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Title: Welfare-Oriented Manual Restraint Technique for Substance Administration and Blood Sampling in Mice

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

- 4. Testimonials (optional):** Would you be open to filming two short testimonial statements **live during your JoVE shoot**? These will **not appear in your JoVE video** but may be used in JoVE's promotional materials. **no**

Current Protocol Length

Number of Steps: 20

Number of Shots: 48

Interviews

Videographer: Obtain headshots for all authors available at the filming location.

INTRODUCTION:

~~REQUIRED: Summarize your research focus and the main questions it aims to answer.~~

- 1.1. **Linda Hammerich**: Our research focuses on effective rodent restraint methods to improve animal and handler safety while minimizing stress during biomedical procedures.
 - 1.1.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

~~Describe the research gap or limitation in existing methods and how this protocol addresses them.~~

- 1.2. **Katarina Riesner**: Traditional mouse restraint can induce stress and affect experimental outcomes. This protocol provides safer, less stressful restraint while maintaining procedural effectiveness.
 - 1.2.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

CONCLUSION:

~~What does this protocol allow researchers to study or measure?~~

- 1.3. **Linda Hammerich**: Our novel application tunnel enables low-stress, secure mouse restraint for routine injections and blood sampling, improving welfare and handling.
 - 1.3.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

~~Describe the most important consideration or challenge when performing this protocol.~~

- 1.4. **Linda Hammerich**: A key consideration is that this method is limited to conscious animals and provides access only to the tail and lower abdomen.

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

~~How can future studies build on this work? Describe the questions or research directions that can be investigated next.~~

- 1.5. **Katarina Riesner**: Future studies can adapt this method for rats and explore its effectiveness across diverse biomedical procedures, animal models, and research applications.

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

Videographer: Obtain headshots for all authors available at the filming location.

Ethics Title Card

This research has been approved by the competent local authority Landesamt für
Gesundheit und Soziales Berlin (LAGeSo)

Protocol

***Videographer:** Please always record an audio slate while filming each shot mentioned in the shotlist.*

2. Preparation of the Working Area and Application Tunnel

Demonstrator: Katarina Riesner

- 2.1. To begin, clean the application tunnel with 70 percent ethanol and allow it to evaporate completely for approximately 5 minutes [1].
 - 2.1.1. WIDE: Talent cleaning the application tunnel with 70 percent ethanol..
- 2.2. Place a rolled piece of washed and autoclaved lining inside the application tunnel [1]. Using the fingers, gently compress the lining at the tunnel entrance until the remaining opening is approximately the width of 2 adjacent fingers [2].
 - 2.2.1. Talent placing the rolled piece of washed and autoclaved lining inside the application tunnel.
 - 2.2.2. Talent gently compressing the lining at the tunnel entrance with the fingers.
- 2.3. Then, position the application tunnel on the working surface [1] and place an additional piece of flat lining in front of the tunnel entrance [2].
 - 2.3.1. Talent positioning the application tunnel on the working surface. ***Videographer:** Please film multiple reusable shots to use them later in the protocol*
 - 2.3.2. Talent placing an additional piece of flat lining in front of the tunnel entrance.
- 2.4. Prepare the injection syringe fitted with a 27-gauge needle containing the desired injection solution [1] and place it next to the application tunnel [2]. Ensure that swabs and disinfectant spray are readily available [3].
 - 2.4.1. Talent preparing the injection syringe fitted with a 27-gauge needle containing the injection solution.
 - 2.4.2. Talent places the prepared injection syringe next to the application tunnel.
 - 2.4.3. Talent arranging the swabs and disinfectant spray beside the application tunnel.

3. Tunnel-Based Restraint for Animal Welfare

- 3.1. To transfer the mouse from the home cage, position the handling tube in front of the application tunnel, allowing the mouse to enter the tunnel voluntarily [1].
 - 3.1.1. Talent positions the handling tube directly in front of the application tunnel opening as the mouse voluntarily enters the tunnel. ***Videographer:** Please film multiple reusable shots to use them later in the protocol*

~~3.1.2. Shot of the mouse entering the tunnel.~~ **NOTE: Merged with the previous shot**

3.2. Once inside, allow the mouse to remain positioned within the rolled lining without turning around [1].

3.2.1. Shot of the mouse positioned within the rolled lining inside the application tunnel without turning around.

4. Intravenous Tail Vein Injection

4.1. Gently straighten the mouse's tail by holding the tip [1] and disinfect the intended injection site [2]. Rotate the tail approximately 90 degrees to position the lateral tail veins dorsally [3].

4.1.1. Talent gently straightening the tail by holding the tip.

4.1.2. Talent disinfecting the intended injection site on the tail.

4.1.3. Talent rotating the tail approximately 90 degrees to position the lateral tail veins dorsally.

4.2. Begin the injection at the distal end of the tail, inserting the needle at a 15-to-20-degree angle nearly parallel to the tail to preserve proximal sites for additional attempts if needed [1]. Advance the needle only slightly beyond the bevel [3]. Inject the desired volume at a slow, steady rate of no more than 5 milliliters per kilogram over 13 seconds [5].

4.2.1. Close-up shot of the needle being inserted at an angle of approximately 15 to 20 degrees relative to the tail. **NOTE: 4.3.1.2 the end was a perfect run (4.2.1-4.3.2)**

4.2.2. Close-up shot of the needle being advanced only slightly beyond the bevel.

4.2.3. Talent slowly injects the solution into the lateral tail vein.

4.3. Then, withdraw the needle [1] and apply gentle pressure to the injection site with a sterile swab to prevent bleeding [2].

4.3.1. Talent withdrawing the needle from the tail vein.

4.3.2. Talent applying gentle pressure to the injection site with a sterile swab.

5. Positive Reinforcement

5.1. Return the application tunnel containing the mouse to the home cage [1] and allow the mouse to voluntarily exit from the opposite end of the application tunnel [2].

5.1.1. Talent returning the application tunnel containing the mouse to the home cage.

5.1.2. Shot of the mouse voluntarily exiting from the opposite end of the application tunnel into the home cage. *Videographer: Please film multiple reusable shots to use them later in the protocol*

- 5.2. Provide treats in the home cage to support positive reinforcement and habituation to the procedure [1].

5.2.1. Talent placing treats in the home cage for the mouse.

6. Tunnel Maintenance

- 6.1. Remove the inner lining from the application tunnel [1] and clean it with 70 percent ethanol [2].

6.1.1. Talent removing the inner lining from the application tunnel.

6.1.2. Talent cleaning the application tunnel with 70 percent ethanol. *Videographer: Please film multiple reusable shots to use them later in the protocol*

- 6.2. Reuse the lining if it is clean. Otherwise, wash and autoclave it before reusing [1].

6.2.1. Shot of the clean lining being set aside for reuse.

7. Welfare-Oriented Intraperitoneal Injection Using an Application Tunnel

- 7.1. After preparing the application tunnel [1], position it on top of the wedge on the working surface [3] and place an additional flat lining in front of the tunnel entrance [4].

7.1.1. *Reuse 2.3.1*

7.1.2. Talent positioning the application tunnel on top of the wedge on the working surface.

7.1.3. Talent placing an additional flat lining in front of the application tunnel entrance.

- 7.2. Allow the mouse to enter the application tunnel as demonstrated earlier [1]. Hold the base of the mouse's tail between the thumb and forefinger of one hand [2]. Apply gentle pressure to the sacral area using the middle finger of the same hand and slightly turn the tail upwards [3]. Stabilize the mouse to provide clear access to the caudal abdominal region [4].

7.2.1. *Reuse 3.1.2*

7.2.2. Talent holding the base of the tail between the thumb and forefinger. **NOTE: Wrong clapperboard! 8.2.2**

7.2.3. Talent applying gentle pressure to the sacral area with the middle finger while slightly turning the tail upwards. **NOTE: Wrong clapperboard! 8.2.3**

7.2.4. Close-up shot of the stabilized mouse with the caudal abdominal region clearly exposed. **NOTE: Wrong clapperboard! 8.2.4**

- 7.3. Perform the injection by holding the syringe parallel to the working surface [1] and inserting the needle into the clearly visible caudal abdominal region [2].

- 7.3.1. Talent holding the syringe parallel to the working surface.
- 7.3.2. Talent inserting the needle into the caudal abdominal region.
- 7.4. After injection, gently release the pressure and the tail [1]. Then, allow the mouse to exit the tunnel [2] and clean the application tunnel as demonstrated earlier [3].
 - 7.4.1. Talent gently releasing the pressure applied to the sacral area and the tail
 - 7.4.2. [5.1.2](#).
 - 7.4.3. [6.1.2](#)

8. Blood Sampling from the Tail Vein

- 8.1. After tunnel preparation and voluntary entry of the mouse into the application tunnel [1], gently straighten the mouse's tail by holding the tip [1] and disinfect the intended sampling site [2]. Rotate the tail approximately 90 degrees to position the lateral tail veins dorsally [3].
 - 8.1.1. [Reuse 3.1.2](#)
 - 8.1.2. WIDE: Talent gently straightening the tail by holding the tip. **NOTE: Use 8.2.1.7 (for 8.2.1-8.3.1)**
 - 8.1.3. Talent disinfecting the intended blood sampling site on the tail.
 - 8.1.4. Talent rotating the tail approximately 90 degrees to position the lateral tail veins dorsally.
- 8.2. Puncture the lateral tail vein using a lancet [1] and collect the blood drops using a microtube or capillary [2].
 - 8.2.1. Talent puncturing the lateral tail vein using a lancet.
 - 8.2.2. Talent collecting blood drops using a microtube or capillary.
- 8.3. Afterward, apply gentle pressure to the puncture site with a sterile swab to control bleeding [1], followed by positive reinforcement [2] and tunnel cleaning as previously demonstrated [3].
 - 8.3.1. Talent applying gentle pressure to the puncture site with a sterile swab.
 - 8.3.2. [Reuse 5.1.2](#)
 - 8.3.3. [Reuse 6.1.2](#)

Results

9. Results

9.1. Both female and male mice showed a significant reduction in mouse grimace scale score when blood sampling was performed in the application tunnel compared to the conventional restrainer [1].

9.1.1. LAB MEDIA: Figure 2A and B. *Video editor: Highlight the box plot labeled 'Tunnel'.*

9.2. In both female and male mice, a lower occurrence of audible vocalizations [1] and attempts to turn around were observed when intravenous injections were performed inside the application tunnel compared to a conventional restrainer [2].

9.2.1. LAB MEDIA: Figure 3. *Video editor: Highlight the upper-left and lower-left charts titled 'vocalization' and the bar labeled 'Restrainer'.*

9.2.2. LAB MEDIA: Figure 3. *Video editor: Highlight the upper-right and lower-right charts titled 'attempts to turn around' and emphasize bar labeled 'Restrainer'.*

1. Ethanol
Pronunciation link: <https://www.merriam-webster.com/dictionary/ethanol>
IPA: /'εθə,nɔ:l/
Phonetic Spelling: ETH·uh·nawl
2. Autoclaved
Pronunciation link: <https://www.merriam-webster.com/dictionary/autoclave>
IPA: /'ɔ:tou,kleɪvd/
Phonetic Spelling: AW·toh·klayvd
3. Syringe
Pronunciation link: <https://www.merriam-webster.com/dictionary/syringe>
IPA: /sə'rɪndʒ/
Phonetic Spelling: suh·RINJ
4. Disinfectant
Pronunciation link: <https://www.merriam-webster.com/dictionary/disinfectant>
IPA: /,dɪsɪn'fektənt/
Phonetic Spelling: dis·in·FEK·tuhnt
5. Intravenous
Pronunciation link: <https://www.merriam-webster.com/dictionary/intravenous>
IPA: /,ɪntrə'veɪnəs/
Phonetic Spelling: in·truh·VEE·nuhs
6. Lateral
Pronunciation link: <https://www.merriam-webster.com/dictionary/lateral>
IPA: /'lætərəl/
Phonetic Spelling: LAT·uh·ruhl
7. Dorsally
Pronunciation link: <https://www.merriam-webster.com/dictionary/dorsal>
IPA: /'dɔ:rsəli/
Phonetic Spelling: DOR·suh·lee
8. Distal
Pronunciation link: <https://www.merriam-webster.com/dictionary/distal>
IPA: /'dɪstəl/
Phonetic Spelling: DIS·tuhl
9. Proximal
Pronunciation link: <https://www.merriam-webster.com/dictionary/proximal>
IPA: /'prɔ:ksəməl/
Phonetic Spelling: PROK·suh·muhl
10. Bevel
Pronunciation link: <https://www.merriam-webster.com/dictionary/bevel>
IPA: /'bevəl/
Phonetic Spelling: BEV·uhl
11. Habituation
Pronunciation link: <https://www.merriam-webster.com/dictionary/habituation>
IPA: /hə,bɪtʃu'eɪʃən/
Phonetic Spelling: huh·bich·oo·AY·shuhn

12. Intraperitoneal
Pronunciation link: <https://www.merriam-webster.com/medical/intraperitoneal>
IPA: /ˌɪntrəˌpɛrətəˈniːəl/
Phonetic Spelling: in·truh·peh·ruh·tuh·NEE·uhl
13. Sacral
Pronunciation link: <https://www.merriam-webster.com/dictionary/sacral>
IPA: /ˈseɪkrəl/
Phonetic Spelling: SAY·kruhl
14. Caudal
Pronunciation link: <https://www.merriam-webster.com/dictionary/caudal>
IPA: /ˈkɔːdəl/
Phonetic Spelling: KAW·duhl
15. Abdominal
Pronunciation link: <https://www.merriam-webster.com/dictionary/abdominal>
IPA: /æbˈdɑːmənəl/
Phonetic Spelling: ab·DOM·uh·nuhl
16. Lancet
Pronunciation link: <https://www.merriam-webster.com/dictionary/lancet>
IPA: /ˈlænsət/
Phonetic Spelling: LAN·sit
17. Microtube
Pronunciation link: No confirmed link found
IPA: /ˈmaɪkroʊˌtuːb/
Phonetic Spelling: MY·kroh·toob
18. Capillary
Pronunciation link: <https://www.merriam-webster.com/dictionary/capillary>
IPA: /ˈkæpəˌlɛri/
Phonetic Spelling: KAP·uh·lair·ee
19. Grimace
Pronunciation link: <https://www.merriam-webster.com/dictionary/grimace>
IPA: /ˈɡrɪməs/, /ɡrɪˈmeɪs/
Phonetic Spelling: GRIM·uhs / grih·MAYS
20. Vocalizations
Pronunciation link: <https://www.merriam-webster.com/dictionary/vocalization>
IPA: /ˌvɒkələˈzeɪʃənz/
Phonetic Spelling: voh·kuh·luh·ZAY·shuhnz