Submission ID #: 62641
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Project Page Link: https://www.jove.com/account/file-uploader?src=19105553

Title: Direct Cannula Implantation in the Cisterna Magna of Pigs

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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 wear masks until videographer steps away (≥6 ft/2 m) and begins filming, then the interviewee removes the mask for line delivery only. When take is captured, the interviewee puts the mask back on. Statements can be filmed outside if weather permits.

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

Current Protocol Length

Number of Steps: 22
Number of Shots: 47

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Iben Lundgaard:** This protocol is important because it facilitates the investigation of the glymphatic system in a large mammal which brings us a little closer to understanding human glymphatics.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *B-roll: 3.5.1.*

1.2. **Nicholas Bechet:** There are 2 advantages to this technique. Introduction of tracers into the cisterna magna avoids any direct damage to the brain, in contrast to interventricular cannulations. Secondly the direct approach for the cannulation allows for direct visual feedback leading to higher success rates.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *B-roll: 3.3.1.*

Ethics Title Card

1.3. Procedures involving animal subjects were carried out in accordance with the European directive 2010/63/EU and were approved by Malmö-Lund Ethical committee on Animal Research and conducted according to the CODEX guidelines of the Swedish Research Council.

Protocol

2. Surgery

2.1. Begin by placing the pig in a prone position **[1].** Palpate the back of its head and neck **[2]** to locate and mark the occipital crest, the spine of the first thoracic vertebrae **[3],** and the base of each ear **[4]**.

2.1.1. WIDE: Establishing shot of the talent with the pig in a prone position.

2.1.2. Talent palpating the back of the head and neck of the pig.

2.1.3. Talent marking the occipital crest and spine of the first thoracic vertebrae.

2.1.4. Talent marking the base of each ear.

2.2. Draw a straight line between the crest and the vertebrae along the longitudinal axis **[1]**. Then, following the base of the skull, draw two lines from the crest to the base of each ear **[2]**.

2.2.1. Talent drawing a line between the crest and the vertebrae.

2.2.2. Talent drawing two lines from the crest to the base of each ear.

2.3. Carefully clamp the animal's tail and watch for a reflex to check if it is in a deep sleep **[1]**.

2.3.1. Talent clamping the tail.

2.4. Begin by making a dermal incision along the longitudinal line down to the muscle using a scalpel with a Number 21 blade **[1]**. Then, extend two perpendicular incisions further along the shoulders, each 10 to 15 centimeters in length **[2]**.

2.4.1. Talent making a dermal incision down to the muscle.

2.4.2. Talent making two perpendicular incisions.

2.5. Starting from the occipital crests, make dermal incisions along the line down to the base of each ear **[1]**.

2.5.1. Talent making dermal incisions from the occipital crests.

2.6. Use anatomical forceps to grip the skin corners formed at the occipital crest **[1]**. Then, run the scalpel blade lightly over the fascia to separate the skin from the underlying

muscle **[2-TXT]**. Resect the skin along each of the five incisions to visualize parts of the trapezius muscle **[3]**.

2.6.1. Talent gripping the skin using anatomical forceps.
2.6.2. Talent resecting the skin along one incision. **TEXT: From the rostral to caudal**
2.6.3. A shot of the exposed trapezius muscle

2.7. Use the scalpel to make a longitudinal incision approximately one centimeter deep where the trapezius comes together at the midline **[1]**.

2.7.1. Talent making the longitudinal incision.

2.8. Then, perform a blunt dissection along the longitudinal cut in the muscles using a combination of straight and curved surgical forceps, which will separate the bellies of the trapezius and underlying semispinalis capitus biventer muscle **[1]**.

2.8.1. Talent performing the blunt dissection.

2.9. Sever any persisting muscle fibers with a scalpel **[1]** and continue to perform blunt dissection until the semispinalis capitus complexus becomes visible **[2]**.

2.9.1. Talent severing the muscle fibers. 2.9.1 and 2.9.2 not filmed.
2.9.2. Talent performing blunt dissection.

2.10. Move along the posterior aspect of the skull and sever the origins of the trapezius and semispinalis capitus biventer muscles **[1].** To separate the two muscles longitudinally, use the scalpel to perform blunt dissection until the semispinalis capitus complexus is fully visible **[2]**.

2.10.1. Talent severing origins of the trapezius and semispinalis capitus biventer muscles. Videographer NOTE: 2.10.1 is slated as 2.9.1
2.10.2. Talent performing blunt dissection to separate the muscles. Videographer NOTE: 2.10.2 is slated as 2.9.2

2.11. Then, use the self-retaining retractors to retract the trapezius and semispinalis capitus biventer muscles **[1]**. Use the scalpel to make a 1-centimeter-deep longitudinal incision where the bellies of the semispinalis capitus complexus come together at the midline **[2]**.

2.11.1. Talent retracting the two muscles.
2.11.2. Talent making the longitudinal incision.

2.12. Perform a blunt dissection using surgical forceps, working along the longitudinal cut between the muscle bellies until both the atlas and axis are palpable **[1]**.

2.12.1. Talent performing a blunt dissection until atlas and axis are palpable.

2.13. Move along the posterior aspect of the skull severing the origins of the semispinalis capitus complexus muscles **[1].** Use the scalpel and blunt dissection to separate the muscle longitudinally from the underlying vertebrae **[2]**.

2.13.1. Talent skull severing the origins of the semispinalis capitus complexus muscles.
2.13.2. Talent separating the muscle from the underlying vertebrae.

2.14. Then, use another set of self-retaining retractors to retract the semispinalis capitus complexus muscles **[1]**. Use a scalpel to carefully remove any remaining tissue overlying the region where the atlas meets the skull base **[2]**.

2.14.1. Talent retracting the muscles using the retractors.
2.14.2. Talent removing the remaining tissue.

2.15. Place one arm under the animal's neck **[1]** and one finger at the juncture of the atlas and the skull **[2]**. Then, simultaneously elevate the head and flex the neck while palpating with the finger **[3]** to reveal the cisterna magna **[4]**.

2.15.1. Talent placing an arm under the animal's neck.
2.15.2. Talent placing the finger at the juncture of the atlas and the skull.
2.15.3. Talent elevating the head and flexing the neck while palpating with the finger.
2.15.4. A shot of the cisterna magna of the animal.

3. Cannulation and Injection

3.1. Ensure that one person elevates and flexes the head and neck of the animal while the other palpates for the cisterna magna, making a note of its anatomical location **[1]**.

3.1.1. Talent flexing the head and neck of the animal while the assistant palpates to find the location of the cisterna magna.

3.2. Introduce a 22-gauge cannula slowly and carefully into the cisterna magna through the dura at an angle oblique to the longitudinal axis **[1]**. Then, retract the needle from the cannula and place a cap on the lock **[2]**.

3.2.1. Talent introducing the 22 G cannula into the cisterna magna.
3.2.2. Talent retracting the needle from the cannula and placing a cap on the lock.

3.3. Start with applying superglue and an accelerator where the cannula enters the tissue **[1]**. Then, apply the dental cement **[2]**. Wait for 5 minutes for the cement to harden **[3]**.

3.3.1. Talent applying superglue and the accelerator.
3.3.2. Talent applying the dental cement. 3.3.2 and 3.3.3 not filmed.
3.3.3. A shot of the hardened cement.

3.4. Remove the cap carefully from the cannula **[1].** Attach the cannula to the male end of the IV line tap with the tracer using a 10-centimeter extension **[2]**.

3.4.1. Talent removing the cap from the cannula. Videographer NOTE: 3.4.1- 3.5.3 is combined in two takes
3.4.2. Talent attaching the IV line tap with the tracer to the cannula.

3.5. Inject the tracer slowly at a rate of 100 microliters per minute, either by hand or using a micro-infusion pump **[1]**. Remove the IV line tap and replace it with the cap **[2]**. Check if the tracer is visible pulsating at the base of the cannula **[3]**.

3.5.1. Talent injecting the tracer.
3.5.2. Talent removing the 3-way IV line tap and replacing the cap. 3.5.2 not filmed.
3.5.3. Talent checking the tracer.

3.6. Then, place sandbags under the neck of the animal to maintain some flexion **[1]**. Release the head and leave the animal in a resting prone position **[2].**

3.6.1. Talent placing the sandbags under the neck.
3.6.2. WIDE: A shot of the animal resting prone position.

3.7. Release the self-retaining retractors **[1]** and replace the muscles **[2]**. Use the surgical towel clamps to bring the skin together over the muscles **[3]**. First, use gauze and then

a blanket to cover the towel clamps and the incision to limit heat loss. Allow the tracer to circulate for the desired amount of time **[4]**.

3.7.1. Talent releasing the self-retaining retractors.
3.7.2. Talent placing the muscles as before. Videographer NOTE: 3.7.2-3.7.4 are combined
3.7.3. Talent using surgical towel clamps to bring the skin together.
3.7.4. Talent covering the towel clamps and the incision with gauze and blanket.

Results

4. Results: Distribution of the Tracer Inside the Pig Brain

4.1. Stitched macroscopic images of the brain's dorsal surface can provide detailed insights into the distribution patterns of tracer across the sulci and fissures **[1]**.

4.1.1. LAB MEDIA: Figure 1E *Video Editor: Emphasize the leftmost image.*

4.2. Similar images from the brain's ventral and lateral surfaces can provide information about the tracer distribution in the temporal lobe **[1]** and the lateral fissure **[2]**.

4.2.1. LAB MEDIA: Figure 1E *Video Editor: Emphasize the middle image.*
4.2.2. LAB MEDIA: Figure 1E *Video Editor: Emphasize the rightmost image.*

4.3. Images of the brain surface of higher magnification produced using a stereoscope can help visualize the tracer in the PVS along the arteries **[1]**.

4.3.1. LAB MEDIA: Figure 1F and Figure 1G.

4.4. Macroscopic coronal brain sections provide insight into the depth of tracer penetration in the interhemispheric fissure **[1]** and subcortical tracer distribution in structures such as the hippocampus **[2]** and striatum **[3]**.

4.4.1. LAB MEDIA: Figure 1H *Video Editor: Emphasize the parts of the image labelled as IHS.*
4.4.2. LAB MEDIA: Figure 1H *Video Editor: Emphasize the parts of the image labelled as HPC.*
4.4.3. LAB MEDIA: Figure 1H *Video Editor: Emphasize the part of the image labelled as STR.*

4.5. Immunohistochemical staining for AQP4 **[1]**, glial-fibrillary acidic protein **[2],** and smooth muscle actin **[3]** showed that the tracer localized within the PVS as well as moved into the brain parenchyma **[4]**.

4.5.1. LAB MEDIA: Figure 1I.

4.5.2. LAB MEDIA: Figure 1L.

4.5.3. LAB MEDIA: Figure 1N.

4.5.4. LAB MEDIA: Figure 1N, 1L ,1I.

4.6. Astrocyte foot processes that form the outer surface of the PVS are identified using AQP4 **[1]** and glial-fibrillary acidic protein staining **[2]**. The endothelial cells that form the inner surface of the PVS are stained using lectin **[3]** and glut-1 stain **[4]**.

4.6.1. LAB MEDIA: Figure 1J. *Video Editor: Emphasize the orange stain in Figure 1J.*

4.6.2. LAB MEDIA: Figure 1M. *Video Editor: Emphasize the orange stain in Figure 1M.*

4.6.3. LAB MEDIA: Figure 1M. *Video Editor: Emphasize the green stain in Figure 1M.*

4.6.4. LAB MEDIA: Figure 1J. *Video Editor: Emphasize the green stain in Figure 1J.*

4.7. SMA staining identifies arteries and arterioles and can be used to show that PVS influx occurs along arteries instead of veins, which constitutes the basic physiology of normal glymphatic function **[1]**.

4.7.1. LAB MEDIA: Figure 1N.

Conclusion

5. Conclusion Interview Statements

5.1. **Nagesh Shanbhag:** Going forward, we hope to explore the glymphatic system at a high resolution in a large mammal in the context of neuropathologies such as stroke and traumatic brain injury.

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