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Title: An Olfactory Preference Test for Measuring Olfactory Hedonic Biases in Mouse Models of Depression

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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, all done**

3. **Filming location:** Will the filming need to take place in multiple locations? **Yes, 300 m apart**

Current Protocol Length
Number of Steps: 16
Number of Shots: 33

Introduction

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

1.1. **Mariana Alonso:** A negative bias in emotional processing is a major characteristic of depressive disorders. This bias is particularly interesting to better understand the physiopathology of the disease.

1.1.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:3.3.1*

What advantage does your protocol offer compared to other techniques?

1.2. **Claire-Hélène de Badts**: Here, we propose a protocol for the reliable assessment of innate odor valuation, using odors associated with various valence, in both male and female mouse models of depression.

1.2.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:2.9*

How will your findings advance research in your field?

1.3. **Claire-Hélène de Badts**: Assessing hedonic bias in mouse models is essential, both to better evaluate the induced depressive-like phenotype, and as a tool to investigate neural mechanisms behind these changes in valence assignment.

1.3.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:3.3.2*

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

1.4. **Mariana Alonso**: Overall, this protocol provides new avenues for translational research to understand the mechanisms underlying depression and treatment efficacy.

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

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

1.5. **Claire-Hélène de Badts:** In the future, we will try to decipher the brain circuits behind this disrupted emotional processing, and potential sex-specific mechanisms, along with the action of antidepressants onto these pathways.

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

All animal care and experimental procedures followed national and European (2010/63/EU) guidelines and were approved by the French Ministry of Research (APAFIS #51636-2024101718013761 v4)

Protocol

2. Mouse Odor Preference Test to Measure Negative Bias in Depression Models

Demonstrator: Claire-Hélène de Badts

2.1. To begin, prepare the experimental setup using a two-compartment arena measuring 45 centimeters by 50 centimeters by 25 centimeters, with grey plexiglass walls and a white floor **[1]**. Divide the arena into two equal compartments of 25 centimeters by 45 centimeters using a grey plexiglass wall measuring 37 centimeters by 25 centimeters **[2]**.

2.1.1. WIDE: Talent assembling the two-compartment arena, showing the measurements. AUTHOR'S NOTE: Use 2sd part + CU

2.1.2. Talent placing the central grey plexiglass divider.

2.2. Secure perforated Petri dish with adhesive and place them at the bottom of the wall in each compartment **[1]**. Then use dual-sided adhesive or Blu-tack to fixate each Petri dish vertically onto the wall of the arena **[2]**.

2.2.1. Talent applying adhesive to secure perforated Petri dishes and aligning them against the wall of each compartment

2.2.2. Talent aligning them against the wall using dual-sided adhesive or Blu-tack. AUTHOR'S NOTE: Use take 2

2.3. Next, position a camera two meters above the arena to record the behavior of the animals and enable automatic tracking of their movements throughout the experiment **[1]**. Now, divide the mice into at least two experimental groups, the control mice and test group mice **[2]**.

2.3.1. Talent mounting a camera above the arena and adjusting its angle for overhead recording.

2.3.2. Talent labeling cages and separating mice into two experimental groups. AUTHOR's NOTE: MED + CU

2.4. Transfer each mouse to the behavioral testing room thirty minutes before the experiment begins **[1-TXT]**. Using a luxmeter, set the lighting in the arena to approximately 40 lux and verify that the illumination is evenly distributed **[2]**.

2.4.1. Talent gently moving a mouse cage into the behavioral testing room and placing it on a table. **TXT: Ensure standard conditions are maintained (23 ± 1 °C; humidity: 40%) and that the room is free from sound, lighting and odor**

2.4.2. Talent using a luxmeter to measure and adjust lighting evenly across the arena.

2.5. Next, add a clean filter paper measuring 42.5 millimeters into each of the two Petri dishes in the arena **[1]**. ~~Use transparent tape to secure the lid of each Petri dish to its base to maintain vertical placement **[2].**~~

AUTHOR'S NOTE: Move 2.5 after 2.1

~~2.5.1.~~ Talent placing a clean piece of filter paper into each Petri dish.

2.5.2. ~~Talent taping down the lid of each Petri dish to keep it attached while vertical.~~

NOTE: 2.5.2: Deleted by authors

2.6. On the first day, gently place all mice from the same housing cage together into the arena for 10 minutes to reduce neophobia **[1]**.

2.6.1. Talent releasing multiple mice from a single housing cage into the arena simultaneously.

2.7. On the day of testing, prepare in a glass vial, the odor under a chemical hood using the correct dilution **[1]**. Dilute the odorant in mineral oil or distilled water, depending on its chemical properties **[2]**. Then store the mineral oil separately in a ventilated room to avoid contamination **[3-TXT]**.

2.7.1. Talent setting up the chemicals in a chemical hood.

~~2.7.2.~~ Talent adding either mineral oil or distilled water to the solution. AUTHOR'S NOTE: Take 1 add odor, take 2 add up oil

2.7.3. ~~Talent placing the mineral oil bottle inside a separate ventilated cabinet. **TXT: Use a new bottle of mineral oil for each new experiment**~~

Added shot: 2.7.4: Set the glass vial

~~2.8. Store the prepared odorant solution in a glass vial to prevent contamination from plastic materials **[1].**~~

2.8.1. ~~Talent pouring the prepared solution into a clean glass vial and sealing it. AUTHOR'S NOTE: Shot deleted.~~

2.9. Just before testing each mouse, use a micropipette with a filter tip to collect 200 microliters of the odor solution under the hood **[1]**. Dispense the solution onto the filter paper in one of the Petri dishes **[2]**. Leave the second filter paper in the other Petri dish untreated **[3]**.

2.9.1. Talent using a micropipette with a filter tip to draw the odor solution.

AUTHOR'S NOTE: mix take 1 and 2

2.9.2. Talent placing the solution onto the filter paper inside one of the Petri dishes.

AUTHOR'S NOTE: Filmed with wth 2.9.1

2.9.3. Shot of the untouched filter paper in the control Petri dish.

2.10. Transfer the Petri dishes to the testing room **[1].** Then vertically fix them onto the wall of the arena **[2]**.

2.10.1. Talent carrying the Petri dishes into the testing room. AUTHOR'S NOTE:

Use2nd part

2.10.2. Talent securing the Petri dishes onto the wall in the arena. AUTHOR's NOTE: MED + CU

2.11. Place each mouse individually into the arena at the center, between the two compartments **[1]**. Record the behavior for 10 minutes using the overhead camera **[2]**. Remove the mouse from the arena after the observation period ends **[3]**.

2.11.1. Talent gently introducing a single mouse at the center point between the compartments.

2.11.2. Shot of the Mouse exploring both compartments of the arena as the overhead camera records. AUTHOR'S NOTE: Filmed with 2.11.1

2.11.3. Talent lifting the mouse out of the arena using both hands. AUTHOR'S NOTE: Use last one

2.12. Remove the odor Petri dish from the arena **[1]**. Then clean the arena using a 70 percent ethanol disinfectant solution and dry it thoroughly **[2]**.

2.12.1. Talent detaching and removing the Petri dish that contained the odor sample.

2.12.2. Talent spraying the arena walls and floor with ethanol and wiping it dry with a paper towel.

2.13. Discard the used filter paper **[1]** and prepare the next odor sample for the next mouse **[2-TXT]**.

2.13.1. Talent throwing away the filter paper.

2.13.2. Talent adding 2nd odor to a new filter paper. **TXT: Ideally test 1 odor/day starting from neutral then positive and negative odors**

2.14. Use an automatic tracking software to record the mouse's position throughout the test **[1]**. Define distinct zones within the arena for analysis **[2]**.

2.14.1. SCREEN: 68391_Screenshot_1.mp4 00:02-00:20

2.14.2. SCREEN: 68391_Screenshot_2.mp4. 00:02-00:17,00:38-00:42

Results

3. Results

3.1. During habituation, all mice spent at least 50 seconds in both the odor and control zones, indicating adequate task engagement **[1]**. Importantly mice exposed to UCMS *(U-C-M-S)* spent the same time in the odor zone than controls, although some difference could be observed in other zones **[2-TXT].**

3.1.1. LAB MEDIA: Figure 2C*. Video editor: Highlight the bars showing time in the odor and control zones*

3.1.2. LAB MEDIA: Figure 2C. Video editor: Highlight the red bar for UCMS in the control zone **TXT: UCMS: Unpredictable Chronic Mild Stress**

3.2. Control female mice moved significantly more than UCMS females when exposed to male urine, but not during habituation or TMT *(T-M-T)* exposure **[1-TXT].**

3.2.1. LAB MEDIA: Figure 2D. *Video editor: Highlight the gray bars in the MU section* **TXT: TMT: 2,4,5-trimethylthiazole**

3.3. All readouts of hedonic response show significant differences or a statistical tendency between Control and UCMS females **[1]**. This effect is also visible through the global odor exploration index, which is a combined measure of hedonic and locomotor response **[2]**.

3.3.1. LAB MEDIA: Figure 2E–G *Video editor: Highlight the red UCMS bars*

3.3.2. LAB MEDIA: Figure 2H. *Video editor: Highlight the red UCMS bars*

3.4. The odor exploration index positively correlated with the emotionality score for both male urine and TMT in female mice **[1]**.

3.4.1. LAB MEDIA: Figure 2I. *Video editor: Sequentially highlight the two trend lines in the scatter plot, showing upward slopes for both*

3.5. In male mice, no differences were observed during habituation or in total distance moved through the test **[1]**. The time spent in the odor zone shows a significant reduction of female urine and TMT exploration in UCMS compared to Controls **[2]**.

3.5.1. LAB MEDIA: Figure 3C,D.

3.5.2. LAB MEDIA: Figure 3E *Video editor: Please highlight the red bars*

3.6. UCMS mice enter significantly less in the odor zone, or tend to, and present a significantly reduced preference index for both female urine and TMT **[1]**.

3.6.1. LAB MEDIA: Figure 3F, G. *Video editor: Please highlight the red bars*

3.7. The odor exploration index shows a negatively shifted behavioral response to female urine and TMT of UCMS mice compared to Controls **[1]** which tend to be positively correlated with the animals' emotionality score **[2]**.

3.7.1. LAB MEDIA: Figure 3H *Video editor: Please highlight the red bars*

3.7.2. LAB MEDIA: Figure 3I *Video editor: Sequentially highlight the upward trend lines in the pink (FU) and blue (TMT) sections.*

Pronunciation Guide:

1. Olfactory

- **Pronunciation link**: https://www.merriam-webster.com/dictionary/olfactory
- **IPA**: /ˈɑːlˌfæktəri/ or /ˈoʊlˌfæktəri/
- **Phonetic Spelling**: AWL-fak-tuh-ree or OHL-fak-tuh-ree

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2. Hedonic

- **Pronunciation link**: https://www.merriam-webster.com/dictionary/hedonic
- **IPA**: /hɪˈdɑːnɪk/
- **Phonetic Spelling**: hi-DAH-nik

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3. Valence

- **Pronunciation link**: https://www.merriam-webster.com/dictionary/valence
- **IPA**: /ˈveɪləns/
- **Phonetic Spelling**: VAY-luhns

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4. Neophobia

- **Pronunciation link**: https://www.merriam-webster.com/dictionary/neophobia
- **IPA**: /ˌniːəˈfoʊbiə/
- **Phonetic Spelling**: nee-uh-FOH-bee-uh

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5. Micropipette

- **Pronunciation link**: https://www.howtopronounce.com/micropipette
- **IPA**: /ˌmaɪkroʊpaɪˈpɛt/
- **Phonetic Spelling**: MY-kroh-pie-PET

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6. Petri

- **Pronunciation link**: https://www.merriam-webster.com/dictionary/Petri
- **IPA**: /ˈpiːtri/ or /ˈpɛtri/
- **Phonetic Spelling**: PEE-tree or PEH-tree

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7. Luxmeter

- **Pronunciation link**: https://www.howtopronounce.com/luxmeter
- **IPA**: /ˈlʌksˌmiːtər/
- **Phonetic Spelling**: LUKS-mee-ter

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8. TMT (2,4,5-trimethylthiazole)

- **Pronunciation link**: https://www.howtopronounce.com/2-4-5-trimethylthiazole
- **IPA**: /ˌtraɪˌmɛθəlˈθaɪəzoʊl/
- **Phonetic Spelling**: try-METH-uhl-THY-uh-zohl

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9. Amygdala

- **Pronunciation link**: https://www.merriam-webster.com/dictionary/amygdala
- **IPA**: /əˈmɪgdələ/
- **Phonetic Spelling**: uh-MIG-duh-luh

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10. Neurogenesis

- **Pronunciation link**: https://www.merriam-webster.com/dictionary/neurogenesis
- **IPA**: /ˌnʊroʊˈdʒɛnəsɪs/
- **Phonetic Spelling**: noo-roh-JEN-uh-sis

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11. Anhedonia

- **Pronunciation link**: https://www.merriam-webster.com/dictionary/anhedonia
- **IPA**: /ˌænhiˈdoʊniə/
- **Phonetic Spelling**: an-hee-DOH-nee-uh

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12. Corticosterone

- **Pronunciation link**: https://www.merriam-webster.com/dictionary/corticosterone
- **IPA**: /ˌkɔːrtɪˈkɔːstərəʊn/
- **Phonetic Spelling**: kor-ti-KOR-stuh-rohn

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13. Chemogenetic

- **Pronunciation link**: https://www.howtopronounce.com/chemogenetic
- **IPA**: /ˌkɛmoʊdʒəˈnɛtɪk/
- **Phonetic Spelling**: KEM-oh-jeh-NET-ik

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14. Basolateral

- **Pronunciation link**: https://www.howtopronounce.com/basolateral
- **IPA**: /ˌbeɪsoʊˈlætərəl/
- **Phonetic Spelling**: BAY-soh-LAT-uh-ruhl

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15. Amygdala

- **Pronunciation link**: https://www.merriam-webster.com/dictionary/amygdala
- **IPA**: /əˈmɪgdələ/
- **Phonetic Spelling**: uh-MIG-duh-luh

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16. Nucleus Accumbens

- **Pronunciation link**: https://www.howtopronounce.com/nucleus-accumbens
- **IPA**: /ˈnjuːkliəs əˈkʌmbənz/
- **Phonetic Spelling**: NOO-klee-uhs uh-KUM-benz

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17. Paraventricular

- **Pronunciation link**: https://www.howtopronounce.com/paraventricular
- **IPA**: /ˌpærəˈvɛntrɪkjələr/
- **Phonetic Spelling**: PAR-uh-VEN-trik-yuh-lur

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18. Thalamus

- **Pronunciation link**: https://www.merriam-webster.com/dictionary/thalamus
- **IPA**: /ˈθæləməs/
- **Phonetic Spelling**: THAL-uh-muhs

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19. Neuroplasticity

- **Pronunciation link**: https://www.howtopronounce.com/neuroplasticity
- **IPA**: /ˌnʊroʊplæˈstɪsəti/
- **Phonetic Spelling**: noo-roh-plas-TIS-uh-tee

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20. Chemogenetics

- **Pronunciation link**: https://www.howtopronounce.com/chemogenetics
- **IPA**: /ˌkɛmoʊdʒəˈnɛtɪks/
- **Phonetic Spelling**: KEM-oh-jeh-NET-iks