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Title: A Scalable Model to Study the Effects of Blunt-Force Injury in Adult Zebrafish

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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**

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

   Yes

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

3. **Interview statements:** Considering the COVID-19-imposed mask-wearing and social distancing recommendations, which interview statement filming option is the most appropriate for your group? **Please select one**.

   ☒ Interviewees self-record interview statements. JoVE can provide support for this option.

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

Current Protocol Length

Number of Steps: 19
Number of Shots: 49

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **David Hyde:** This protocol provides a simple, rapid, and reproducible method to induce a scalable blunt-force traumatic brain injury in adult zebrafish.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *B-roll: 2.7.2.*

1.2. **David Hyde:** The adult zebrafish that undergo this blunt-force TBI exhibit many of the characteristics observed in humans that suffer from a blunt-force TBI.

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

Introduction of Demonstrator on Camera

1.3. **David Hyde:** Demonstrating the procedure will be Mr. James Hentig and Ms. Kaylee Cloghessy, two doctoral students from my laboratory.

1.3.1. INTERVIEW: Author saying the above.

1.3.2. The named demonstrator(s) looks up from the workbench or desk, or microscope and acknowledges the camera.

NOTE: All interview statements were filmed by the videographer on the day of the shoot.

Protocol

2. Traumatic Brain Injury Paradigm

2.1. Begin by filling a Petri dish with modeling clay **[1]**, then use the back of a pair of forceps to create a raised platform with additional modeling clay **[2-TXT]**.

2.1.1. WIDE: Establishing shot of the talent filling the Petri dish with clay.

2.1.2. Talent creating the raised platform using clay and forceps. **TEXT: 5 cm x 1.5 cm**

2.2. Divide the raised platform lengthwise into two approximately equal halves using a razor blade **[1]**. Form the two halves into a channel such that it accommodates the length of an adult fish **[2]**. Use additional clay to build walls to secure two-thirds of the fish body with the head exposed **[3]**.

2.2.1. Talent dividing the raised platform.

2.2.2. Talent forming the channel.

2.2.3. Talent building walls to secure the fish body.

2.3. Mold a small support in the exposed head region perpendicular to the walls to avoid rotation or recoil of the head upon injury **[1]**. Ensure that the channel's sides do not impede the dropped weight **[2].** *Videographer: This step is important!*

2.3.1. Talent molding the support perpendicular to the walls.

2.3.2. Talent checking if the channel's sides impede the dropped weight.

2.4. Use a mini-hole punch to create a 3-millimeter steel disk from a 22-gauge steel flashing **[1]**. Place the fish on the clay mold within the channel with its dorsal side up so that its body is secured on the sides **[2]**. Place the 3-milliliter 22-gauge steel disc on the head, centered over the desired impact point **[3]**. *Videographer: This step is important!*

2.4.1. Talent punching the disk in the steel flashing.

2.4.2. Talent placing the fish within the channel.

2.4.3. Talent placing the steel disk on the impact point.

2.5. Align the fish perpendicularly to avoid its head from tilting to one side, which could cause an uneven impact **[1]**. Use a standard ring stand and arm clamp to secure the steel or plastic tubing, ensuring it is straight, so the bottom of the tubing is 1.5 centimeters above the head of the zebrafish **[2]**.

2.5.1. Talent aligning the fish.
2.5.2. Talent securing the tubing with the standard ring stand and arm clamp.

2.6. Look down the tubing to ensure it is aligned above the steel plate **[1]**.

2.6.1. Talent checking the alignment of the tubing.

2.7. Choose the appropriate ball bearing based on the desired severity of injury **[1-TXT].** Drop the ball bearing from a predetermined height down the tubing onto the steel plate **[2-TXT]**. Then, place the injured fish in the recovery tank to be monitored **[3]**.

2.7.1. A shot of the ball bearing to be used. **TEXT: 1.5 g for mild and moderate TBI, and 3.3 g for severe TBI**
2.7.2. Talent dropping the ball bearing onto the steel plate.
2.7.3. Talent placing the injured fish in the recovery tank.

3. Brain Dissection

3.1. Fill a Petri dish with modeling clay and create a small cavity to support the body during dissection **[1]**. Place the fish in the clay mold with its dorsal side up **[2]**. Place two dissection pins, one through the midline halfway down the body and the other around 5 millimeters behind the base of the head **[3]**.

3.1.1. Talent filling the Petri dish with clay and making a small cavity.

3.1.2. Talent placing the fish in the clay mold.

3.1.3. Talent placing the dissection pins.

3.2. Use a pair of number five Dumont forceps to bluntly sever the optic nerve ~~**[1]**~~ and remove the eyes **[2]**.

~~3.2.1. SCOPE: Talent severing the optic nerve.~~

NOTE: The microscope shots uploaded by the authors does not include footage for shot number 3.2.1.

3.2.2. SCOPE: JoVE TBI Rd 1 Brain dissection.mov. 00:06 – 00:08, 00:23 – 00:25.

3.3. Place one end of the number five forceps under the right parietal plate **[1]**. Make a deliberate scissor action moving toward the rostral end **[2-TXT]** and remove the right frontal plate **[3]**. *Videographer: This step is important!*

3.3.1. SCOPE: JoVE TBI Rd 1 Brain dissection.mov. 00:31 – 00:36.

3.3.2. SCOPE: JoVE TBI Rd 1 Brain dissection.mov. 00:37 – 00:40. **TEXT: Keep the forceps at an angle of 45˚ or lower**

3.3.3. SCOPE: JoVE TBI Rd 1 Brain dissection.mov. 00:47 – 00:50.

3.4. Then, rotate the fish 90 degrees clockwise **[1]**. Place one end of the number five forceps under the left parietal plate **[2]** and use the same scissor motion to remove the left parietal and frontal plates, exposing the entire dorsal aspect of the brain **[3]**. *Videographer: This step is important!*

3.4.1. SCOPE: JoVE TBI Rd 1 Brain dissection.mov. 00:58 – 01:02.
3.4.2. SCOPE: JoVE TBI Rd 1 Brain dissection.mov. 01:09 – 01:12.
3.4.3. SCOPE: JoVE TBI Rd 1 Brain dissection.mov. 01:12 – 01:17.

3.5. Use the number 5 forceps to bluntly transect the maxilla such that the olfactory bulbs are preserved and not damaged **[1]**. Using the same forceps, remove the right opercle, preopercle, interopercle, and subopercle **[2]**.

3.5.1. SCOPE: JoVE TBI Rd 1 Brain dissection.mov. 01:21 – 01:26.
3.5.2. SCOPE: JoVE TBI Rd 1 Brain dissection.mov. 01:27 – 01:31.

3.6. Then, bluntly resect the musculature at the caudal end of the calvarium to expose the spinal cord **[1]**. Using the forceps bluntly transect the spinal cord **[2].** Place the forceps carefully under the brain **[3]** and gently remove the brain from the calvarium **[4-TXT]**. *Videographer: This step is important!*

3.6.1. SCOPE: JoVE TBI Rd 1 Brain dissection.mov. 01:38 – 01:41, 01:48 – 01:54.
3.6.2. SCOPE: JoVE TBI Rd 1 Brain dissection.mov. 02:02 – 02:06.
3.6.3. SCOPE: JoVE TBI Rd 1 Brain dissection.mov. 02:14 – 02:17.
3.6.4. SCOPE: JoVE TBI Rd 1 Brain dissection.mov. 02:19 – 02:22, 02:27- 02:28 **TEXT: Do not pinch the brain**

4. Edema Studies in the Zebrafish Brain

4.1. Dissect the whole brain or the region of interest using the instructions in the text manuscript **[1]** and place the brain immediately on a small weigh boat using fine forceps, taking care not to stab or scrape the brain **[2]**.

4.1.1. Talent at the microscope dissecting the whole brain or the region of interest.
4.1.2. Talent placing the brain on the weigh boat.

4.2. Transfer the brain to the tared drying weigh boat **[1]** and record the wet weight of the brain **[2]**. Orient the brains to lay them flat on the weigh boat with the dorsal side facing up **[3]**, then place the brain and the drying weigh boat in a hybridization oven set to 60 degrees Celsius for 8 hours **[4]**.

4.2.1. Talent transferring the brain to the weigh boat.
4.2.2. Talent recording the wet weight of the brain.
4.2.3. Talent orienting the brain on the weigh boat.
4.2.4. Talent Placing the weigh boat in the hybridization oven.

4.3. To transfer the brain to a new tared small weigh boat once it is dry, pinch the fine forceps together **[1]** and, starting at the ventral side of the brain, scoop in an upward motion **[2]**.

4.3.1. Talent pinching the forceps together.
4.3.2. Talent scooping the brain to transfer it to a new weigh boat.

5. Labeling Cellular Proliferation Across the Neuroaxis and Preparing Fixed Tissue

5.1. Make a partial incision on a wet sponge **[1]**. Place one fish at a time in the opening with the ventral side up **[2]** and use a 30-gauge needle to inject approximately 40 microliters of 10 millimolar EdU into the fish's body **[3-TXT]**. Then, return the fish to the holding tank filled with system water **[4]**.

5.1.1. Talent making incisions on the wet sponge.
5.1.2. Talent placing the fish in the opening.
5.1.3. Talent injecting EdU into the fish's body. **TEXT: 5-ethynyl-2'-deoxyuridine - EdU**
5.1.4. Talent returning the fish to the holding tank.

5.2. Collect the brains as mentioned previously **[1]** and place them as a group in a 5-milliliter glass vial containing 2 milliliters of 9 parts 100 percent ethanol to 1 part 37 percent formaldehyde **[2]**. Fix the brains at 4 degrees Celsius on a rocker platform **[3]**.

5.2.1. Talent collecting the brains.
5.2.2. Talent placing the brains in the vial with the fixing solution.
5.2.3. Talent placing the vial on a rocker platform.

5.3. Use a cryostat chuck to embed the brains in TFM in the desired orientation on dry ice **[1-TXT]**.

5.3.1. Talent embedding the brains in TFM. **TEXT: Tissue freezing medium - TFM**

Results

6. Results: Effects of Blunt-Force Injury in Adult Zebrafish

6.1. Vascular injury was found to be one of the easiest and most prominent pathologies to identify successful injury via this model **[1]**. The ability to identify the indicator changed with the strain of fish used during injury **[2]**.

6.1.1. LAB MEDIA: Figure 3L

6.1.2. LAB MEDIA: Figure 3D, 3H, and 3L.

6.2. Identification of vascular injury in *wild-type AB* was difficult to distinguish between either mild or moderate TBI **[1]** and undamaged control fish due to the pigmentation **[2]**.

6.2.1. LAB MEDIA: Figure 3A-D. *Video Editor: Emphasize Figure 3B and 3C.*
6.2.2. LAB MEDIA: Figure 3-D. *Video Editor: Emphasize Figure 3A.*

6.3. Following injury, the mild TBI fish displayed minimal surface abrasions **[1],** while the moderate TBI fish exhibited limited cerebral hemorrhaging **[2]**. The extent of the injury was apparent in severe TBI fish **[3]**.

6.3.1. LAB MEDIA: Figure 3A-D. *Video Editor: Emphasize Figure 3B.*
6.3.2. LAB MEDIA: Figure 3A-D. *Video Editor: Emphasize Figure 3C*
6.3.3. LAB MEDIA: Figure 3A-D. *Video Editor: Emphasize Figure 3D*

6.4. In contrast, vascular injury can be easily identified when using *albino* **[1]** or *casper* fish **[2]**.

6.4.1. LAB MEDIA: Figure 3E-H.
6.4.2. LAB MEDIA: Figure 3I-L.

6.5. Swelling of the cerebrum due to the injury was assessed using edema **[1]**.

6.5.1. LAB MEDIA: Figure 4.

6.6. In contrast, both moderate TBI and severe TBI had significant edema 1 dpi and 3 dpi **[1],** but fluid content of both moderate TBI and severe TBI returned to levels resembling undamaged controls by 5 dpi **[2]**.

6.6.1. LAB MEDIA: Figure 4. *Video Editor: Emphasize the 1 dpi and 3dpi boxplots for moTBI and sTBI.*
6.6.2. LAB MEDIA: Figure 4. *Video Editor: Emphasize the 5 dpi boxplots for moTBI and sTBI.*

6.7. This blunt-force injury resulted in a robust cell proliferation response spanning the neuroaxis**[1]**. Increased EdU labeling was observed in the ventricular and subventricular zones of the forebrain compared to undamaged controls **[2]**.

6.7.1. LAB MEDIA: Figure 5.
6.7.2. LAB MEDIA: Figure 5B.

6.8. The injured brains displayed increased EdU labeling in the periventricular grey zone, the optic tectal lobes, and aspects of the anterior hypothalamus **[1]** compared to the undamaged fish brain **[2]**.

6.8.1. LAB MEDIA: Figure 5C and 5D. *Video Editor: Emphasize Figure 5D.*
6.8.2. LAB MEDIA: Figure 5C and 5D. *Video Editor: Emphasize Figure 5C.*

6.9. Following severe TBI, neurogenic regions in the hindbrain exhibited increased cell proliferation **[1]** as compared to the undamaged brain **[2]**.

6.9.1. LAB MEDIA: Figure 5E-H. *Video Editor: Emphasize Figure 5F and 5H.*
6.9.2. LAB MEDIA: Figure 5E-H. *Video Editor: Emphasize Figure 5E and 5G.*

Conclusion

7. Conclusion Interview Statements

7.1. **James Hentig:** For proper induction of injury, ensure the fish are secured and stable in the mold and that tubing is straight to avoid off-center impact or alter the weight drop's trajectory.

7.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested b-roll: 2.3.1, 2.5.2.*

7.2. **James Hentig:** This procedure provides a rapid and cost-effective blunt-force injury induction method applied to a regenerative model that would be especially applicable for repeated head trauma or injury-induced regenerative studies.

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

NOTE: All interview statements were filmed by the videographer on the day of the shoot.