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Title: Surgical Protocols for Deep Cervical Lymphovenous Anastomosis in a Rat Model: Lymph Node and Lymphatic Vessel Anastomoses

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Author Questionnaire

1. We have marked your project as author-provided footage, meaning you film the video yourself and provide JoVE with the footage to edit. JoVE will not send the videographer. Please confirm that this is correct.

√ Correct

2. Interview statements: 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.

3. Proposed interview filming date: Please indicate the proposed date that your group will **self-film** interviews: **26/12/2025**

When you are ready to submit your video files, please contact our China Location Producer, Yuan Yue.

Current Protocol Length

Number of Steps: 24
Number of Shots: 31

Introduction

INTRODUCTION:

1.1. **Songyu Yang:** Our DcLVA technique shows promise for treating Alzheimer's, though its mechanisms remain unclear. Animal models are key to uncovering them.

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

What are the current experimental challenges?

1.2. **Songyu Yang:** A key challenge is the anatomical difference between rats and humans. Standardizing surgical landmarks and techniques is crucial for reliable results.

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

CONCLUSION:

How will your findings advance research in your field?

1.3. **Nannan Zhao**: Our study provides specific references and a standardized framework for dcLVA, including dcLnVA and dcLaVA, supporting both basic research and clinical application.

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

What questions will future research focus on?

1.4. **Nannan Zhao:** We will investigate the efficacy and mechanisms of this surgical procedure in AD rat models in the future.

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

Ethics Title Card

This research has been approved by the Institutional Animal Care and Use Committee at the Harbin Medical University

Protocol

2. Surgical Incision and Preparation of the Posterior Facial Vein (PFV)

Demonstrator: Nannan Zhao

2.1. To begin, disinfect the midline of the rat's neck three times with iodine tincture to ensure a sterile surgical field **[1]**.

2.1.1. LAB MEDIA: 69201_Video 1.MOV 00:00 – 00:08

2.2. Then, make a midline incision approximately 3 centimeters in length using surgical scissors **[1]**.

2.2.1. LAB MEDIA: 69201_Video 1.MOV 00:18 – 00:25

2.3. Take care not to damage the submaxillary gland and blood vessels during dissection **[1]**.

2.3.1. LAB MEDIA: 69201_Video 1.MOV 01:01 – 01:08

2.4. Retract the surgical incision to expose the sternothyroid muscle, sternocleidomastoid muscle, and the omohyoid muscle **[1]**.

2.4.1. LAB MEDIA: 69201_Video 1.MOV 01:57 – 02:09

2.5. Identify the preauricular facial vein, a tributary of the external jugular vein, along the lateral margin of the sternocleidomastoid muscle **[1]**.

2.5.1. LAB MEDIA: 69201_Video 1.MOV 02:25 – 02:33

2.6. At the bifurcation between the preauricular facial vein and the external jugular vein, occlude the preauricular facial vein using a microvascular clip **[1]**.

2.6.1. LAB MEDIA: 69201_Video 1.MOV 02:54 – 03:01

2.7. Continue to bluntly dissect the preauricular facial vein to obtain sufficient length, and clamp it at the distal end **[1]**. Ensure the preauricular facial vein segment remains as loose as possible to allow for subsequent traction during anastomosis with the deep cervical lymph node **[2]**.

2.7.1. LAB MEDIA: 69201_Video 1.MOV 04:42 – 05:00

2.7.2. LAB MEDIA: 69201_Video 1.MOV 05:00 – 05:20

2.8. Now, create a longitudinal incision parallel to the vessel axis using microvascular scissors **[1]**.

2.8.1. LAB MEDIA: 69201_Video 1.MOV 05:31 – 05:38

2.9. Make sure the preauricular facial vein incision is longitudinal, as the transverse incision can be enlarged and ruptured by traction **[1]**.

2.9.1. LAB MEDIA: 69201_Video 1.MOV 05:38 – 05:45

2.10. Irrigate the isolated vascular segment thoroughly with heparinized saline, prepared as 0.1 percent sodium heparin in 0.9 percent sodium chloride, until intravascular erythrocytes are completely cleared **[1]**.

2.10.1. LAB MEDIA: 69201_Video 1.MOV 05:48 – 06:05

3. Lymph Node-Vein Anastomosis

Demonstrator: Nannan Zhao

3.1. Explore and fully expose the deep cervical lymph node and its afferent lymphatic vessel in the middle of the sternothyroid muscle and sternocleidomastoid muscle **[1]**.

3.1.1. LAB MEDIA: 69201_Video 1.MOV 03:02 – 03:12

3.2. Dissect the deep cervical lymph node gradually using a blunt technique **[1]**. Carefully incise the fascia between the deep cervical lymph node and surrounding tissues, avoiding damage to both the node and its afferent lymphatic vessel **[2]**.

3.2.1. LAB MEDIA: 69201_Video 1.MOV 03:35 – 03:41

3.2.2. LAB MEDIA: 69201_Video 1.MOV 03:41 – 03:50

3.3. Ensure that the preauricular facial vein and the deep cervical lymph node are close to each other to minimize tension after anastomosis **[1]**.

3.3.1. LAB MEDIA: 69201_Video 1.MOV 04:10 – 04:19

3.4. Next, make an incision at the distal end of the deep cervical lymph node **[1]**. Ensure that the width of the incision is smaller than or similar to the diameter of the preauricular facial vein, so that the blood flow in the vein is not significantly affected **[2]**.

3.4.1. LAB MEDIA: 69201_Video 1.MOV 06:56 – 07:03

3.4.2. LAB MEDIA: 69201_Video 1.MOV 07:03 – 07:13

3.5. Using a 12-0 *(12-oh)* nylon suture, anastomose the proximal end of the preauricular facial vein incision to the proximal end of the deep cervical lymph node incision **[1]**.

3.5.1. LAB MEDIA: 69201_Video 1.MOV 07:45 – 07:55

3.6. Continue to expand the incision length of the preauricular facial vein to the distal end so that it is slightly larger than the length of the deep cervical lymph node incision **[1]**.

3.6.1. LAB MEDIA: 69201_Video 1.MOV 09:18 – 09:28

3.7. Then, pass the needle sequentially through the outer edge of the deep cervical lymph node transection and the inner edge of the preauricular facial vein incision, thereby pulling the lymph node transection into the lumen of the vein **[1]**.

3.7.1. LAB MEDIA: 69201_Video 1.MOV 09:40 – 09:80 and 10:25-10:36

3.8. Perform a continuous suture from the inferior to the superior edge of the deep cervical lymph node transection **[1]**. Administer heparin sodium once more just prior to completing the final suture to prevent thrombus formation within the preauricular facial vein lumen **[2]**. Maintain appropriate tension on the suture during the continuous closure to ensure a secure closure and prevent subsequent leakage **[3]**.

3.8.1. LAB MEDIA: 69201_Video 1.MOV 14:50 – 15:00

3.8.2. LAB MEDIA: 69201_Video 1.MOV 15:20 – 15:29

3.8.3. LAB MEDIA: 69201_Video 1.MOV 17:06 – 17:15

4. Lymphatic Vessel-Vein Anastomosis

Demonstrator: Nannan Zhao

4.1. Transect the afferent lymphatic vessel and preserve the surrounding tissue to serve as a suture anchor point **[1]**.

4.1.1. LAB MEDIA: 69201_Video 2.MOV 00:43 – 00:50

4.2. Using a 12-0 suture needle, insert it about 2 millimeters proximal to the preauricular facial vein incision and advance it to exit through the opening of the vein incision **[1]**.

4.2.1. LAB MEDIA: 69201_Video 2.MOV 04:40 – 04:51

4.3. Enter the tissue near the stump of the afferent lymphatic vessel with the suture needle to catch the vessel **[1]**.

4.3.1. LAB MEDIA: 69201_Video 2.MOV 05:18 – 05:24

4.4. Now, pass the needle into the preauricular facial vein incision and out through its proximal edge **[1]**, then tie the suture to fix the afferent lymphatic vessel stump within the lumen of the vein **[2]**.

4.4.1. LAB MEDIA: 69201_Video 2.MOV 05:58 – 06:20

4.4.2. LAB MEDIA: 69201_Video 2.MOV 06:36 – 06:45

4.5. Completely insert the afferent lymphatic vessel stump into the lumen of the preauricular facial vein, orienting it toward the proximal direction to maintain physiological drainage **[1]**.

4.5.1. LAB MEDIA: 69201_Video 2.MOV 06:24 – 06:35

4.6. Finally, suture the surrounding tissue near the stump of the afferent lymphatic vessel to the inner edge of the preauricular facial vein incision to provide external fixation **[1]** and give heparin sodium before the last stitch to keep the preauricular facial vein lumen free of clot **[2]**.

4.6.1. LAB MEDIA: 69201_Video 2.MOV 08:05 – 08:13

4.6.2. LAB MEDIA: 69201_Video 2.MOV 08:13 – 08:17

Results

5. Results

5.1. In deep cervical lymph node-vein anastomosis, the average hue angle of the post-anastomosis posterior facial vein was biased towards the blue tone at 278.2 degrees **[1]**, compared to the adjacent anterior facial vein at 327.9 degrees **[2]**, and showed a greater difference than the pre-anastomosis posterior facial vein at 24.8 degrees **[3]**.

5.1.1. LAB MEDIA: Figure 3E. *Video editor: Highlight the value labeled "PFV-2=278.2°"*

5.1.2. LAB MEDIA: Figure 3E. *Video editor: Highlight the value labeled AFV=327.9°.*

5.1.3. LAB MEDIA: Figure 3E. *Video editor: Highlight the value labeled PFV-1=24.8°.*

5.2. For deep cervical lymphatic vessel-vein anastomosis, the average hue angle of post-anastomosis posterior facial vein was 282.4 degrees, showing a stronger blue tone **[1]** than the adjacent anterior facial vein at 303.7 degrees **[2]**, and the difference was more distinct than the pre-anastomosis posterior facial vein at 39.6 degrees **[3]**.

5.2.1. LAB MEDIA: Figure 3J. *Video editor: Highlight the value labeled PFV-2=282.4°*.

5.2.2. LAB MEDIA: Figure 3J. *Video editor: Highlight the value labeled AFV=303.7°.*

5.2.3. LAB MEDIA: Figure 3J. *Video editor: Highlight the value labeled PFV-1=39.6°*.

1. Iodine
   Pronunciation link: https://www.merriam-webster.com/dictionary/iodine
   IPA: /ˈaɪəˌdaɪn/
   Phonetic Spelling: eye·uh·dyn
2. Tincture
   Pronunciation link: https://www.merriam-webster.com/dictionary/tincture
   IPA: /ˈtɪŋktʃər/
   Phonetic Spelling: tingk·cher
3. Sterile
   Pronunciation link: https://www.merriam-webster.com/dictionary/sterile
   IPA: /ˈstɛrəl/
   Phonetic Spelling: stair·uhl
4. Dissection
   Pronunciation link: https://www.merriam-webster.com/dictionary/dissection
   IPA: /dɪˈsɛkʃən/
   Phonetic Spelling: dih·sek·shun
5. Submaxillary
   Pronunciation link: https://www.merriam-webster.com/medical/submaxillary

IPA: /ˌsʌbˈmæksəˌlɛri/
Phonetic Spelling: sub·maks·uh·lair·ee

6. Sternothyroid
   Pronunciation link: https://www.merriam-webster.com/medical/sternothyroid
   IPA: /ˌstɝːnoʊˈθaɪˌɔɪd/
   Phonetic Spelling: ster·noh·thye·oyd
7. Sternocleidomastoid
   Pronunciation link: https://www.merriam-webster.com/dictionary/sternocleidomastoid
   IPA: /ˌstɝːnoʊˌklaɪdəˈmæsˌtɔɪd/
   Phonetic Spelling: ster·noh·klyde·uh·mas·toyd
8. Omohyoid
   Pronunciation link: https://www.merriam-webster.com/medical/omohyoid
   IPA: /ˌoʊmoʊˈhaɪˌɔɪd/
   Phonetic Spelling: oh·moh·hye·oyd
9. Preauricular
   Pronunciation link: https://www.merriam-webster.com/medical/preauricular
   IPA: /ˌpriːəˈrɪkjələr/
   Phonetic Spelling: pree·uh·rik·yuh·lur
10. Tributary
    Pronunciation link: https://www.merriam-webster.com/dictionary/tributary
    IPA: /ˈtrɪbjəˌtɛri/
    Phonetic Spelling: trib·yuh·tair·ee
11. Jugular
    Pronunciation link: https://www.merriam-webster.com/dictionary/jugular
    IPA: /ˈdʒʌgjələr/
    Phonetic Spelling: jug·yuh·lur
12. Bifurcation
    Pronunciation link: https://www.merriam-webster.com/dictionary/bifurcation
    IPA: /ˌbaɪfərˈkeɪʃən/
    Phonetic Spelling: bye·fer·kay·shun
13. Microvascular
    Pronunciation link: https://www.merriam-webster.com/medical/microvascular
    IPA: /ˌmaɪkroʊˈvæskjələr/
    Phonetic Spelling: mye·kroh·vas·kyuh·lur
14. Occlude
    Pronunciation link: https://www.merriam-webster.com/dictionary/occlude
    IPA: /əˈkluːd/
    Phonetic Spelling: uh·klood
15. Longitudinal
    Pronunciation link: https://www.merriam-webster.com/dictionary/longitudinal
    IPA: /ˌlɑːndʒəˈtuːd(ə)nəl/
    Phonetic Spelling: lon·juh·too·duh·nul
16. Transverse
    Pronunciation link: https://www.merriam-webster.com/dictionary/transverse

IPA: /ˈtrænzˌvɝːs/
Phonetic Spelling: tranz·vers

17. Heparinized
Pronunciation link: https://www.merriam-webster.com/medical/heparinized
IPA: /hɪˈpærəˌnaɪzd/
Phonetic Spelling: huh·pair·uh·nyzd
18. Intravascular
Pronunciation link: https://www.merriam-webster.com/medical/intravascular
IPA: /ˌɪntrəˈvæskjələr/
Phonetic Spelling: in·truh·vas·kyuh·lur
19. Erythrocytes
Pronunciation link: https://www.merriam-webster.com/dictionary/erythrocyte
IPA: /ɪˈrɪθrəˌsaɪt/
Phonetic Spelling: ih·rith·ruh·site
20. Lymphatic
Pronunciation link: https://www.merriam-webster.com/dictionary/lymphatic
IPA: /lɪmˈfætɪk/
Phonetic Spelling: lim·fat·ik
21. Afferent
Pronunciation link: https://www.merriam-webster.com/dictionary/afferent
IPA: /ˈæfərənt/
Phonetic Spelling: af·uh·runt
22. Fascia
Pronunciation link: https://www.merriam-webster.com/dictionary/fascia
IPA: /ˈfæʃ(ə)/
Phonetic Spelling: fash·uh
23. Anastomosis
Pronunciation link: https://www.merriam-webster.com/dictionary/anastomosis
IPA: /əˌnæstəˈmoʊsəs/, /ˌænəstəˈmoʊsəs/
Phonetic Spelling: uh·nas·tuh·moh·suhs / an·uh·stuh·moh·suhs
24. Transection
Pronunciation link: https://www.merriam-webster.com/medical/transection
IPA: /trænˈsɛkʃən/
Phonetic Spelling: tran·sek·shun
25. Lumen
Pronunciation link: https://www.merriam-webster.com/dictionary/lumen
IPA: /ˈluːmən/
Phonetic Spelling: loo·mun
26. Thrombus
Pronunciation link: https://www.merriam-webster.com/dictionary/thrombus
IPA: /ˈθrɑːmbəs/
Phonetic Spelling: thrahm·bus
27. Physiological
Pronunciation link: https://www.merriam-webster.com/dictionary/physiological

IPA: /ˌfɪziəˈlɑːdʒɪkəl/
Phonetic Spelling: fiz·ee·uh·lah·ji·kul

28. Erythrocytes
Pronunciation link: https://www.merriam-webster.com/dictionary/erythrocyte
IPA: /ɪˈrɪθrəˌsaɪt/
Phonetic Spelling: ih·rith·ruh·site
29. Hue
Pronunciation link: https://www.merriam-webster.com/dictionary/hue
IPA: /hjuː/
Phonetic Spelling: hyoo
30. Anastomosis (post-anastomosis / pre-anastomosis)
Pronunciation link: https://www.merriam-webster.com/dictionary/anastomosis
IPA: /əˌnæstəˈmoʊsəs/, /ˌænəstəˈmoʊsəs/
Phonetic Spelling: uh·nas·tuh·moh·suhs / an·uh·stuh·moh·suhs