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Title: Cerebellar Regional Dissection for Molecular Analysis

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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? **no**

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: 27
Number of Shots: 55

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Katie Hamel:** Traditional molecular studies are done on whole cerebellar extracts, which can mask any distinctions across specific cerebellar regions. This protocol makes it possible to assess distinct regions of the cerebellum separately and allows for the exploration of molecular mechanisms that may underlie their unique contributions to a variety of behaviors.

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

1.2. **Katie Hamel:** The main advantage of this technique is that it allows for the reproducible and quick dissection of four cerebellar regions: the deep cerebellar nuclei, anterior and posterior vermal cerebellar cortex, and the cerebellar cortex of the hemispheres.

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) at the University of Minnesota.

Protocol

2. Brain extraction and dissection

2.1. After decapitating the euthanized mouse **[1]**, make an incision with a razor blade along the medial sagittal line of the head, starting at the nose and continuing all the way back **[2]**. Separate the skin and use the razor blade to cut away the muscle on each side, cutting down past the ear canals **[3]**.

2.1.1. WIDE: Establishing shot of talent preparing to dissect the mouse.

2.1.2. Talent making the incision.

2.1.3. Talent separating the skin and cutting the muscle.

2.2. Using dissecting scissors, trim any spinal cord regions, up to where the brain stem meets the cerebellum, taking care to not damage the cerebellum **[1]**.

2.2.1. Talent trimming the spinal cord regions.

2.3. Insert one of the vascular scissor blades into the space between the brainstem and vertebral column and cut toward the ear canal, lifting upward while cutting to limit damage to the tissue **[1]**.

2.3.1. Talent inserting the scissors and cutting the bone.

2.4. Continue to cut along the edge of the skull up toward the olfactory bulbs **[1]**. Using the blunt forceps, gently peel off the back of the skull to uncover the posterior region of the brain and cerebellum **[2]**.

2.4.1. Talent cutting along the skull to the olfactory bulbs.

2.4.2. Talent peeling off the back of the skull.

2.5. Position the blunt forceps along the cut edge of the skull and peel the skull up and over the brain **[1]**. Trim the rest of the skull with the vascular scissors and blunt forceps, clearing most of it from the top of the brain **[2]**.

2.5.1. Talent peeling the rest of the skull up and over the brain.

2.5.2. Talent trimming the rest of the skull.

2.6. Slightly lift the brain with the microspatula to remove the olfactory bulbs from the remaining skull and disconnect optic tract fibers **[1]**. The brain should come free easily at this point **[2]**. Place the brain into the Petri dish sitting on ice and remove any remaining skull or other debris **[3]**.

2.6.1. Talent removing the olfactory bulbs and disconnecting the optic fibers.

2.6.2. Talent freeing the brain.

2.6.3. Talent placing the brain in the Petri dish.

2.6.4. ~~Talent removing remaining skull from the brain~~.

2.7. Gently place the brain into the brain matrix with the dorsal side up. Make sure that it is set level in the matrix and that the midline is in the center **[1]**. Place one razor blade along the sagittal midline, making sure that the blade pushes all the way to the bottom of the matrix **[2]**. *Videographer: This step is important!*

2.7.1. Talent positioning the brain in the matrix.

2.7.2. Talent placing the razor blade.

2.8. Place another razor blade 1-millimeter to the side of the first blade. Place two more blades, resulting in three blades placed on one side of the brain, all 1-millimeter apart **[1]**. Repeat this on the other side **[2]**. *Videographer: This step is important!*

2.8.1. Talent placing the next 3 razor blades.

2.8.2. Talent placing razor blades on the other side.

2.9. Carefully grab the front and back ends of the razor blades and lift them straight up out of the matrix. Discard the tissue on the outside of the razor blades **[1]**. Slowly, separate one razor blade at a time from the others, being careful not to damage the tissue sections **[2]**.

2.9.1. Talent lifting the razor blades.

2.9.2. Talent separating the razor blades.

2.10. Slide the tissue section off the razor blade and onto the glass slide with the microspatula. Six sagittal brain sections should be collected **[1]**.

2.10.1. Talent sliding the tissue onto a glass slide. NOTE: This step was only done for some tissue, the rest were left on the razor blades for easier viewing

2.11. The 4 most lateral sections will have DCN visible **[1]**. To isolate the DCN, hold a trimmed 200-microliter pipette tip perpendicularly over the DCN and push down through the tissue firmly, rocking in all directions **[2]**. Lift the pipette up and visually confirm the presence of the tissue in the tip **[3]**. *Videographer: This step is important!*

2.11.1. Sections of the DCN.

2.11.2. Talent pushing the pipette into the DCN.

2.11.3. Talent lifting the pipette with the tissue visible.

2.12. Place one finger at the top of the tip and push down, causing the tissue to bulge out **[1]**. Insert the tip into a correctly labeled microfuge tube and ensure that the tissue punch is placed in the bottom of the tube **[2]**.

2.12.1. Talent pushing on the top of the pipette tip.

2.12.2. Talent placing the tissue into a microfuge tube.

2.13. Repeat this for the remaining three sections, placing the DCN punches in the same tube **[1]**. Flash freeze the tube in liquid nitrogen **[2]**.

2.13.1. Talent adding more tissue to the tube.

2.13.2. Talent flash freezing the tube.

2.14. Push away the rest of the brain tissue around the cerebellum in the sections where the DCN was extracted **[1]**. Use blunt forceps to gently pick up these hemisphere cerebellar cortex sections **[2]** and place them into respective microfuge tubes, then flash freeze them **[3]**.

2.14.1. Talent pushing away the rest of the brain tissue around the cerebellum.

2.14.2. Talent picking up the sections.

2.14.3. Talent placing the sections in a tube.

2.15. For the last two vermal sections, push away surrounding brain tissue to leave only the cerebellum **[1]**. Using a razor blade, make a cut separating the anterior lobules from the posterior lobules. Ensure that the cut is just after the formation of lobule 6 and does not include lobule 10 **[2]**. *Videographer: This step is important!*

2.15.1. Talent pushing away the surrounding tissue.

2.15.2. Talent cutting to separate lobules.

2.16. Using blunt forceps, carefully place the anterior cerebellar cortex sections and posterior cerebellar cortex sections into their respective microfuge tubes **[1]** and flash freeze them by leaving the tubes in liquid nitrogen for 5 minutes **[2-TXT]**.

2.16.1. Talent placing sections into microfuge tubes.

2.16.2. Talent flash freezing the tubes. **TEXT: Store at -80 degrees Celsius**

3. RNA extraction

3.1. Place the microfuge tubes on ice to keep the tissue from thawing too quickly and apply 150 microliters of cold RNA isolation solution in the microfuge tube **[1]**, then homogenize with a sterilized pestle **[2]**. NOTE: Shots 3.1.1, 3.1.2, 3.2.1, 3.2.2 and 3.3.1 are combined in one video footage. Two samples were filmed.

3.1.1. Talent adding RNA isolation reagent to a tube on ice.

3.1.2. Talent homogenizing the sample.

3.2. Once the tissue is homogenized, pipette the solution up and down to ensure there is no remaining intact tissue **[1]**. Further break up any small tissue pieces by pulling it up into an insulin syringe a few times **[2]**.

3.2.1. Talent pipetting the solution up and down.

3.2.2. Talent pulling the solution into an insulin syringe.

3.3. Add another 350 microliters of the reagent, pipette up and down to mix thoroughly, and let it sit at room temperature for 5 minutes **[1]**. Add 150 microliters of chloroform to the tube and shake it vigorously, then let it rest for 2 to 3 minutes **[2]**. Centrifuge at 12,000 x *g*, at 15 degrees Celsius for 10 minutes **[3]**.

3.3.1. Talent adding more reagent and pipetting.

3.3.2. Talent adding chloroform and shaking the tube.

3.3.3. Talent putting the tube in the centrifuge and closing the lid.

3.4. Carefully remove the tubes from the centrifuge and set the temperature of the centrifuge to 4 degrees Celsius **[1]**. Transfer only the clear aqueous phase **[2]** into a new tube, taking care to not disrupt the opaque interphase. Save or discard the remaining solution in the tubes **[3]**. *Videographer: This step is important!*

3.4.1. Talent removing the tubes and setting the centrifuge temperature.

3.4.2. Talent removing the clear phase.

3.4.3. Talent placing the clear phase into a new tube.

3.5. Add 100% isopropyl alcohol at a 1 to 2 ratio and mix thoroughly by pipetting up and down **[1]**. Let the sample rest at room temperature for 10 minutes to precipitate the RNA out of the solution **[2]**.

3.5.1. Talent adding isopropyl to a sample tube, with the isopropyl container in the shot.

3.5.2. Sample sitting at room temperature.

3.6. Centrifuge at 12,000 times *g* and 4 degrees Celsius for 10 minutes, placing all tubes in the same orientation to make it easier to visualize the pellet **[1]**.

3.6.1. Talent putting the tubes in the centrifuge and starting the centrifugation.

3.7. After centrifugation, carefully remove the tubes **[1]** and remove the supernatant with a pipette, making sure to not disrupt the pellet. The pellet is gel-like and difficult to see, so estimate where it is based on the orientation of the tubes in the centrifuge **[2]**. *Videographer: This step difficult!*

3.7.1. Talent taking the tubes out of the centrifuge.

3.7.2. Talent removing the supernatant from a tube.

3.8. After removing all supernatant, add 500 microliters of 75% ethanol **[1]**, vortex briefly **[2]**, and centrifuge at 7,500 times *g* and 4 degrees Celsius for 5 minutes **[3]**.

3.8.1. Talent adding 75% ethanol to a sample, with the ethanol container in the shot.

3.8.2. Talent vortexing the sample.

3.8.3. Talent putting the sample in the centrifuge and closing the lid.

3.9. Remove the supernatant carefully, without disrupting the pellet **[1]**. Leave the caps open to dry the sample for 5 to 10 minutes **[2]**.

3.9.1. Talent removing the supernatant.

3.9.2. Talent leaving the tubes on the lab bench to dry.

3.10. Once dry, resuspend the pellet in DNase free water. Add 20 microliters to the samples for DCN and 30 microliters for all others **[1]**. Store the samples at -80 degrees Celsius until further analysis **[2]**.

3.10.1. Talent resuspending a sample.

3.10.2. Talent putting the samples in the freezer and closing the door.

Results

4. Results: RT qPCR relative gene expression in isolated specific regions of the cerebellum

4.1. This protocol was used to dissect four distinct regions of the mouse cerebellum and to explore regional differences in gene expression. The expression levels of aldolase C, parvalbumin, and Kcng4 *(spell out 'K-C-N-G-4')* were assessed using real time quantitative PCR **[1]**.

4.1.1. LAB MEDIA: Figure 2.

4.2. Aldolase C is more highly expressed in the posterior cerebellar vermis **[1]** but lower in the DCN and the anterior region of the vermis when compared to the bulk cerebellar dissection **[2]**.

4.2.1. LAB MEDIA: Figure 2 A. Video Editor: Emphasize the purple bar.

4.2.2. LAB MEDIA: Figure 2 A. Video Editor: Emphasize the pink and green bars.

4.3. Parvalbumin is similarly present in the DCN, anterior vermis, posterior vermis, and hemisphere cerebellar cortices as in the bulk cerebellar extracts **[1]**.

4.3.1. LAB MEDIA: Figure 2B.

4.4. Kcng4 is significantly enriched in the DCN and the anterior vermis **[1]** but not in the posterior vermis or hemispheres when compared to the bulk extraction **[2]**.

4.4.1. LAB MEDIA: Figure 2 C. *Video Editor: Emphasize the green and pink bars.*

4.4.2. LAB MEDIA: Figure 2 C. *Video Editor: Emphasize the purple and blue bars.*

4.5. To directly compare the expression of aldolase C across the cerebellar cortex, the expression levels were compared to the anterior vermis **[1].** The expression level of aldolase C was significantly higher in the posterior vermis **[2]** and trending higher in the cerebellar hemispheres **[3].**

4.5.1. LAB MEDIA: Figure 3.

4.5.2. LAB MEDIA: Figure 3. *Video Editor: Emphasize the purple bar.*

4.5.3. LAB MEDIA: Figure 3. *Video Editor: Emphasize the blue bar.*

Conclusion

5. Conclusion Interview Statements

5.1. **Katie Hamel:** The RNA extracted from these regions can also be used in RNAsequencing experiments. RNAseq comparing these four cerebellar regions would provide more detailed information on molecular mechanisms underlying discrete functions of these different regions as well as potential differences in their vulnerability in disease.

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