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TITLE: Electrophysiological and Behavioral Evaluation of C-LTMR Plasticity Induced by Spinal Cord Injury: Transformation from Pleasure to Pain Afferents

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14. ABSTRACT

C-low threshold mechanoreceptors (C-LTMRs) are a sub-population of cutaneous afferents that innervate hairy skin and encode pleasant touch. The study tests the hypothesis that C-LTMRs are transformed into allodynia-encoding nociceptors after spinal cord injury (SCI). In rodents, C-LTMRs can be selectively identified by their expression of tyrosine hydroxylase (TH). Using transgenic TH-Cre mice, we proposed to examine whether (i)SCI modifies C-LTMRs' recruitment and activation properties, (ii) C-LTMR plasticity after SCI contributes to at-level mechanical allodynia and (iii) sympathetic activity modulates C-LTMR activity and the expression of neuropathic pain. Overall, we acquired data that align with or fully support the general hypothesis. First, we report that using a tamoxifen-inducible strain of TH-Cre mice, neural responses are evoked by selective activation of TH+ C-LTMRs in the sciatic nerve and trunk skin. Second, we show that SCI and mechanical truncal stimulation (only after SCI) induce short-lasting increases in respiratory rates (RRs) in adult mice. Third, using two place preference behavioral paradigms, TH-Cre mice with a SCI show a significant increase in time spent in the non-stimulated (escape chamber) immediately after mechanical or optical stimulation of the trunk. This effect developed at 3 weeks and persisted to at least 5 weeks after SCI. Interestingly, SCI mice also showed significant hind-paw hypersensitivity compared to pre-stimulation and/or sham and naïve control mice. Overall, these observations suggest that mechanical truncal stimulation, that is consistent with C-LTMRs recruitment, elicits an aversive (not pleasurable) response after SCI, which strongly supports our hypothesis. Moreover, the findings suggest that C-LTMRs may indeed signal pain after SCI.

15. SUBJECT TERMS
Spinal cord injury; receptive field; tyrosine hydroxylase (TH); mechanical allodynia; pain; respiratory rate

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TABLE OF CONTENTS

1. INTRODUCTION:

C-low threshold mechanoreceptors (C-LTMRs) are a sub-population of cutaneous afferents that innervate hairy skin and encode pleasant touch. The study tests the hypothesis that C-LTMRs are transformed into allodynia-encoding nociceptors after spinal cord injury (SCI). In rodents, C-LTMRs can be selectively identified by their expression of tyrosine hydroxylase (TH). Using transgenic TH-Cre mice, the study proposed to investigate electrophysiological, cellular and functional plasticity of C-LTMRs after SCI. The objectives of the study were to show (i) SCI modifies C-LTMRs' recruitment and activation properties, (ii) C-LTMR plasticity after SCI contributes to at-level mechanical allodynia and (iii) sympathetic activity modulates C-LTMR activity and the expression of neuropathic pain. Overall, we acquired data that align with or fully support our general hypothesis. First, we provide preliminary data obtained in a tamoxifen-inducible strain of TH-Cre mice that show neural responses evoked by selective activation of TH^+ C-LTMRs by optogenetic stimulation of the sciatic nerve and trunk skin. These results were obtained from both SCI and sham operated mice. Second, we show that SCI and mechanical truncal stimulation (*only after SCI*) induce short-lasting increases in respiratory rates (RRs) in adult mice. Third, in two behavioral paradigms [Place Escape/Avoidance Paradigm (PEAP), and conditioned place aversion (CPA)], we show that TH-Cre mice with a SCI show a significant increase in time spent in the non-stimulated (escape chamber) immediately after mechanical or optical stimulation of the trunk. This effect developed at 3 weeks and persisted to at least 5 weeks after SCI. Interestingly, SCI mice also showed significant hind-paw hypersensitivity compared to pre-stimulation and/or sham and naïve control mice. Overall, these observations suggest that mechanical truncal stimulation, that is consistent with C-LTMRs recruitment, elicits an aversive (not pleasurable) response after SCI, which strongly supports our hypothesis. Moreover, the findings suggest that C-LTMRs may indeed signal pain after SCI.

2. KEYWORDS:

Allodynia; Brush stimulation; Channelrhodopsin; C-LTMRs; Ex-vivo skin-nerve; Low-threshold mechanoreceptors; Pain; Place escape/avoidance paradigm (PEAP); Sympathetic; Respiratory rate; Spinal cord injury (SCI); Tamoxifen; TH-Cre; Trunk

3. ACCOMPLISHMENTS:

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

Table 1: Our original statement of work, including the projected milestones

Specific Aim 1: Investigate the stimulation forces and velocities that engage C-LTMRs:	**0-8 months**	**Garraway**	**Hochman**
Subtask 1 & 2: Optogenetic and mechanical characterization of C-LTMRs using the Skin-Nerve Electrophysiology	0-3		Hochman Watkins
Subtask 3 & 4: Electrophysiological recording after SCI and pharmacology	4-8	Garraway	Watkins
Other: Construction and optimization of programmable brush stimulator		Goolsby	
Milestone(s) Achieved: (i) Construction on mechanical brush and optimization of the preparation			

(2) Establish the electrophysiological characterization and parameters that engage C-LTMRs in naïve and SCI mice.			
Specific Aim 2: Investigate the behavioral effect of C-LTMR activation in intact and SCI mice:	**9-34 months**	**Garraway**	**Hochman**
Subtask 2.1, 2&4: Mechanical and optogenetic stimulation of the trunk skin in naïve and SCI mice Behavioral tests: At level and below level pain tests	9-24	Grad Stud Martin	Watkins
Subtask 2:3 Behavioral effects of decreased sympathetic activity with β-blocker, propranolol	18-34	Grad Stud Martin	Watkins
Other: Maintenance of animal colony and post-surgical care of mice		Martin	Sawchuk
Milestone(s) Achieved: Establish the specific effects C-LTMRs have on pain and responses after SCI, the relationship between changes in HR and RR on the expression of pain and the behavioral effect of decreased sympathetic activity.			
Specific Aim 3: Assess whether activation of C-LTMRs result in central sensitization and increase expression of pain genes:	**18-36 months**	**Garraway**	**Hochman**
Subtask: RNA, protein extraction and cellular assays	18-36	Grad Stud Martin	Sawchuk
Other: Maintenance of animal colony and post-surgical care of mice		Grad Stud Martin	Sawchuk
Milestone(s) Achieved: Show the effect of C-LTMR plasticity after SCI has on the expression of nociceptive genes in the spinal cord. Data presentation			

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

During the three years funding period, we made significant progress with this project, some of which have been reported in previously-submitted reports. Below, we provide a detailed description of goals accomplished for each specific aim (SA).

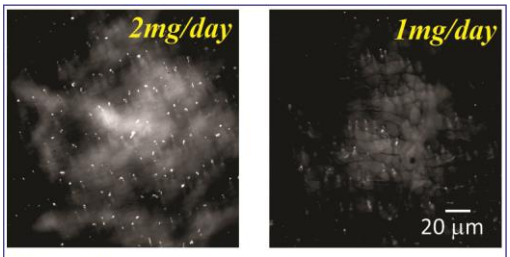

Figure 1. Shown is RFP labeling from littermate TH^{ER}::tdTomato mice given either 2 mg (left) or 1 mg doses (right) of subcutaneous tamoxifen at postnatal days 12 and 14.

Findings related to SA1: Investigate the stimulation forces and velocities that engage C-LTMRs:

Technological: We have successfully developed and verified the TH-Cre^{C} (constitutively expressed) and TH-Cre^{ER} (inducible) mice lines as well as crosses with CHR2-expressing Ai32 mice. Furthermore, we have now successfully recorded neural impulses in response to mechanical and optical stimulation of the

skin, using the fully-optimized skin-nerve electrophysiological preparation. Additional specific advances we have made are described and shown below:

1. We were able to take advantage of variable dosages of tamoxifen to undertake sparse labeling studies that will allow assessment of afferent plasticity both when crossed with tdTomato reporter (**Figure 1**). Some of these mice (sham and SCI) are being prepared for additional immunohistological studies

2. We previously demonstrated optical recruitment of TH+ afferents in TH^{C}::CHR2 mice. We have now verified that the TH-Cre^{ER} line can be used for optogenetic studies on C-LTMR afferent recruitment in the periphery as well as in the spinal cord (**Figure 2**).

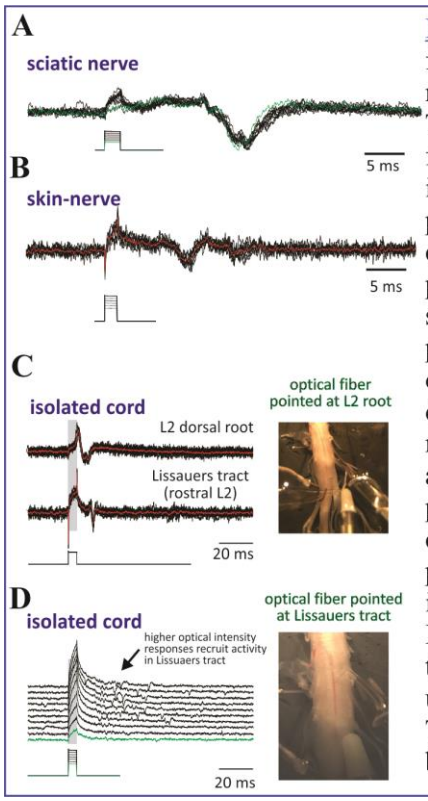

Figure 2. Shown are recordings from an adult female (P160) mouse with recruitment of TH::CHR2 C-LTMR axons following optogenetic activation in sciatic nerve (A), a skin nerve preparation (B), and in the spinal cord (C & D). The mouse was provided with 2 mg doses of subcutaneous tamoxifen at postnatal days 12 and 14. Based on our anatomical studies, we determined that this dose was maximal for genetic labeling of all TH -positive afferents. In panels A and D, green trace coincides with smallest optical pulse delivered in a series of increasing illumination intensities. In panels B and C the red trace is the superimposed average of underlying individual events. Timing of optical pulses is shown below events in each panel.

3. ***Optogenetic characterization of C-LTMR [using the Skin-nerve preparation].*** As shown in **Figure 2** we are now finally capable of selectively activating TH^{+} C-LTMRs using optogenetics in an inducible strain. We use a 2W 445nm copper module laser with a 405-G-2 glass lens. A fiber optic cable delivers blue light that, when directed at the epidermal side of the skin, activates the CHR2 channels on our neurons of interest. Frequency, duration and intensity of the light stimulus will be controlled by the computer program Clampex. Fiber optic illumination is swept across the skin to determine receptive fields characteristics. The fully-optimized recording chamber allows for simultaneous epidermal stimulation and dorsal nerve recording.

Results: We have just completed recordings from 10 TH::CHR2 mice [3 TH^{C}::CHR2 and 7 TH^{ER}::CHR2]. Animals used for these recording were also used for behavioral experiments reported on below (**SA2**). Optogenetic stimulation of the trunk skin evoked neural activity in several thoracic nerves. Examples of the representative recording and an illustration of the experimental setup are shown in **Figure 3**. Additionally, pharmacological manipulations targeting several catecholaminergic systems [acetylcholine, norepinephrine and serotonin] were incorporated in these studies, to assess the modifiability properties of C-LTMRs.

Next steps: Although, we have completed electrophysiological recording in this cohort of TH::CHR2 animals, the data have not yet been fully analyzed and thus we can't report on whether SCI changes the overall properties of C-LTMRs. As described previously, we will use spike sorting software and cluster analysis in Spike2 (Cambridge Electronic Design) to distinguish simultaneously recruited units. Evoked neural impulses will be further characterized

by spike width, conduction velocity, fatigability, and adaptation. In analyzing the data, comparisons will be also made across drug treatments and between Sham and SCI subjects.

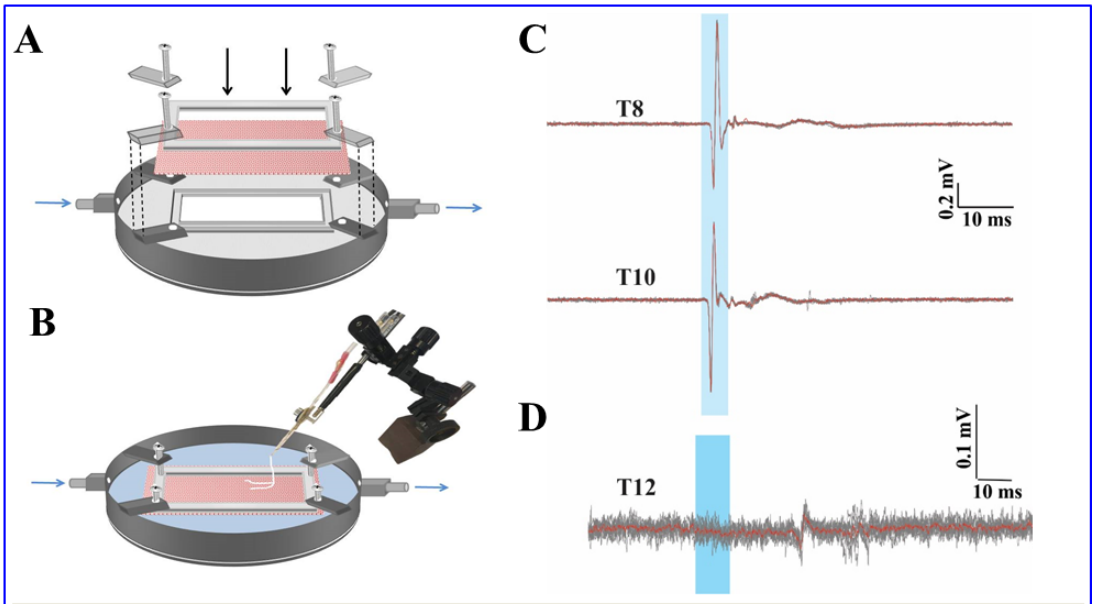

Figure 3. Diagram of recording set-up and C-LTMR-evoked neural responses. **A**) The recording dish. The skin-nerve prep (pink) is placed epidermal-side down over a hole in the dish and held in place by the cap and screws. The skin forms a seal and the dish is filled with a circulating bath (blue arrows). **B**) The distal end of the nerve is attached to a tight-fitting suction electrode. Electrophysiological recordings using the *ex-vivo* skin-nerve preparation validated successful activation of TH^{+} afferents. Examples in **C**) are obtained from a TH-Cre^{C} x CH2R mouse and the recording in **D**) is obtained from a tamoxifen -treated TH-Cre^{ER} x CH2R mouse, at least 4 weeks after SCI. In both recordings, we show recording from the identified dorsal cutaneous nerve (T8, T10 & T12) during optical stimulation of the attached trunk skin. Stimulation is administered via fiberoptic blue light pulses (blue bars; 2.5V, 5 Hz stim., 10 5-ms pulses) to the skin. For each nerve, the gray traces are 10 identical recordings stacked together; and the colored overlay is the average of all ten recordings. Recruited afferents display characteristics of C-LTMRs, including a conduction velocity of ~0.37m/s.

Findings related to SA2: Investigate the behavioral effect of C-LTMR activation in intact and SCI mice:

During the funding period, we significantly advanced the behavioral portions of the study.

1. Effect of SCI and mechanical stimulation on RRs: As was previously reported, SCI TH-Cre^{C} mice had elevated respiratory rates (RR) 1 day after injury. In this group, the RRs were significantly increased compared to their pre-surgery RRs and to values observed in sham-operated controls ($p < .05$, *t test*). The increase in RRs was not maintained to 1-4 weeks after SCI (**Figure 4**).

As it relates to the effect brush stimulation of the trunk has on RRs, we obtained results that marginally align with our general hypothesis. Briefly, mechanical stimulation to the trunk produced a small, short-lasting increase in RR, only in mice with a SCI. However, this effect was not significant when compared to the pre-stimulation RRs. This is unlike results obtained in adult

rats, which showed robust increases in RRs in response to mechanical stimulation of the trunk [*Noble DJ et al - J Neurotrauma. 2018 Nov 29. doi: 10.1089/neu.2018.5936*].

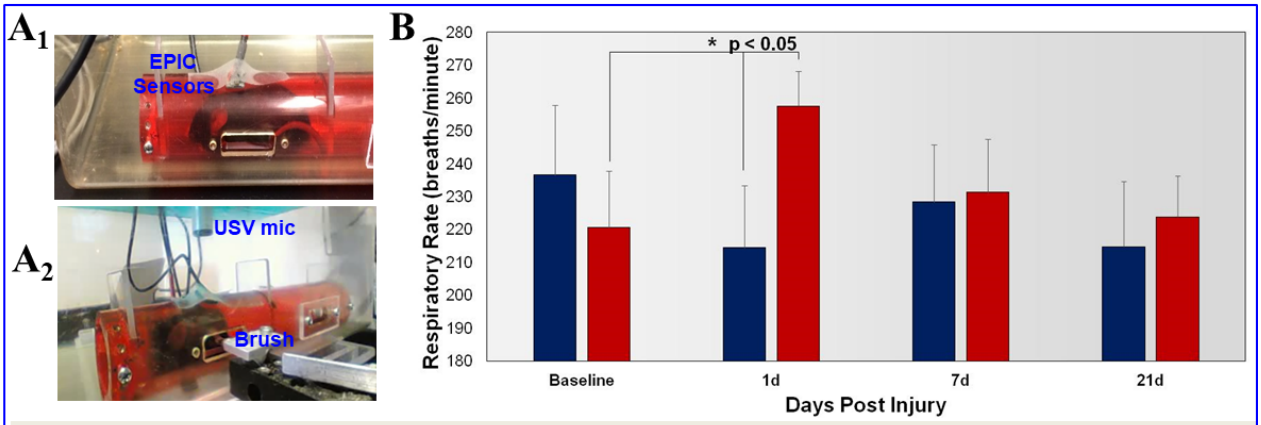

Figure 4. Assessment of Respiratory Rates (RRs) and effect of brush stimulation. B_1) A semi-restrained setup permits the additional detection of respiratory rate in response to stimulation, using remote sensors affixed to the sides of a cylindrical tube. **B_2)** Screenshot demonstrates the placement of microphone for USV recording and use of computerized robotic arm for mechanical brush. Stimuli are swept through a window across the side of a semi-restrained mouse at different frequencies, to recruit unique populations of sensory afferents. Increased RRs after SCI. RRs are significantly increased 1 day after T10 contusion SCI, compared to their pre-surgery RRs or the RRs of their sham counterparts on day 1. This increase in RRs was not observed at 1 or 3 weeks after SCI; nor did brush stimulation produce additional increase in RRs (not shown).

2. Brush-induced chamber preferences using a Place escape/avoidance paradigm. We then modified our behavioral apparatus to better assess the behavioral effects of truncal stimulation. This involved the inclusion of a two-chamber (light-dark) Place escape/avoidance paradigm (PEAP, **Figure 5A**) [LaBuda CJ, Fuchs PN (2000) A behavioral test paradigm to measure the aversive quality of inflammatory and neuropathic pain in rats. *Exp. Neurol. 163(2): 490-4*]. The incorporation of this new approach allowed us to examine 'preference' for the escape (light) chamber before and after truncal stimulation in freely behaving mice.

Brief Methods: Mice were first acclimated to the environment and light-dark chambers. After acclimation and at the start of the experiments, they were placed in the dark chamber but allowed to freely access both chambers for 30 minutes. Once baseline chamber preferences and number of transitions between chambers were established, the TH-Cre mice were divided into two groups for surgical procedures. They received a moderate contusion injury at T10 or a sham surgical procedure. The wildtype animals did not receive surgery. At weekly time points, 1 to 5 weeks after SCI, the animals were returned to the PEAP. They were allowed to freely access both chambers for 10 min (pre-stimulation period). Then the trunk was stimulated with a small camel hair brush (one stroke/minute) for 10 minutes, while mice were confined to the dark chamber with a small plastic barrier. Immediately thereafter, the barrier was removed and the mice were again free to access both light and dark chambers for 10 minutes (post-stimulation period). Infrared video recording was performed to visualize mice behavior while in the dark chamber and for manual analyses of time spent in the light chamber and number of transitions during the 10-min pre- and post-stimulation periods.

Results: We have been able to assess place preference in uninjured wild-type mice (n=12, 6 males and 6 females). This first step was important to validate the paradigm and establish

whether sex played a role in baseline and post-stimulation chamber preferences. The results indicated that there were no sex differences in chamber preferences over 3 weeks (not shown).

We also tested a total of 14 TH-Cre mice (7 sham and 7 SCI). As shown in **Figure 5B**, there were no differences in baseline chamber preferences between the sham and SCI mice. In general, both groups distributed their time 48:52% in the light and dark chambers (*also true for wildtype uninjured mice*). When the post-stimulation chamber preferences were examined, SCI mice showed a significant increase in preference for the light "escape" chamber that developed over the course of 3-5 weeks (*, $p < .05$), compared to day 1 after SCI. Stimulation was first administered 7 days after SCI. Overall, these studies show that SCI mice develop a preference for the escape (light) chamber in response to trunk stimulation. Furthermore, this effect emerges in parallel with SCI-induced hindpaw allodynia. At 21 days after SCI, mice showed a significant

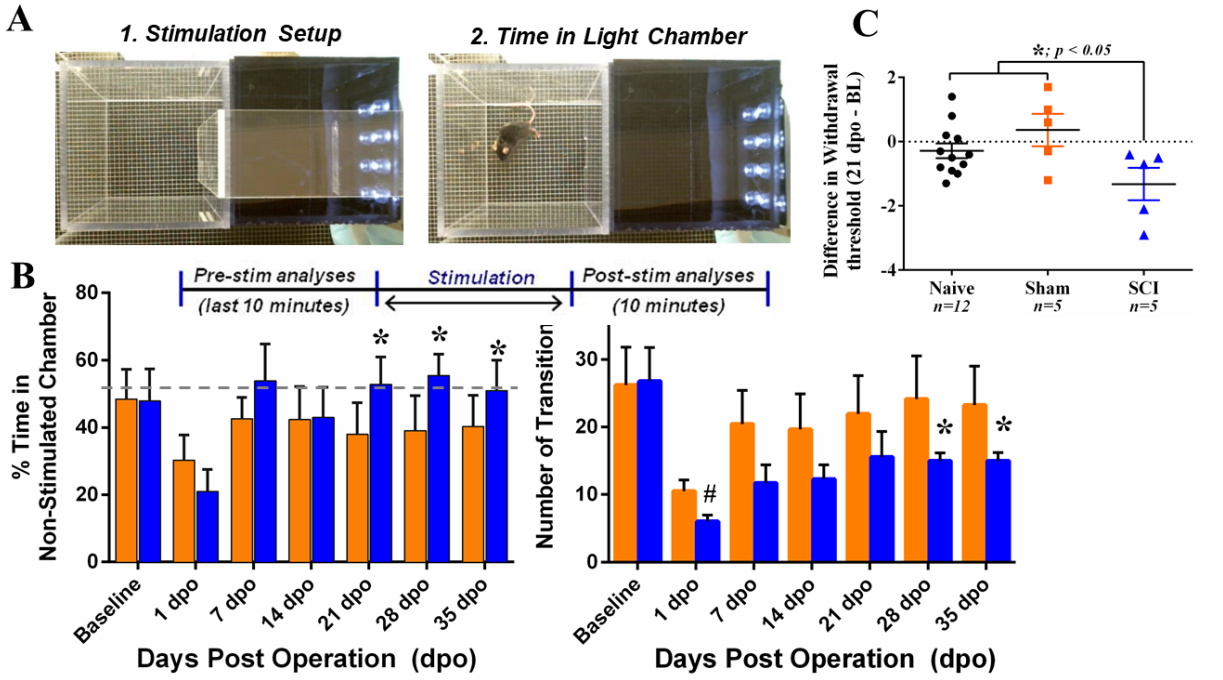

Figure 5. Overview of the PEAP behavioral set-up and results: **A**) Illustration shows an aerial view of the behavioral set-up (1). It also shows how we assess time spent in light chamber (2). **B**). Top depicts the 30 minute experimental procedure. Analyzed data cover the last 10 minutes of the pre-stimulation period and the post-stimulation period (10 minutes). Stimulation is administered for 10 minutes (1/min), with mice being confined to the dark chamber. Note, stimulation is first administered on 7 dpo. Results: Left: SCI mice (n=7) avoid a context associated with at-level brush stimulation following injury. Compared to shams (n=7), SCI show an increase in preference for the light "escape" chamber during the post-stimulation period compared to day 1 preferences, that developed at 21 days and persisted to 35 days (*, $p < .05$). The horizontal dotted line indicates the average time spent in the light chamber during the baseline pre-stimulation period in both SCI and SCI groups. Right: At 1 day post SCI, SCI mice displayed significantly less side-to-side transitions compared to sham mice (#, $p < .05$), probably due to SCI-induced impairment of locomotion. However, SCI mice showed a significant increase in the number of of side-to-side transitions at 28 and 35 days compared to 1 day (*, $p < .05$), an effect that reflects the gradual recovery of locomotor functions. **C**) At 21 days, a subset of SCI mice (n=5) also showed increased hindpaw sensitivity to von Frey stimulation compared to shams and naïve subjects (* $p<.05$).

reduction in hindpaw mechanical threshold on the von Frey test compared to naïve and sham controls (**Figure 5C**).

3. Behavioral effect evoked by optogenetic stimulation of C-LTMRs using a modified Conditioned Place Aversion Paradigm. In the last year, we successfully undertook studies with

TH-Cre^{ER} mice crossed with CHR2 expressing Ai32 mice [TH::ChR mice] to assess the effect of optical stimulation of the trunk on (i) breathing rates and (ii) chamber preferences using a modified 5-day conditioned place aversion (CPA) paradigm (**Figure 6**).

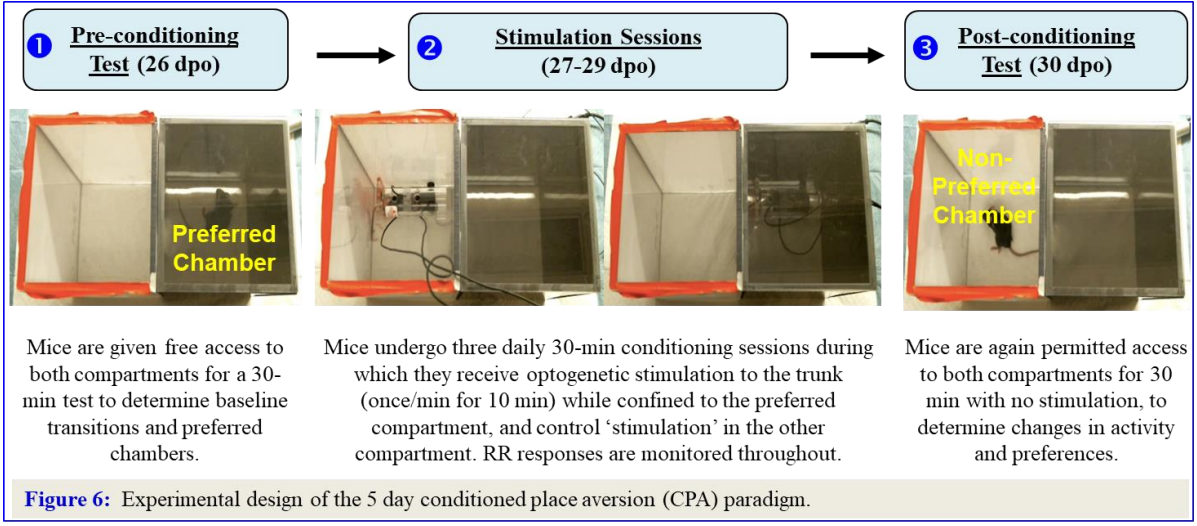

Figure 6: Experimental design of the 5 day conditioned place aversion (CPA) paradigm.

Basic description of CPA experimental methods: Mice received a T10 contusion SCI (70 kdynes, IH impactor) or sham surgery. Starting at 26 days post-surgery, a 5-day CPA paradigm was run to assess affective pain responses to optical stimulation. Video recordings were collected during testing and non-contact electric field sensors (EPIC, Plessey Semiconductors) were used to continuously monitor RR before, during, and after stimulation from semi-restrained mice in small cylindrical tubes. The CPA experimental design is shown in **Figure 6**.

Results: The overall results are shown in **Figure 7**. (**A**) Previous studies revealed a transient elevation in resting RR at acute time points following SCI. Here, we observed that SCI mice undergoing the 5-day CPA paradigm had a higher overall RR than sham controls over conditioning sessions 1-3. RRs were not consistently greater during optogenetic vs. control stimulation (*not shown*), suggesting a dissociation between evoked RR and the changes in chamber preference. (**B**) Scatterplot RR responses to optogenetic stimulation in an individual mouse are also shown, along with the stimulation setup. (**C**) SCI mice showed dramatic aversion to a context associated with optogenetic stimulation targeting truncal C-LTMRs 4 weeks after SCI. Specifically, SCI showed a significant increase in time spent in the non-stimulated (not preferred) chamber, compared to pre-stimulation. This effect was not seen in shams. This effect mimicked the long-lasting aversion to manually applied brush stimulation we observed previously (**Figure 5**). The significant change in chamber preference seen in SCI mice occurred despite maintained impairment of locomotor activity (**Right**), i.e. decreased side-to-side transitions ($p < 0.001$ *vs. sham*). (**D**) A subset of mice underwent the von Frey test of hindpaw sensitivity at baseline and 28 days post operation (dpo).

Overall, these findings show (i) SCI mice breathe faster during CPA conditioning sessions but not acutely following C-LTMR activation and (ii) SCI mice avoid a context associated with at-level C-LTMR stimulation following injury.

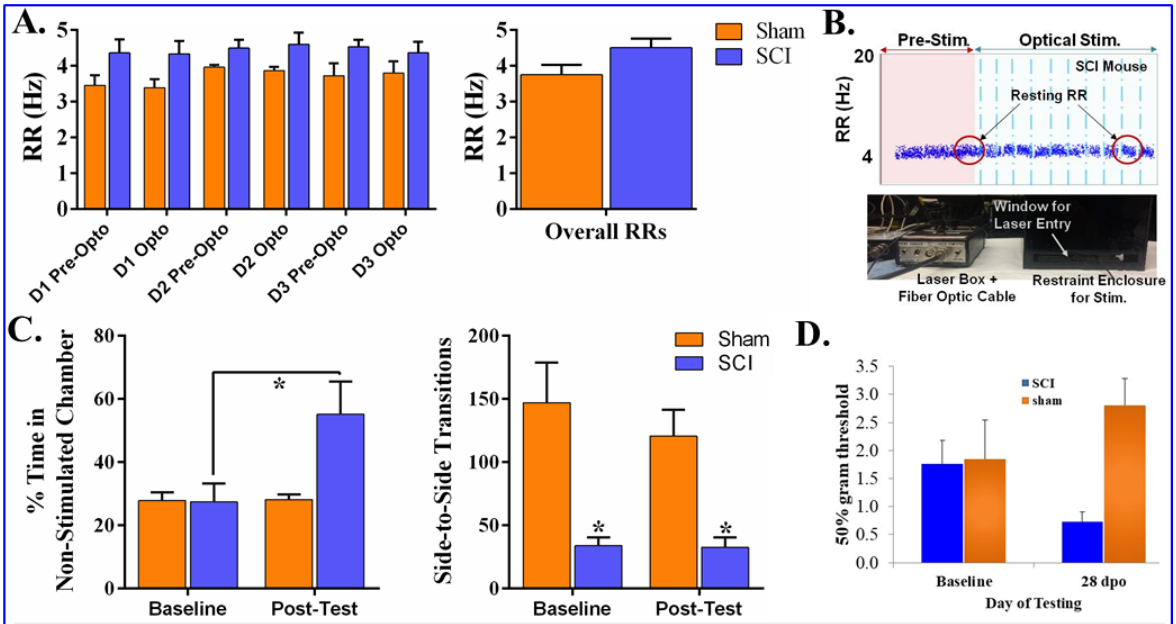

Figure 7: SCI mice exhibit stimulation-induced CPA. **A**) During the 5 day Conditioned place aversion paradigm, SCI had higher overall RR than sham controls over the 3 conditioning sessions. However, stimulation did not acutely increase RR in neither shams nor SCI subjects. **B**) A picture illustrating the stimulation setup (**below**) and an example of scatterplot RR responses to optogenetic stimulation in an individual mouse (**top**) are shown. **C**) As shown in Figure 5, mechanical brush stimulation to the trunk of SCI mice while confined to their preferred chamber later developed a preference for the non-stimulated "escape" chamber that peaked 3-5 weeks after injury. Here, we found similar changes in chamber preference following selective optical stimulation of TH-expressing sensory afferents, the C-LTMRs ($p = 0.01$ *SCI baseline vs. post-test*). **Right:** This occurred despite maintained impairment of locomotor activity (seen as a decrease in side-to-side transitions) in SCI mice compared to sham controls ($p < 0.001$ *vs. sham*). **D**) A subset of mice showed a trend toward increased von Frey sensitivity in SCI mice at 28 dpo compared to baseline.

Next steps:

3. Assessment of spontaneous or stimulation-induced USVs. One of our objectives is to identify ultrasonic vocalizations (USVs) that may be indicative of spontaneous or evoked pain. We are working with several colleagues at Emory to improve our current set-up to record and analyze USV in adult mice in response to C-LTMRs stimulation before and after SCI.

Findings related to SA3: Assess whether activation of C-LTMRs results in central sensitization and increased expression of pain genes:

We have completed the cellular assessment of pERK and NFkB expression in the spinal cord. Our results reveal no significant increases in either pERK or NFkB in the lesioned spinal cord, 35 days after SCI (**Figure 8**). While disappointing, these results do not necessarily reflect the effect C-LTMRs stimulation has on these pain-related genes. In fact, we have previously shown that both pERK and NFkB are decreased in the lesion spinal cord after SCI [Garraway et al, Pain 155(11):2344-59;2014 & Garraway et al, Neurosci 199:86-102, 2011].

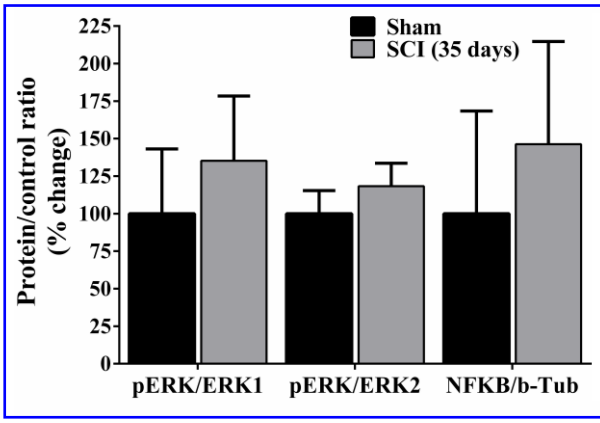

Figure 8. No significant increases in pERK or NFkB in the spinal cord 35 days after injury
Experimental design: At the end of behavioral experiments, Th-Cre animals were sacrificed. The lesioned spinal cord was removed and subsequently processed for extraction of total protein. 30 μg of total protein were subjected to SDS-PAGE, followed by immunoblotted for pERK, ERK, NFκB and β-tubulin.
Results: There were no differences in the expression of pERK1 & 2 or NFkB in the lesioned spinal cord at 35 days.

However, it is apparent that western blot analyses may not be the best tool to assess changes in pain gene expression as it relates to C-LTMRs. As we progress with this line of study, some sham and SCI TH-CHR2 mice will be paraformaldehyde-perfused immediately after the last optogenetic behavioral procedure. The spinal cord will be processed for pERK and c-fos immununohistochemistry.

- **What opportunities for training and professional development has the project provided?**

  Nothing to Report

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

  Nothing to Report

- **What do you plan to do during the next reporting period to accomplish the goals?**

  Nothing to Report

4. **IMPACT:**

- **What was the impact on the development of the principal discipline(s) of the project?**

  Nothing to Report

- **What was the impact on other disciplines?**

  Nothing to Report

- **What was the impact on technology transfer?**

  Nothing to Report

- **What was the impact on society beyond science and technology?**

Nothing to Report

5. CHANGES/PROBLEMS:

- **Changes in approach and reasons for change**

As was previously stated, we made slight changes to our behavioral paradigm. In fact, we included the Place Escape/Avoidance Paradigm (PEAP) and Conditioned Place Aversion (CPA) paradigms, which provided a better readout of the effect of brush and optogenetic stimulation in mice. This change did not impede the study in any way and instead has provided valuable data that support our hypothesis.

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

We previously reported on the lack of specificity with the constitutively expressed TH-Cre mice. We have since identified and obtained a tamoxifen-inducible Cre strain. Both behavioral and electrophysiological studies have been successfully undertaken in these mice.

- **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**

  Nothing to Report

- **Significant changes in use or care of human subjects**

  N/A

- **Significant changes in use or care of vertebrate animals**

  Nothing to Report

- **Significant changes in use of biohazards and/or select agents**

  Nothing to Report

6. PRODUCTS: List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."

- **Publications, conference papers, and presentations**
  - **Journal publications.**

    Nothing to Report

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

    Nothing to Report

  - **Other publications, conference papers and presentations**. (3 poster presentations)

1 Noble DJ, Dongmo R and Garraway SM. (2018) Behavioral conditioning approaches to investigate and reverse effects of peripheral afferent stimulation in a mouse model of neuropathic pain after spinal cord injury. Soc Neurosci Abstr Program No. 568.15, 2018

2 Noble DJ, Dongmo R and Garraway SM. (2017) Spinal cord injured mice develop a long-lasting aversive memory of at-level tactile stimulation Soc. Neurosci. Abstr. # 143.06 Poster Presentation.

3 Noble DJ, Martin KK, Dongmo R and Garraway SM. (2017). Development of a novel technique to investigate respiratory dysfunction and the emergence of chronic pain following spinal cord injury. Emory University Postdoctoral Research Symposium. Poster Presentation. ***Winner of Best Poster Award***

- **Website(s) or other Internet site(s)**

  Nothing to Report

- **Technologies or techniques**

  Nothing to Report

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

  Nothing to Report

- **Other Products**

  Nothing to Report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

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

Table 2: List of Personnel who are currently working on the project

(1) PDs/PIs

Name:	**Sandra M Garraway**
Project Role:	PI
Nearest person month worked:	48/month
Contribution to the project:	I have been directly involved in many aspects of the experiments and have supervised the direction of all experiments performed to date.
Name:	**Shawn Hochman**
Project Role:	Co-I
Nearest person month worked:	24/month
Contribution to the project:	Dr. Hochman supervises all the electrophysiology-related components of the study.

(2) Other persons

Name:	**Donald Noble**
Project Role:	Postdoctoral Fellow (Garraway)
Nearest person month worked:	160/month
Contribution to the project:	Dr. Noble is responsible for all behavioral studies to be undertaken
Name:	**Michael Sawchuk**
Project Role:	Lab Manager (Hochman)
Nearest person month worked:	32/month
Contribution to the project:	Mr. Sawchuk performs most of the histology.
Name:	**Karmarcha Martin**
Project Role:	Research Specialist (Garraway)
Nearest person month worked:	48/month
Contribution to the project:	She works on establishing animal colonies, performs surgery and assists with behavioral studies. She performs general lab duties associated with the project.
Name:	**Mallika Halder** and **Makalele Gorsich** (substitute for Kevin Watkins)
Project Role:	Research Specialist (Hochman) Gorsich- Graduate student (Hochman/Garraway)
Nearest person month worked:	160/month
Contribution to the project:	Both have been training under Dr. Hochman's supervision to undertake the electrophysiological studies.
Name:	**William Goolsby**
Project Role:	Engineer
Nearest person month worked:	16 hrs/month
Contribution to the project:	Mr. Goolsby builds and maintains many of apparati needed for the studies. These include the mechanical brush, diode lasers, CO2 incubation chamber and mPEAP chambers.

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

  Nothing to Report

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

  Nothing to Report

8. SPECIAL REPORTING REQUIREMENTS

- **COLLABORATIVE AWARDS:**
- **QUAD CHARTS:**

9. APPENDICES:

Spinal cord injured mice develop a long-lasting aversive memory of at-level tactile stimulation

***D. J. NOBLE**, R. DONGMO, S. M. GARRAWAY; Physiol., Emory Univ., Atlanta, GA

Pain, which can be experienced above, at, and below the level of injury, is a clinically relevant outcome of spinal cord injury (SCI). Recently our lab has focused on developing novel assessments of at-level allodynia using rodent models of SCI. We previously observed that 1 week after injury mechanical brush stimulation to the trunk causes an acute increase in respiratory rate (RR) in adult SCI mice, suggesting that at-level pain could be modulated by acute autonomic dysfunction following SCI. Here, we sought to establish a more complete profile of the physio-behavioral changes following injury and their relation to the development and expression of at-level mechanical hypersensitivity. Studies were undertaken in adult C57BL/6 mice with a T10 contusion SCI (70 kdynes, IH impactor) or sham surgery. Using a modified light-dark chamber conditioned place aversion paradigm, we assessed side preferences (% time spent in each compartment) and side-to-side crosses before and at time points ranging from 1 day to 5 weeks after surgery. Starting 1 week after surgery, the mice were given truncal stimulation with a small brush at approximately the level of injury (once/min for 5 mins, at ~ 1 cm/s) while confined to the dark chamber. Preferences and crosses were also monitored during the 10-min periods immediately preceding (pre-stimulation) and following (post-stimulation) stimulation, during which the mice could freely access both chambers. Equivalent 10-min time points were scored before and 1 day following surgery but without any stimulation in the intervening 5 mins. SCI mice showed a selective increase in preference for the light “escape” chamber during the pre-stimulation period at later vs. earlier weeks. The change in preference was first observed 1 week after the initial stimulation, developed gradually, and reached significance by 4 weeks after injury ($p < 0.05$), consistent with the typical timeline of chronic at-level allodynia. SCI mice also displayed a trend toward increased crosses during later weeks as they recovered from injury. There were no changes in either outcome measure in sham mice over the 5-week testing period. Surprisingly, both groups responded similarly to the stimulation on a more acute timescale, with indistinguishable post-stimulation preferences across the 5 weeks. We conclude that mice develop a long-lasting memory of manually applied brush stimulation following SCI that reflects the transition of mechanical stimulation from innocuous to aversive. Together with early autonomic dysfunction, long-term avoidance of repeated stimulation of the trunk may serve as a marker for the development of at-level mechanical allodynia.

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*__D. J. NOBLE__, R. DONGMO, S. M. GARRAWAY; Physiol., Emory Univ., Atlanta, GA.
Behavioral conditioning approaches to investigate and reverse effects of peripheral afferent stimulation in a mouse model of neuropathic pain after spinal cord injury.

Recently, our lab has been investigating cutaneous afferents known as C-LTMRs that innervate hairy skin and normally encode for pleasant, affiliative touch. These afferents may be converted to transduce mechanical allodynia following spinal cord injury (SCI). We recently found that mechanical stimulation delivered at the level of injury and tuned to selectively recruit C-LTMRs evoked acute increases in respiratory rate (RR) in adult mice 1 week after SCI ($p < .05$). We have now shown that mice with a contusion SCI also avoid a context associated with this stimulation in a conditioned place aversion (CPA) setup. This increase in preference for the light “escape” chamber progressively developed over the course of 5 weeks, reaching significance at 21, 28, and 35 days post injury ($p < .05$ in each case). Given the different timelines of RR and behavioral changes, early RR increases could predict the emergence of affective pain. Here, we performed a series of pilot studies to assess the efficacy of a novel feedback-based strategy to reverse RR increases after SCI. Adult C57BL/6 or Th::ChR transgenic (for optogenetic targeting of C-LTMRs) mice received a T10 contusion SCI (70 kdynes, IH impactor) or sham surgery and were assessed starting 1 week after surgery. At weekly time points, repeated truncal stimulation (once/min for 10 mins) was administered in a modified CPA paradigm, either with a small brush or blue laser to mechanically or optically activate C-LTMRs. We then tested the feasibility of slow respiratory rate (SRR) training using a paradigm developed in uninjured rats to lower RRs over time and potentially reduce reactivity to stressful and nociceptive stimuli. Mice underwent 10-15 daily 2-hour SRR training sessions, during which RR was continuously recording via remote electric field sensors. Recorded data was processed by a customized interface in LabVIEW to monitor breathing and provide real-time LED feedback (aversive strobe light) that turned off whenever RR $\leq$ 240 breaths/min. SCI mice significantly decreased their RR from baseline by the second SRR training session ($p < .05$) and spent ~80% of each session below the target RR. Control animals trained using reversal conditioning procedures (rewarded for RR $\geq$ 220 breaths/min) did not experience a similar decrease. Furthermore, post-training RRs in SCI mice were statistically indistinguishable from resting RRs in a cohort of age-matched, experimentally naïve mice. These results demonstrate adaptability of SRR conditioning procedures to mice for studies into neuropathic pain following SCI. Ongoing studies are examining the impact of SRR training on C-LTMR-mediated pain aversion and stress-associated behaviors.

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