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Title: Induction of Complete Transection-Type Spinal Cord Injury in Mice

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Author Questionnaire

1. Microscopy: Does your protocol involve video microscopy, such as filming a complex dissection or microinjection technique? **N**

2. Software: Does the part of your protocol being filmed demonstrate software usage? **N**

3. Filming location: Will the filming need to take place in multiple locations (greater than walking distance)? **N**

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Ronak Reshamwala**: This protocol delivers a precise, surgically controlled injury to induce full spinal cord transection in mice with high a reproducibility and very high survival rates **[1]**.

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

REQUIRED:

1.2. **Ronak Reshamwala**: The use of a fine drill for the bone injury minimizes the damage to the surrounding tissue, allowing the injury and treatment responses to be modelled with more accuracy **[1]**.

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

Ethics Title Card

1.3. Procedures involving animal subjects have been approved by the Institutional Animal Care and Use Committee (IACUC) or at Griffith University.

Protocol

Videographer NOTE: Due to the nature of the protocol (a procedure on a live mouse), we filmed the majority of it in one clip, looking over the surgeon's shoulder. He gave some narration on the steps he was performing, but it's wasn't feasible to start and stop clip, and mark each clip based on the shot number in the script. I anticipate it will be easy enough for the editor to follow what's happening.

2. Laminectomy

2.1. To perform a laminectomy, after confirming a lack of response to pedal reflex in an anesthetized, 8-10-week-old female C57BL/6 mouse **[1-TXT]**, shave the back fur to expose the surgical area over the dorsal spine **[2]** and sterilize the shaved area with sterile cotton swabs soaked in povidone iodine antiseptic liquid and surgical spirit **[3-TXT]**.

2.1.1. WIDE: Talent pinching toe *Videographer: More Talent than mouse in shot*
TEXT: Anesthesia: 5% -> 1.5-2% isoflurane; Analgesia: buprenorphine 0.03 mg/kg

2.1.2. Fur being shaved, with heat pad visible in frame

2.1.3. Skin being wiped **TEXT: Antiseptic and spirit containers visible in frame**

2.2. Using a scalpel, make a vertical, midline incision at the T10 vertebral level **[1]** and use straight forceps to lift the skin from the underlying fascia **[2]** to facilitate the retractor placement **[3]**.

2.2.1. Incision being made
2.2.2. Skin being lifted
2.2.3. Skin being reflected and retracted/Retractor being placed

2.3. To expose the spines of the T9-T11 vertebrae, use the blunt edge of the scalpel to make a small midline incision in the subcutaneous tissue and underlying fascia **[1]** and use fine tip, non-sharp forceps to blunt dissect and reflect the fascia **[2]**.

2.3.1. Incision being made
2.3.2. Tissue being dissected and reflected

2.4. To expose the laminae, use the blunt tip of the scalpel to split the dorsal trunk and para-spinous muscles along the spines of the T9-T11 vertebrae **[1]** and use the blunt fine tip forceps to blunt dissect the muscles in layers to expose the laminae of the vertebrae **[2-TXT]**.

2.4.1. Muscles being split
2.4.2. Muscles being dissected **TEXT: Use 37 °C- saline and swabs to control and clear any bleeding as necessary**

2.5. Use the same forceps to make small pockets around the transverse processes of the T10 vertebra **[1]** and hook the prongs of curved forceps under the transverse processes within the created pockets to stabilize the T10 vertebral body **[2]**.

2.5.1. Pocket(s) being created *Videographer: Important step*
2.5.2. Forceps being hooked into pocket(s) *Videographer: Important step*

2.6. Thoroughly rinse the T10 laminae with warm saline **[1]** and use cotton swabs to gently wipe the bony surface clean, taking care that no muscle or ligament attachments remain along the bilateral surface **[2]**.

2.6.1. Laminae being rinsed
2.6.2. Bony surface being wiped/muscle and/or ligament being removed

2.7. To break the laminae bilaterally, use a 0.55-millimeter-diameter, 7-millimeter length drill bit tip to trace a vertical path from the T9-T10 intervertebral space to the T10-T11 intervertebral space along both T10 laminae with the drill off **[1]**.

2.7.1. Path being traced

2.8. When the path has been traced, turn on the drill **[1]** and slowly and carefully make a vertical trench on the right lamina of the T10 vertebra, using the curved forceps to keep the vertebra stable **[2-TXT]**.

2.8.1. Talent turning on drill *Videographer: Important/difficult step*
2.8.2. Trench being made *Videographer: Important/difficult step; Video Editor: please emphasize forceps when mentioned* **TEXT: Caution: Do not penetrate through bone or injure spinal cord**

2.9. After repeating the process on the left side of the lamina **[1]**, irrigate the tissue with warm saline to wash away any remaining bone fragments **[2]**.

2.9.1. Second trench being made *Videographer: Important/difficult step*
2.9.2. Tissue being irrigated *Videographer: Important/difficult step*

2.10. **Ronak Reshamwala**: Maintaining a good grip on the spine with one hand is essential for this step. It is also important to become comfortable and confident with using the drill **[1]**.

2.10.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

2.11. Next, use the angled fine tip forceps to grip the spinous process **[1]** and remove the whole dorsal segment of the laminae separated by the bilateral drilling **[2]**.

2.11.1. Process being gripped *Videographer: Important step*
2.11.2. Segment being removed *Videographer: Important step*

2.12. Then irrigate and swab as necessary to allow a clear visualization of the spinal cord within the laminectomy window **[1]**.

2.12.1. Spine being irrigated

3. Transection and Closure

3.1. To induce the spinal cord injury within the exposed cord, use a narrow, round cutting edged blade **[1]** to slice the cord at the center of the laminectomy window, taking care to sweep the lateral recesses of the spinal column to induce a complete transection injury **[2]**.

3.1.1. WIDE: Talent selecting blade *Videographer: Important step*
3.1.2. Cord being sliced *Videographer: Important step*

3.2. To confirm completeness of the transection injury, use the blunt fine tip forceps to remove any remaining connections at the transection site **[1]**.

3.2.1. Connection(s) being removed *Videographer: Important step*

3.3. Once hemostasis has been achieved at the transection site **[1]**, release the curved forceps grip on the T10 vertebrae **[2]** and bring the edges of the dissected muscles together along the midline to achieve good apposition **[3]**.

3.3.1. Tissue being swabbed
3.3.2. Forceps being released
3.3.3. Muscle being apposed

3.4. Using 5-0 polyglactin 910 absorbable sutures, suture the muscles in layers, making sure that the natural curvature of the spine does not cause any tension at the suture line or open up the sutures **[1]**.

3.4.1. Muscle sutures being placed

3.5. Then use 5-0 non-absorbable silk sutures to close the subcutaneous tissue and skin, taking care that there is no bleeding, clots, or debris remaining under the skin before closure **[1-TXT]**.

3.5.1. Skin sutures being placed **TEXT: Perform final warm saline irrigation as necessary**

Protocol Script Questions

A. Which steps from the protocol are the most important for viewers to see?
2.5., 2.8., 2.9., 2.11., 3.1., 3.2.

B. What is the single most difficult aspect of this procedure and what do you do to ensure success?
2.8., 2.9. This procedure must be first performed on cadavers to practice of achieving a steady grip and stable drilling before attempting a live surgery.

Results

4. Results: Representative Transection Type Injury Results

4.1. To assess whether this method for inducing transection-type spinal cord injury in mice is reproducible and consistent **[1]**, the injured spinal cord can be analyzed by immunohistochemistry **[2]** and behavioral testing **[3]**.

4.1.1. LAB MEDIA: Figure 3
4.1.2. LAB MEDIA: Figure 3 *Video Editor: please emphasize Figures 3A and Figure 3B Injury Size Measurement graph*
4.1.3. LAB MEDIA: Figure 3 *Video Editor: please emphasize Figures 3B Motor Behaviour Scores graph and Figures 3D and 3E images*

4.2. Immunolabeling against the astrocyte marker glial fibrillary acidic protein **[1]** facilitates demarcation of the boundary of the intact spinal cord **[2]**. In this longitudinal section, the injury site located can be visualized between the cord stumps as expected **[3]**.

4.2.1. LAB MEDIA: Figure 3A
4.2.2. LAB MEDIA: Figure 3A *Video Editor: please emphasize red signal in left and right of image*
4.2.3. LAB MEDIA: Figure 3A *Video Editor: please emphasize space between red signals and/or Injury site text and arrow*

4.3. A consistent-sized defect can be induced at the transection site **[1]** with an average minimal distance of 550.4 plus or minus 17.3 micrometers **[2]**.

4.3.1. LAB MEDIA: Figure 3B
4.3.2. LAB MEDIA: Figure 3B *Video Editor: please add/emphasize horizontal line through middle of data box*

4.4. Behavioral data deploying the Basso Mouse Scale of an open field test shows that the injured mice exhibit no hind limb movement after the injury **[1]**.

4.4.1. LAB MEDIA: Figure 3C *Video Editor: please emphasize data line starting from 0 BMS data point to end of graph*

4.5. Thus, the protocol produces a complete and reliable transection-type injury **[1]** that results in a complete loss of function below the injury level that does not lead to spontaneous reversal of the paralysis **[2]**.

4.5.1. LAB MEDIA: Figures 3D and 3E
4.5.2. LAB MEDIA: Figures 3D and 3E *Video Editor: please emphasize Figure 3E*

Conclusion

5. Conclusion Interview Statements

5.1. **Ronak Reshamwala**: Take care to drill in a straight line with a stable hand, because it can be tricky to repair the laminectomy window once it has been drilled incorrectly **[1]**.

5.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera (2.8., 2.9.)

5.2. **Ronak Reshamwala**: After spinal cord injury induction, various treatment options can be applied, including drug delivery and cell transplantation, to identify potential therapies that improve spinal cord injury recovery **[1]**.

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