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Title: Accessing the Subdural Space of the Rodent Spinal Cord for Treatment Delivery

Authors and Affiliations:
Salvador Lopez[1], Chien Kow[2], Manju Ganesh[1], Darren Svirskis[1], Bruce Harland[1]

[1]School of Pharmacy, University of Auckland
[2] Neurosurgical Department, Auckland City Hospital

Corresponding Authors:
Salvador Lopez salvador.lopez@auckland.ac.nz

Email Addresses for All Authors:
Chien Kow chienyew@gmail.com
Darren Svirskis d.svirskis@auckland.ac.nz
Manju Ganesh manju.ganesh@auckland.ac.nz
Bruce Harland bruce.harland@auckland.ac.nz
Salvador Lopez salvador.lopez@auckland.ac.nz

Author Questionnaire

1. **Microscopy**: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **Yes, all done**

   If **Yes**, can you record movies/images using your own microscope camera?

   SCOPE shots: 3.1.1b, 3.1.2, 3.2.1, 3.2.2, 3.3.1, 3.3.2, 3.3.3, 3.4.1, 3.4.2, 3.5.1, 3.6.1, 3.6.2, 4.1.1, 4.1.2, 4.1.3, 4.2.1, 4.2.2, 4.3.1, 4.4.1, 4.5.1, 4.6.1, 5.1.1.1, 5.2.1, 5.2.2, 5.3.1, 5.3.2, 5.5.1, 5.6.1, 5.6.2, 5.7.1

2. **Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **No**

3. **Filming location:** Will the filming need to take place in multiple locations? **No**

Current Protocol Length
Number of Steps: 25
Number of Shots: 46 (30 Scope)

Introduction

Videographer: Obtain headshots for all authors available at the filming location.

1.1. **Salvador Lopez**: We're exploring whether delivering treatment directly onto injured spinal tissue, via subdural administration, can improve recovery and function after spinal cord injury.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.3.1*

What are the current experimental challenges?

1.2. **Manju Ganesh:** Current challenges for our research revolve around broadening the scope of our treatment modalities. We want to create a reproducible and reliable treatment paradigm that can be applied in multiple scenarios.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll:3.2.1*

What significant findings have you established in your field?

1.3. **Bruce Harland:** Recently, our group has shown improvements in motor and sensory function in spinal cord injured rodents following electric field stimulation treatment.

1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll:4.3.1*

What advantage does your protocol offer compared to other techniques?

1.4. **Manju Ganesh :** Our protocol delivers treatments into the intrathecal space, bypassing the blood-spinal cord barrier, offering potentially greater efficacy than standard epidural delivery methods.

1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll:5.2.1*

What research questions will your laboratory focus on in the future?

1.5. **Salvador Lopez:** Moving forward, our group plans to utilize this method in larger animal models as well as examine its effects on bladder function following SCI.

1.5.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll:6.3.1*

Videographer: Obtain headshots for all authors available at the filming location.

Testimonial Questions (OPTIONAL):

Videographer: Please capture all testimonial shots in a wide-angle format with sufficient headspace, as the final videos will be rendered in a 1:1 aspect ratio. Testimonial statements will be presented live by the authors, sharing their spontaneous perspectives.

How do you think publishing with JoVE will enhance the visibility and impact of your research?

1.6. **Salvador Lopez, Research Fellow/Did Not answer**: (authors will present their testimonial statements live)

1.6.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll:6.3.1*

Can you share a specific success story or benefit you've experienced—or expect to experience—after using or publishing with JoVE? (This could include increased collaborations, citations, funding opportunities, streamlined lab procedures, reduced training time, cost savings in the lab, or improved lab productivity.)

1.7. **Bruce Harland, Senior Research Fellow/Did Not answer**: (authors will present their testimonial statements live)

1.7.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll:5.2.1*

Author's NOTE: Did not answer the testimonials

Ethics Title Card

This research has been approved by the University of Auckland's Institution Animal Ethics Committee (AEC) and the University Animal Welfare Officers (AWO)

Protocol

NOTE: The authors have reviewed the videographer's footage and have given filenames and timestamps for each talent shot to be used in the video. The author indicated timestamps have been added in red italicized font against each shot and the writer has not reviewed the footage for these regular talent shots.

2. Preparation of the Rat for the Surgical Procedure

Demonstrator: Manju Ganesh

2.1. To begin, position the anesthetized rat on a clean table **[1]** and shave the fur along the back of the animal, starting at the base of the neck and ending at the top of the hips **[2]**. After shaving, administer preoperative subcutaneous injections **[3].**

2.1.1. WIDE: Talent placing the anesthetized animal on a clean workbench.**TXT: Anesthesia: 3% Isoflurane** *Timestamp: 0:05-0:35/ Video: P1003919*

2.1.2. Talent shaving the back of the rat from the base of the neck to the hips using electric clippers. *Timestamp: start 0:25-0:50/ Video: P1003920*

2.1.3. **Added Shot**: Talent administers preoperative subcutaneous injections of Temgesic, Baytril, Metacam, and saline (described in protocol). *Timestamp: 0:16-0:41/ Video: P1003921*

2.2. Using a chlorhexidine scrub, disinfect the shaved area in a circular motion, moving outward from the center. Then apply chlorhexidine tincture in the same manner **[1]**. Administer subcutaneous Marcaine and repeat previous steps for disinfection **[2].** Apply ophthalmic lubricant to the eyes to prevent drying out **[3]**.

2.2.1. Talent scrubbing the center of the shaved area with chlorhexidine scrub and tincture using a circular motion. *Timestamp: 0:10-25 & 0:30-0:45/ Video: P1003923*

2.2.2.5 Added Shot: Talent administering subcutaneous Marcaine in several locations along the site of surgical incision. Timestamp: 0:15-0:40/ Video: P1003924

2.2.3 Talent applying lubricant to both eyes of the rat. *Timestamp: 0:15-0:35/ Video: P1003926*

2.3. Now, transfer the rat onto a heating pad placed under the microscope **[1]** and organize all sterile tools **[2]**. Drape a press-and-seal film over the shaved area **[3].** Cut an opening just large enough to expose the surgical field without revealing unshaved fur **[4]**.

2.3.1. Talent placing the rat on a heating pad under the surgical microscope. *Timestamp: 0:20-0:32/ P1003928*

2.3.1.1 (Added shot): Talent being handed sterile surgical tools to place in sterile field. *Timestamp: 0:24-0:36 & 1:13-1:23/ Video: P1003929*

2.3.2. Talent laying a press-and-seal drape over the rat's back and cutting circular window to expose the surgical region. *Timestamp: 0:06-0:13/ Video: P1003930*

2.3.3. Talent cutting a circular window in the drape to expose only the surgical region. *Timestamp: 0:13-0:29/ Video: P1003930*

2.4. Insert a rectal temperature probe to monitor the rat's temperature **[1]**. Adjust the heating pad as needed to maintain a temperature of approximately 37 degrees Celsius **[2]** and perform animal welfare checks every 5 to 10 minutes **[3]**.

2.4.1. Talent inserting the rectal temperature probe into the rat. *Timestamp: 0:06-0:26/ Video: P1003932*

2.4.2. Talent adjusting heating pad settings while monitoring temperature. *Timestamp: 0:18-0:26/ Video: P1003933*

2.4.3. Talent checking the rat's respiration. *Timestamp: 0:10-0:20/ Video: P1003934*

2.5. Next, administer a pedal reflex test to confirm the animal has reached a surgical plane of anesthesia. Once confirmed, begin the surgical procedure **[1]**.

2.5.1. Talent pinching the hind paw of the rat with fingers to assess pedal reflex. *Timestamp: 0:09-0:16/ Video: P1003935*

~~2.5.2. Please move shot 2.5.2 (talent being handed sterile surgical tools to place in sterile field) directly after 2.3.1~~

3. Tissue Dissection to Access the Spine

Demonstrator: Salvador Lopez

3.1. Using a scalpel blade, make a 6 to 8-centimeter-long linear incision in a rostral to caudal direction, beginning just below the base of the neck and extending over the thoracic hump to expose vertebrae T9 to T13 **[1] [2]**. Then, with Graefe forceps and micro spring scissors, gently retract the connective tissue beneath the incision to expose the spinal muscle tissue **[3]**.

3.1.1. Talent making a straight incision from the base of the neck to the thoracic hump using a scalpel blade. *Timestamp: 0:13-0:22/ Video: P1003936 Video editor: Please use split screen to show 3.1.1 and 3.1.1b simultaneously.*

Added shot 3.1.1b SCOPE: 3.1.1b

3.1.2. SCOPE: 3.1.2.

3.2. Now, identify the T13 and T12 spinous processes by locating the two white V *(we)*-shaped tendons in the caudal half of the incision. The spinous process just rostral to this second V, moving head to tail, is T13 **[1]**.

3.2.1. SCOPE: 3.2.1.

3.3. Starting between T13 and T12, moving toward T9, use micro scissors to make small incisions in the connective tissue between each vertebral process to separate them **[1]**. Then, cut a parallel channel along the side of the spinous processes to expose the lamina of each segment **[2]**. Repeat this dissection on both sides of the spine **[3]**.

3.3.1. SCOPE: 3.3.1 00:05-00:20.

3.3.2. SCOPE: 3.3.2.

3.3.3. SCOPE: 3.3.3.

3.4. Next, attach serrefine clamps to the muscle walls to hold the surgical site open **[1]**. Place one pair at T13, another at the T11-T12 border, and a third at the T11-T10 border **[2]**.

3.4.1. SCOPE: 3.4.1.

3.4.2. SCOPE: 3.4.2.

3.5. Once the channels are defined, use micro scissors or rongeurs to remove the connective tissue between each spinous process **[1]**.

3.5.1. SCOPE: 3.5.1.

3.6. Continue clearing until all connective tissue and muscle is dissected from the lamina between rostral T13 and caudal T9 **[1-TXT]**.

3.6.1. SCOPE: 3.6.1 00:05-00:20. **TXT: Ensure the laminae are clearly exposed on both sides**

4. Laminectomy Procedure
Demonstrator: Salvador Lopez

4.1. Locate the caudal portion of the T12 lamina that overlaps the rostral portion of T13 **[1]**.

Position the tip of the rongeurs underneath this overlapping area **[2]** and gently remove small pieces of the lamina **[3]**.

4.1.1. SCOPE: 4.1.1.

4.1.2. SCOPE: 4.1.2.

4.1.3. SCOPE: 4.1.3. 00:03-00:15

4.2. Alternate from one side to the other, continuing to remove portions of the T12 lamina **[1]**. To enlarge the opening, extend the cut into the midline of the process and expose the spinal cord, forming a hole approximately 5 millimeters across **[2]**.

4.2.1. SCOPE: 4.2.1.

4.2.2. SCOPE: 4.2.2. 00:06-00:17

4.3. Then, position the rongeurs under the lamina on both sides and carefully create a uniform channel extending toward the cranial end of T12 **[1]**.

4.3.1. SCOPE: 4.3.1.

4.4. Repeat the same procedure to remove the lamina from T11 **[1]** and T10 **[2]**.

4.4.1. SCOPE: 4.4.1.

4.4.2. SCOPE: 4.4.2. 00:11-00:15

4.5. Once the laminectomy exposes an adequate area from T10 to T12, gently flush the thecal sac with saline **[1]**.

4.5.1. SCOPE: 4.5.1. 00:05-00:15.

5. Durotomy for Treatment Application

5.1. Perform the durotomy using the dissection microscope to access the subdural space of the spinal cord **[1]**. Confirm that the chosen site for the durotomy and the region directly in front of it are free of branching blood vessels from the midline vessel **[2]**.

5.1.1. Talent gathering instruments for durotomy. *Timestamp: 0:05-0:17/ P1003937*

5.1.2. SCOPE**:** 5.1.2

5.2. Bend a 27-gauge needle to a 90-degree angle with the bevel facing upward **[1]**.

5.2.1. SCOPE: 5.2.1.

5.3. At the desired entry point, pierce the dura mater gently with the tip of the bent needle. Once the dura is punctured, carefully lift the needle dorsally to tear a circular hole in the dura, no more than 2 millimeters on either side of the midline blood vessel **[1]**. Look for a slight leakage of cerebrospinal fluid to confirm successful durotomy **[2]**.

5.3.1. SCOPE: 5.3.1.

5.3.2. SCOPE: 5.3.2.

5.4. Use the holes made in the dura to deliver treatment or insert a device into the subdural space **[1]**. For this protocol, insert intrathecal catheters either to guide a stimulation device or to inject hydrogel.

5.4.1. SCOPE: 5.4.1.

5.5. Confirm that the catheter tips are visible under the dura mater before proceeding. A successful placement is evident if the catheters glide beneath the dural membrane and over the small spinal blood vessels **[1]**. Once in place, use the catheters to deliver the desired treatment **[2]** **[3]**.

5.5.1. SCOPE: 5.5.1.

5.5.2. SCOPE: 5.5.2 *Video editor: Please show 5.5.2 and 5.5.3 simultaneously*

5.5.3. Added shot: SCOPE: 5.5.3 00:04-00:18

5.6. After delivering the treatment, insert a piece of sterile surgical gel-foam into the laminectomy cavity to promote haemostasis, aid healing, and maintain spinal structure **[1]**.

5.6.1. SCOPE: 5.6.1.

5.7. Finally, use 4-0 *(4-oh)* polydioxanone absorbable suture to close the muscle **[1]** and skin layers above the spine **[2]**.

5.7.1. Talent stitching the muscle layers closed using absorbable sutures. *Timestamp (muscle): 0:18-0:34/ P1003941*

5.7.2. **Added shot:** Talent stitching the skin layer closed using absorbable suture. *Timestamp (skin): 0:20-0:40/ P1003943*

Results

6. Results

6.1. A bioelectronic device was implanted **[1]** or a hydrogel was delivered into 6-to-8-week-old Sprague-Dawley rats **[2]**.

6.1.1. LAB MEDIA: Figure 4A

6.1.2. LAB MEDIA: Figure 4B

6.2. Following each procedure, all animals maintained normal motor function with no difference in BBB score between groups 7 days post-procedure, indicating the safe implementation of the described protocol **[1]**.

6.2.1. LAB MEDIA: Figure 4C *Video editor: Highlight the black, blue and yellowish dot for D7*

1. Anesthetized

Pronunciation link:
https://www.merriam-webster.com/dictionary/anesthetized
IPA: /ˈænəsθəˌtaɪzd/
Phonetic Spelling: an-uhs-thuh-tized

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2. Isoflurane

Pronunciation link:
https://www.merriam-webster.com/dictionary/isoflurane
IPA: /ˌaɪsəˈflʊˌreɪn/
Phonetic Spelling: eye-soh-floo-rain

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3. Temgesic

Pronunciation link:
https://www.howtopronounce.com/temgesic

IPA: /ˈtɛmˌdʒɛsɪk/
Phonetic Spelling: tem-jes-ik

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4. Baytril

Pronunciation link:
https://www.howtopronounce.com/baytril
IPA: /ˈbeɪˌtrɪl/
Phonetic Spelling: bay-tril

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5. Metacam

Pronunciation link:
https://www.howtopronounce.com/metacam
IPA: /ˈmɛtəˌkæm/
Phonetic Spelling: meh-tuh-cam

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6. Chlorhexidine

Pronunciation link:
https://www.merriam-webster.com/dictionary/chlorhexidine
IPA: /klɔːrˈhɛksɪˌdiːn/
Phonetic Spelling: klor-hek-suh-deen

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7. Marcaine

Pronunciation link:
https://www.howtopronounce.com/marcaine
IPA: /ˈmɑːrˌkeɪn/
Phonetic Spelling: mar-kane

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8. Ophthalmic

Pronunciation link:
https://www.merriam-webster.com/dictionary/ophthalmic
IPA: /ɑfˈθælmɪk/
Phonetic Spelling: off-thal-mik

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9. Rostral

Pronunciation link:
https://www.merriam-webster.com/dictionary/rostral
IPA: /ˈrɑːstrəl/
Phonetic Spelling: rahs-truhl

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10. Caudal

Pronunciation link:
https://www.merriam-webster.com/dictionary/caudal
IPA: /ˈkɔːdəl/
Phonetic Spelling: kaw-duhl

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11. Lamina

Pronunciation link:
https://www.merriam-webster.com/dictionary/lamina
IPA: /ˈlæmɪnə/
Phonetic Spelling: lam-ih-nuh

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12. Serrefine (clamps)

Pronunciation link:
https://www.howtopronounce.com/serrefine
IPA: /ˈsɛrəˌfaɪn/
Phonetic Spelling: seh-ruh-fine

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13. Laminectomy

Pronunciation link:
https://www.merriam-webster.com/dictionary/laminectomy
IPA: /ˌlæməˈnɛktəmi/
Phonetic Spelling: lam-uh-neck-tuh-mee

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14. Thecal (sac)

Pronunciation link:
https://www.merriam-webster.com/dictionary/thecal
IPA: /ˈθiːkəl/
Phonetic Spelling: thee-kuhl

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15. Durotomy

Pronunciation link:
https://www.howtopronounce.com/durotomy
IPA: /dʊˈrɑːtəmi/
Phonetic Spelling: doo-rah-tuh-mee

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16. Dura mater

Pronunciation link:
https://www.merriam-webster.com/dictionary/dura%20mater
IPA: /ˈdʊrə ˌmeɪtər/
Phonetic Spelling: doo-rah may-ter

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17. Subdural

Pronunciation link:
https://www.merriam-webster.com/dictionary/subdural
IPA: /ˌsʌbˈdjʊrəl/
Phonetic Spelling: sub-dyur-uhl

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18. Intrathecal

Pronunciation link:
https://www.merriam-webster.com/dictionary/intrathecal
IPA: /ˌɪntrəˈθiːkəl/
Phonetic Spelling: in-truh-thee-kuhl

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19. Hydrogel

Pronunciation link:
https://www.merriam-webster.com/dictionary/hydrogel
IPA: /ˈhaɪdrəˌdʒɛl/
Phonetic Spelling: hy-droh-jel

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20. Polydioxanone

Pronunciation link:
https://www.howtopronounce.com/polydioxanone
IPA: /ˌpɑːlɪdaɪˈɑːksəˌnoʊn/
Phonetic Spelling: pah-lee-dye-ox-uh-nohn

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21. Absorbable

Pronunciation link:
https://www.merriam-webster.com/dictionary/absorbable
IPA: /əbˈzɔrbəbəl/
Phonetic Spelling: ub-zor-buh-buhl

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22. Haemostasis

Pronunciation link:
https://www.merriam-webster.com/dictionary/hemostasis
(Alternate spelling: haemostasis = hemostasis)
IPA: /ˌhiːməˈsteɪsɪs/
Phonetic Spelling: hee-moh-stay-sis