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TITLE:

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

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neurologic severity score, NSS, rats, spatial working memory task, traumatic brain injury, TBI

SUMMARY:

Traumatic brain injury (TBI) is commonly associated with memory impairment. Here, we present a protocol to assess spatial working memory after TBI via a metric task. A metric test is a useful tool to study spatial working memory impairment after TBI.

ABSTRACT:

Impairments to sensory, short-term, and long-term memory are common side effects after traumatic brain injury (TBI). Due to the ethical limitations of human studies, animal models

provide suitable alternatives to test treatment methods, and to study the mechanisms and related complications of the condition. Experimental rodent models have historically been the most widely used due to their accessibility, low cost, reproducibility, and validated approaches. A metric test, which tests the ability to recall the placement of two objects at various distances and angles from one another, is a technique to study impairment in spatial working memory (SWM) after TBI. The significant advantages of metric tasks include the possibility of dynamic observation, low cost, reproducibility, relative ease of implementation, and low stress environment. Here, we present a metric test protocol to measure impairment of SWM in adult rats after TBI. This test provides a feasible way to evaluate physiology and pathophysiology of brain function more effectively.

INTRODUCTION:

The prevalence of neurological deficits such as attention, executive function, and certain memory deficits after moderate traumatic brain injury (TBI) is more than 50 percent[1–8]. TBI can lead to severe impairments in spatial short-term, long-term, and working memory[9]. These memory impairments have been observed in rodent models of TBI. Rodent models have enabled the development of techniques to test memory, allowing for deeper examinations into the effect of TBI on memory processing in neural memory systems.

Two tests, related to topological and metric spatial information processing respectively, assist with measuring spatial working memory (SWM). The topological test depends on changing the size of environmental space or related spaces of connection or enclosure around an object, while the metric test assesses changes in angles or distance between objects[10,11]. Goodrich-Hunsaker et al. first adapted the human topological test for rats[10] and applied the metric task to dissociate the roles of the parietal cortex (PC) and dorsal hippocampus in spatial information processing[11]. Similarly, Gurkoff and colleagues evaluated metric, topological, and temporal ordering memory tasks after lateral fluid percussion injury[9]. There is a correlation between damage to certain regions of the brain and impairment of metric or topological memory. It has been suggested that metric memory impairment is related to lesions in bilateral dorsal dentate gyrus and cornu ammonis (CA) sub-region CA3 of the hippocampus, and that topological memory impairment is related to bilateral parietal cortex lesions[10,12].

The purpose of this protocol is to assess spatial memory deficit in a rat population via a metric task. This method is a suitable alternative to investigate mechanisms of SWM after brain injury, and its advantages include the relative ease of implementation, high sensitivity, low cost of reproducibility, the possibility of dynamic observation, and a low stress environment. Compared to other behavioral tasks such as the Barnes maze[13,14], Morris water navigation task[15–17], or spatial maze tasks[18,19], this metric test is less complicated. Due to its ease of implementation, the metric test requires a shorter and less stressful training period and takes place over only 2 days[9]: 1 day for habituation and 1 day for the task. Moreover, our proposed test is easier to perform than other low stress tests, such as the novel object recognition (NOR) task, and does not require the extra day of habituation[20].

This paper provides a straightforward model for evaluating SWM after brain injury. This

assessment of post-TBI SWM may assist in a more comprehensive investigation of its pathophysiology.

PROTOCOL:

The experiments were performed following the recommendations of the Declarations of Helsinki and Tokyo and the Guidelines for the Use of Experimental Animals of the European Community. The experiments were approved by the Animal Care Committee of Ben-Gurion University of the Negev. A protocol timeline is illustrated in **Figure 1**.

1. Surgical procedures and fluid percussion TBI

1.1. Select male and female adult Sprague–Dawley rats, housed at a room temperature of 22 ± 1 °C, and humidity of 40%–60%, with 12–12 h light-dark cycles.

1.2. Provide food as chow and water *ad libitum*. Perform experiments between morning hours, i.e., 6:00 a.m. and 12:00 p.m.

1.3. Perform a baseline neurological assessment for both the control and TBI groups prior to the start of the experiment (see section 2 below).

1.4. Anesthetize the rats with inhaled 4% isoflurane for induction and 1.5% for maintenance of anesthesia. Ensure that rat is immobilized by testing pedal reflex or movement in response to an irritant.

NOTE: Use a continuous isoflurane administration system for anesthesia. Perform all procedures in aseptic conditions.

1.5. Perform parasagittal fluid-percussion injury as previously described[21,22].

1.6. Perform 0.1% bupivacaine infiltration subcutaneously and intradermally prior to closing the wound. Transfer the rat to the recovery room and continue monitoring the neurological (e.g., paralysis), respiratory (e.g., respiratory arrest) and cardiovascular state (e.g., decreases in soft tissue perfusion, changes in color of pupils, and bradycardia) for 24 h. Prior to emergence from anesthesia, administer 0.01 mg/kg intramuscular buprenorphine as postoperative analgesia. Repeat doses every 12 h for at least 48 h.

2. Evaluation of Neurological Severity Score (NSS)

NOTE: Assessment of neurological deficit was performed and graded using an NSS, as previously described[23,24]. The maximum score of alteration in motor function and behavior is 24 points. A score of 0 indicates an intact neurological status and 24 indicates severe neurological dysfunction, as previously described[24].

2.1. Test the rat's inability to leave a circle (50 cm in diameter) when placed in its center. Perform this task three times, with each session lasting 30 min, 60 min, and more than 60 min each.

2.2. Test the rat for a loss of righting reflex.

2.2.1. Place the animal on its back in the palm of the researcher's hand. Give a score of 1 if the animal is able to right itself[25] (standing on all four paws).

2.3. Test the rat for hemiplegia, the inability of the rat to resist forced positioning.

2.4. Raise the rat by its tail to test the reflexive bending of the hindlimb.

2.5. Put the rat on the floor to test its ability to walk straight.

2.6. Perform testing for three reflexive behaviors: the pinna reflex, the corneal reflex, and the startle reflex.

2.6.1. For the pinna reflex, perform light tactile stimulation to test ear retraction as previously described[25].

2.6.2. To test the corneal reflex, monitor blink response when applying a needle lightly to the eye and measure on a scale of 0 (no response) to triple eye blink (3), as previously described[25].

2.6.3. For the startle reflex, drag a pen across the top of the wire cage and record response with a scale from 0 (no response) to 3 (1 cm jump or more), as previously described[25].

2.7. Grade the rat based on loss of seeking behavior and prostration (not moving their antennae, sniffing, or running after being transferred to a new environment)[24].

2.8. Test limb reflexes for the placement on the left and right forelimbs, and then the left and right hindlimbs.

2.9. Analyze functionality via the beam balancing task with a beam that is 1.5 cm wide. Perform the test for sessions lasting 20 seconds, 40 seconds, and more than 60 seconds.

2.10. Run the beam walking test with three different beams: 8.5 cm wide, 5 cm wide, and 2.5 cm wide.

3. Preparing for the metric task

3.1. Equipment

3.1.1. Place a white circular platform 200 cm in diameter and 1 cm thick on a table. The height

of the table should be 80 cm above the floor.

3.1.2. Establish two different objects in the center of circular platform 68 cm away from each other.

NOTE: In this experiment, two glass bottles were used for objects, one round bottle with a height of 13.5 cm and another faceted bottle with a height of 20 cm. Fill bottles with water to ensure stability.

3.1.3. Prepare a camera and install the required computer software for capturing, saving, and processing data. Install the camera at a height of 290 cm from the floor.

NOTE: The distance between the platform and camera depends on the camera specifications. The camera frame should cover the entire area of the arena in which the test is being conducted. The distance for our experiment between the platform and the camera was 210 cm.

3.2. Habituation

3.2.1. On the day before the task, habituate the rat to the new environment for 10 min by placing on the arena without video recording.

NOTE: During habituation two different objects were removed from the platform.

NOTE: Do not perform the neurological tasks and the metric task on the same day. If for some reason the tasks must be performed on the same day, it is better to perform the metric task followed by the neurological tests.

4. Performing the metric task

NOTE: Performing the metric task consists of two periods: 1) habituation (15 min) and 2) test (5 min) period.

4.1. Habituation period

4.1.2. Establish two different objects in the center of the circular platform 68 cm away from each other.

4.1.3. Place the rat on the end of the platform equidistant from the objects for a 15 min period, and record the video.

4.1.4. Remove the rat from the platform and place in an individual cage for 5 min.

4.1.5. Clean the platform with 5%–10% alcohol.

NOTE: Up to 70% alcohol may be used to clean the platform in well-ventilated areas.

4.2. Test period

4.2.1. Reduce the distance between objects to 34 cm.

4.2.2. Place the rat on the platform for 5 min and record the rat's exploration activity on video.

4.2.3. Clean the platform with 5%–10% alcohol.

5. Data analysis

NOTE: Data analysis is performed by video tracking software specifically designed for animal behavior studies that automatically records animal activity and movement (see **Table of Materials**). This software automates a range of behavioral variables, including mobility, activity, and explorative behavior.

5.1. Prior to analyzing the video files, insert the software hardware key. Start the video tracking software and open preset **Template**.

5.2. In the **Setup** section, verify settings as follows: **Arena**, **Trial Control**, and **Detecting Settings** (see **Figure 2a**).

NOTE: For this experiment, parameters for the exploration area are defined as 6 cm around the object of interest. The time the rat entered into this area was measured.

5.3. After verifying the settings, duplicate and rename them.

5.4. On the general screen of the program, **Grab Background** by right clicking on the mouse.

5.5. Select a video file for the background image. In the **Browse** menu, select the location of the video file.

5.6. Capture the image and mark the investigated areas and zones, calibrating the image for analysis. Perform the same steps for **Trial Control** and **Detecting Settings**.

5.7. In the general menu, select **Trial List** and download the list of video files for analysis.

5.8. Add the videos and indicate the location with the required settings.

5.9. Select acquisition and **Start Trial** (see **Figure 2b,c**). Export all data as Excel files (see **Figure 2d**).

NOTE: Perform all calculations for the habituation and test periods. Metric task assessment is

prepared with an advanced template.

REPRESENTATIVE RESULTS:

The significance of comparisons between groups was determined using the Mann-Whitney test. Statistical significance of results was considered at $P < 0.05$, while statistically high relevance was measured at $P < 0.01$.

The results showed no differences in NSS between all groups before intervention and 28 days after TBI. Each group consisted of 12 female or 12 male rats. The NSS scores obtained 48 h after TBI are presented in **Table 1**. Rats from the TBI group that showed significant neurological deficit on day 28 after injury were excluded from the experiment. The data is measured as counts and presented as median ± range.

The sham-operated control group did not show any neurological deficit at 48 h after the first day of the study (NSS-0). Neurological deficit at 48 h after TBI was significantly greater for the male TBI rats than for the male sham-operated rats (5.5(4-7) vs. 0(0-0), $U = 0$, $p < 0.01$, $r = -0.89$), and for the female TBI rats than for the female sham-operated rats (4.5(3.25-6) vs. 0(0-0), $U = 0$, $p < 0.01$, $r = -0.91$), according to the Mann-Whitney test (**Table 1**).

A Mann-Whitney test indicated that object exploration time during the metric task was significantly shorter for the male TBI rats vs. male sham-operated rats (130% ± 44.3% vs. 1978% ± 59.2%), $U = 0$, $p < 0.01$, $r = -0.85$ (see **Figure 3a**,**b**). The data is measured as seconds expressed in % of baseline point and presented as mean ± SEM. Baseline is measured as the time of exploration during the first 5 min of the habituation period. The remaining three time points (5–10 min, 10–15 min, and 20–25 min) were calculated as a percentage of the baseline.

A Mann-Whitney test indicated that object exploration time during the metric task was significantly shorter for the female TBI rats vs. female sham-operated rats (89% ± 43.5% vs. 2160% ± 43.6%), $U = 0$, $p < 0.01$, $r = -0.85$ (see **Figure 4a,b**). The data is measured as seconds expressed in % of baseline point and presented as mean ± SEM. Baseline is measured as the time of exploration during the habituation period.

There was no significant difference found between male and female groups.

FIGURE AND TABLE LEGENDS:

Figure 1: Protocol schematic with timelines. This figure shows protocol timeline. Groups of rats at different times included a sham-operated control group and TBI group and were assessed by NSS score at -1 h, 48 h, and 28 days after injury.

Figure 2: Representative data analysis. Screen captures of the video tracking software for (**A**) Trial control settings (**B**) Trial list and (**C**) Acquisition, and example data exported into Excel (**D**). See text and video for details.

Figure 3: Metric task for male rats. The object exploration time during the metric task was significantly shorter for the male TBI rats vs. the male sham-operated rats (see **Figure 3a**,**b**, which illustrates the data on different y-axis scales).

Figure 4: Metric task for female rats. The object exploration time during the metric task was significantly shorter for female TBI rats vs. female sham-operated rats (see **Figure 4a, b**, which illustrates the data on different y-axis scales).

Table 1: Determination of neurological performance. Neurological deficit at 48 h after TBI was significantly greater for the male TBI rats than for the male sham-operated rats and female TBI rats than for the female sham-operated rats.

DISCUSSION:

By specifically targeting the metric spatial information process, this metric test provides a necessary tool toward understanding memory deficiency after TBI. The protocol presented in this paper is a modification of previously described behavioral tasks[11]. One previously described metric task used two different paradigms, each consisting of three habituation sessions and one testing session. The first paradigm consisted of moving the familiar objects closer together after habituation and the second paradigm moved the objects farther away[11].

Compared to the Barnes maze, which is performed over five[13] or fourteen[14] days, the metric task presented here is performed within 2 days, the first day for habituation and the second day for the task[9]. The task in this protocol is less stressful than comparable behavioral tasks such as the Morris water maze, due to the stress induced by swimming in the maze and the longer duration of the task[15–17]. Maze tests for spatial memory require a significant learning period; even a simple T maze requires at least 5 days of training[18]. For more complex radial mazes, 15–20 days of daily testing is recommended[19].

This protocol contains several critical steps. One crucial component is the need to treat the arena with an alcohol solution as well as the objects on it. It is also necessary that the surface of the arena is dry and clean, since the smell of alcohol and scents left over from previous animals can change the behavior of the animal under study. In addition, constantly adequate ventilation of the behavior room is vital. Since noise is one of the stress factors that can change the behavior of animals, we recommend proper soundproofing. Additionally, the platform height of 80 cm and the relative distance of the platform from other objects is necessary in order for the rat not to jump or climb onto another object. Further, maintaining consistent settings in processing recorded video files during set-up will help avoid incorrect interpretation of the data.

The neurological deficit that develops as a result of TBI must be considered in the assessment of memory. Neurological deficits after head trauma are a contributing factor that is part of this disease. Assessment of neurological deficits is very important in the rodent model of brain injury and is a highly-sensitive and frequently-used outcome[26]. However, severe neurological deficits can have an effect on behavioral tests, especially on tests that measure memory assessment[27].

The comparable Morris water maze task also assesses memory impairment[28]. A low score on the Morris test in TBI or stroked rats is highly correlated with neurological deficits and, in fact, reflects not memory or cognitive impairment, but rather neurological performance and the ability to withstand stress.

To minimize the effect of TBI-related neurological deficits on memory scores, we used the following approaches: 1) we used models of TBI of mild to moderate severity, which spontaneously recover neurological performance after 1 month. 2) Rats that showed neurological deficit 28 days after TBI were excluded from behavioral experiments, based on our observations that all rats with mild injury recover. In groups of 10–20 rats affected with severe TBI, one rat on average has a significant neurological deficit which may affect mobility. 3) To assess memory after trauma, we did not use tests related to movement, the results of which may be influenced by neurological deficiency (as in the Morris water maze). While the Barnes test and related tests are useful to assess memory in models of TBI and stroke, the metric test is better suited to assess SWM. Thus, the metric test is the test of choice for assessing the SWM of rats after TBI.

A limitation of this protocol is the use of a metric test alone rather than a topological test. We envision future studies that also incorporate topological tests to measure other aspects of SWM. Surprisingly, according to our results, no statistically significant difference was found between male and female rats. A large number of studies show sex differences after TBI[29], many based on the difference in concentrations of reproductive hormones. Estrogen and progesterone play a neuroprotection role after TBI, which are shown to decrease intracranial pressure and improve neurological function score respectively[30]. According to a meta-analysis study, men more frequently suffer from TBI, but women have worse prognoses[31]. Cognitive impairments, the most common complication after TBI, trend toward gender differences, with women showing greater improvement on spatial positioning tasks and men performing better on verbal tasks[32–34]. Our results, however, indicate the possibility for uncertainty about gender-related spatial memory differences.

Among the various types of TBI models, the model of fluid percussion induced TBI is well documented and described, is easily reproducible, and has lower variability than other models[35,36]. However, it is important to note that the metric test has broad utility and may be used effectively with other TBI models. The metric test described in this protocol also allows for further research into memory impairment in comparable models of neurological damage, such as models of diffuse axonal brain injury[24,37] and stroke[38]. This protocol may also be useful for studying the efficacy of various treatment modalities in restoring SWM after TBI.

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DISCLOSURES:

The authors have nothing to disclose.

REFERENCES:

1. Binder, L. M. Persisting symptoms after mild head injury: A review of the postconcussive syndrome. *Journal of Clinical and Experimental Neuropsychology*. **8** (4), 323–346 (1986).
2. Binder, L. M. A review of mild head trauma. Part II: Clinical implications. *Journal of Clinical and Experimental Neuropsychology*. **19** (3), 432–457 (1997).
3. Binder, L. M., Rohling, M. L., Larrabee, G. J. A review of mild head trauma. Part I: Meta-analytic review of neuropsychological studies. *Journal of Clinical and Experimental Neuropsychology*. **19** (3), 421–431 (1997).
4. Leininger, B. E., Gramling, S. E., Farrell, A. D., Kreutzer, J. S., Peck, E. A. Neuropsychological deficits in symptomatic minor head injury patients after concussion and mild concussion. *Journal of Neurology, Neurosurgery & Psychiatry*. **53** (4), 293–296 (1990).
5. Levin, H. S. et al. Neurobehavioral outcome following minor head injury: a three-center study. *Journal of Neurosurgery*. **66** (2), 234–243 (1987).
6. McMillan, T. M. Minor head injury. *Current Opinion in Neurology*. **10** (6), 479–483 (1997).
7. Millis, S. R. et al. Long-term neuropsychological outcome after traumatic brain injury. *The Journal of Head Trauma Rehabilitation*. **16** (4), 343–355 (2001).
8. Stuss, D. et al. Reaction time after head injury: fatigue, divided and focused attention, and consistency of performance. *Journal of Neurology, Neurosurgery & Psychiatry*. **52** (6), 742–748 (1989).
9. Gurkoff, G. G. et al. Evaluation of metric, topological, and temporal ordering memory tasks after lateral fluid percussion injury. *Journal of Neurotrauma*. **30** (4), 292–300 (2013).
10. Goodrich-Hunsaker, N. J., Howard, B. P., Hunsaker, M. R., Kesner, R. P. Human topological task adapted for rats: Spatial information processes of the parietal cortex. *Neurobiology of Learning and Memory*. **90** (2), 389–394 (2008).
11. Goodrich-Hunsaker, N. J., Hunsaker, M. R., Kesner, R. P. Dissociating the role of the parietal cortex and dorsal hippocampus for spatial information processing. *Behavioral Neuroscience*. **119** (5), 1307 (2005).
12. Goodrich-Hunsaker, N. J., Hunsaker, M. R., Kesner, R. P. The interactions and dissociations of the dorsal hippocampus subregions: how the dentate gyrus, CA3, and CA1 process spatial information. *Behavioral Neuroscience*. **122** (1), 16 (2008).
13. Rosenfeld, C. S., Ferguson, S. A. Barnes maze testing strategies with small and large rodent models. *Journal of Visualized Experiments: JoVE*. (84), e51194 (2014).
14. O'leary, T. P., Brown, R. E. The effects of apparatus design and test procedure on learning and memory performance of C57BL/6J mice on the Barnes maze. *Journal of Neuroscience Methods*. **203** (2), 315–324 (2012).
15. Bromley-Brits, K., Deng, Y., Song, W. Morris water maze test for learning and memory deficits in Alzheimer's disease model mice. *Journal of Visualized Experiments: JoVE*. (53), e2920 (2011).

16. Smith, C., Rose, G. M. Evidence for a paradoxical sleep window for place learning in the Morris water maze. *Physiology & Behavior*. **59** (1), 93–97 (1996).
17. Roof, R. L., Zhang, Q., Glasier, M. M., Stein, D. G. Gender-specific impairment on Morris water maze task after entorhinal cortex lesion. *Behavioural Brain Research*. **57** (1), 47–51 (1993).
18. Deacon, R. M., Rawlins, J. N. P. T-maze alternation in the rodent. *Nature Protocols*. **1** (1), 7 (2006).
19. Penley, S. C., Gaudet, C. M., Threlkeld, S. W. Use of an eight-arm radial water maze to assess working and reference memory following neonatal brain injury. *Journal of Visualized Experiments: JoVE.* (82), e50940 (2013).
20. Davis, A. R., Shear, D. A., Chen, Z., Lu, X.-C. M., Tortella, F. C. A comparison of two cognitive test paradigms in a penetrating brain injury model. *Journal of Neuroscience Methods*. **189** (1), 84–87 (2010).
21. Jones, N. C. et al. Experimental traumatic brain injury induces a pervasive hyperanxious phenotype in rats. *Journal of Neurotrauma*. **25** (11), 1367–1374 (2008).
22. Kabadi, S. V., Hilton, G. D., Stoica, B. A., Zapple, D. N., Faden, A. I. Fluid-percussion–induced traumatic brain injury model in rats. *Nature Protocols*. **5** (9), 1552 (2010).
23. Ohayon, S. et al. Cell-free DNA as a marker for prediction of brain damage in traumatic brain injury in rats. *Journal of Neurotrauma*. **29** (2), 261–267 (2012).
24. Frank, D. et al. Induction of Diffuse Axonal Brain Injury in Rats Based on Rotational Acceleration. *Journal of Visualized Experiments: JoVE*. (159), e61198 (2020).
25. Hunter, A. et al. Functional assessments in mice and rats after focal stroke. *Neuropharmacology*. **39** (5), 806–816 (2000).
26. Yarnell, A. M. et al. The revised neurobehavioral severity scale (NSS-R) for rodents. *Current Protocols in Neuroscience*. **75**, 9.52.1–9.52.16 (2016).
27. Fujimoto, S. T., Longhi, L., Saatman, K. E., McIntosh, T. K. Motor and cognitive function evaluation following experimental traumatic brain injury. *Neuroscience & Biobehavioral Reviews*. **28** (4), 365–378 (2004).
28. Hausser, N. et al. Detecting behavioral deficits in rats after traumatic brain injury. *Journal of Visualized Experiments: JoVE*. (131), e56044 (2018).
29. Ma, C. et al. Sex differences in traumatic brain injury: a multi-dimensional exploration in genes, hormones, cells, individuals, and society. *Chinese Neurosurgical Journal*. **5** (1), 1–9 (2019).
30. Shahrokhi, N., Khaksari, M., Soltani, Z., Mahmoodi, M., Nakhaee, N. Effect of sex steroid hormones on brain edema, intracranial pressure, and neurologic outcomes after traumatic brain injury. *Canadian Journal of Physiology and Pharmacology*. **88** (4), 414–421, (2010).
31. Farace, E., Alves, W. M. Do women fare worse: a metaanalysis of gender differences in traumatic brain injury outcome. *Journal of Neurosurgery*. **93** (4), 539–545 (2000).
32. Basso, M. R., Harrington, K., Matson, M., Lowery, N. FORUM sex differences on the WMS-III: findings concerning verbal paired associates and faces. *The Clinical Neuropsychologist*. **14** (2), 231–235 (2000).
33. Janowsky, J. S., Chavez, B., Zamboni, B. D., Orwoll, E. The cognitive neuropsychology of sex hormones in men and women. *Developmental Neuropsychology*. **14** (2–3), 421–440 (1998).

34. Halari, R. et al. Sex differences and individual differences in cognitive performance and their relationship to endogenous gonadal hormones and gonadotropins. *Behavioral Neuroscience*. **119** (1), 104 (2005).
35. Rowe, R. K., Griffiths, D., Lifshitz, J. in *Pre-Clinical and Clinical Methods in Brain Trauma Research*. Springer. 97–110 (2018).
36. Kabadi, S. V., Hilton, G. D., Stoica, B. A., Zapple, D. N., Faden, A. I. Fluid-percussion-induced traumatic brain injury model in rats. *Nature Protocols*. **5** (9), 1552–1563 (2010).
37. Losurdo, M., Davidsson, J., Sköld, M. K. Diffuse axonal injury in the rat brain: axonal injury and oligodendrocyte activity following rotational injury. *Brain Sciences*. **10** (4), 229 (2020).
38. Kuts, R. et al. A novel method for assessing cerebral edema, infarcted zone and blood-brain barrier breakdown in a single post-stroke rodent brain. *Frontiers in Neuroscience*. **13**, 1105 (2019).

Figure 1

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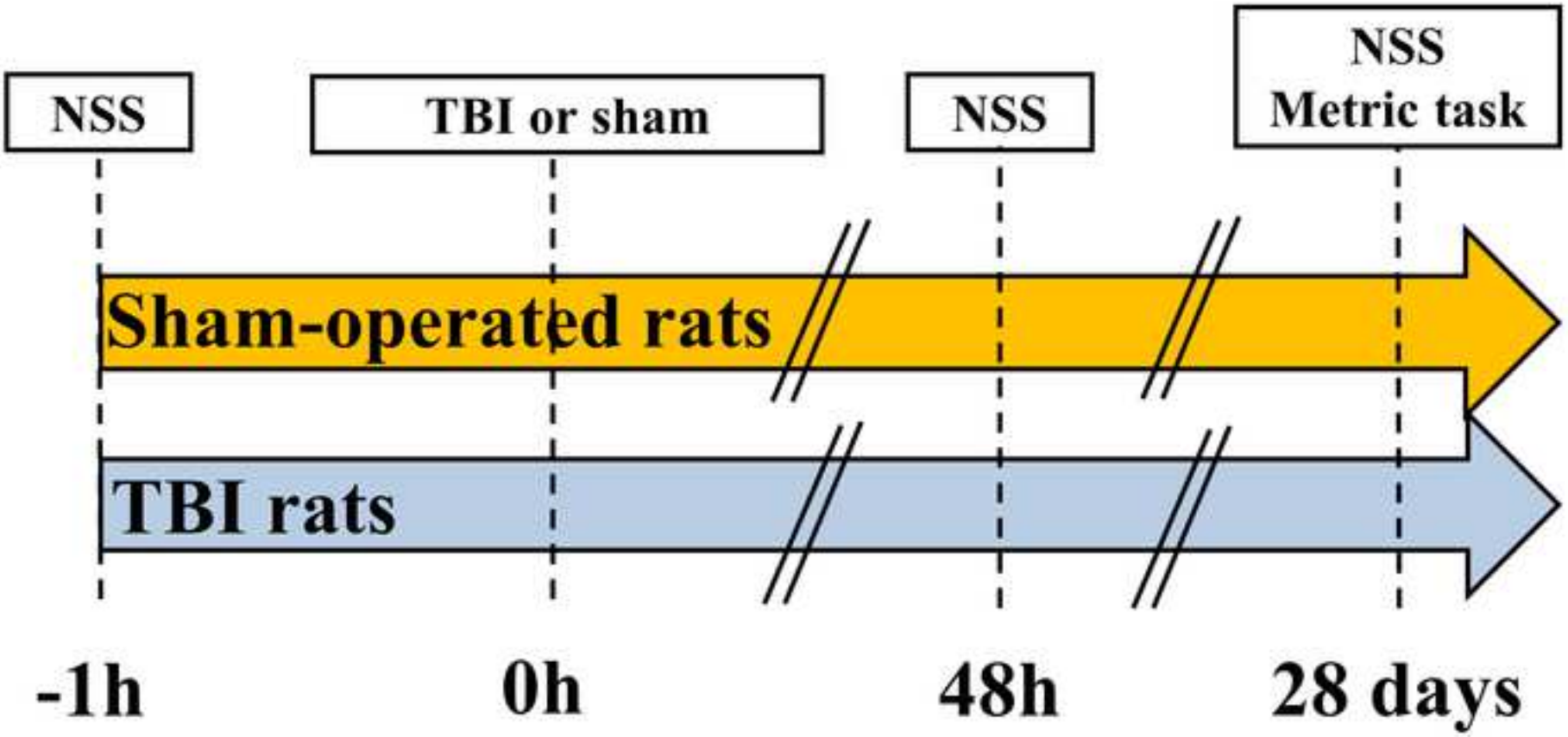

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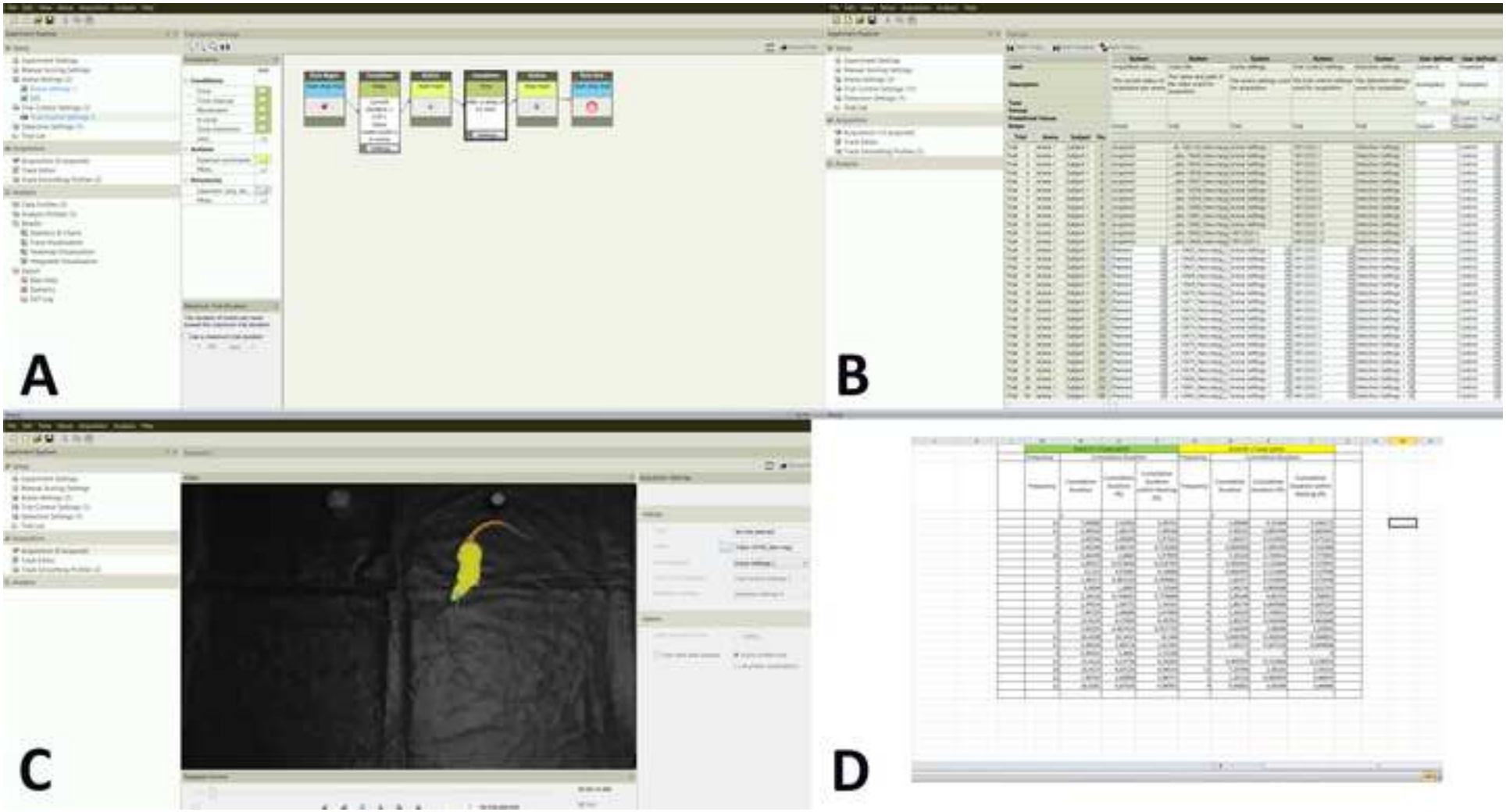

Figure 3

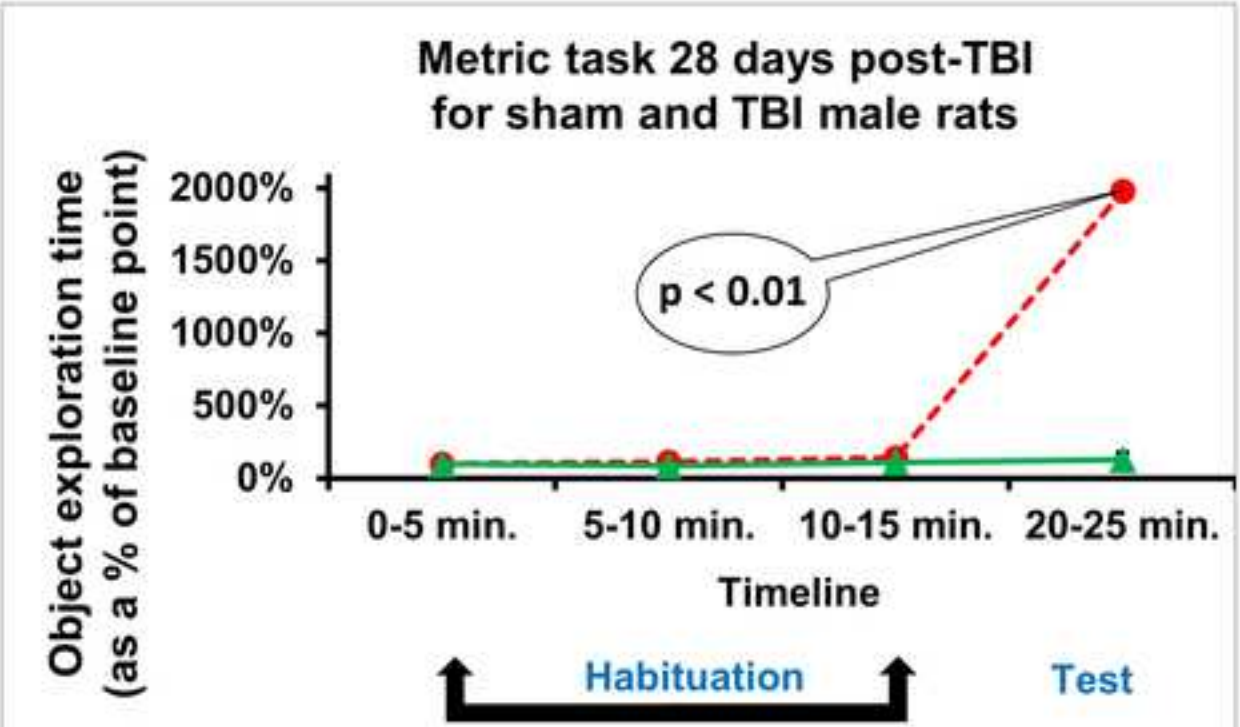

Fig. 3.a

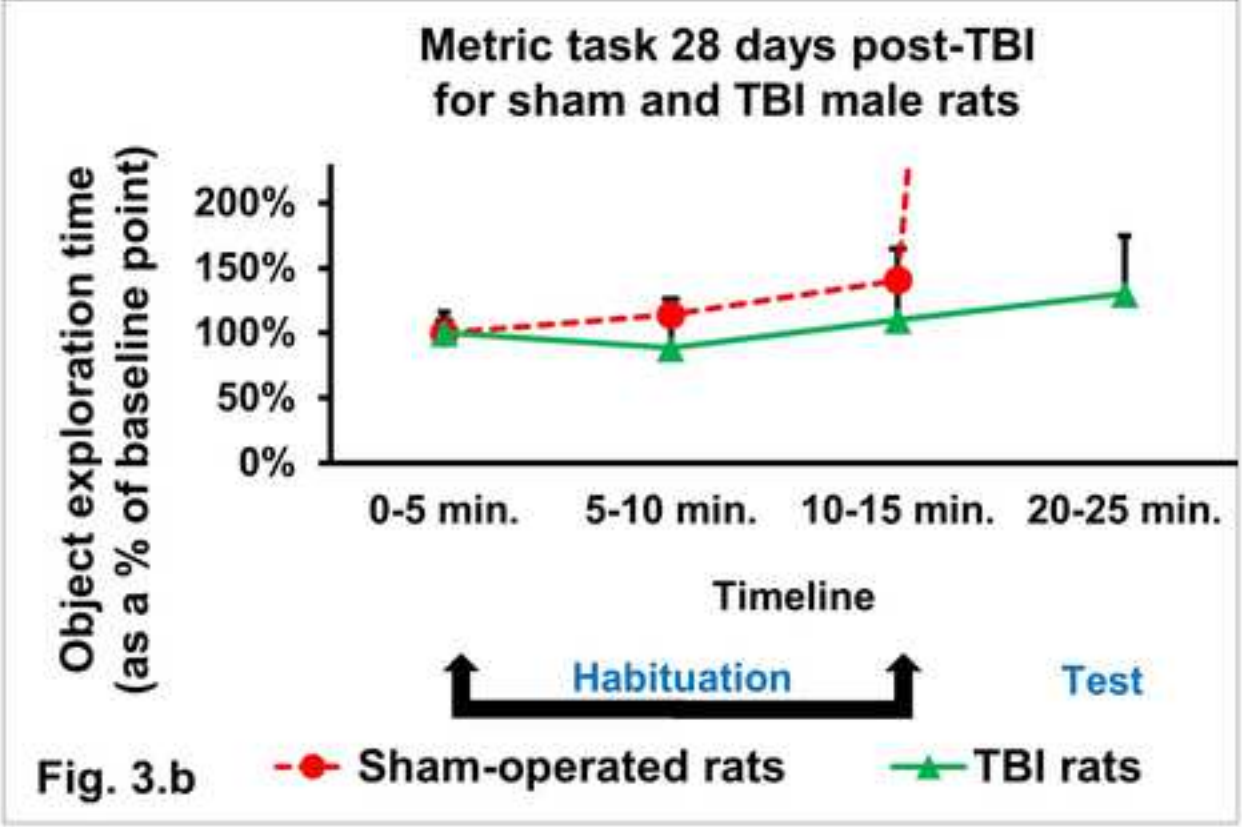

Fig. 3.b

Figure 4

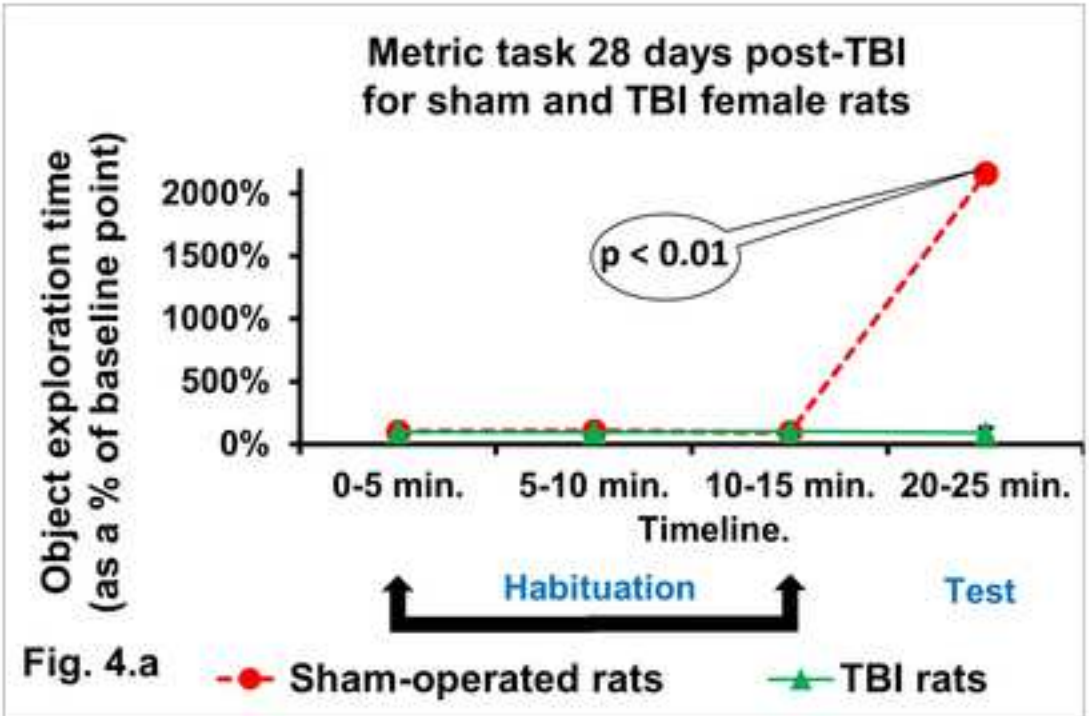

Fig. 4.a

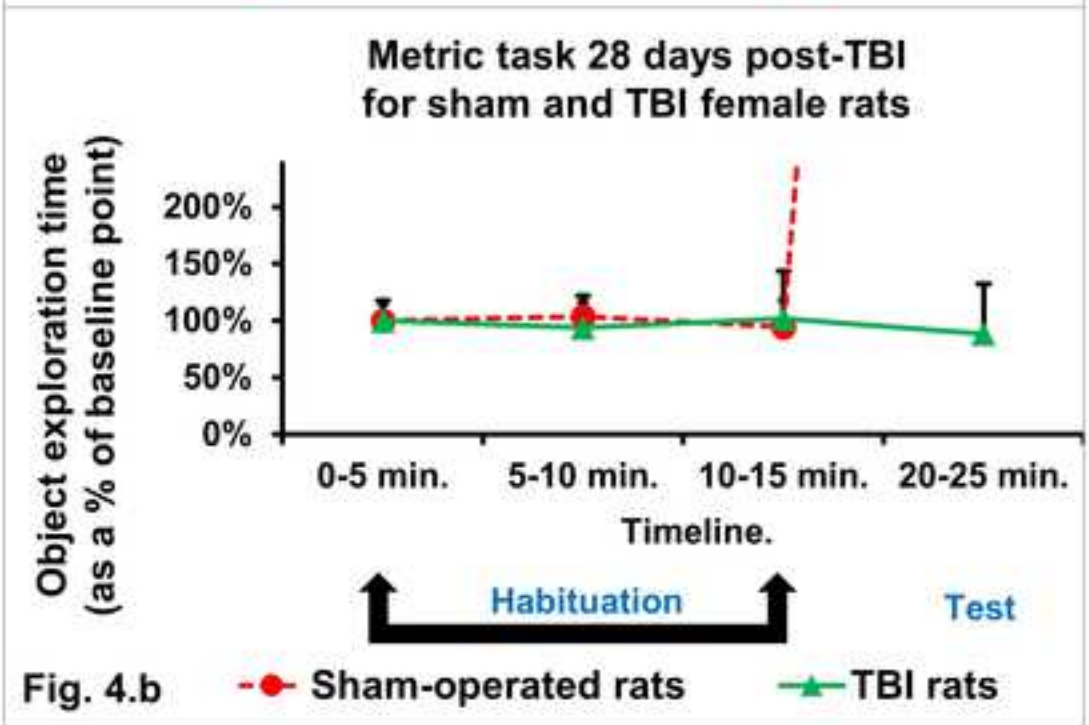

Fig. 4.b

Table 1

NSS values of the study groups at 48 h after TBI			M
Animal Group	**N**	**Baseline**	**48h**
Sham-operated female/male rats	12	0(0-0)	0(0-0)
TBI male rats	12	0(0-0)	5.5(4-7)*
TBI female rats	12	0(0-0)	4.5(3.25-6)*

edian (range)

1w	2w	4w
0(0-0)	0(0-0)	0(0-0)
2(1-6)*	1.5(0-2)*	0(0-2)
1.5(0.25-2.8)*	1(0-2)*	0(0-0.8)

Table of Materials

Click here to access/download;Table of Materials;JoVE_ Tableof_Materials (Spatial memory) .xlsx

Name of Material/Equipment	Company	Catalog Number
2% chlorhexidine in 70% alcohol solution	SIGMA - ALDRICH	500 cc
Bupivacaine 0.1 %		
4 boards of different thicknesses (1.5cm, 2.5cm, 5cm and 8.5cm)		
4-0 Nylon suture	4-00	
Bottles Bottlses (four) for topological an metric tasks	Techniplast	ACBT0262SU
Diamond Hole Saw Drill 3mm diameter		Glass Hole Saw Kit
Digital Weighing Scale	SIGMA - ALDRICH	Rs 4,000
Dissecting scissors	SIGMA - ALDRICH	Z265969
Ethanol 99.9 %	Pharmacy	
EthoVision XT (Video software)	Noldus, Wageningen, Netherlands	
Fluid-percussion device	custom-made at the university workshop	
Gauze Sponges	Fisher	22-362-178
Gloves (thin laboratory gloves)		
Heater with thermometer	Heatingpad-1	Model: HEATINGPAD-1/2
Horizon-XL	Mennen Medical Ltd	

Isofluran, USP 100%	Piramamal Critical Care, Inc	NDC 66794-017	
Office 365 ProPlus	Microsoft	-	
Olympus BX 40 microscope	Olympus		
Operating forceps	SIGMA - ALDRICH		
Operating Scissors	SIGMA - ALDRICH		
PC Computer for USV recording and data analyses	Intel		
Plexiglass boxes linked by a narrow passage			
Purina Chow	Purina		5001
Rat cages (rat home cage or another enclosure)	Techniplast	2000P	
Scalpel blades 11	SIGMA - ALDRICH	S2771	
SPSS	SPSS Inc., Chicago, IL, USA	20 package	
Stereotaxic Instrument	custom-made at the university workshop		
Timing device	Interval Timer:Timing for recording USV's		
Topological and metric tasks device	Self made in Ben Gurion University of Negev		
Video camera	Logitech	C920 HD PRO WEBCAM	
Windows 10	Microsoft		

Comments/Description

For general antisepsis of the skin in the operatory field

This is to evaluate neurological defect

150 ml bottles filled with 100 ml of water and 100 ml 1%(w/v) sucrose solution

For objects used two little bottles, first round (height 13.5 cm) and second faceted (height 20 cm) shape and two big faceted bottles

Optional.

5%-10% solution used to clean equipment and remove odors

Optional

No specific brand is recommended.

Optional.

No specific brand is recommended.

Anesthetic liquid for inhalation

Microsoft Office Excel

Intel® core i5-6500 CPU @ 3.2GHz, 16 GB RAM, 64-bit operating system

Two transparent 30 cm × 20 cm × 20 cm plexiglass boxes linked by a narrow 15 cm × 15 cm × 60 cm passage
Rodent laboratory chow given to rats, mice and hamster is a life-cycle nutrition that has been used in biomedical researc for over 5

No specific brand is recommended

No specific brand is recommended

Optional. Any timer will do, although it is convenient to use an interval timer if you are tickling multiple rats

White circular platform 200 cm in diameter and 1 cm thick on table

Digital video camera for high definition recording of rat behavior under plus maze test

s, first 9x6 cm (height 21 cm) and second 7x7 cm (height 21 cm).

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March 12, 2021

Attn: Vineeta Bajaj, Ph.D.

Review Editor

Journal of Visualized Experiments (JoVE)

JoVE62291R1

Title: A metric test for assessing spatial working memory in adult rats following traumatic brain injury

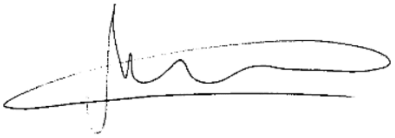
Dear Dr. Bajaj,

Please find attached a revised version of the manuscript JoVE62291R1. In this revised manuscript, we have taken into consideration all the valuable and relevant comments of the reviewers. We have extensively rephrased and clarified parts of the manuscript and made all the corrections as requested. The authors' responses to reviewers' suggestions below are in bold font, and the requested edits are tracked in the revised manuscript.

On behalf of the authors, I would like to express our sincere appreciation for the reviewers' and editor's feedback and suggestions. We sincerely hope that this revised manuscript is now suitable for publication in JoVE.

We thank you and the reviewers for your consideration.

Sincerely,



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Answers to the Editorial and Reviewers' Comments

[Editorial comments]:
1. The editor has formatted the manuscript to match the journal's style. Please retain and use the attached file for revision.
2. Please address all the specific comments marked in the manuscript.
3. Please address all the reviewers' comments as well.

[Reviewer #2]:
Manuscript Summary:
In the revised manuscript, the authors fully addressed the comments concerning the original manuscript.

Major Concerns:
none

Minor Concerns:
I suggest that the authors rewrite 2 sentences:
Lines 57-58: "…ability to recall the placement of two objects of relative distance and angles…"
Lines 371-372: "In groups of 10-20 rats affected with severe TBI containing 10-20 rats…"

Thank you. These have been rewritten.

[Reviewer #3]:
Manuscript Summary:
In the manuscript, "A metric test for assessing spatial working memory in adult rats following traumatic brain injury" Frank et al detail a modified metric task protocol that can effectively assess spatial working memory following a model of mild to moderate TBI. The results of the metric test indicate clear working memory impairment in both male and female rats, several weeks following injury when neurological signs appear normal. Furthermore, the authors describe a clear and reproducible protocol and also explain advantages of this test including reduced stress on the rodent, short test duration, as well as its effectiveness in various injury models. Overall, this is a well-written methods manuscript with a detailed approach.

Major Concerns:
None

Minor Concerns:
1. The description of how the baseline point is calculated is unclear. It is written in the manuscript "Baseline is measured as the time of exploration during the habituation period." It is unclear whether this baseline point is calculated from just the first 5 minutes of habituation, or the entire 15 minute habituation trial? It would be very helpful for a more precise description within the manuscript. While I believe only the first 5 minutes were used, it would be best if this was clearly written in the text.

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Thank you for this important point. You are right that the measurements taken in the first 5 time points (0-5 minutes) were taken as the baseline and presented as 100% of the exploration time. The remaining 3 time points (5-10 minutes, 10-15 minutes and 20-25 minutes) were calculated as a percentage of the baseline. This has been clarified in the text.

2. Table 1 only includes NSS values from 48 hours post TBI. For more clarity, it would be best if the table included NSS scores from before, 48 hours post, and 28 days post TBI, even if neurological impairments were most apparent at the 48 hour time point. This may not be necessary if values were 0, pre- and 28 days post TBI, however this should be stated.

Thank you. We have changed Table 1 to include those figures.

3. On lines 371-372 in the discussion, the words "10-20 rats" is repeated twice in the same sentence.

This has been changed.

[Reviewer #4]:
Revised Manuscript in present form is suitable for publication.
On behalf of the authors, we would like to thank you for support and previous recommendations.