, M. C., and Alvarez, F. J. (2000). Postnatal maturation of gephyrin/glycine receptor clusters on developing Renshaw cells. *J. Comp. Neurol.* 426, 130–142. doi: 10.1002/1096-9861(20001009)426:1<130::AID-CNE9>3.0.CO;2-7
- Glasgow, S. M., Henke, R. M., Macdonald, R. J., Wright, C. V., and Johnson, J. E. (2005). *Ptf1a* determines GABAergic over glutamatergic neuronal cell fate in the spinal cord dorsal horn. *Development* 132, 5461–5469. doi: 10.1242/dev.02167
- Gosgnach, S., Lanuza, G. M., Butt, S. J., Saueressig, H., Zhang, Y., Velasquez, T., et al. (2006). V1 spinal neurons regulate the speed of vertebrate locomotor outputs. *Nature* 440, 215–219. doi: 10.1038/nature04545
- Gould, T. W., Buss, R. R., Vinsant, S., Prevette, D., Sun, W., Knudson, M., et al. (2006). Complete dissociation of motor neuron death from motor dysfunction by *Bax* deletion in a mouse model of ALS. *J. Neurosci.* 26, 8774–8786. doi: 10.1523/JNEUROSCI.2315-06.2006
- Gould, T. W., Yonemura, S., Oppenheim, R. W., Ohmori, S., and Enomoto, H. (2008). The neurotrophic effects of glial cell line-derived neurotrophic factor on spinal motoneurons are restricted to fusimotor subtypes. *J. Neurosci.* 28, 2131–2146. doi: 10.1523/JNEUROSCI.5185-07.2008
- Goulding, M. (2009). Circuits controlling vertebrate locomotion: moving in a new direction. *Nat. Rev. Neurosci.* 10, 507–518. doi: 10.1038/nrn2608
- Goulding, M., Lanuza, G., Sapir, T., and Narayan, S. (2002). The formation of sensorimotor circuits. *Curr. Opin. Neurobiol.* 12, 508–515. doi: 10.1016/S0959-4388(02)00371-9
- Goulding, M., and Pfaff, S. L. (2005). Development of circuits that generate simple rhythmic behaviors in vertebrates. *Curr. Opin. Neurobiol.* 15, 14–20. doi: 10.1016/j.conb.2005.01.017
- Gowan, K., Helms, A. W., Hunsaker, T. L., Collisson, T., Ebert, P. J., Odom, R., et al. (2001). Crossinhibitory activities of *Ngn1* and *Math1* allow specification of distinct dorsal interneurons. *Neuron* 31, 219–232. doi: 10.1016/S0896-6273(01)00367-1
- Griener, A., Zhang, W., Kao, H., Wagner, C., and Gosgnach, S. (2015). Probing diversity within subpopulations of locomotor-related V0 interneurons. *Dev. Neurobiol.* doi: 10.1002/dneu.22277 [Epub ahead of print].
- Grillner, S., and Jessell, T. M. (2009). Measured motion: searching for simplicity in spinal locomotor networks. *Curr. Opin. Neurobiol.* 19, 572–586. doi: 10.1016/j.conb.2009.10.011
- Gross, M. K., Dottori, M., and Goulding, M. (2002). *Lbx1* specifies somatosensory association interneurons in the dorsal spinal cord. *Neuron* 34, 535–49. doi: 10.1016/S0896-6273(02)00690-6
- Gross, M. K., Moran-Rivard, L., Velasquez, T., Nakatsu, M. N., Jagla, K., and Goulding, M. (2000). *Lbx1* is required for muscle precursor migration along a lateral pathway into the limb. *Development* 127, 413–424.
- Hanson, M. G., and Landmesser, L. T. (2004). Normal patterns of spontaneous activity are required for correct motor axon guidance and the expression of specific guidance molecules. *Neuron* 43, 687–701. doi: 10.1016/j.neuron.2004.08.018
- Hegarty, S. V., O'Keefe, G. W., and Sullivan, A. M. (2013). BMP-Smad 1/5/8 signalling in the development of the nervous system. *Prog. Neurobiol.* 109, 28–41. doi: 10.1016/j.pneurobio.2013.07.002
- Helms, A. W., Battiste, J., Henke, R. M., Nakada, Y., Simplicio, N., Guillemot, F., et al. (2005). Sequential roles for *Mash1* and *Ngn2* in the generation of dorsal spinal cord interneurons. *Development* 132, 2709–2719. doi: 10.1242/dev.01859
- Helms, A. W., and Johnson, J. E. (1998). Progenitors of dorsal commissural interneurons are defined by *MATH1* expression. *Development* 125, 919–928.
- Helms, A. W., and Johnson, J. E. (2003). Specification of dorsal spinal cord interneurons. *Curr. Opin. Neurobiol.* 13, 42–49. doi: 10.1016/S0959-4388(03)00010-2
- Henke, R. M., Savage, T. K., Meredith, D. M., Glasgow, S. M., Hori, K., Dumas, J., et al. (2009). *Neurog2* is a direct downstream target of the *Ptf1a-Rbpj* transcription complex in dorsal spinal cord. *Development* 136, 2945–2954. doi: 10.1242/dev.035352
- Hinckley, C. A., Hartley, R., Wu, L., Todd, A., and Ziskind-Conhaim, L. (2005). Locomotor-like rhythms in a genetically distinct cluster of interneurons in the mammalian spinal cord. *J. Neurophysiol.* 93, 1439–1449. doi: 10.1152/jn.00647.2004
- Hinckley, C. A., and Ziskind-Conhaim, L. (2006). Electrical coupling between locomotor-related excitatory interneurons in the mammalian spinal cord. *J. Neurosci.* 26, 8477–8483. doi: 10.1523/JNEUROSCI.0395-06.2006
- Holz, A., Kollmus, H., Ryge, J., Niederkofler, V., Dias, J., Ericson, J., et al. (2010). The transcription factors *Nkx2.2* and *Nkx2.9* play a novel role in floor plate development and commissural axon guidance. *Development* 137, 4249–4260. doi: 10.1242/dev.053819
- Hultborn, H., Jankowska, E., and Lindstrom, S. (1971). Recurrent inhibition of interneurons monosynaptically activated from group Ia afferents. *J. Physiol.* 215, 613–636. doi: 10.1113/jphysiol.1971.sp009488
- Hultborn, H., and Udo, M. (1972). Convergence of large muscle spindle (Ia) afferents at interneuronal level in the reciprocal Ia inhibitory pathway to motoneurons. *Acta Physiol. Scand.* 84, 493–499. doi: 10.1111/j.1748-1716.1972.tb05199.x
- Jankowska, E. (2001). Spinal interneuronal systems: identification, multifunctional character and reconfigurations in mammals. *J. Physiol.* 533, 31–40. doi: 10.1111/j.1469-7793.2001.0031b.x
- Jessell, T. M. (2000). Neuronal specification in the spinal cord: inductive signals and transcriptional codes. *Nat. Rev. Genet.* 1, 20–29. doi: 10.1038/35049541
- Ji, S. J., Zhuang, B., Falco, C., Schneider, A., Schuster-Gossler, K., Gossler, A., et al. (2006). Mesodermal and neuronal retinoids regulate the induction and maintenance of limb innervating spinal motor neurons. *Dev. Biol.* 297, 249–261. doi: 10.1016/j.ydbio.2006.05.015
- John, A., Wildner, H., and Britsch, S. (2005). The homeodomain transcription factor *Gbx1* identifies a subpopulation of late-born GABAergic interneurons in the developing dorsal spinal cord. *Dev. Dyn.* 234, 767–771. doi: 10.1002/dvdy.20568
- Jung, H., Lacombe, J., Mazzoni, E. O., Liem, K. F. Jr., Grinstein, J., Mahony, S., et al. (2010). Global control of motor neuron topography mediated by the repressive actions of a single *hox* gene. *Neuron* 67, 781–796. doi: 10.1016/j.neuron.2010.08.008
- Kang, K., Lee, D., Hong, S., Park, S. G., and Song, M. R. (2013). The E3 ligase *Mind bomb-1* (*Mib1*) modulates Delta-Notch signaling to control neurogenesis and gliogenesis in the developing spinal cord. *J. Biol. Chem.* 288, 2580–2592. doi: 10.1074/jbc.M112.398263
- Kiehn, O. (2006). Locomotor circuits in the mammalian spinal cord. *Annu. Rev. Neurosci.* 29, 279–306. doi: 10.1146/annurev.neuro.29.051605.112910
- Kimura, Y., Okamura, Y., and Higashijima, S. (2006). *alx*, a zebrafish homolog of *Chx10*, marks ipsilateral descending excitatory interneurons that participate in the regulation of spinal locomotor circuits. *J. Neurosci.* 26, 5684–5697. doi: 10.1523/JNEUROSCI.4993-05.2006
- Kimura, Y., Satou, C., and Higashijima, S. (2008). V2a and V2b neurons are generated by the final divisions of pair-producing progenitors in the zebrafish spinal cord. *Development* 135, 3001–3005. doi: 10.1242/dev.024802
- Kriks, S., Lanuza, G. M., Mizuguchi, R., Nakafuku, M., and Goulding, M. (2005). *Gsh2* is required for the repression of *Ngn1* and specification of dorsal interneuron fate in the spinal cord. *Development* 132, 2991–3002. doi: 10.1242/dev.01878
- Kullander, K., Croll, S. D., Zimmer, M., Pan, L., McClain, J., Hughes, V., et al. (2001). Ephrin-B3 is the midline barrier that prevents corticospinal tract axons from recrossing, allowing for unilateral motor control. *Genes Dev.* 15, 877–888. doi: 10.1101/gad.868901
- Kwan, A. C., Dietz, S. B., Webb, W. W., and Harris-Warrick, R. M. (2009). Activity of Hb9 interneurons during fictive locomotion in mouse spinal cord. *J. Neurosci.* 29, 11601–11613. doi: 10.1523/JNEUROSCI.1612-09.2009

- Ladle, D. R., Pecho-Vrieseling, E., and Arber, S. (2007). Assembly of motor circuits in the spinal cord: driven to function by genetic and experience-dependent mechanisms. *Neuron* 56, 270–283. doi: 10.1016/j.neuron.2007.09.026
- Landmesser, L. (1978). The distribution of motoneurons supplying chick hind limb muscles. *J. Physiol.* 284, 371–389. doi: 10.1113/jphysiol.1978.sp012545
- Lanuza, G. M., Gosgnach, S., Pierani, A., Jessell, T. M., and Goulding, M. (2004). Genetic identification of spinal interneurons that coordinate left-right locomotor activity necessary for walking movements. *Neuron* 42, 375–386. doi: 10.1016/S0896-6273(04)00249-1
- Le Dréau, G., Garcia-Campmany, L., Rabadán, M. A., Ferronha, T., Tozer, S., Briscoe, J., et al. (2012). Canonical BMP7 activity is required for the generation of discrete neuronal populations in the dorsal spinal cord. *Development* 139, 259–268. doi: 10.1242/dev.074948
- Lee, K. J., Dietrich, P., and Jessell, T. M. (2000). Genetic ablation reveals that the roof plate is essential for dorsal interneuron specification. *Nature* 403, 734–740. doi: 10.1038/35001507
- Lee, K. J., Mendelsohn, M., and Jessell, T. M. (1998). Neuronal patterning by BMPs: a requirement for GDF7 in the generation of a discrete class of commissural interneurons in the mouse spinal cord. *Genes Dev.* 12, 3394–3407. doi: 10.1101/gad.12.21.3394
- Lee, S., Lee, B., Joshi, K., Pfaff, S. L., Lee, J. W., and Lee, S. K. (2008). A regulatory network to segregate the identity of neuronal subtypes. *Dev. Cell* 14, 877–889. doi: 10.1016/j.devcel.2008.03.021
- Lee, S. K., and Pfaff, S. L. (2001). Transcriptional networks regulating neuronal identity in the developing spinal cord. *Nat. Neurosci.* 4(Suppl.), 1183–1191. doi: 10.1038/nm750
- Li, S., Misra, K., and Xiang, M. (2010). A Cre transgenic line for studying V2 neuronal lineages and functions in the spinal cord. *Genesis* 48, 667–672. doi: 10.1002/dvg.20669
- Liem, K. F. Jr., Tremml, G., and Jessell, T. M. (1997). A role for the roof plate and its resident TGFβ-related proteins in neuronal patterning in the dorsal spinal cord. *Cell* 91, 127–138. doi: 10.1016/S0092-8674(01)80015-5
- Liem, K. F. Jr., Tremml, G., Roelink, H., and Jessell, T. M. (1995). Dorsal differentiation of neural plate cells induced by BMP-mediated signals from epidermal ectoderm. *Cell* 82, 969–979. doi: 10.1016/0092-8674(95)90276-7
- Lin, J. H., Saito, T., Anderson, D. J., Lance-Jones, C., Jessell, T. M., and Arber, S. (1998). Functionally related motor neuron pool and muscle sensory afferent subtypes defined by coordinate ETS gene expression. *Cell* 95, 393–407. doi: 10.1016/S0092-8674(00)81770-5
- Liu, J. P., Laufer, E., and Jessell, T. M. (2001). Assigning the positional identity of spinal motor neurons: rostrocaudal patterning of Hox-c expression by FGFs, Gdf11, and retinoids. *Neuron* 32, 997–1012. doi: 10.1016/S0896-6273(01)00544-X
- Liu, Z., Hu, X., Huang, C., Zheng, K., Takebayashi, H., Cao, C., et al. (2014). Olig3 is not involved in the ventral patterning of spinal cord. *PLoS ONE* 9:e111076. doi: 10.1371/journal.pone.0111076
- Lundfald, L., Restrepo, C. E., Butt, S. J., Peng, C. Y., Droho, S., Endo, T., et al. (2007). Phenotype of V2-derived interneurons and their relationship to the axon guidance molecule EphA4 in the developing mouse spinal cord. *Eur. J. Neurosci.* 26, 2989–3002. doi: 10.1111/j.1460-9568.2007.05906.x
- Marion, J. F., Yang, C., Caqueret, A., Boucher, F., and Michaud, J. L. (2005). Sim1 and Sim2 are required for the correct targeting of mammillary body axons. *Development* 132, 5527–5537. doi: 10.1242/dev.02142
- Matise, M. P., and Joyner, A. L. (1997). Expression patterns of developmental control genes in normal and Engrailed-1 mutant mouse spinal cord reveal early diversity in developing interneurons. *J. Neurosci.* 17, 7805–7816.
- McLean, D. L., Masino, M. A., Koh, I. Y., Lindquist, W. B., and Fetcho, J. R. (2008). Continuous shifts in the active set of spinal interneurons during changes in locomotor speed. *Nat. Neurosci.* 11, 1419–1429. doi: 10.1038/nn.2225
- Megason, S. G., and McMahon, A. P. (2002). A mitogen gradient of dorsal midline Wnts organizes growth in the CNS. *Development* 129, 2087–2098.
- Mentis, G. Z., Alvarez, F. J., Bonnot, A., Richards, D. S., Gonzalez-Forero, D., Zerda, R., et al. (2005). Noncholinergic excitatory actions of motoneurons in the neonatal mammalian spinal cord. *Proc. Natl. Acad. Sci. U.S.A.* 102, 7344–7349. doi: 10.1073/pnas.0502788102
- Meredith, D. M., Masui, T., Swift, G. H., MacDonald, R. J., and Johnson, J. E. (2009). Multiple transcriptional mechanisms control Ptf1a levels during neural development including autoregulation by the PTF1-J complex. *J. Neurosci.* 29, 11139–11148. doi: 10.1523/JNEUROSCI.2303-09.2009
- Miesegaes, G. R., Klisch, T. J., Thaller, C., Ahmad, K. A., Atkinson, R. C., and Zoghbi, H. Y. (2009). Identification and subclassification of new Atoh1 derived cell populations during mouse spinal cord development. *Dev. Biol.* 327, 339–351. doi: 10.1016/j.ydbio.2008.12.016
- Misra, M., Shah, V., Carpenter, E., McCaffery, P., and Lance-Jones, C. (2009). Restricted patterns of Hoxd10 and Hoxd11 set segmental differences in motoneuron subtype complement in the lumbosacral spinal cord. *Dev. Biol.* 330, 54–72. doi: 10.1016/j.ydbio.2009.03.009
- Mizuguchi, R., Kriks, S., Cordes, R., Gossler, A., Ma, Q., and Goulding, M. (2006). Ascl1 and Gsh1/2 control inhibitory and excitatory cell fate in spinal sensory interneurons. *Nat. Neurosci.* 9, 770–778. doi: 10.1038/nn1706
- Moran-Rivard, L., Kagawa, T., Saueressig, H., Gross, M. K., Burrill, J., Goulding, M., et al. (2001). Evx1 is a postmitotic determinant of v0 interneuron identity in the spinal cord. *Neuron* 29, 385–399. doi: 10.1016/S0896-6273(01)00213-6
- Morikawa, Y., Hisaoka, T., and Senba, E. (2009). Characterization of Foxp2-expressing cells in the developing spinal cord. *Neuroscience* 162, 1150–1162. doi: 10.1016/j.neuroscience.2009.05.022
- Muhr, J., Graziano, E., Wilson, S., Jessell, T. M., and Edlund, T. (1999). Convergent inductive signals specify midbrain, hindbrain, and spinal cord identity in gastrula stage chick embryos. *Neuron* 23, 689–702. doi: 10.1016/S0896-6273(01)80028-3
- Muller, T., Anlag, K., Wildner, H., Britsch, S., Treier, M., and Birchmeier, C. (2005). The bHLH factor Olig3 coordinates the specification of dorsal neurons in the spinal cord. *Genes Dev.* 19, 733–743. doi: 10.1101/gad.326105
- Muller, T., Brohmann, H., Pierani, A., Heppenstall, P. A., Lewin, G. R., et al. (2002). The homeodomain factor lbx1 distinguishes two major programs of neuronal differentiation in the dorsal spinal cord. *Neuron* 34, 551–562. doi: 10.1016/S0896-6273(02)00689-X
- Muroyama, Y., Fujihara, M., Ikeya, M., Kondoh, H., and Takada, S. (2002). Wnt signaling plays an essential role in neuronal specification of the dorsal spinal cord. *Genes Dev.* 16, 548–553. doi: 10.1101/gad.937102
- Myers, C. P., Lewcock, J. W., Hanson, M. G., Gosgnach, S., Aimone, J. B., Gage, H. F., et al. (2005). Cholinergic input is required during embryonic development to mediate proper assembly of spinal locomotor circuits. *Neuron* 46, 37–49. doi: 10.1016/j.neuron.2005.02.022
- Nornes, H. O., and Carry, M. (1978). Neurogenesis in spinal cord of mouse: an autoradiographic analysis. *Brain Res.* 159, 1–6. doi: 10.1016/0006-8993(78)90105-1
- Novitsch, B. G., Chen, A. I., and Jessell, T. M. (2001). Coordinate regulation of motor neuron subtype identity and pan-neuronal properties by the bHLH repressor Olig2. *Neuron* 31, 773–789. doi: 10.1016/S0896-6273(01)00407-X
- Okigawa, S., Mizoguchi, T., Okano, M., Tanaka, H., Isoda, M., Isoda, M., Jiang, Y. J., et al. (2014). Different combinations of Notch ligands and receptors regulate V2 interneuron progenitor proliferation and V2a/V2b cell fate determination. *Dev. Biol.* 391, 196–206. doi: 10.1016/j.ydbio.2014.04.011
- Panayi, H., Panayiotou, E., Orford, M., Genethliou, N., Mean, R., Lapathitis, G., et al. (2010). Sox1 is required for the specification of a novel p2-derived interneuron subtype in the mouse ventral spinal cord. *J. Neurosci.* 30, 12274–12280. doi: 10.1523/JNEUROSCI.2402-10.2010
- Peng, C. Y., Yajima, H., Burns, C. E., Zon, L. I., Sisodia, S. S., Pfaff, S. L., et al. (2007). Notch and MAML signaling drives Scl-dependent interneuron diversity in the spinal cord. *Neuron* 53, 813–827. doi: 10.1016/j.neuron.2007.02.019
- Pfaff, S. L., Mendelsohn, M., Stewart, C. L., Edlund, T., and Jessell, T. M. (1996). Requirement for LIM homeobox gene Isl1 in motor neuron generation reveals a motor neuron-dependent step in interneuron differentiation. *Cell* 84, 309–320. doi: 10.1016/S0092-8674(00)80985-X
- Pierani, A., Brenner-Morton, S., Chiang, C., and Jessell, T. M. (1999). A sonic hedgehog-independent, retinoid-activated pathway of neurogenesis in the ventral spinal cord. *Cell* 97, 903–915. doi: 10.1016/S0092-8674(00)80802-8
- Pierani, A., Moran-Rivard, L., Sunshine, M. J., Littman, D. R., Goulding, M., and Jessell, T. M. (2001). Control of interneuron fate in the developing spinal

- cord by the progenitor homeodomain protein Dbx1. *Neuron* 29, 367–384. doi: 10.1016/S0896-6273(01)00212-4
- Pillai, A., Mansouri, A., Behringer, R., Westphal, H., and Goulding, M. (2007). Lhx1 and Lhx5 maintain the inhibitory-neurotransmitter status of interneurons in the dorsal spinal cord. *Development* 134, 357–366. doi: 10.1242/dev.02717
- Qian, Y., Shirasawa, S., Chen, C. L., Cheng, L., and Ma, Q. (2002). Proper development of relay somatic sensory neurons and D2/D4 interneurons requires homeobox genes *Rnx/Tlx-3* and *Tlx-1*. *Genes Dev.* 16, 1220–1233. doi: 10.1101/gad.982802
- Quiroga, A. C., Stolt, C. C., Del Corral, R. D., Dimitrov, S., Perez-Alcala, S., Sock, E., et al. (2015). Sox5 controls dorsal progenitor and interneuron specification in the spinal cord. *Dev. Neurobiol.* 75, 522–538. doi: 10.1002/dneu.22240
- Ramos, C., Rocha, S., Gaspar, C., and Henrique, D. (2010). Two Notch ligands, Dll1 and Jag1, are differentially restricted in their range of action to control neurogenesis in the mammalian spinal cord. *PLoS ONE* 5:e15515. doi: 10.1371/journal.pone.0015515
- Richards, D. S., Griffith, R. W., Romer, S. H., and Alvarez, F. J. (2014). Motor axon synapses on renshaw cells contain higher levels of aspartate than glutamate. *PLoS ONE* 9:e97240. doi: 10.1371/journal.pone.0097240
- Roelink, H., Augsburger, A., Heemskerk, J., Korch, V., Norlin, S., Ruiz i Altaba, A., et al. (1994). Floor plate and motor neuron induction by *vhh-1*, a vertebrate homolog of hedgehog expressed by the notochord. *Cell* 76, 761–775. doi: 10.1016/0092-8674(94)90514-2
- Sander, M., Paydar, S., Ericson, J., Briscoe, J., Berber, E., German, M., et al. (2000). Ventral neural patterning by *Nkx* homeobox genes: *Nkx6.1* controls somatic motor neuron and ventral interneuron fates. *Genes Dev.* 14, 2134–2139. doi: 10.1101/gad.820400
- Sapir, T., Geiman, E. J., Wang, Z., Velasquez, T., Mitsui, S., Yoshihara, Y., et al. (2004). *Pax6* and *engrailed 1* regulate two distinct aspects of renshaw cell development. *J. Neurosci.* 24, 1255–1264. doi: 10.1523/JNEUROSCI.3187-03.2004
- Saueressig, H., Burrill, J., and Goulding, M. (1999). *Engrailed-1* and *netrin-1* regulate axon pathfinding by association interneurons that project to motor neurons. *Development* 126, 4201–4212.
- Scardigli, R., Schuurmans, C., Gradwohl, G., and Guillemot, F. (2001). Crossregulation between *Neurogenin2* and pathways specifying neuronal identity in the spinal cord. *Neuron* 31, 203–217. doi: 10.1016/S0896-6273(01)00358-0
- Sharma, K., Leonard, A. E., Lettieri, K., and Pfaff, S. L. (2000). Genetic and epigenetic mechanisms contribute to motor neuron pathfinding. *Nature* 406, 515–519. doi: 10.1038/35020078
- Sharma, K., Sheng, H. Z., Lettieri, K., Li, H., Karavanov, A., Potter, S., et al. (1998). LIM homeodomain factors *Lhx3* and *Lhx4* assign subtype identities for motor neurons. *Cell* 95, 817–828. doi: 10.1016/S0092-8674(00)81704-3
- Shirasaki, R., and Pfaff, S. L. (2002). Transcriptional codes and the control of neuronal identity. *Annu. Rev. Neurosci.* 25, 251–281. doi: 10.1146/annurev.neuro.25.112701.142916
- Shneider, N. A., Brown, M. N., Smith, C. A., Pickel, J., and Alvarez, F. J. (2009). Gamma motor neurons express distinct genetic markers at birth and require muscle spindle-derived GDNF for postnatal survival. *Neural Dev.* 4, 42. doi: 10.1186/1749-8104-4-42
- Skaggs, K., Martin, D. M., and Novitsch, B. G. (2011). Regulation of spinal interneuron development by the Olig-related protein *Bhlhb5* and Notch signaling. *Development* 138, 3199–3211. doi: 10.1242/dev.057281
- Sockanathan, S., and Jessell, T. M. (1998). Motor neuron-derived retinoid signaling specifies the subtype identity of spinal motor neurons. *Cell* 94, 503–514. doi: 10.1016/S0092-8674(00)81591-3
- Sockanathan, S., Perlmann, T., and Jessell, T. M. (2003). Retinoid receptor signaling in postmitotic motor neurons regulates rostrocaudal positional identity and axonal projection pattern. *Neuron* 40, 97–111. doi: 10.1016/S0896-6273(03)00532-4
- Stam, F. J., Hendricks, T. J., Zhang, J., Geiman, E. J., Francius, C., Labosky, P. A., et al. (2012). Renshaw cell interneuron specialization is controlled by a temporally restricted transcription factor program. *Development* 139, 179–190. doi: 10.1242/dev.071134
- Stepien, A. E., and Arber, S. (2008). Probing the locomotor conundrum: descending the 'V' interneuron ladder. *Neuron* 60, 1–4. doi: 10.1016/j.neuron.2008.09.030
- Stepien, A. E., Tripodi, M., and Arber, S. (2010). Monosynaptic rabies virus reveals premotor network organization and synaptic specificity of cholinergic partition cells. *Neuron* 68, 456–472. doi: 10.1016/j.neuron.2010.10.019
- Tanabe, Y., William, C., and Jessell, T. M. (1998). Specification of motor neuron identity by the *MNR2* homeodomain protein. *Cell* 95, 67–80. doi: 10.1016/S0092-8674(00)81783-3
- Thaler, J., Harrison, K., Sharma, K., Lettieri, K., Kehrl, J., and Pfaff, S. L. (1999). Active suppression of interneuron programs within developing motor neurons revealed by analysis of homeodomain factor *HB9*. *Neuron* 23, 675–687. doi: 10.1016/S0896-6273(01)80027-1
- Thaler, J. P., Koo, S. J., Kania, A., Lettieri, K., Andrews, S., Cox, C., et al. (2004). A postmitotic role for *Isl*-class LIM homeodomain proteins in the assignment of visceral spinal motor neuron identity. *Neuron* 41, 337–350. doi: 10.1016/S0896-6273(04)00011-X
- Thaler, J. P., Lee, S. K., Jurata, L. W., Gill, G. N., and Pfaff, S. L. (2002). LIM factor *Lhx3* contributes to the specification of motor neuron and interneuron identity through cell-type-specific protein-protein interactions. *Cell* 110, 237–249. doi: 10.1016/S0092-8674(02)00823-1
- Timmer, J. R., Wang, C., and Niswander, L. (2002). BMP signaling patterns the dorsal and intermediate neural tube via regulation of homeobox and helix-loop-helix transcription factors. *Development* 129, 2459–2472.
- Tracey, D. P. (1985). "The somatosensory system." in *The Rat Nervous System*, ed. G. Paxinos (San Diego, CA: Academic Press), 129–152.
- Tsushima, T., Ensini, M., Morton, S. B., Baldassare, M., Edlund, T., Jessell, T. M., et al. (1994). Topographic organization of embryonic motor neurons defined by expression of LIM homeobox genes. *Cell* 79, 957–970. doi: 10.1016/0092-8674(94)90027-2
- Vallstedt, A., and Kullander, K. (2013). Dorsally derived spinal interneurons in locomotor circuits. *Ann. N. Y. Acad. Sci.* 1279, 32–42. doi: 10.1111/j.1749-6632.2012.06801.x
- Vallstedt, A., Muhr, J., Pattyn, A., Pierani, A., Mendelsohn, M., Sander, M., et al. (2001). Different levels of repressor activity assign redundant and specific roles to *Nkx6* genes in motor neuron and interneuron specification. *Neuron* 31, 743–755. doi: 10.1016/S0896-6273(01)00412-3
- Wang, Z., Li, L., Goulding, M., and Frank, E. (2008). Early postnatal development of reciprocal Ia inhibition in the murine spinal cord. *J. Neurophysiol.* 100, 185–196. doi: 10.1152/jn.90354.2008
- Watterson, R. L. (1965). *Organogenesis*. New York, NY: Holt, Rinehart and Winston, 129–159.
- Wenner, P., Matisse, M. P., Joyner, A., and O'Donovan, M. J. (1998). Physiological and molecular characterization of interneurons in the developing spinal cord. *Ann. N. Y. Acad. Sci.* 860, 425–427. doi: 10.1111/j.1749-6632.1998.tb09066.x
- Wenner, P., O'Donovan, M. J., and Matisse, M. P. (2000). Topographical and physiological characterization of interneurons that express *engrailed-1* in the embryonic chick spinal cord. *J. Neurophysiol.* 84, 2651–2657.
- Wichterle, H., Lieberam, I., Porter, J. A., and Jessell, T. M. (2002). Directed differentiation of embryonic stem cells into motor neurons. *Cell* 110, 385–397. doi: 10.1016/S0092-8674(02)00835-8
- Wickersham, I. R., Lyon, D. C., Barnard, R. J., Mori, T., Finke, S., Conzelmann, K. K., et al. (2007). Monosynaptic restriction of transsynaptic tracing from single, genetically targeted neurons. *Neuron* 53, 639–647. doi: 10.1016/j.neuron.2007.01.033
- Wilson, J. M., Hartley, R., Maxwell, D. J., Todd, A. J., Lieberam, I., Kaltschmidt, J. A., et al. (2005). Conditional rhythmicity of ventral spinal interneurons defined by expression of the *Hb9* homeodomain protein. *J. Neurosci.* 25, 5710–5719. doi: 10.1523/JNEUROSCI.0274-05.2005
- Wilson, S. I., Shafer, B., Lee, K. J., and Dodd, J. (2008). A molecular program for contralateral trajectory: *Rig-1* control by LIM homeodomain transcription factors. *Neuron* 59, 413–424. doi: 10.1016/j.neuron.2008.07.020
- Yang, X., Tomita, T., Wines-Samuelson, M., Beglopoulos, V., Tansey, M. G., Kopan, R., et al. (2006). Notch1 signaling influences v2 interneuron and motor neuron development in the spinal cord. *Dev. Neurosci.* 28, 102–117. doi: 10.1159/000090757
- Yokoyama, N., Romero, M. I., Cowan, C. A., Galvan, P., Helmbacher, F., Charnay, P., et al. (2001). Forward signaling mediated by ephrin-B3 prevents contralateral corticospinal axons from recrossing the spinal cord midline. *Neuron* 29, 85–97. doi: 10.1016/S0896-6273(01)00182-9

- Zagoraiou, L., Akay, T., Martin, J. F., Brownstone, R. M., Jessell, T. M., and Miles, G. B. (2009). A cluster of cholinergic premotor interneurons modulates mouse locomotor activity. *Neuron* 64, 645–662. doi: 10.1016/j.neuron.2009.10.017
- Zhang, J., Lanuza, G. M., Britz, O., Wang, Z., Siembab, V. C., Zhang, Y., et al. (2014). V1 and v2b interneurons secure the alternating flexor-extensor motor activity mice require for limbed locomotion. *Neuron* 82, 138–150. doi: 10.1016/j.neuron.2014.02.013
- Zhang, Y., Narayan, S., Geiman, E., Lanuza, G. M., Velasquez, T., Shanks, B., et al. (2008). V3 spinal neurons establish a robust and balanced locomotor rhythm during walking. *Neuron* 60, 84–96. doi: 10.1016/j.neuron.2008.09.027
- Zhong, G., Droho, S., Crone, S. A., Dietz, S., Kwan, A. C., Kwan, A. C., et al. (2010). Electrophysiological characterization of V2a interneurons and their locomotor-related activity in the neonatal mouse spinal cord. *J. Neurosci.* 30, 170–182. doi: 10.1523/JNEUROSCI.4849-09.2010
- Zou, M., Luo, H., and Xiang, M. (2015). Selective neuronal lineages derived from Dll4-expressing progenitors/precursors in the retina and spinal cord. *Dev. Dyn.* 244, 86–97. doi: 10.1002/dvdy.24185

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2015 Lu, Niu and Alaynick. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) or licensor are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.