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Title: A Metric Test for Assessing Spatial Working Memory in Adult Rats Following Traumatic Brain Injury

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? **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?

☒ 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: 15

Number of Shots: 35

Introduction

1. Introductory Interview Statements

REQUIRED:

- 1.1. **Dmitry Frank:** Traumatic brain injury is commonly associated with memory impairment. Here, we present a protocol to assess spatial working memory after traumatic brain injury via a metric task.
 - 1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested b-roll: 3.4.4.*
- 1.2. **Dmitry Frank:** The main advantages of metric tasks include the possibility of dynamic observation, low cost, reproducibility, relative ease of implementation, and low stress environment.
 - 1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

Ethics Title Card

- 1.3. Procedures involving animal subjects have been approved by the Animal Care Committee of Ben-Gurion University of the Negev.

Protocol

2. Evaluation of Neurological Severity Score

- 2.1. To evaluate the neurological severity score after parasagittal fluid-percussion injury, place the adult Sprague-Dawley rat at the center of a 50-centimeter diameter circle **[1-TXT]** three times and monitor its inability to exit the circle **[2-TXT]**.
 - 2.1.1. WIDE: Establishing shot of talent placing the rat at the center of the circle.
TEXT: Include a control group
 - 2.1.2. Rat moving around in the circle/getting out of the circle. **TEXT: i.e., 30 min, 60 min, >60 min session**
- 2.2. To test for a loss of righting reflex, place the animal on its back in the palm of the hand **[1]** and give a score of 1 if the rat is able to right itself **[2]**.
 - 2.2.1. Talent placing the animal on its back on hand.
 - 2.2.2. The animal righting itself (standing on all four paws).
- 2.3. To test for hemiplegia, evaluate the inability of the rat to resist forced positioning **[1]**. To test the reflexive bending of the hindlimb, raise the animal by its tail **[2]**. To test the ability to walk straight, place the rat onto the floor **[3]**.
 - 2.3.1. Talent testing the inability of the rat to resist forced positioning.
 - 2.3.2. Talent raising the rat by its tail.
 - 2.3.3. Talent placing the rat on the floor.
- 2.4. To test the pinna reflex, perform light tactile stimulation to observe the ear retraction **[1]**. To test the corneal reflex, lightly apply a needle to the eye and measure the blink response **[2-TXT]**. To test the startle reflex, drag a pen across the top of the wire cage and record the animal's response **[3-TXT]**.
 - 2.4.1. Talent performing light tactile stimulation.
 - 2.4.2. Needle being applied/shot of response. **TEXT: 0 = no response; 3 = triple blink**
 - 2.4.3. Pen being dragged/shot of response. *Videographer: Can split action into two separate shots to get a good view of rat reaction as necessary* **TEXT: 0 = no response; 3 = jump ≥1 cm**

- 2.5. To grade the loss of seeking behavior and prostration, place the rat into a new environment and observe whether the rat moves its antennae, sniffs, or runs [1]. Then, test the limb reflexes for placement on the left and right forelimbs followed by the left and right hindlimbs [2].

- 2.5.1. Talent placing the rat in a new environment/shot of rat response

- 2.5.2. Talent testing limb reflexes.

- 2.6. To test balance, perform a beam balancing task by placing the rat onto a 1.5-centimeter-wide beam for one 20-, 40-, and greater than 60-second session [1]. Then, run a beam walking test using three beams of different widths [2-TXT].

- 2.6.1. Rat being placed onto beam/rat balancing on beam.

- 2.6.2. Rat being placed onto beam/rat walking on beam. **TEXT: i.e., 8.5, 5, and 2.5 cm**

3. Metric Task

- 3.1. Before performing the metric task, place a 200-centimeter-diameter, 1-centimeter-thick white circular platform onto an 80-centimeter-tall table [1] and place two objects in the center of the platform 68 centimeters apart [2].

- 3.1.1. WIDE: Talent placing circular platform on the table.

- 3.1.2. Talent placing two objects on the platform.

- 3.2. Install a camera with the required computer software for capturing, saving, and processing data at a height of 290 centimeters from the floor [1].

- 3.2.1. Talent installing the camera, with platform in view.

- 3.3. One day before the analysis, place the rat on the platform for 10 minutes to habituate the animal to the new environment [1]. On the day of the task, place the rat on the end of the platform equidistant from the objects for a 15-minute habituation period [2] and start recording [3].

- 3.3.1. Talent placing a rat on the platform.

- 3.3.2. Talent placing the rat on the platform.

- 3.3.3. Talent starting recording.

- 3.4. At the end of the experimental habituation period, transfer the rat to an individual cage for 5 minutes [1] and clean the platform with 5 to 10% alcohol [2]. Reduce the

distance between the objects to 34 centimeters [3], then place the rat on the platform for 5 minutes [4] while recording its exploration activity [5].

3.4.1. Talent placing the rat in a cage.

3.4.2. Talent cleaning the platform. *Videographer: Obtain multiple usable takes because this will be used again.*

3.4.3. Talent reducing the distance between the objects.

3.4.4. Talent placing the rat on the platform.

3.4.5. Talent recording video.

3.5. Clean the platform again before proceeding with the next animal [1].

3.5.1. *Use 3.4.2. Talent cleaning platform.*

4. Data Analysis

4.1. Prior to analyzing the video files, insert the software hardware key [1]. Then start the video tracking software [2] and open the preset **Template** [3].

4.1.1. WIDE: Talent inserting software hardware key.

4.1.2. Talent starting video tracking software, with monitor visible in frame.
Videographer: Obtain a few shots of talent clicking the mouse and typing on the keyboard to use as b-roll throughout the video

4.1.3. SCREEN: 4.1.3. 0:12-0:16.

4.2. After verifying, duplicating, and renaming the **Arena**, **Trial Control**, and **Detection** settings [1], in the main field of view, right-click the mouse to select **Grab Background**. In the **Browse** menu, select the location of the video file to be used as the background image [2].

4.2.1. SCREEN: 4.1.3. 0:18-0:24.

4.2.2. SCREEN: 4.2.1. 0:08-0:19.

4.3. Capture the image and mark the investigated areas and zones to calibrate the image for analysis [1]. Perform the same setup for the **Trial Control** and **Detection** settings [2].

4.3.1. SCREEN: 4.3.1. 0:05-0:36. *Video Editor: Speed up, as necessary*

4.3.2. SCREEN: 4.3.1. 0:37-0:42, then skip to 0:46-0:50.

4.4. After downloading the list of video files for analysis from the **Trial List**, add the videos and indicate the location with the required settings. Then select acquisition and **Start Trial [1]** and export all data into a spreadsheet **[2]**.

4.4.1. SCREEN: 4.4.1 (1) 0:06-0:57. *Video Editor: Speed up, as necessary*

4.4.2. LAB MEDIA: Figure 2D.

Results

5. Results: Neurological Deficit After Traumatic Brain Injury (TBI)

5.1. The control group does not show any neurological deficits 48 hours after the sham injury [1]. After TBI (T-B-eye), neurological deficits are significantly greater in both male and female injured animals [2].

5.1.1. LAB MEDIA: Table 1. *Video Editor: Emphasize 48 h sham-operated data [0(0-0)]*

5.1.2. LAB MEDIA: Table 1. *Video Editor: Emphasize 48 h TBI male and female data [5.5(4-7) and 4.5(3.25-6)]*

5.2. During the metric task, the object exploration time [1] is significantly shorter for the male TBI rats [2] than for the male sham-operated rats [3]. The same pattern is observed [4] for the female TBI [5] and female sham-operated rats [6].

5.2.1. LAB MEDIA: Figure 3a.

5.2.2. LAB MEDIA: Figure 3a. *Video Editor: Emphasize green triangle at 20-25 min*

5.2.3. LAB MEDIA: Figure 3a. *Video Editor: Emphasize the red dot at 20-25 min*

5.2.4. LAB MEDIA: Figure 4a.

5.2.5. LAB MEDIA: Figure 4a. *Video Editor: Emphasize green triangle 20-25 min*

5.2.6. LAB MEDIA: Figure 4a. *Video Editor: Emphasize red dot at 20-25 min*

Conclusion

6. Conclusion Interview Statements

6.1. **Dmitry Frank:** Following the metric task, we can perform other methods for memory assessment, such as the Barnes maze and topological and water maze tasks.

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

6.2. **Dmitry Frank:** The metric test also allows for further research into memory impairment in comparable models of neurological damage, such as models of diffuse axonal brain injury and stroke.

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