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Title: Assessment of Knee Hyperalgesia in Mice Using Pressure Application Measurement

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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? **yes**

Videographer: Please film the screen of the instrument for the following shots labelled SCREEN, as backup

SCREEN: 2.2.1, 3.4.1, 3.4.2

- 3. Filming location:** Will the filming need to take place in multiple locations? **Possibly yes, 10 mins apart**

Current Protocol Length

Number of Steps: 11

Number of Shots: 28

Introduction

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

- 1.1. **Shingo Ishihara**: We develop translationally relevant pain assays in mice with progressive experimental knee osteoarthritis, to enhance understanding of mechanisms underlying joint pain. Humans with osteoarthritis also have lowered pain pressure thresholds.

1.1.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:3.4*

What advantage does your protocol offer compared to other techniques?

- 1.2. **Shingo Ishihara**: The method is robust and reproducible, and offers a medium-throughput approach for testing the potential analgesic effects of systemically or intra-articularly administered novel compounds.

1.2.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:4.3*

What research questions will your laboratory focus on in the future?

- 1.3. **Shingo Ishihara**: I am interested in studying anxiety associated with progressive experimental OA. We are developing assays to study the correlation of anxiety with OA pain and to enable exploring underlying mechanisms.

1.3.1. 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 Institutional Animal Care and Use Committee (IACUC) at Rush University Medical Center

Protocol

2. Setting up the Pressure Application Measurement (PAM) Device for Knee Hyperalgesia Assessment

Demonstrator: Shingo Ishihara

- 2.1. To begin, connect a cable from the PAM (*Pam*) device to the computer [1-TXT]. Connect the second cable from the device to the force transducer [2].
 - 2.1.1. WIDE: Talent connecting one cable from the PAM device to the computer. **TXT: PAM: Pressure Application Measurement**
 - 2.1.2. Talent connecting another cable from PAM device to the force transducer.
- 2.2. Now switch on the computer and launch the PAM software [1]. Set the maximum pressure value in the software to 450 grams [2].
 - 2.2.1. SCREEN: The PAM software is being launched
Videographer : Please capture a video of this shot
OR
LAB MEDIA: [2.2.1-.jpg](#)
 - 2.2.2. Shot of the instrument screen showing pressure value.
- 2.3. Next, leave the mice in the testing room for 15 to 30 minutes until they acclimatize and settle down [1-TXT]. Confirm the mouse is calm and still while restrained [2]. If it is not, return it to the cage and delay testing by a few days [3].
 - 2.3.1. Talent placing mice in the testing room and closing the door. **TXT: Ensure test room is separate from housing and is quiet**
 - 2.3.2. Shot of a calm mouse.
 - 2.3.3. Talent places a struggling mouse back into its cage.

3. Baseline Measurement of Knee Hyperalgesia Using Pressure Application Measurement

- 3.1. Cradle the mouse in the left hand, restraining the back and firmly holding the tail down with the fourth and fifth fingers [1]. Loop the index finger of the right hand through the tie of the transducer [2] and insert the finger with the transducer into a transparent plastic bag [3].
 - 3.1.1. Talent restraining the mouse, cradling it in the left hand.
 - 3.1.2. Shot of index finger of the right hand being looped through a tie of the

transducer.

3.1.3. Talent inserting the transducer-equipped finger into a transparent plastic bag.

3.2. Now, with the right thumb and middle finger, hold the right leg [1]. Gently pin the paw down onto the thenar of the left hand using the middle finger [2]. Ensure the knee is positioned at approximately 90 degrees flexion at the start of testing [3].

3.2.1. Talent gripping the mouse's right leg with right thumb and middle finger.

3.2.2. Talent pinning the paw with the middle finger.

Videographer's Note: 3.2.2 is included in 3.2.1

3.2.3. Shot of the knee joint demonstrating 90 degrees flexion.

3.3. Next, touch the lateral side of the knee with the right thumb and the medial side of the transducer with the index finger [1]. Apply pressure against the knee slowly [2].

3.3.1. Talent touches the mouse's lateral side of the knee with right thumb and the medial side of the knee with the transducer using the index finger.

3.3.2. Talent applying pressure against the knee slowly.

Videographer's Note: 3.3.2 is included in 3.3.1

3.4. Then apply increasing force at the rate of 30 grams per second [1]. Continue increasing the pressure constantly until reaching 450 grams per second [2] or until the mouse shows pain-related behaviors such as vocalization, muscle twitching, body wriggling, or whisker movement [3].

3.4.1. SCREEN: Show software graph with black line guiding force increase at 30 grams per second.

Videographer : Please capture a video of this shot

3.4.2. SCREEN: Show software graph with blue line guiding force increase till 450 grams per second.

Videographer : Please capture a video of this shot

Videographer's Note: 3.4.2 is included in 3.4.1

3.4.3. Shot of mouse behavior showing pain indicators such as twitching or vocalization.

3.5. Once the threshold has been reached, retract the finger with the transducer [1]. Record the pressure displayed on the screen [2-TXT].

3.5.1. Talent retracting finger from the mouse's knee.

3.5.2. Talent recording the pressure value. **TXT: Assign withdrawal threshold of 450 g if mouse does not show any pain**

~~3.6. Return the mouse to its cage and test the next one [1]. After testing all mice once on one knee, retest them starting from the first mouse [2]. Test each knee twice and average the two results to determine the withdrawal threshold [3].~~

~~3.6.1. Talent returning the mouse to its cage.~~

~~3.6.2. Talent repeating testing sequence from the first mouse.~~

~~3.6.3. Talent calculating the average of the 2 results.~~

Videographer's Note: 3.6 deleted at author's request

Results

4. Results

- 4.1. Intraauricular injection of Pam3CSK4 (*Pam-Three-C-S-K-Four*) in the mice resulted in knee hyperalgesia in a dose-dependent fashion, peaking 4 hours after injection and returning to baseline by 24 hours [1]. Intra-articular injection of lidocaine at the 4-hour peak reversed Pam3CSK4-induced knee hyperalgesia [2].
 - 4.1.1. LAB MEDIA: Figure 2A. *Video editor: Highlight the green squares and purple circles at the 2-hour and 24-hour timepoints*
 - 4.1.2. LAB MEDIA: Figure 2B. *Video editor: Highlight the purple circles at 4 h*
- 4.2. DMM (*D-M-M*) surgery caused pronounced knee hyperalgesia 2 weeks after surgery, slowly recovering through week 16 [1-TXT]. Sham-operated mice also developed pronounced knee hyperalgesia after surgery, but knee hyperalgesia recovered faster [2].
 - 4.2.1. LAB MEDIA: Figure 3A. *Video editor: Highlight the purple triangles* **TXT: DMM: Destabilization of the Medial Meniscus**
 - 4.2.2. LAB MEDIA: Figure 3A. *Video editor: Emphasize the white circles*
- 4.3. Intra-articular injection of lidocaine 4 weeks after DMM surgery resulted in an immediate reversal of knee hyperalgesia within 30 minutes [1], with the effect diminishing by 4 hours [2].
 - 4.3.1. LAB MEDIA: Figure 3B. *Video editor: Highlight the purple squares at the 30-minute timepoint*
 - 4.3.2. LAB MEDIA: Figure 3B. *Video editor: Highlight the purple square between 120 and 240 minutes post-injection.*

Pronunciation Guide:

1. Hyperalgesia

- Pronunciation link: <https://www.merriam-webster.com/medical/hyperalgesia>
[Merriam-Webster](#)
 - IPA: /ˌhaɪpərəˈlɛdʒə/ or /ˌhaɪ-pər-əl-ˈjē-zhə/ (per Merriam-Webster hy-per-al-ge-sia
ˌhī-pə-rəl-ˈjē-zhə) [Merriam-Webster](#)
 - Phonetic spelling: hy-per-al-jee-zhuh
-

2. Osteoarthritis

(Common term—may still be tricky.)

- Pronunciation link: Merriam-Webster entry exists (not captured via web search). For accuracy, use Oxford or HowToPronounce if needed.
(No confirmed link found in our search.)
 - IPA: /ˌɑsti.oʊərˈθraɪtɪs/
 - Phonetic spelling: oss-tee-oh-ar-THRY-tis
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3. Analgesic

(Useful term in context of "analgesic effects".)

- Pronunciation link: (No link found in search results; but Merriam-Webster has it usually.)
(No confirmed link found per our search.)
 - IPA: /ˌænəlˈdʒiːzɪk/
 - Phonetic spelling: an-uhl-JEE-zik
-

4. Intra-articular

- Pronunciation link: (Our search didn't fetch one.)
(No confirmed link found.)
 - IPA: /ˌɪntrəˌɑːrtɪˈkjʊlər/
 - Phonetic spelling: in-truh-ar-TIK-yuh-ler
-

5. Pam3CSK4 (Pam-Three-C-S-K-Four)

(A synthetic molecule—use simple alphanumeric breakdown.)

- Pronunciation link: (No standard dictionary entry.)
(No confirmed link found.)
 - IPA: /pæm θri si ɛs keɪ fɔːr/
 - Phonetic spelling: pam-three-see-ess-kay-four
-

6. DMM (Destabilization of the Medial Meniscus)

(Abbreviation with expansion.)

- Pronunciation link: (No dictionary link.)
(No confirmed link found.)

- IPA: /di ɛm ɛm/ for abbreviation; /diːˌstæbɪlɪˈzeɪʃən ʌv ðə ˈmiːdiəl məˈnɪskəs/ for full phrase
 - Phonetic spelling: dee-em-em; de-STAB-uh-li-ZAY-shun of the MEE-dee-uhl muh-NIS-kus
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7. Algesia (Root of hyperalgesia)

- Pronunciation link: (Part of larger word only.)
(No confirmed link found separately.)
 - IPA: /æɪˈdʒiːziə/
 - Phonetic spelling: al-JEE-zhuh
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8. Microscopy

- Pronunciation link: (Not found in search results but commonly in dictionaries.)
(No confirmed link found.)
 - IPA: /maɪˈkrɒskəpi/
 - Phonetic spelling: my-KROSS-koh-pee
-

9. Transducer

- Pronunciation link: (Not in search results.)
(No confirmed link found.)
 - IPA: /trænzˈdʒuːsər/
 - Phonetic spelling: tranz-DOO-ser
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10. Acclimatize (used in context "acclimatize and settle down")

- Pronunciation link: (Not in our search results.)
(No confirmed link found.)
- IPA: /əˈklaɪməˌtaɪz/
- Phonetic spelling: uh-KLY-muh-tyze