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Title: Development of an Uncomplicated Mild Traumatic Brain Injury Model Modified by Weight-Drop Method and Evidenced by Magnetic Resonance Imaging

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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? **YES**

   If **Yes**, how far apart are the locations? **1.3 km**

Current Protocol Length
Number of Steps: 25
Number of Shots: 52

Introduction

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

1.1. **Pin-Hui Kuo:** Our research focuses on developing a closed-head injury animal model that mimics neuroimaging outcomes of uncomplicated mild traumatic brain injury. We aim to determine whether different impact parameters in rmTBI lead to distinct imaging, behavioral, and pathological changes.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.2.1*

What significant findings have you established in your field?

1.2. **Tzu-Hsuan Tang:** We developed an animal model that replicates the radiological changes of uncomplicated mTBI, demonstrating significant behavioral deficits and long-term brain atrophy.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.8.1*

What research gap are you addressing with your protocol?

1.3. **Pin-Hui Kuo:** This work connects cross-sectional human studies with animal pathology research, providing translational and longitudinal neuroimaging assessments to better understand mTBI progression.

1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.13.1*

What new scientific questions have your results paved the way for?

1.4. **Tzu-Hsuan Tang:** Our results open new avenues for investigating disease progression and outcome changes after uncomplicated mTBI. They also highlight the crucial role of injury parameters in shaping post-injury outcomes.

1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 4.1.1*

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

1.5. **Pin-Hui Kuo:** Our lab focuses on pediatric and adolescent mTBI, the largest patient cohort. We'll apply our controllable CHI model to study developmental brain injury

and track long-term changes using MRI, behavioral, and neuropathological assessments.

1.5.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 4.1.2*

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 the National Yang Ming Chiao Tung University

Protocol

2. Induction of Closed-Head Injury (CHI)

Demonstrator: Pin-Hui Kuo

2.1. To begin, place the anesthetized rat on a heating pad **[1-TXT]** and secure it in the stereotaxic frame using a tooth bar **[2]**.
- 2.1.1. WIDE: Talent placing the rat on the heating pad. **TXT: Anesthesia: 2% Isoflurane with medical air (2.5 - 3 L/min)**
- 2.1.2. Talent securing the rat in the stereotaxic frame using the tooth bar.

2.2. Position the ear bars to stabilize the head **[1]**. Ensure the rat is centered and symmetrical in the stereotaxic frame **[2]**.
- 2.2.1. Talent positioning the ear bars on the rat.
- 2.2.2. Shot of the rat centered in the stereotaxic frame.

2.3. Now, attach the sensor of the pulse oximeter to the hind paw to monitor respiration rate, heart rate, blood oxygen level, and body temperature **[1]**. Then, inject 1 milliliter per kilogram body weight of lidocaine subcutaneously into the rat's neck as an analgesic **[2-TXT]**.
- 2.3.1. Talent attaching the pulse oximeter sensor to the rat's hind paw and inserting the rectal probe.
- 2.3.2. Talent injecting lidocaine into the rat's neck. **TXT: lidocaine: 20 mg/mL**

2.4. Apply hair removal cream to the head **[1]**. After 3 minutes, wipe off the cream using 70% isopropyl alcohol swabs **[2]**.
- 2.4.1. Talent applying hair removal cream to the rat's head. **Videographer's NOTE**: scene 2.4.1 is slated 2.4.2 .
- 2.4.2. Talent wiping off the cream using alcohol swabs. **Videographer's NOTE**: scene 2.4.2 is now slated 2.4.3.

2.5. Clean the shaved area multiple times using a sterile cotton swab soaked in iodine **[1]**. Then, remove the iodine residue with a cotton swab soaked in 70% ethanol **[2]**.
- 2.5.1. Talent cleaning/wiping the shaved area with iodine soaked swab.
- 2.5.2. Talent removing/wiping off the iodine residue with ethanol-soaked swabs.

2.6. Using sterile surgical blade, create a midline incision approximately 2 to 2.5 centimeters in length to access the skull surface **[1]**.
- 2.6.1. Talent making a midline incision with sterile surgical blade.

2.7. Next, remove the soft tissue from the skull using a cotton pad **[1]**. Clean the skull surface with a cotton swab soaked in 0.9% saline **[2]**, followed by a dry cotton pad **[3]**.
2.7.1. Talent removing soft tissue using a cotton pad.
2.7.2. Talent cleaning the skull with saline-soaked swabs.
2.7.3. Talent dabbing the skull gently with a cotton pad.

2.8. Now, identify the bregma as the reference point to determine the impact area **[1]**.
2.8.1. Shot of talent pointing to the bregma on the skull.

2.9. After identifying the desired coordinates, cement a circular stainless steel helmet over the designated area using dental cement **[1]**. Then, remove the heating pad and pulse oximeter **[2]**. Move the stereotactic device and the rat onto the lift table under the closed-head injury impactor **[3]**.
2.9.1. Talent cementing the stainless steel helmet in place.
2.9.2. Talent removing the heating pad and pulse oximeter.
2.9.3. Talent moving the rat and stereotactic device onto the lift table under the impactor.

2.10. Elevate the rat's body using a foam sponge **[1]**. Remove the rat from the ear bars while keeping it secured on the tooth bar connected to a nose cone delivering 2% isoflurane **[2]**.
2.10.1. Shot of the foam sponge elevating the rat's body.
2.10.2. Talent removing the ear bars while maintaining the rat's position.

2.11. Ensure the rat's head and body are level in the rostral-caudal direction **[1]**.
2.11.1. Shot of the rat's head and body aligned properly.

2.12. Adjust the lift table to ensure there is no gap between the CHI *(C-H-I)* impactor and the helmet **[1]**. Then, turn off isoflurane 5 seconds before impact **[2]**.
2.12.1. Talent adjusting the lift table to position the rat correctly.
2.12.2. Talent turning off the isoflurane pump.

2.13. Now, drop a 600-gram brass weight from a height of 1 meter through a stainless steel tube **[1]** to the secured impactor with a round tip aiming at the metal helmet **[2]**.
2.13.1. Shot of the brass weight dropping through the tube.
2.13.2. Shot of the impact of the weight striking the metal helmet.

2.14. After striking, lower the lift table **[1]**. Remove the rat from the stereotaxic frame **[2]** and place it in a supine position on a heating pad **[3]**.
2.14.1. Talent lowering the lift table.
2.14.2. Talent removing the rat from the frame.
2.14.3. Talent placing the rat in a supine position on a heating pad.

2.15. Record the righting reflex time, which is the time when the rat attempts to move from the supine position to the prone position **[1]**. Now, re-immobilize the rat in the stereotaxic frame **[2]**.
2.15.1. Shot of the rat moving from the supine position to the prone position.
2.15.2. Talent securing the rat back into the stereotaxic frame.

2.16. Remove the helmet **[1]** and clean all the connective tissue before cementing from the skull surface **[2]**.
2.16.1. Talent removing the helmet.
2.16.2. Talent cleaning the skull using a cotton pad.

2.17. Now, cover the skull with dental cement and allow it to dry **[1]**. Using the back of a tweezer, check that the cement is stiff and hard **[2]**.
2.17.1. Talent applying dental cement to cover the skull.
2.17.2. Talent checking the dried cement hardness with the back of a tweezer.

2.18. Then, close the incision using 4-0 *(4-oh)* nylon surgical sutures with 4 to 5 independent knots **[1]** and apply topical antibiotics to the surgical site to prevent infections **[2]**. Inject 1 milliliter per kilogram body weight of carprofen subcutaneously into the neck as a post-surgery analgesic **[3-TXT]**.
2.18.1. Talent suturing the incision with 4-0 nylon surgical sutures.
2.18.2. Talent applying antibiotics to the incision site.
2.18.3. Talent injecting carprofen into the rat's neck. **TXT: Carprofen: 50 mg/mL**

2.19. Place the rat in a clean cage on a heating pad until it regains consciousness **[1]**. Once the rat sits upright, return it to the home cage **[2]**.
2.19.1. Talent placing the rat in a clean cage with a heating pad.
2.19.2. Shot of the rat regaining consciousness and being taken out.

2.20. Orally administer 5 milliliters of acetaminophen mixed in 200 milliliters of water daily for 3 consecutive days as an analgesic **[1-TXT]**.
2.20.1. Talent administering acetaminophen solution to the rat. **TXT: Acetaminophen: 24 mg/mL**

3. Preparation for MRI of the Rat Brain

3.1. Position the anesthetized rat in the head holder **[1-TXT]** and connect to a nose cone for maintenance of anesthesia **[2-TXT]**.
3.1.1. Talent securing the rat in the head holder. **TXT: Anesthesia induction: 5% Isoflurane and medical air (2.5 - 3 L/min)**
3.1.2. Talent connecting the nose cone to the rat. **TXT: Anesthesia maintenance: 2% Isofluraneand medical air (1.5 - 2 L/min)**

3.2. Fixate the head with a small piece of tape to prevent movement during scanning **[1]**.
   3.2.1. Talent applying tape to secure the rat's head.

3.3. Then, place a pressure pad under the thorax to monitor respiration **[1]**, insert the electrodes and tape the oximeter clips to the hind limb to check the heart rate **[2]**.
   3.3.1. Talent positioning the pressure pad under the rat's thorax.
   3.3.2. Talent inserting the needle electrodes to the rat's limbs. **NOTE**: The action was modified

3.4. Insert the rectal probe to measure the rectal temperature **[1]**. Cover the rat with a heating blanket with circulating warm water and tissue wrap to maintain body temperature during the experiment **[2]**.
   3.4.1. Talent inserting the rectal probe.
   3.4.2. Talent covering the rat with a heating blanket and tissue wrap. **Videographer's NOTE**: scene 3.4.2 was shot separately, and not at the time of shooting 3.4.1

3.5. Use the laser positioning system of the PET/MR *(Pet-M-R)* scanner to mark the center of the head for precise alignment **[1]**. Move the rat into the MRI bore using the motorized animal transport system until the center of the head aligns with the iso-center of the scanner **[2]**. Finally, obtain the MRI images and analyse them **[3]**.
   3.5.1. Shot of the screen showing Laser positioning system marking the center of the rat's head.
   3.5.2. Shot of the screen showing the motorized animal transport system moving the rat into the MRI bore.
   3.5.3. Talent working at the MRI station.

Results

4. Representative Results

4.1. No significant skull fracture, brain contusion, white matter edema, or deformation was observed in T2-weighted images and fractional anisotropy maps at 1 **[1]** and 50 days post-CHI, confirming minimal structural damage in the CHI model **[2]**.

4.1.1. LAB MEDIA: Figure 2. *Video editor: Highlight the images for "SMCX/2hits" corresponding to day 1*.

4.1.2. LAB MEDIA: Figure 2. *Video editor: Highlight the images for "SMCX/2hits" corresponding to day 50*.

4.2. A significant reduction in cortical volume was observed at 50 days post-CHI and repetitive CHI resulted in greater cortical loss compared to single CHI **[1]**. The most substantial cortical volume reduction was noted in slices at Bregma minus 4 to plus zero and Bregma minus 5 to plus 1 after repetitive CHI with different impact parameters **[2]**. A significantly smaller cortical volume was observed at Bregma 0 after central brain CHI compared to SMCx *(S-M-C-X)* CHI **[3]**.

4.2.1. LAB MEDIA: Figure 4C.

4.2.2. LAB MEDIA: Figure 4C. *Video editor: Highlight dark grey and black bars from -5 to +1.*

4.2.3. LAB MEDIA: Figure 4C. *Video editor: Highlight blue bar corresponding to "0" on X-axis*

4.3. Immunostaining at day 50 post-injury demonstrated astrocyte accumulation in the ipsilesional SMCx, irrespective of CHI severity and impact site **[1]**.

4.3.1. LAB MEDIA: Figure 6. *Video editor: Sequentially Highlight the 3 images for "SMCx" and then the image "central"*

Pronunciation guide

1. Stereotaxic

Pronunciation link:
https://www.merriam-webster.com/dictionary/stereotaxic
IPA: /ˌsterēəˈtaksɪk/
Phonetic Spelling: stair-ee-oh-tak-sik

2. Isoflurane

Pronunciation link:
https://www.merriam-webster.com/dictionary/isoflurane
IPA: /ˌaɪsəˈflʊreɪn/
Phonetic Spelling: eye-suh-floo-rayn

3. Lidocaine

Pronunciation link:
https://www.merriam-webster.com/dictionary/lidocaine
IPA: /ˈlaɪdəˌkeɪn/
Phonetic Spelling: lie-duh-kayn

4. Bregma

Pronunciation link:
https://www.howtopronounce.com/bregma
IPA: /ˈbrɛgmə/
Phonetic Spelling: breg-muh

5. Carprofen

Pronunciation link:
https://www.howtopronounce.com/carprofen
IPA: /ˈkɑːrproʊfɛn/
Phonetic Spelling: kar-pro-fen

6. Acetaminophen

Pronunciation link:
https://www.merriam-webster.com/dictionary/acetaminophen
IPA: /əˌsiːtəˈmɪnəfən/
Phonetic Spelling: uh-see-tuh-min-uh-fen

7. MRI (Magnetic Resonance Imaging)

Pronunciation link:
https://www.merriam-webster.com/dictionary/MRI
IPA: /ˌɛmˌɑːrˈaɪ/
Phonetic Spelling: em-ar-eye

8. Iso-center

Pronunciation link:
https://www.howtopronounce.com/isocenter
IPA: /ˈaɪsoʊˌsɛntər/
Phonetic Spelling: eye-so-sen-ter

9. Anisotropy (as in "fractional anisotropy")

Pronunciation link:
https://www.merriam-webster.com/dictionary/anisotropy
IPA: /ˌænəˈsɑːtrəpi/
Phonetic Spelling: an-uh-sah-truh-pee

10. Contusion

Pronunciation link:
https://www.merriam-webster.com/dictionary/contusion
IPA: /kənˈtuːʒən/
Phonetic Spelling: kuhn-too-zhun

11. Edema

Pronunciation link:
https://www.merriam-webster.com/dictionary/edema
IPA: /ɪˈdiːmə/
Phonetic Spelling: ih-dee-muh

12. Ipsilesional

Pronunciation link:
https://www.howtopronounce.com/ipsilesional
IPA: /ˌɪpsɪˈliːʒənəl/
Phonetic Spelling: ip-sih-lee-zhuh-nuhl

13. Astrocyte

Pronunciation link:
https://www.merriam-webster.com/dictionary/astrocyte
IPA: /ˈæstrəˌsaɪt/
Phonetic Spelling: as-troh-syte