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Title: Sucrose Preference and Novelty-Induced Hypophagia Tests in Rats Using an Automated Food Intake Monitoring System

Authors and Affiliations:

Martha Anna Schalla[1*], Stephanie Gladys Kühne[1*], Tiemo Friedrich[1], Vivien Hanel[1], Peter Kobelt[1], Miriam Goebel-Stengel[1,2,3], Matthias Rose[1,4], Andreas Stengel[1,3]

[1]Charité Center for Internal Medicine and Dermatology, Department for Psychosomatic Medicine, Charité-Universitätsmedizin, Corporate Member of Freie Universität Berlin, Humboldt-Universität zu Berlin and Berlin Institute of Health, Berlin, Germany
[2]Department of Internal Medicine, Helios Clinic, Rottweil, Germany
[3]Department of Psychosomatic Medicine and Psychotherapy, University Hospital Tübingen, Tübingen, Germany
[4]Department of Quantitative Health Sciences, Medical School University of Massachusetts, Worcester, MA, USA

*These authors contributed equally

Corresponding Authors:

Andreas Stengel (andreas.stengel@med.uni-tuebingen.de)

Email Addresses for Co-authors:

Martha Anna Schalla (martha.schalla@charite.de)
Stephanie Gladys Kühne (stephanie.kuehne@charite.de)
Tiemo Friedrich (tiemo.friedrich@charite.de)
Peter Kobelt (peter.kobelt@charite.de)
Miriam Goebel-Stengel (miriam.goebel-stengel@gmx.de)
Matthias Rose (matthias.rose@charite.de)
Vivien Hanel (vivien.hanel@charite.de)

Author Questionnaire

1. **Microscopy**: Does your protocol involve video microscopy, such as filming a complex dissection or microinjection technique? **No**
2. **Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **Yes, all set.**
3. **Filming location:** Will the filming need to take place in multiple locations? **No**

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Martha Schalla:** The sucrose-preference and novelty-induced-hypophagia tests are established techniques for evaluating anhedonia. Their combination with the automated intake monitoring system allows a detailed analysis with high accuracy.

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

1.2. **Vivien Hanel:** This protocol reduces the incidence of errors by integrating spillage into the data analysis, preventing hoarding and reducing unintentional fluid loss. Additionally, the protocol makes it possible to assess the intake microstructure.

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

OPTIONAL:

1.3. **Vivien Hanel:** This technique aims to meet the need for valid tests detecting anhedonia in rodents, considering the rising prevalence of depressive disorders. It can also be used to study anti-depressive effects of drugs.

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

1.4. **Martha Schalla:** When using the automated intake monitoring system, it is important to remember that it needs to be maintained and cleaned properly on a daily basis in order to provide accurate data.

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

Ethics Title Card

1.5. Procedures involving animal subjects followed the specific institutional ethics guidelines and were approved by the state authority for animal research.

Protocol

2. Sucrose Preference Test

2.1. To conduct the training, close all gates **[1]** and remove the water bottle and food container from the microbalances **[2]**. Place pre-weighed food on top of the cage and document its weight daily **[3]**. Fill a clean water bottle with about 100 milliliters of water and place it back on the hopper **[4]**.

2.1.1. Talent closing the gates.

2.1.2. Talent removing the water bottle and food container.

2.1.3. Talent placing food on top of the cage.

2.1.4. Talent putting a filled water bottle in the hopper, with the bottle clearly labeled.

2.2. Fill a second bottle with 100 milliliters of freshly made 1% sucrose solution **[1]** and place it on the hopper, making sure to mark the bottles and document their locations **[2]**. Document the start of training in the monitoring system **[3]** and open all gates **[4]**. *Videographer: This step is important!*

2.2.1. Talent filling a bottle with sucrose solution.

2.2.2. Talent placing the bottle on the hopper, with the bottle clearly labeled.

2.2.3. SCREEN: 24h.mp4. 0:07 – 0:18. *Video Editor: Speed this up as needed.*

2.2.4. Gates opening. *Videographer: Obtain multiple reusable takes of this shot because it will be reused in 2.5.5.*

2.3. Leave the gates open for 24 hours **[1]**, resulting in *ad libitum* access to both bottles **[2]**, then document the end of training **[3]** and close the gates **[4]**. Prepare fresh sucrose solution daily and make sure to clean and refill bottles every 24 hours **[5]**, switching the position of the bottles to avoid habituation **[6]**. *Videographer: This step is important!*

2.3.1. SCREEN: SPT Training (1).mp4, 0:44 – 1:00. *Video Editor: Speed this up.*

2.3.2. Cage with gates open and rats accessing the bottles. *Videographer: Obtain multiple reusable takes of this shot because it will be reused in 2.4.1 and 2.6.1.*

2.3.3. SCREEN: SPT Training (2).mp4.

2.3.4. Gates closing.

2.3.5. Talent cleaning the bottles.

2.3.6. Talent putting the bottles back on the hoppers, with the positions switched compared to shots 2.1.4 and 2.2.2.

2.4. Conduct the training for at least 48 hours **[1]**, only proceeding with testing when the sucrose preference ratios reach approximately 1 **[2]**. Twenty-four hours before the test, remove the bottle with the sucrose solution so that the rat has access to standard chow and water only **[3]**.

2.4.1. *Use 2.3.2.*

2.4.2. Talent using the Data Viewer to see the preference ratio.

2.4.3. Talent removing the sucrose bottle.

2.5. Prior to testing, prepare one fresh bottle filled with tap water and another filled with 1% sucrose **[1-TXT]**. Close all gates **[2]**, remove the bottle from the hopper, and replace it with the prepared bottles of water and sucrose **[3]**. Then, document the start of the test in the monitoring system **[4]** and open all gates **[5]**. *Videographer: This step is important!*

2.5.1. Talent filling the bottles. **TEXT: 100 mL per bottle**

2.5.2. Gates closing. *Videographer: Obtain multiple reusable takes of this shot because it will be reused in 3.6.3.*

2.5.3. Talent replacing bottles.

2.5.4. SCREEN: SPT (1).mp4. 0:36 – 0:43.

2.5.5. *Use 2.2.4.*

2.6. Leave the gates open for 60 minutes **[1]**, then document the end of the test **[2]** and close them **[3]**.

2.6.1. *Use 2.3.2.*

2.6.2. SCREEN: SPT (2).mp4. 0:03 – 0:09.

2.6.3. *Use 2.5.2.*

3. Novelty-induced Hypophagia Test

3.1. To prepare for the training period, close all gates and remove the hopper with the standard chow **[1]**. Fill a fresh hopper with a palatable snack, inserting the crackers carefully to prevent crumbling **[2]**, then place the hopper on the cage mount on top of the microbalance **[3]**.

3.1.1. Talent closing the gates and removing the chow hopper.

3.1.2. Talent filling the hopper with crackers.

3.1.3. Talent placing the hopper on the cage mount.

3.2. Document the beginning of the training in the monitoring system **[1]** and open the gates for 30 minutes so that the rat has *ad libitum* access to the snack and water **[2]**.

After 30 minutes, document the end of training **[3]** and close the gates **[4]**, then replace the snack with standard chow **[5]**.

3.2.1. SCREEN: NIH Training (1).mp4. 0:38 – 0:47. *Video Editor: Speed this up.*

3.2.2. Gates opening. *Videographer: Obtain multiple reusable takes of this shot because it will be reused in 3.5.2.*

3.2.3. SCREEN: NIH Training (2).mp4. 0:02 – 0:12. *Video Editor: Speed this up.*

3.2.4. Gates closing.

3.2.5. Talent replacing the food hopper for one with standard chow.

3.3. Repeat the training daily until a stable baseline of palatable snack intake is achieved and does not statistically differ between training days **[1]**.

3.3.1. Cage with gates open and rat eating the snack.

3.4. When ready to perform the test, prepare an empty, freshly cleaned cage without bedding or enrichment and attach it to the automated food intake monitoring system **[1]**. Place a hopper with a bottle of tap water and a hopper with a palatable snack on the cage mounts **[2]**. *Videographer: This step is important!*

3.4.1. Talent attaching fresh cage to the automated monitoring system.

3.4.2. Talent placing water and food on the cage mounts.

3.5. Remove the rat from the home cage and place it in the novel cage **[1]**. Open the gates for 30 minutes **[2]**, making sure to document the start and end of testing **[3]**. When finished, place the rat back in its home cage **[4, 5]**. *Videographer: This step is important!*

3.5.1. Talent putting the rat in the novel cage.

3.5.2. Talent opens the gates of the novel cage with rat inside.

3.5.3. SCREEN: NIH (1).mp4. 0:37 – 0:46

3.5.4. Talent exchanging the novel cage with home cage.

3.5.5. Added Rat being transferred in home cage. Video Editor: can show both 3.5.4. and 3.5.5. or just 3.5.5. as time allows

Results

4. Results: Sucrose Preference and Novelty-induced Hypophagia measurements

4.1. A sucrose preference test administered to eight rats **[1]** showed an increase in consumption of sucrose **[2]** and decrease in water intake over the testing period **[3]**. The sucrose preference ratio increased from an average of 0.8 on the first day to 0.99 on the second day **[4]**.

4.1.1. LAB MEDIA: Figure 3.

4.1.2. LAB MEDIA: Figure 3. *Video Editor: Emphasize A.*

4.1.3. LAB MEDIA: Figure 3. *Video Editor: Emphasize B.*

4.1.4. LAB MEDIA: Figure 3 C.

4.2. Sucrose intake microstructure was assessed automatically using the monitoring system **[1]**. Meal size **[2]**, meal duration **[3]**, meal frequency **[4]**, and 9 other parameters were automatically measured **[5]**.

4.2.1. LAB MEDIA: Figure 4. *Video Editor: Rearrange the 12 graphs horizontally so all of them fit on the screen without zooming out too much (maybe 2 rows and 6 columns).*

4.2.2. LAB MEDIA: Figure 4. *Video Editor: Emphasize A.*

4.2.3. LAB MEDIA: Figure 4. *Video Editor: Emphasize B.*

4.2.4. LAB MEDIA: Figure 4. *Video Editor: Emphasize E.*

4.2.5. LAB MEDIA: Figure 4.

4.3. To demonstrate the advantages of using the automated intake monitoring system **[1]**, automated measurements were compared **[2]** to those obtained using conventional manual assessments **[3]**. All measurements were different between groups, possibly due to erroneously high measurement or spillage when assessed manually **[4]**.

4.3.1. LAB MEDIA: Table 1.

4.3.2. LAB MEDIA: Table 1. *Video Editor: Emphasize the automated monitoring column.*

4.3.3. LAB MEDIA: Table 1. *Video Editor: Emphasize the manual assessment column.*

4.3.4. LAB MEDIA: Table 1.

4.4. During the training period for the novelty-induced hypophagia test, overall intake of the palatable snack steadily increased, indicating an adaptation during the first 2 to 3 days **[1]**. Similarly, meal size tended towards an increase between training days **[2]** while meal duration did not **[3]**.

4.4.1. LAB MEDIA: Figure 5. *Video Editor: Rearrange the 8 graphs horizontally so all of them fit on the screen without zooming out too much (maybe 2 rows and 4 columns).*

4.4.2. LAB MEDIA: Figure 5. *Video Editor: Emphasize A.*

4.4.3. LAB MEDIA: Figure 5. *Video Editor: Emphasize B.*

4.5. On the test day, naïve rats exposed to the snack in a novel environment **[1]** showed a palatable snack intake of about 1 gram **[2]**. Other parameters of food intake microstructure such as meal duration **[3]**, time spent in meals **[4]**, and latency to first bout were also assessed automatically **[5]**.

4.5.1. LAB MEDIA: Figure 6. *Video Editor: Rearrange the 8 graphs horizontally so all of them fit on the screen without zooming out too much (maybe 2 rows and 4 columns).*

4.5.2. LAB MEDIA: Figure 6. *Video Editor: Emphasize A.*

4.5.3. LAB MEDIA: Figure 6. *Video Editor: Emphasize B.*

4.5.4. LAB MEDIA: Figure 6. *Video Editor: Emphasize C.*

4.5.5. LAB MEDIA: Figure 6. *Video Editor: Emphasize D.*

Conclusion

5. Conclusion Interview Statements

5.1. **Martha Schalla:** When performing this protocol, document all the procedures in the comment section of the monitoring software to save the time of the beginning and the end of a session. Always document which substance is placed on which balance.

5.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: LAB MEDIA SPT (1).mp4.*

5.2. **Vivien Hanel:** Since this method is not associated with stress, the test can be repeated after an appropriate time of training in-between testing.

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

5.3. **Martha Schalla:** After establishing this technique, it was used to explore anhedonic features of different neuropeptides in undisturbed animals, expanding the knowledge of their functions.

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