FINAL SCRIPT: APPROVED FOR FILMING

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Title: Administration of Δ^9-Tetrahydrocannabinol (THC) in Adolescent and Adult Mice

Authors and Affiliations:

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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? **Very close. They are separate rooms in the same floor of the same building.**

Current Protocol Length
Number of Steps: 24
Number of Shots: 36

Introduction

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

REQUIRED:

1.1. **Daniele Piomelli:** We study the body's own cannabis—the so-called endocannabinoid system— and one of our main objectives is to understand how THC—the intoxicating component in cannabis—interacts with this system.

1.1.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: LAB MEDIA: Figure 1.*

What are the current experimental challenges?

1.2. **Kwang-Mook Jung:** One major challenge in working on cannabis is to design animal experiments that are relevant to the human condition. Factors like dose and route of administration are important.

1.2.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 3.2.2.*

What significant findings have you established in your field?

1.3. **Kwang-Mook Jung:** Using mice, we found that THC exposure during adolescence has pronounced effects on adult physiology, even long after exposure is stopped. For example, it alters how the brain responds to social stress.

1.3.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: LAB MEDIA: Figure 6C, 6D*.

What advantage does your protocol offer compared to other techniques?

1.4. **Alex Mabou Tagne:** This article describes a protocol for preparing THC for parenteral administration and assessing its acute pharmacodynamic effects in mice of both sexes at two developmental stages: adolescence and young adulthood.

1.4.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: LAB MEDIA: Figure 4.*

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

1.5. **Alex Mabou Tagne:** Understanding how age, genetics, and health conditions affect THC responses can guide targeted prevention and treatment. Notably, adolescent cannabis use requires further study, as exposure during this critical neurodevelopmental phase may cause lasting negative effects.

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.

Testimonial Questions:

Videographer: Please capture all testimonial shots in a wide-angle format with sufficient headspace, as the final videos will be rendered in a 1:1 aspect ratio. Testimonial statements will be presented live by the authors, sharing their spontaneous perspectives.

How do you think publishing with JoVE will enhance the visibility and impact of your research?

1.6. **Daniele Piomelli, Distinguished professor of anatomy and neurobiology, University of California, Irvine**: (authors will present their testimonial statements live)

1.6.1. 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 (IACUC) of the University of California, Irvine

Protocol

2. Procedure for THC Administration in Mice

Demonstrator: Hye-Lim Lee

2.1. To begin, use a pipette to withdraw an appropriate volume of tetrahydrocannabinol or THC *(T-H-C)* based on the dose in milligrams per kilogram and the body weight of the mice in grams, keeping the container on ice **[1]**. Carefully add the solution to the bottom of an 8 milliliter glass vial **[2]**.

2.1.1. WIDE: Talent withdrawing tetrahydrocannabinol using a pipette while keeping the container on ice.

2.1.2. Talent dispensing tetrahydrocannabinol to the bottom of an 8 milliliter glass vial.

2.2. After moving the vial to a chemical fume hood, gently evaporate the solvent to dryness using a gentle stream of nitrogen gas **[1]**.

2.2.1. A shot of the nitrogen gas stream directed at the vial as the solvent evaporates. **TXT: Ensure the solution does not splash on the sides of the vial**

2.3. Record the amount of THC used in the Controlled Substance I *(one)* Usage Log located in the Controlled Substance Binder **[1]**.

2.3.1. Talent recording the amount of tetrahydrocannabinol used into the Controlled Substance I Usage Log.

2.4. Once the solvent is completely dried, add the volume of Tween-80 *(eighty)* required to achieve a final 5 percent concentration in saline **[1]**.

2.4.1. Talent pipetting Tween-80 into the dried vial.

2.5. Warm the vial in a beaker containing hot water at or below 60 degrees Celsius **[1]**. Vortex the vial to fully dissolve THC and repeat the process if needed **[2]**.

2.5.1. Talent placing the vial into a beaker of hot water on a heating plate.

2.5.2. Talent vortexing the vial.

2.6. Now, gradually add sterile saline in small increments **[1-TXT]**. Vortex **[2]** and heat the vial between each addition **[3]**.

2.6.1. Talent pipetting and adding sterile saline into the vial. **TXT: Volume of saline added: 200 µL, 300 µL, 500 µL, and 1 mL**

2.6.2. Talent vortexing the vial.

2.6.3. Talent heating the vial.

2.7. Repeat these steps until the required volume of saline has been added to make a final THC solution in 5% Tween-80 and 95% saline, and the solution is fully dissolved **[1]**.

2.7.1. CU: A shot of the homogenous final tetrahydrocannabinol solution in 5% Tween-80 and 95% saline.

2.8. Sonicate the vial in an ultrasonic water bath set to approximately 100 percent power at 40 kilohertz and 37 degrees Celsius for 5 minutes **[1]**. Confirm that the solution is clear and free from any undissolved material **[2-TXT]**.

2.8.1. Talent placing the vial in the sonicator.

2.8.2. Close-up of the clear tetrahydrocannabinol solution after sonication. **TXT: The color of the liquid may vary depending on the vehicle used**

2.9. Next, weigh the mouse to calculate the appropriate volume of THC solution to inject, based on 10 milliliters per kilogram per mouse **[1-TXT]**.

2.9.1. Talent placing the mouse on a digital scale. **TXT: Freshly prepare the solutions on the day of use**

3. Assessing the Pharmacological Effects of THC: Catalepsy Test

Demonstrator: Heidi C. Avalos

3.1. Prepare a horizontal bar 7 centimeters in length and elevated 4 centimeters above the surface of a clean lab bench under ambient illumination of 160 lux **[1]**. Place an electronic timer within reach **[2]**.

3.1.1. A shot of the horizontal bar apparatus on a clean lab bench.

3.1.2. Talent placing the electronic timer next to the test setup.

3.2. Using clean latex gloves, remove the mouse from the cage of the testing lab **[1]**, administer the calculated dose of THC or vehicle **[2]**, and return it to the cage **[3]**.

3.2.1. Talent wearing latex gloves, removing the mouse from the cage. **TXT: Move the mouse to this cage 60 min before testing; Wear a clean lab coat and no perfume**

3.2.2. Talent injecting tetrahydrocannabinol into the mouse.

3.2.3. Talent placing the mouse back into its cage.

3.3. After approximately 45 minutes post-injection, gently place the mouse's front paws on the horizontal bar **[1]**.

3.3.1. Talent gently positioning the mouse with its front paws resting on the horizontal bar.

3.4. Start the electronic timer to record the duration of immobility **[1]**. Stop the timer when the mouse removes one or both paws or when 10 seconds have passed **[2-TXT]**.

3.4.1. Talent starting the timer.

3.4.2. Talent stopping the timer and the timer reading showing 10 seconds have passed. **TXT Repeat the test 2x per mouse; Rest interval between trials: 2 min**

3.5. After repeating the test, use the average of the two immobility times, measured in seconds, as the indicator of catalepsy for final data analysis **[1]**.

3.5.1. Talent calculating the average immobility times on a spreadsheet. *Videographer: Please film the screen for this shot.*

4. Assessing the Pharmacological Effects of THC: Open-Field Test

Demonstrator: Dominick D'Agosta

4.1. For the open-field test, use an arena equipped with video tracking equipment, ensuring it is clean and free of any odor cues **[1]**.

4.1.1. A shot of the open-field arena.

4.2. Approximately 30 minutes after injecting THC, gently place the mouse in the center of the arena, minimizing handling stress **[1]**.

4.2.1. Talent placing the mouse in the center of the open-field arena with a gentle motion.

4.3. Record a 10-minute session using a camcorder and behavioral tracking software **[1]**.

4.3.1. SCREEN: 4_3_1_Control_VEH.mp4 00:00-00:20 and 4_3_1_10-mgkg-THC.mp4 00:15-00:35. *Video Editor: Play the two videos side by side and for the video 4_3_1_Control_VEH.mp4, add the label "Control" and for the video 4_3_1_10-mgkg-THC.mp4 add the label "THC-induced" on the top or bottom (whichever looks better).*

4.4. At the end of the test, return the mouse to its home cage **[1]** and clean the open-field arena thoroughly with the cleaning solution **[2]**.

4.4.1. Talent gently placing the mouse back into its home cage.

4.4.2. Talent spraying and wiping down the arena with a cleaning solution.

4.5. Calculate the total distance traveled, measured in meters, as the primary parameter to quantify locomotor activity **[1]**.

4.5.1. Talent observing or analysing a table showing the total distance traveled by the mouse in meters.

5. Assessing the Pharmacological Effects of THC: Tail Immersion Test

Demonstrator: Alex Mabou Tagne

5.1. For the tail immersion test, prepare a water bath set to 54 degrees Celsius under ambient lighting of 160 lux **[1]**. Position an electronic timer nearby and ready for use **[2]**.

5.1.1. A shot of the water bath that is used for the tail immersion test.

5.1.2. Talent placing an electronic timer beside the water bath.

5.2. Gently restrain the mouse in a soft tissue pocket made from pet training pads, leaving the tail accessible **[1]**.

5.2.1. Talent wrapping the mouse securely in a soft tissue pocket with the tail exposed.

5.3. Immerse the distal one-third of the tail into the water bath **[1]** and start the timer simultaneously **[2]**.

5.3.1. Talent lowering the mouse's tail into the hot water bath.

5.3.2. Talent activating the timer.

5.4. Record the latency to tail withdrawal, defined by a reflexive tail flick, with a cutoff time of 10 seconds to prevent tissue damage **[1-TXT]**.

5.4.1. Software interface showing the tail flick latency being captured in seconds. **TXT: Repeat this 2x per mouse at 60, 180, and 300 min post-THC injection; Interval between trials: 5 min** NOTE: Videographer filmed this shot.

5.5. After repeating the test, average the two latency measurements, recorded in seconds, to calculate the primary outcome for evaluating antinociceptive effects **[1]**.

5.5.1. Talent calculating the average of the two latency measurements. *Videographer: Please film the screen for this shot.*

Results

6. Results

6.1. THC *(T-H-C)* induced dose-dependent catalepsy in both adolescent and adult male mice **[1]**, with stronger responses at 10 milligrams per kilogram **[2]**.

6.1.1. LAB MEDIA: Figure 4A.

6.1.2. LAB MEDIA: Figure 4A. *Video Editor: Highlight the bars at 10 in both "Adolescent" and "Adult" panels.*

6.2. Female mice also showed catalepsy at 10 milligrams per kilogram **[1]**, but adult females responded less than adolescents **[2]**.

6.2.1. LAB MEDIA: Figure 4B. *Video Editor: Highlight the bars at 10 in both "Adolescent" and "Adult" panels.*

6.2.2. LAB MEDIA: Figure 4B. *Video Editor: Emphasize the yellow bar at 10 when the VO says "adult females" and the blue bar at 10 when the VO says "adolescents".*

6.3. Adolescent females exhibited lower catalepsy **[1]** than males **[2]**, indicating a modest sex difference **[3]**.

6.3.1. LAB MEDIA: Figure 4C. *Video Editor: Highlight the taller red bar.*

6.3.2. LAB MEDIA: Figure 4C. *Video Editor: Highlight the taller blue bar.*

6.3.3. LAB MEDIA: Figure 4C.

6.4. In adults, males showed much higher catalepsy **[1]** than females at 10 milligrams per kilogram **[2]**.

6.4.1. LAB MEDIA: Figure 4D. *Video Editor: Highlight the taller blue bar.*

6.4.2. LAB MEDIA: Figure 4D. *Video Editor: Highlight the taller red bar.*

6.5. THC reduced locomotor activity in male mice **[1]**, with adolescents being more sensitive than adults **[2]**.

6.5.1. LAB MEDIA: Figure 5A.

6.5.2. LAB MEDIA: Figure 5A. *Video Editor: Emphasize the "Adolescent" panel.*

6.6. Female mice showed reduced movement at higher doses, with overall significant effects of both dose and age **[1]**.

6.6.1. LAB MEDIA: Figure 5B.

6.7. THC increased tail flick latency in adolescent males and females at 5 and 10 milligrams per kilogram **[1]**.

6.7.1. LAB MEDIA: Figure 6A and 6B. *Video editor: Highlight 6A when the VO says males and 6B when the VO says females.*

6.8. In adults, antinociceptive effects were limited to the highest dose at 60 minutes post-injection **[1]**.

6.8.1. LAB MEDIA: Figure 6C and 6D. *Video Editor: Highlight the blue bars at 10.*

6.9. At 300 minutes, only adolescent mice still showed antinociceptive effects **[1]**.

6.9.1. LAB MEDIA: Figure 6F. *Video editor: Highlight the tallest red and blue bars at 10. Make sure to include the legends that indicate which color corresponds to which bar.*