Submission ID #: 62458
Scriptwriter Name: Domnic Colvin
Supervisor Name: Anastasia Gomez
Project Page Link: https://www.jove.com/account/file-uploader?src=19049738

Title: A Within-Subject Experimental Design Using an Object Location Task in Rats

Authors and Affiliations:

Gülberk Bayraktar[1,2*], Kristoffer Højgaard[1,3*§], Luc Nijssen[1,4], Tomonori Takeuchi[1,5§]

[1]Danish Research Institute of Translational Neuroscience (DANDRITE), Department of Biomedicine, Aarhus University, Denmark
[2]Universitätsklinikum Würzburg, Institut für Klinische Neurobiologie, Germany
[3]Translational Neuropsychiatry Unit, Department of Clinical Medicine, Aarhus University, Denmark
[4]Faculty of Science, Radboud University, the Netherlands
[5]Center for Proteins in Memory - PROMEMO, Danish National Research Foundation, Department of Biomedicine, Aarhus University, Denmark

Corresponding Authors:
Kristoffer Højgaard (khog@clin.au.dk)
Tomonori Takeuchi (tomonori.takeuchi@biomed.au.dk)

Email Addresses for All Authors:
gulberkbayraktar@gmail.com
l.nijssen@student.ru.nl
khog@clin.au.dk
tomonori.takeuchi@biomed.au.dk

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**

3. **Interview statements:** Considering the COVID-19-imposed mask-wearing and social distancing recommendations, which interview statement filming option is the most appropriate for your group? **Please select one**.

   ☒ Interviewees wear masks until videographer steps away (≥6 ft/2 m) and begins filming, then the interviewee removes the mask for line delivery only. When take is captured, the interviewee puts the mask back on. Statements can be filmed outside if weather permits.

4. **Filming location:** Will the filming need to take place in multiple locations? **No**

Current Protocol Length

Number of Steps: 22
Number of Shots: 48 (7 SC)

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Kristoffer Højgaard:** The object location task allows us to investigate various mechanisms of the formation or retention of spatial memory by taking advantage of the spontaneous explorative behavior in rodents **[1].**

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.3.1., 2.4.1., and 2.5.1.*

1.2. **Kristoffer Højgaard:** This protocol allows for the repetition of an object location task up to four times, helping to increase statistical power and the efficiency and output of studies that involve surgical procedures **[1]**.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 3.1., 3.3., 3.5., 8.3, and 8.4.*

Ethics Title Card

1.3. All methods described here have been approved by the Danish National Authorities in accordance with Danish and EU animal welfare legislations.

Protocol

2. Experimental Setup and Preparation of Distinct Contexts

2.1. To begin, obtain a square arena made of opaque, non-porous hard plastic with a minimum 60-centimeter width and 50-centimeter height. For successful recording of rat movements by the automated software, make sure that the floor color contrasts with that of the rat **[1].**

2.1.1. Square arena.

2.2. Place the arena either inside a box or on a platform that is enclosed by a curtain **[1].** To create a context, insert a second layer of insertable walls, in different colors or patterns, into the arena **[2].**

2.2.1. Talent placing the arena inside a box.

2.2.2. Talent inserting a second layer of walls into the arena.

2.3. Hang 3D spatial cues that have distinct geometric shapes and colors high enough so that the rats cannot reach them **[1-TXT].**

2.3.1. Talent hanging the cues on the walls. **TEXT: See text manuscript for cue dimensions**

2.4. Attach different pairs of objects that are non-porous, non-chewable, and easy to clean, with distinct geometric shapes and textures to the floor of the arena **[1-TXT]**.

2.4.1. Talent attaching objects to the floor of the arena. **TEXT: 5 to 15 cm in width and height**

2.5. Fill a non-square shaped bucket with bedding material **[1-TXT]**. Randomly place 5 to 10 objects of different shapes and sizes, which are different from the objects that are going to be used in the experiment, into the bucket **[2].**

2.5.1. Talent filling bucket with bedding material. **TEXT: >50 cm in diameter**

2.5.2. Talent placing the objects randomly in the bucket.

3. Object Habituation

3.1. For session 1, bring all home cages to the experiment room, and let the rats habituate to the room and settle for at least 30 minutes **[1]**. Put 2 to 4 rats from the same cage together in the bucket for 20 minutes **[2]**. Clean the bucket by removing any fecal matter between each group of rats **[3].** *Videographer: This step is important!*

3.1.1. Talent bringing the home cages to the experiment room. *Videographer: Obtain multiple usable takes, this will be reused in 3.3.1, 3.5.1, 3.6.2, 4.1.1., and 5.1.1.*

3.1.2. Talent putting 2-4 rats in the bucket.

3.1.3. Talent cleaning the bucket.

3.2. Put all rats in their home cages and return them to the housing room **[2].**

3.2.1. Talent putting rats in their home cages.

3.3. For session 2, bring all cages to the experiment room and leave for at least 30 minutes **[1]**. Put each rat individually in the bucket for 10 minutes **[2]**. Place the rat back into the home cage **[3]** and clean the bucket after each rat **[4]**.

3.3.1. *Use 3.1.1.*

3.3.2. Talent putting the rats in the bucket.

3.3.3. Talent placing the rat back in the cage.

3.3.4. Talent cleaning the bucket.

3.4. When finished, return all cages to the housing room **[1-TXT].**

3.4.1. Talent returning the cages to the housing room **TEXT: Repeat the protocol for session 3 on a separate day.**

3.5. For session 4, bring all cages to the experiment room and leave for at least 30 minutes **[1]**. Place 2 to 4 rats from the same cage together in the empty arena with no context or spatial cues for 20 minutes **[2].** Place all rats back into the home cage **[3]** and wipe the arena with 70% ethanol after each group of rats **[4-TXT].** *Videographer: This step is important!*

3.5.1. *Use 3.1.1.*

3.5.2. SCREEN: 3-4 Grey box habituation (litter mates).mkv 00:01-00:30. NOTE: 3.5.1-3.5.2 shot together as screen capture recording from the shooting

3.5.3. Talent putting all the rats back to the home cage.

3.5.4. Talent wiping the arena with 70% ethanol. **TEXT: Empty box habituation.**

3.6. Modify the empty arena to create the first context without putting the objects in the arena **[1]**. For session 1 of context habituation, bring all cages to the experiment room and leave for at least 30 minutes **[2]**.

3.6.1. Talent creating the first context of empty arena. *Video Editor: Use brief shots from 2.2.2., 2.3.1., and 2.4.1.*

3.6.2. *Use 3.1.1.*

3.7. Start the recorder **[1]**, then place the first rat in the center of the arena and allow the rat to explore the arena for 10 minutes **[2]**. Then, stop the recorder and place the rat back into the home cage **[3-TXT]**. Return all cages to the housing room when finished **[4].** *Videographer: This step is important!*

3.7.1. SCREEN: 3-6 Context habituation (single rat).mkv 00:01-00:20. NOTE: Video recording is available for this shot showing same process as the screen capture

3.7.2. Talent placing the rats in the center of the arena. *Videographer: Obtain multiple usable takes, this shot will be reused in 3.7.2*.

3.7.3. Talent placing the rat back into the home cage. **TEXT: Wipe the arena with 70% ethanol after each rat.**

3.7.4. Talent returning all the cages to the housing room**.**

3.8. For each rat, repeat this process for sessions 2 and 3 over two consecutive days such that there are 3 sessions of context habituation per rat in total **[1]**.

3.8.1. *Video Editor: Use brief shots from 3.6.2., 3.7.2., 3.7.3, and 3.7.4.*

4. Encoding Trial

4.1. Bring all cages to the experiment room and leave for at least 30 minutes **[1]**. Using the schedule prepared beforehand, place the first identical pair of objects in the designated locations by using sticky mats or double-sided tape **[2].** *Videographer: This step is important!*

4.1.1. *Video Editor: Use 3.1.1. and place Figure 2B in the inset.*

4.1.2. Talent placing the objects in designated locations using sticky mats or tape. **TEXT: At 2 corners and a distance of >10 cm from each respective wall**

4.2. Start the recorder **[1]** and place the first rat in the arena at an equal distance from each object, facing a wall or a corner that is not occupied by any object **[2].** *Videographer: This step is important!*

4.2.1. Talent starting the recorder.

4.2.2. Talent placing the rat into the arena.

4.3. For weak encoding, allow the rat to explore the arena and objects for 20 minutes for a single trial. For strong encoding, allow the rat to explore for 5 minutes with 3 trials for the same rat **[1].** Stop the recorder **[2]**, NOTE: Please add this VO to the following shot 4.3.3 because 4.3.2 was not recorded place the rat back into the home cage **[3]**, remove the objects, and wipe the arena with ethanol after each recording **[4].**

4.3.1. SCREEN: 4-3 Encoding example.mkv 00:01-00:25. NOTE: Video recording is available for this shot showing same process as the screen capture

~~4.3.2. Talent stopping the recording.~~

4.3.3. Talent placing the rat back into home cage,

4.3.4. Talent removing the objects and wiping the arena.

~~4.4. If the delay time is shorter than 24 hours, keep the cages in the experiment room until the test trial **[1]**. If not, return all cages to the housing room when completed **[2].**~~

~~4.4.1. Cages in experiment room.~~

~~4.4.2. Talent placing the cages in the housing room.~~

NOTE: This shot is not recorded

5. Test Trial

5.1. Bring all cages to the experiment room, leaving sufficient time ahead of the first test so that the rats can be left for at least 30 minutes **[1].** Place the objects in the designated locations **[2].** *Videographer: This step is important!*

5.1.1. *Use 3.1.1.*

5.1.2. Talent placing the objects.

5.2. Start the recorder **[1]** and place the first rat in the arena facing a wall or a corner that is not occupied by any object. Allow the rat to explore the arena and objects for 5 minutes **[2].** *Videographer: This step is important!*

5.2.1. SCREEN: 5-2 Test example.mkv 0:01-00:05.

5.2.2. SCREEN: 5-2 Test example.mkv 0:05-00:20.

5.3. Place the rat back into the home cage **[1]**. Remove the objects, and wipe both the objects and the arena thoroughly with 70% ethanol **[2].** Repeat the recording for each rat, then return the cages back to the housing room **[3].**

5.3.1. Talent placing the rat back into the home cage. NOTE: In video this shot is marked as 5.12

5.3.2. Talent removing the objects and wiping the arena.

5.3.3. Use 4.4.2.

6. Data Analysis

6.1. For each rat, score the exploration time for each object in both the encoding and test trials **[1]**. Score encoding trials for the whole duration **[2]** and the test trials for 2 minutes for best discrimination performance **[3].**

6.1.1. *Videographer: take a shot of talent sitting on the computer clicking the mouse.*

6.1.2. SCREEN: 6-1-2 Manual scoring (Encoding).mkv 00:01-00:30.

6.1.3. SCREEN: 6-1-3 Manual scoring (test).mkv 00:01-00:30.

6.2. Calculate the percentage of exploration for each object or the discrimination index for each rat and calculate mean values for the groups. Use a one-sample *t*-test for detecting a significant preference above the chance level **[1-TXT]**.

6.2.1. *Use 6.1.1.* **TEXT:**

$$\textbf{\% exploration of object 1 (novel location)} = \frac{\textbf{Time spent exploring object 1 (novel loc.)}}{\textbf{Total exploration time}} \times \textbf{100\%}$$

$$\textbf{DI} = \frac{\textbf{Time spent exploring object 1 (novel loc.)} - \textbf{Time spent exploring object 2 (stable)}}{\textbf{Total exploration time}}$$

Results

7. Memory Performance After Strong and Weak Encoding

7.1. The strong encoding protocol led to preference for the object at the novel location with a significantly higher mean percentage exploration than the 50% chance level in tests with both 1-hour and 24-hours delays **[1].**

7.1.1. LAB MEDIA: Figure 4A. *Video editor highlight the 1-h and 24-h bar graph above the dotted horizontal line of 50% chance level.*

7.2. The weak encoding protocol produced a significant increase in the preference for the object at the novel location compared to chance level in tests with a 1-hour delay **[1]**, but not a 24-hour delay **[2].**

7.2.1. LAB MEDIA: Figure 4B. *Video editor highlight the 1-h bar graph above the dotted horizontal line of 50% chance level.*

7.2.2. LAB MEDIA: Figure 4B. *Video editor highlight the 24-h bar graph below the dotted horizontal line of 50% chance level.*

7.3. A significant advantage of this protocol is that it can be performed four times using four distinct contexts with the same cohort of rats **[1].**

7.3.1. LAB MEDIA: Figure 1C.

7.4. The results of one possible way of using counterbalancing with two experimental groups of 1-hour memory and 24-hour memory are demonstrated here. The two groups were counterbalanced over contexts 1 and 3 **[1]**, which was repeated in two additional contexts, 2 and 4 **[2].**

7.4.1. LAB MEDIA: Figure 5A. *Video editor focus on the Session X.*

7.4.2. LAB MEDIA: Figure 5A. *Video editor focus on the Session X +1.*

7.5. The results from these four contexts are presented individually, where the memory for each experimental group was assessed by comparing the preference to chance level in each context **[1].** No significant difference was detected among the four contexts. **[2]**

7.5.1. LAB MEDIA: Figure 5B and 4D.

7.5.2. LAB MEDIA: Figure 5A. *Video editor highlight all the bar graphs of 1h and 24h.*

7.6. For a better representation and within-subject comparison of the data, the results from two counterbalanced contexts were combined and compared using within-subject comparison, namely the paired t-test **[1].**

7.6.1. LAB MEDIA: Figure 5C and 5E.

Conclusion

8. Conclusion Interview Statements

8.1. **Kristoffer Højgaard:** Since this task relies on curiosity, the handling and habituation is crucial so that the animals are not overwhelmed or scared during the test **[1]**.

8.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 3.1.-3.5.*

8.2. **Kristoffer Højgaard:** Further manipulations, such as pharmacological and optogenetic interventions, can be implemented in this protocol, as well as in vivo imaging for studying short-term and long-term memories and for enhancing or impairing these memories **[1]**.

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