Barnes maze test for spatial memory: A new, sensitive scoring system for mouse search strategies

Rodríguez Peris, Laura ; Scheuber, Myriam Ilona ; Shan, Huimin ; Braun, Marie ; Schwab, Martin E

DOI: https://doi.org/10.1016/j.bbr.2023.114730

Behavioural Brain Research 458 (2024) 114730

Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Barnes maze test for spatial memory: A new, sensitive scoring system for mouse search strategies

Laura Rodríguez Peris [*], Myriam Ilona Scheuber, Huimin Shan, Marie Braun, Martin E. Schwab

Institute for Regenerative Medicine, University of Zurich, Zurich, Switzerland

ARTICLE INFO

Keywords:
Spatial learning
Spatial memory
Barnes maze test
Search strategies
Traumatic brain injury
Neurodegenerative disease

ABSTRACT

The Barnes maze is a task used to assess spatial learning and memory in rodents. It requires animals to learn the position of a hole that can be used as an escape from a bright and open arena. The often-used parameters of latency and path length to measure learning and memory do not reflect the different navigation strategies chosen by the animals. Here, we propose an 11-point scoring scheme to classify the search strategies developed by the animals during the initial training as well as after the change of the escape target to a new position. Strategy scores add an important dimension to time and path length to assess the behavior in this popular maze.

1. Introduction

There are numerous behavioral tests to assess navigational learning and memory in rodents, in particular the T-maze, the Radial arm maze, the Morris water maze, and the Barnes maze [1,2]. These tests use mostly visual cues to facilitate navigation and positive environmental cues like food or negative environmental cues like intense light to stimulate the animals to find the target.

The Morris water maze is well-known and broadly used, it consists in placing the animals in a circular tank filled with milky water; the task is to rapidly find the submerged escape platform. An important disadvantage of this test is the response to stress triggered in the animals; stress is known to have negative effects on cognitive performance [3,4].

Recently, the Barnes maze has become a popular tool to assess spatial working memory, spatial reference memory, and cognitive flexibility in mice [5]. The Barnes maze is a circular, 1 m diameter platform with 20 holes around its perimeter with a small dark escape box placed below one of the holes. The animals tend to escape from the open and bright platform to the escape box; the response to enter a small dark protected place or hole is a natural and physiological reaction with minimal external stress [6]. The Barnes maze is less aversive, less anxiogenic, and less stressful than the water maze [3]. Therefore, this task enables the assessment of spatial learning and memory in a short period of time without the need to use strong aversive stimuli or deprivation [5,7].

Different parameters measure the performance of the animals in the Barnes maze like the primary latency (time to find the escape box), movement velocity, the number of errors, the total distance traveled, etc. Primary latency and path length can be determined easily and automatically and are frequently used. A very interesting parameter for cognitive performance, however, is the search strategy used by the animal. In the majority of the published studies, the strategies are divided into three broad categories: (1) 'Direct', i.e. moving directly to the target hole or to 1–2 adjacent holes before visiting the target. (2) 'Serial', i.e. the animals visit serially adjacent holes in a clockwise or counter clockwise manner [3]. (3) 'Mixed', i.e. hole searches separated by crossing through the center of the maze or unorganized search [7].

The assessment of the strategies that the animals adopt is gaining importance over time and many authors state the relevance of this parameter. For instance, T. Illouz el al., [8] emphasize that the quantification of primary latency, mean speed, and distance lead to a limited and partial understanding of the mouse's behavior and cognitive capacity.

In the present study, we test intact adult male and female mice and adult male mice with traumatic brain injury in the Barnes maze test. As previously shown [9] mice injured with repetitive mild traumatic brain injury exhibited significant deficits compared to intact mice. Apart from detecting a deficit in the brain-damaged mice, we also found a wide range of different strategies that these animals adopt over the training sessions. We found that these were poorly reflected by the three-category classification mentioned above. We, therefore, developed a more detailed, robust 11-point scoring system to assess the different strategies used by the mice to find the target escape box as quickly as

* Correspondence to: Institute for Regenerative Medicine, Wagistrasse 12, Schlieren 8952, Switzerland.
E-mail address: laura.rodriguezperis@uzh.ch (L. Rodríguez Peris).

https://doi.org/10.1016/j.bbr.2023.114730
Received 8 August 2023; Received in revised form 4 October 2023; Accepted 22 October 2023
Available online 26 October 2023

possible. We feel that these more elaborate scores describe well the cognitive capabilities, in particular the learning, memory, and behavioral flexibility of animals.

2. Materials and methods

2.1. Animals

All experiments were performed in accordance with the rules and guidelines and with the approval of the Veterinary Office of the canton of Zürich, Switzerland. The mice were housed in groups of four in open airflow cages under 12-h dark/light cycle and provided with food and water ad libitum at the University of Zurich animal facility (LASC) in Schlieren. The animals underwent habituation (one week) as well as two weeks of handling to reduce the stress and were tested during the light phase. There were two main groups: intact mice and animals with mild traumatic brain injury (mTBI).

Adult C57BL/6 J mice (n = 14) were obtained from Charles River Laboratories (Germany). Seven animals were males of 26–34 g body weight and seven were females ranging from 20 to 23 g body weight. Epidemiological clinical studies consistently report that TBI is two-times more frequent in males and in females [10,11]. Therefore, we focused our studies mainly on male mace but also included a comparison of intact males with females. For traumatic brain injury, adult male mice (n = 5) received a weight drop injury on the closed skull protected by a metal plate [12]. The weight of the metal impactor was 120 g, and the height of the guiding tube was 58 cm. The parameters used are known to induce a mild TBI producing a transient neurophysiologic brain dysfunction including symptoms such as impaired cognition, sleep disruption, and changes in consciousness and somatic and emotional functions [13,14]. The animals started the Barnes maze test 21 days after the TBI. All the animals were 17–19 weeks old at the time that the experiments took place. Handling of the animals took place by the experimenters daily during 2–3 weeks prior to the start of the experiment using tunnels and open hand to reduce the animal's distress.

2.2. Barnes maze apparatus and procedure

The maze consists of a circular platform of grey-colored PVC with a 100 cm diameter (see Fig. 1). There are 20 holes allocated in the perimeter of the platform of 5 cm of diameter each. A small, black escape box is placed under the platform at one of the holes. Four visual cues are placed on each of the four sides of the Barnes maze with different shapes and colors. To avoid distracting environmental cues from the experimental room, white walls, and two screens were surrounding the apparatus. The light intensity was set at 1000 lx like in other protocols [15–17] acting as mild aversive stimuli and motivating the mice to search for shelter in the escape box. The experiments were performed in the light phase, from 9 am to 3 pm. A video camera, mounted in the center above the circular arena recorded all movements of the mice.

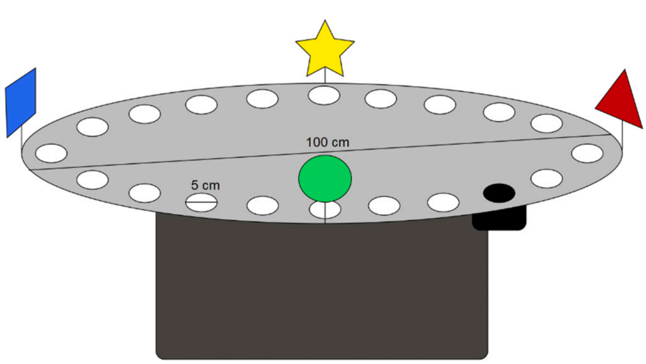

Fig. 1. Barnes maze apparatus appearance and dimensions.

The test consists of a total of nine sessions. Habituation takes place on the first day of the test and consists of placing the animal in the escape box within the Barnes maze for 3 min and in the arena for 1 min [6,7,18]. Immediately afterward, the first training occurs: the animal is placed by the experimenter in the middle of the platform covered by a starting box for 20 s and is then released to find the escape hole/box. If it does not find the escape box within 4 min, the mouse is guided gently to it and kept there for 1 min before returning to the home cage. This training takes place on four consecutive days with four trials each day with 20–30 min intervals and is called the training or acquisition phase. On the fifth day, the first probe trial takes place: the escape box is removed, and the animals can move freely for 3 min before being returned to the home cage. Only one trial is conducted per animal. As indicated [19] in a probe trial longer than 3.5 min the performance of mice is more variable, and the spatial navigation might be underestimated.

The second probe trial takes place three days after the first one to assess long-term memory retention [7]. Training number 5 occurs the day after the probe trial 2 to reinforce the previous learning following the same procedure as the first four trainings. Finally, there are two reversal trials right after training five in which the position of the escape box is rotated for 180º from the old position.

The trials are recorded and analyzed with EthoVision and manual counting, in the following graphs only the first two trials are presented. The parameters measured are stated in Table 1.

2.3. Parameters

Table 1.

2.4. Search strategy scoring system (Fig. 2)

The first three parameters described above assess some aspects of the maze behavior and learning process, yet they only offer a partial understanding of the behavior and cognitive capacity of the mice in this specific environment and task.

We, therefore, developed a more sensitive and specific categorization that is able to identify and distinguish the different behaviors adopted by the animals. We distinguish eleven score levels, from a totally random search (score 1) to a very direct, target-oriented movement trajectory (score 11). The individual scores are described and illustrated in Fig. 2.

The target-finding strategies can be divided into four main groups: direct, with corrections, serial and random.

Direct: animals take a straight direction to the escape box (EB) without checking any other hole. It is the shortest trajectory to get to the target, it represents the maximum level of cognitive performance.

Corrections: Two types of corrections can be found, path corrections happening inside of the escape box sector (meaning inside the area that includes the hole with the escape box and two holes on each side – a total of 5 holes) and corrections happening outside of the escape box sector. Within the first division, we find short correction 1 and 2. The first short correction stands for the strategy where the animals go directly to the escape box after first going to one adjacent hole. Short correction 2 refers to the trajectory in which animals go to the EB after going to two adjacent holes. In the corrections that happen outside the escape box

Table 1
List of measured parameters with the corresponding definitions.

- Total latency – Total time needed to enter the escape box (EB) with the whole body from when the animal is released from the start box.
- Primary latency – Time needed from when the animal leaves the start box to find and enter the EB with the complete head (head poke).
- Path length- Distance traveled from exiting the starting box until entering the EB with the whole body.
- Strategies- Searching strategy that the animals adopt before the first head poke in the escape box.

BARNES MAZE TEST SCORING SYSTEM

Strategy		Types		Description	Score
Direct	Shortest trajectory to get to the target	Direct		**Straight direction** to the escape box (EB) without checking any other hole	11
Corrections	To an incorrect hole/area before self-correcting the trajectory towards the target	Escape box sector	Short correction 1	Directly to EB going through **the adjacent** hole	10
			Short correction 2	Directly to EB going through **the 2 adjacent** holes	9
		Outside the EB sector	Long correction	To the **opposite escape box sector** checking holes and crossing the center (maze) directly to the EB or through adjacent holes	8
			Medium correction	Trajectory **outside the EB sector**, checking holes and then to the EB sector crossing the center	7
Serial	Travels from one hole to the next one in sequence	One direction	Short serial	**3-4** holes in serial strategy before EB	8
			Serial 1	**5-6** holes in serial strategy	7
			Serial 2	**7-10** holes in serial strategy	6
			Serial 3	**11-15** holes in serial strategy	5
			Serial 4	**≥16** holes in serial strategy	4
		Two directions	Combined 1	**holes** serial strategy in two directions	3
		Multiple directions	Combined 2	**holes** in serial strategy with multiple changes in direction	2
Random	Reflects no acquisition of the target location	Random		**Multiple changes** in direction, checking holes nonsequentially, crossing the center once or repeatedly	1

CLARIFICATIONS

In this scoring system, the escape box (EB) is not included in the holes counting.

In the serial strategies if the animal checks one hole in the opposite direction it is counted as exploration. Therefore, the main strategy keeps being serial but the explored and re-checked holes are counted additionally. Likewise, if it skips holes without approaching the center of the maze it stays a serial strategy, but the skipped holes are counted to determine the serial strategies.

The escape box (EB) sector is the area that includes the hole with the escape box and two holes on each side (5 holes).

Directly through/checks: when the animal walks near the holes, the body close by to the circle limits.

Skipping: avoid, pass over the hole with distance, the body is not close to the circumference border.

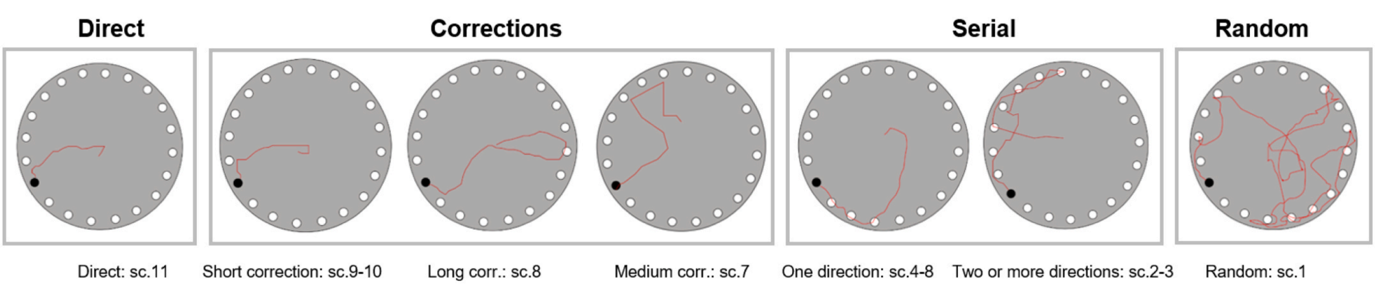

Fig. 2. Classification of search and movement strategies used by mice in the Barnes maze, subdivision into 11 scores. Representation of the different strategies and scores.

sector we can find medium and long correction, depending on how far the animals go before changing the direction of the trajectory. In the medium correction mice start the trajectory going outside of the EB sector and checking holes and then going to the EB sector crossing the center. The long correction strategy is characterized for the animal going to the opposite escape box sector checking holes and crossing the center of the maze directly to the EB or through adjacent holes. These strategies are known for an incorrect start before self-correcting the trajectory towards the target.

Serial: In this strategy division mice travel from one hole to the next one in sequence in a clockwise or counter-clockwise direction. When the strategy only comprises one direction, the strategies are divided depending on the number of holes that mice check on the way to the EB, having a final classification of 5 serial strategies of one direction ranging from 4 to 8 points. When animals have two or more directions, then depending on the number of changes done there are two more serial strategies: combined 1 and 2. The first one refers to when the animal travels the holes in serial strategy in only two directions. The second one, or combined 2, has one point less since it comprises mice travelling the holes in serial strategy having multiple changes in direction.

Finally, the random strategy reflects no acquisition of the target location. This strategy is characterized by multiple changes in direction, animals checking the holes non-sequentially and crossing the center of the maze once or repeatedly.

2.5. Statistical analysis

All statistical tests were completed and represented using GraphPad Prism 8. To assess the sample distribution, a QQ plot was conducted reflecting a non-normal distribution of the data. Each measure of learning (primary latency, total latency, path length, and strategies) was analyzed with the Friedman test to determine if performance improved within the training days. These daily training means were compared using the post hoc test Dunn's multiple comparisons ($p < 0.05$). All correlations were completed with Spearman nonparametric to determine if the strategy scores were similar to the other measures and if it predicted learning and memory performance. Because few significant sex differences were reported, data were pooled across sexes when graphed. To assess differences between TBI and intact mice a two-way ANOVA with Bonferroni's multiple comparisons was conducted in the four different learning parameters. The results are expressed as mean ± SEM and individual values. Significant results were illustrated on the graphs by asterisk representing the different critical P values * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$.

3. Results

3.1. Spatial learning and memory of mice in the Barnes Maze test: Time, distance, and strategies parameters

To evaluate learning and memory of intact, adult mice in the Barnes maze, three parameters were recorded in addition to the strategy scores during the different training and testing days. There were no significant differences between males and females (Mann Whitney U test: $p < 0.05$. Refer to supplementary figure 3) therefore both sexes were pooled but marked with different symbols in all the figures and graphs.

3.1.1. Primary latency

During the acquisition days, there was a small decline in the latency to locate the target/escape hole (=primary latency) from the first day to the second day which then increased slightly on the next training days. On training day 1 the mean was 28.39 s reducing to half on training 5 (15.95 s) and raised in the reversal test where the escape box was relocated (Fig. 3A). Overall, primary latency does reflect learning in this test. The changes in the primary latency over the days were relatively small ($p = .0458$) and there were no significant differences between the testing days (Fig. 3A).

3.1.2. Total latency

Total latency, i.e. the time between the start of the test and the full entry into the escape box diminished gradually over the five training sessions ($p < 0.0001$), decreasing the mean from 142.5 s in training 1–34.65 s in training 5. The latency increased in 'reversal test 1' reaching 69.03 s and decreased again in 'reversal 2' (Fig. 3B). The slope of the learning curve was much steeper for total latency than for primary latency which can also be observed in the significant differences between the first training day and training 5 and reversal 2.

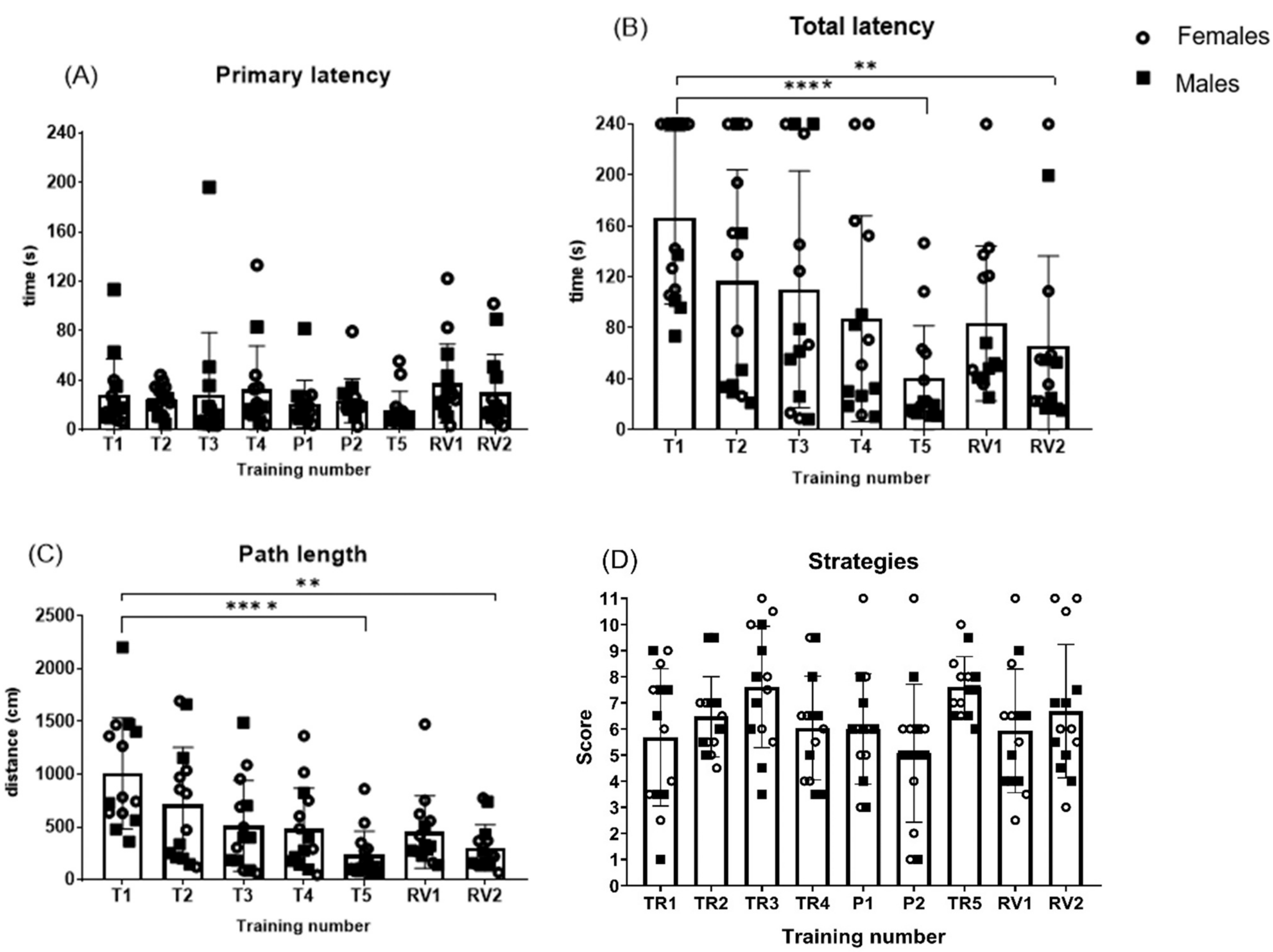

Fig. 3. Performance of adult mice (n = 14/group, 7 males, 7 females) in the Barnes Maze test on the successive days of training and testing as reflected by three parameters: (A) the average, 'primary' latency to find the escape box, (B) the average time to find and fully enter the escape box, (C) the average distance traveled, and (D) the average strategy scores. Bars represent means + /-SEM; points show individual animals in the first trial of the different training days. Friedman test with Dunn's multiple comparisons, significance is illustrated on the graph by asterisks * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$.

3.1.3. Path length

Path length, i.e. the total distance traveled by the mouse, decreased over the training days ($p < 0.0001$), ranging from an average of 890.5 cm on day 1–205.8 cm on training day 5. Target relocation ('reversal 1') increased the path length, which deceased rapidly to the previous level on reversal day 2 (Fig. 3C).

3.1.4. Strategies

The strategy score started around a mean value of 5 points during the first training day to then increase over the acquisition days (TR1-TR4). The score remained stable during the trials where the escape box (EB) was removed (P1 and P2) to get higher in training 5 when the EB was placed again. During the reversal trials, the position of the escape box was shifted to a new one and the intact mice decreased the mean values to raise them again in the second reversal (Fig. 3D).

3.2. Correlation between three time/distance parameters and the strategy score

In order to assess the relationship between the strategy scores and the latency and distance parameters, a Spearman nonparametric correlation was conducted.

Time and distance parameters and search strategy scores were determined for intact males and females (Fig. 4. A, B, and C) and TBI adult mice (Fig. D, E, F) in the Barnes maze. Fig. 4A shows a correlation between the primary latency and the strategy scores ($r = -0.6495$) indicating that when the score gets higher, the primary latency decreases ($p < 0.0001$). The correlation of the total latency and the strategy score was weaker compared with the previous parameter ($r = -0.1820$ and a $p = 0.0729$, Fig. 4B). For the path length, there is a reverse proportional relation, like for the primary latency, with shorter path lengths corresponding to higher strategy scores ($r = -0.3568$, $p < 0.001$) (Fig. 4C).

For the TBI mice ($n = 5$) the slopes of all the correlations are steeper than for the intact mice. For the primary latency, this reversed relation of lower latency times with higher scores gets more noticeable ($r = -0.5759$, $p < 0.0001$, Fig. 4D). For the total latency, this pattern stays consistent, the inclination becomes more pronounced ($r = -0.2991$, $p < 0.001$) contrary to the results obtained with the intact mice. Finally, the path length (Fig. 4F) displays a similar pattern to the previous parameter mentioned, having a pronounced inclination of the slope when correlating the path length to the strategy scores ($r = -0.3568$, $p < 0.001$).

3.3. Spatial learning and memory of mice with traumatic brain injury

3.3.1. Primary latency

The primary latency declined over the acquisition days and remained constantly low from training day 4 on, showing a good learning capacity of these TBI mice (Fig. 5A). In reversal 1, where the position of the escape box was changed, the primary latency increased surpassing that of training 1 since most of the TBI animals did not find the escape box until the end of the trial (240 s) ($p = .0012$, Fig. 5A).

3.3.2. Total latency

The latency was high during the first training days and got reduced to low values at training days 4–5 (Fig. 5B). During reversal 1 the results were very similar to the first training day, the animals took almost the whole duration of the trial to enter the escape box with the whole body. In reversal 2, total latency was reduced to similar values as in training 5 ($p = .0014$, Fig. 5B).

3.3.3. Path length

The learning curve in this parameter mimics that of the previous two parameters ($p = 010$, Fig. 5C).

3.3.4. Strategies

The strategy score increased from a low value around 2 to the maximum value of 10–11 within the first three training days (Fig. 5D). These high scores reflect the ability of the TBI mice to remember the escape box and to use the shortest and most direct way to reach it. These data suggest that mice with mild TBI were able to acquire spatial memory very well ($p = .0004$, Fig. 5D).

3.4. Differences between TBI and intact male mice

We compared intact and TBI mice for the four analyzed parameters of the Barnes maze side by side (Fig. 6). Animals subjected to TBI presented delayed spatial learning as reflected by the slowed decrease of the primary latency over the acquisition days compared to intact animals [$F(8, 80) = 8.440$, $p < 0.0001$, Fig. 6A)]. In the first training days injured animals had double primary latency values compared to the intact ones. On reversal 1 TBI animals' primary latency increased to almost the whole duration of the trial meaning animals were not able to find the escape box within the 240 s of the trial duration.

Regarding the total latency, both groups showed similar results over the different training days except for reversal 1, in which the injured group was essentially unable to find the relocated escape box [$F(6, 60) = 3.672$, $p < 0.01$, Fig. 6B)].

The distance traveled had a similar outcome compared to the parameter of total latency (Fig. 6C), including the high values for the TBI animals at reversal 1 [$F(6, 60) = 2.048$, $p = 0.0731$].

The parameter exhibiting the biggest differences between intact and TBI mice related to the strategy scores (Fig. 6D). Interestingly the groups differed from the beginning of the test: TBI animals began at lower scores than intacts in training 1 and reversal 1 but learned fast and reached higher up to maximal scores at training days 3–5. These data suggest that animals subjected to TBI had significant deficits in starting the task and in initial orientation but, probably due to increased anxiety (data not shown, [20–22] learned faster to hide in the escape box. The lower strategy scores of well-trained intact mice reflect a continuous search behavior with a lower level of urgency to reach the shelter [$F(8, 80) = 6.802$, $p < 0.0001$].

Two-way ANOVA with Bonferroni's multiple comparisons, graph by asterisks * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$.

3.5. Differences between the 3-category and the 11-score point system for the analysis of search strategies

We compared the scores of intact males and females over the training days (Figs. 7A and 7B), as well as for TBI mice (Figs. 7C and 7D) with the 3-category system or our 11-point strategy scoring system. A further comparison of the strategy type instead of the strategy score has been addressed in Supplementary figure 6.

Intact mice showed the flattest curve in the widely used 3 category analysis, with no differences over the training days. In contrast, in the 11-score system, clear differences were detectable over the training days with an increase in the scores from TR1 to TR3 and an unexpected decrease in TR4. There was also a drop from the first to the second probe trial, followed by a rebound in TR5. All these changes could not be perceived using the 3-category strategy analysis.

In the injured mice, the differences between training days were perceivable in both systems but more evident in the 11-score one. Both analysis methods were able to detect the improvement in the performance of the mice over the acquisition days. At reversal 1, when the escape box was shifted and the mice went back to more random search strategies, the decrease in strategy scores was more noted in the 11-score system (Fig. 7C) than in the 3-category system (Fig. 7D).

4. Discussion

The results of the study show the ability of mice to locate the escape

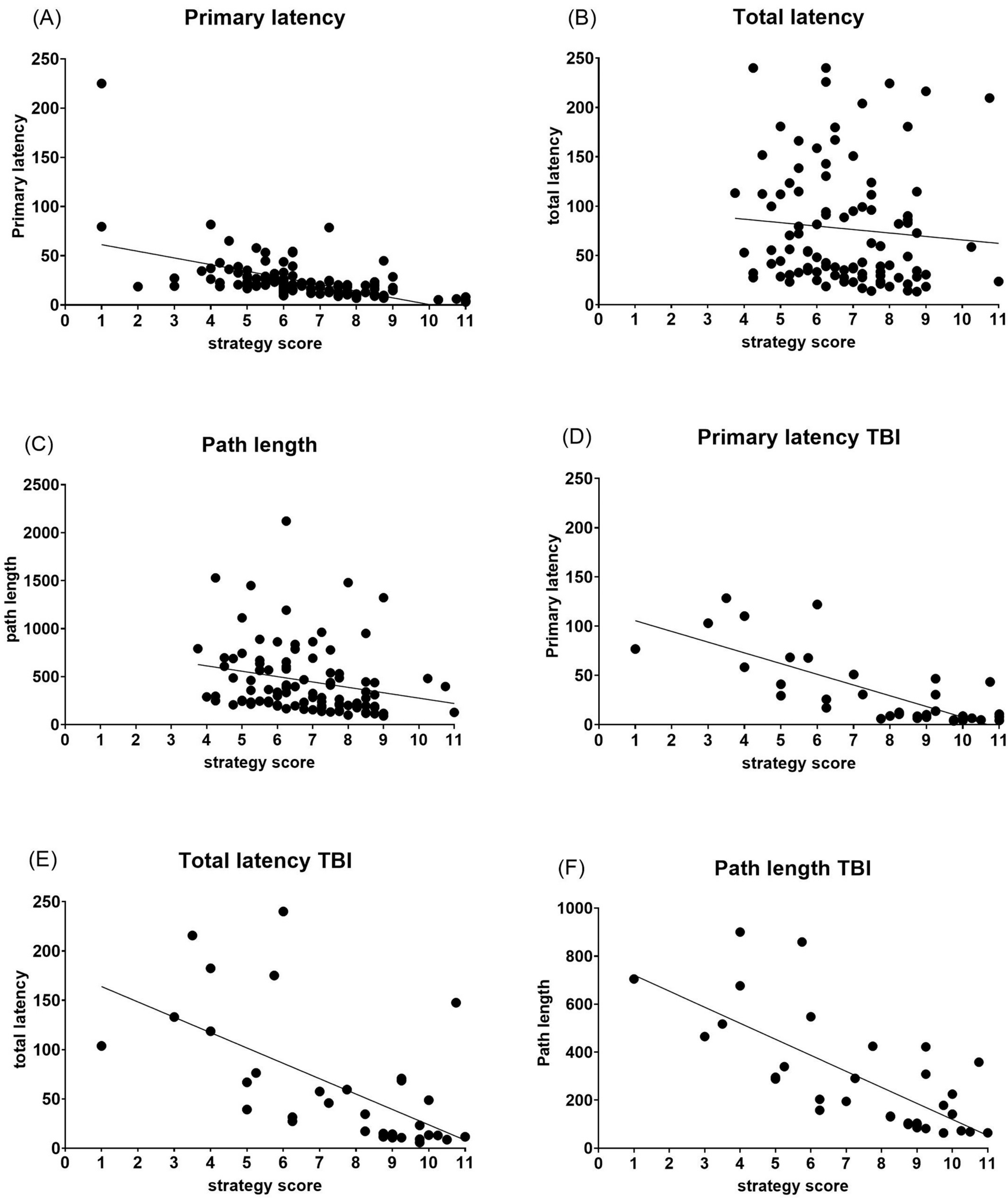

Fig. 4. Scatterplot of three variables measuring latency and path length correlated with the strategy score parameter in intact mice (Fig. 4. A, B, and C), both males and females (n = 14) and TBI males (Fig. D, E, and F) (n = 5). (A) Primary latency ($p < 0.0001$), (B) Total latency ($p > 0.05$), (C) Path length ($p < 0.0003$), (D) Primary latency in TBI ($p < 0.0001$), (E) Total latency TBI ($p < 0.0001$) and (F) Path length in TBI ($p < 0.001$) each correlated with strategy score. Spearman nonparametric correlation with $\alpha = 0.05$.

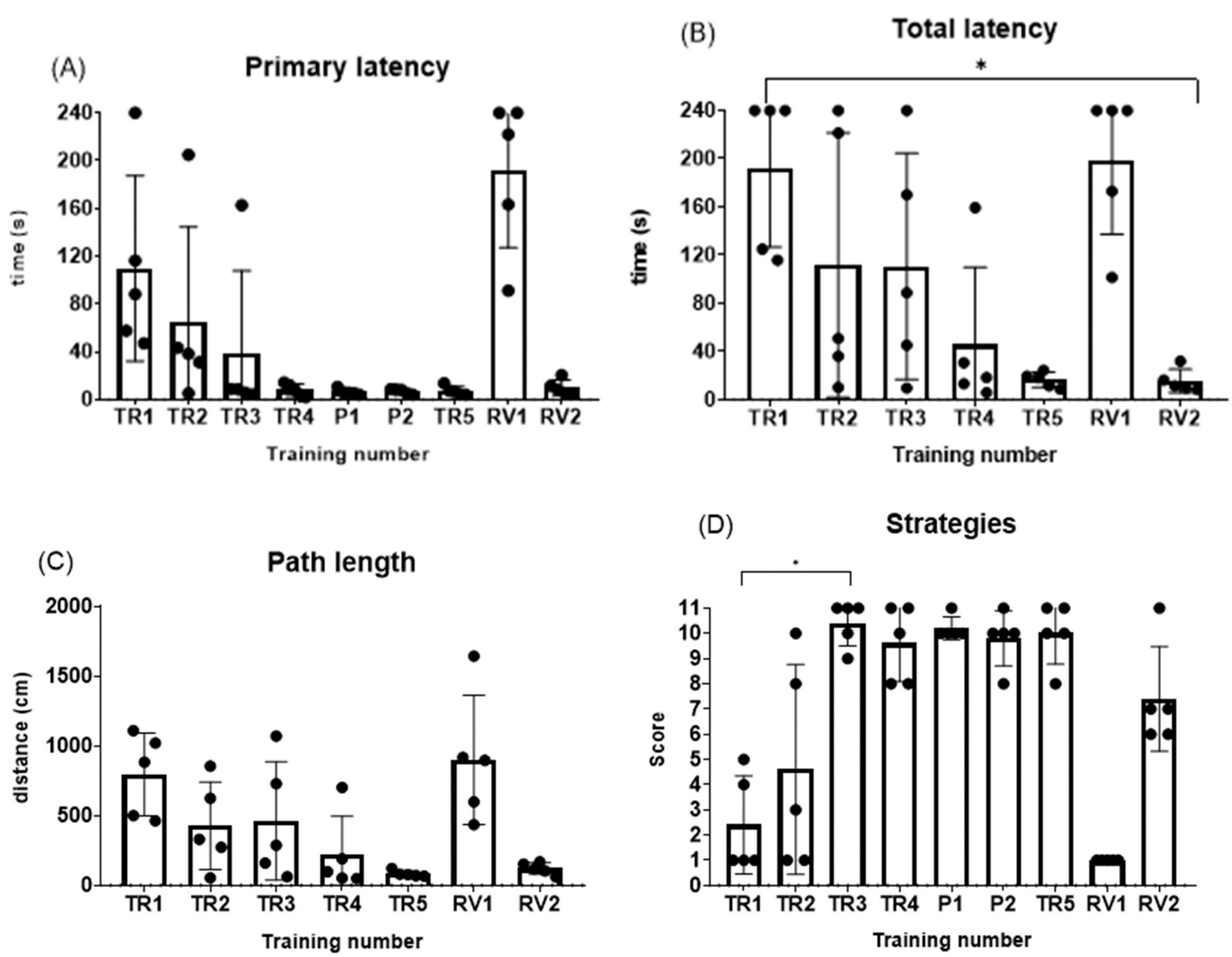

Fig. 5. Performance of TBI mice (n = 5) in the Barnes maze test on the successive days of training and testing as reflected by four parameters: (A) the average, 'primary' latency to find the escape box, (B) the average time to find and fully enter the escape box, (C) the average distance traveled, and (D) the average strategies score. Bars represent means + /-SEM; points show individual animals in the first trial of the different training days. Friedman test with Dunn's multiple comparisons, significance is illustrated on the graph by asterisks * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$.

box faster over the acquisition, testing, and reversal days as reflected by a decrease of the time/distance and an increase of the strategy parameter. Among the three time/distance parameters, total latency and path length are the most sensitive exhibiting the steepest learning curve and being able to detect the changes over the training days in the behavior of the mice.

The latency to locate the escape hole, the so-called 'primary latency', despite being the most widely used measure of learning, was found in the present study to be the least sensitive parameter for detecting spatial learning and memory. This observation is in line with a previous study [19]. The habituation that the animals undergo on the first training day might also be influencing this parameter since the animals encounter the platform and the escape box for a total of 4 min before starting the first trial. Other important factors that can affect the maze behavior and primary latency in particular are anxiety or, on the other hand, lack of motivation. Previous studies have described that unstressed animals familiarize themselves with the environment and the aversive stimuli and are prone to explore the maze instead of entering the escape box immediately [7]. On the other hand, the very fast learning and consistently high strategy scores of TBI animals are in line with an increased level of anxiety often associated with mild TBI [20–22]. Moreover, the low N number used in this study is a factor that could have influenced the results and should be considered. Some experimenters resort to the use of alternative Barnes maze configurations with fewer holes to make serial strategies inefficient but this may not be challenging enough and could therefore increase the probability of the task being performed by chance.

Despite this, the conventional parameters such as latency and path length fail to reflect the complexity of the animals' spatial cognitive abilities and navigation strategies [17,23,24]. Our eleven-point strategy scoring system offers an accurate and sensitive tool to describe the animals' search strategies and their evolution over the training days from totally random to the most target-oriented trajectory. The correlations between time/distance measures and strategy scores were very high, but the curves exhibited typical shallow slopes.

The animals with mild TBI showed a delayed onset of learning compared to the intact animals but learned faster and reached higher strategy scores. An increased level of anxiety may well be the crucial underlying factor for this difference. On the other hand, the problems that TBI animals had in switching back to search after the relocation of the escape box may indicate a lower level of behavioral flexibility. These observations show that the behavior of animals in the Barnes maze reflects more than just learning and memory. Multiple read-outs and careful observations, together with additional tests, e.g. for anxiety, are therefore highly recommended. There is some published evidence showing differences between males and females in spatial and navigational tasks [7,19,25]. However, in the present study, no significant differences between the sexes were found. However, tendencies for higher values for total latency and path length, as well as higher strategy scores for females, were observed.

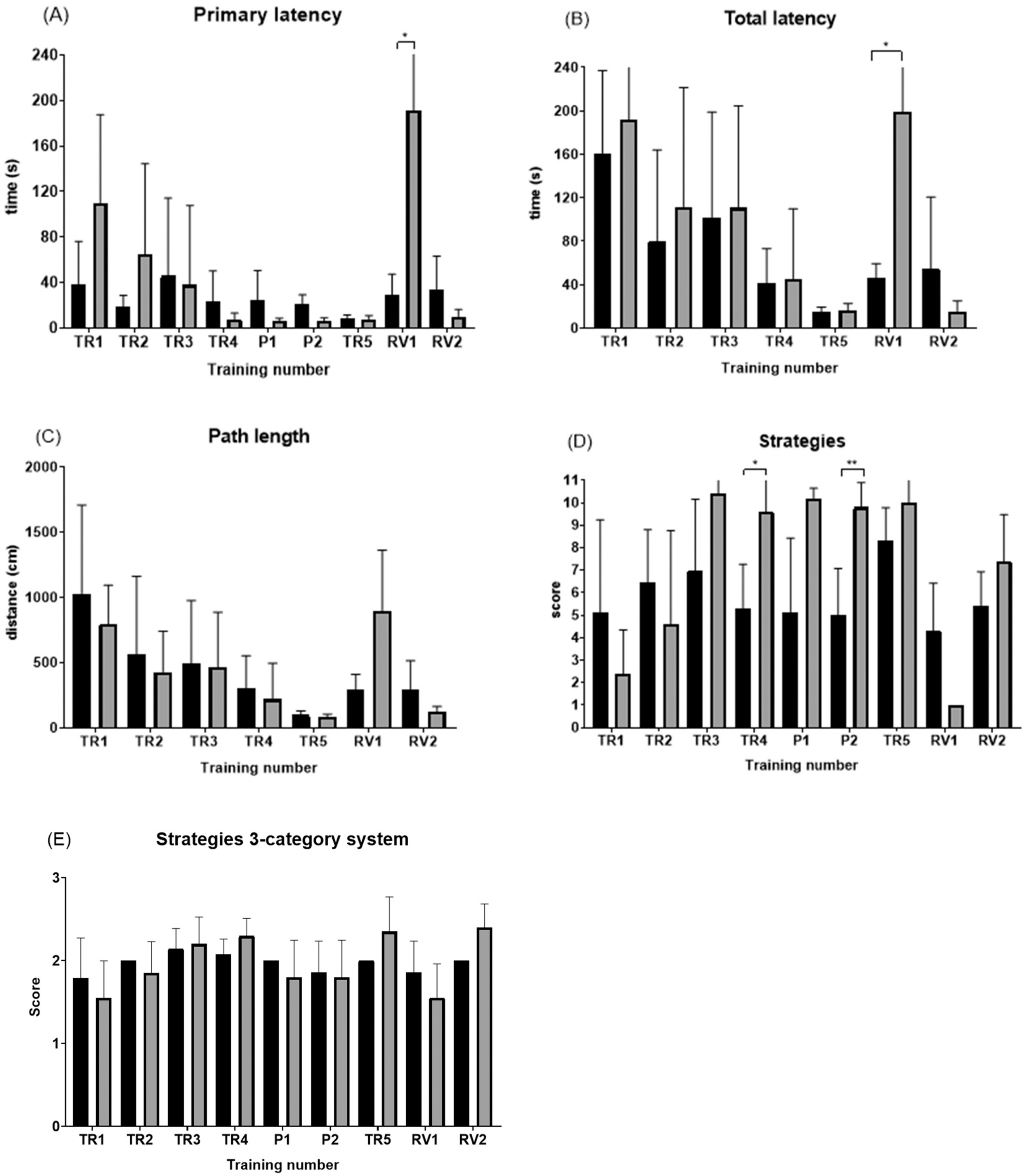

Fig. 6. Performance of TBI male mice (n = 5) and intact males (n = 7) in the Barnes maze test on the successive days of training and testing as reflected by four parameters: (A) primary latency (B) total latency (C) path length, and (D) the average strategies score. Bars represent means + /-SEM; Two way ANOVA with Bonferroni's multiple comparisons, graph by asterisks * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$.

Overall, this study shows that the strategy scoring system is a powerful tool to assess spatial learning in mice. We recommend combining total latency, path length and strategy scores for a comprehensive, sensitive analysis of rodent behavior in the Barnes maze.

CRediT authorship contribution statement

Laura Rodriguez Peris: Conceptualization, Methodology, Investigation, Formal analysis, Writing – original draft. **Myriam Ilona Scheuber:** Methodology, Investigation. **Huimin Shan:** Methodology,

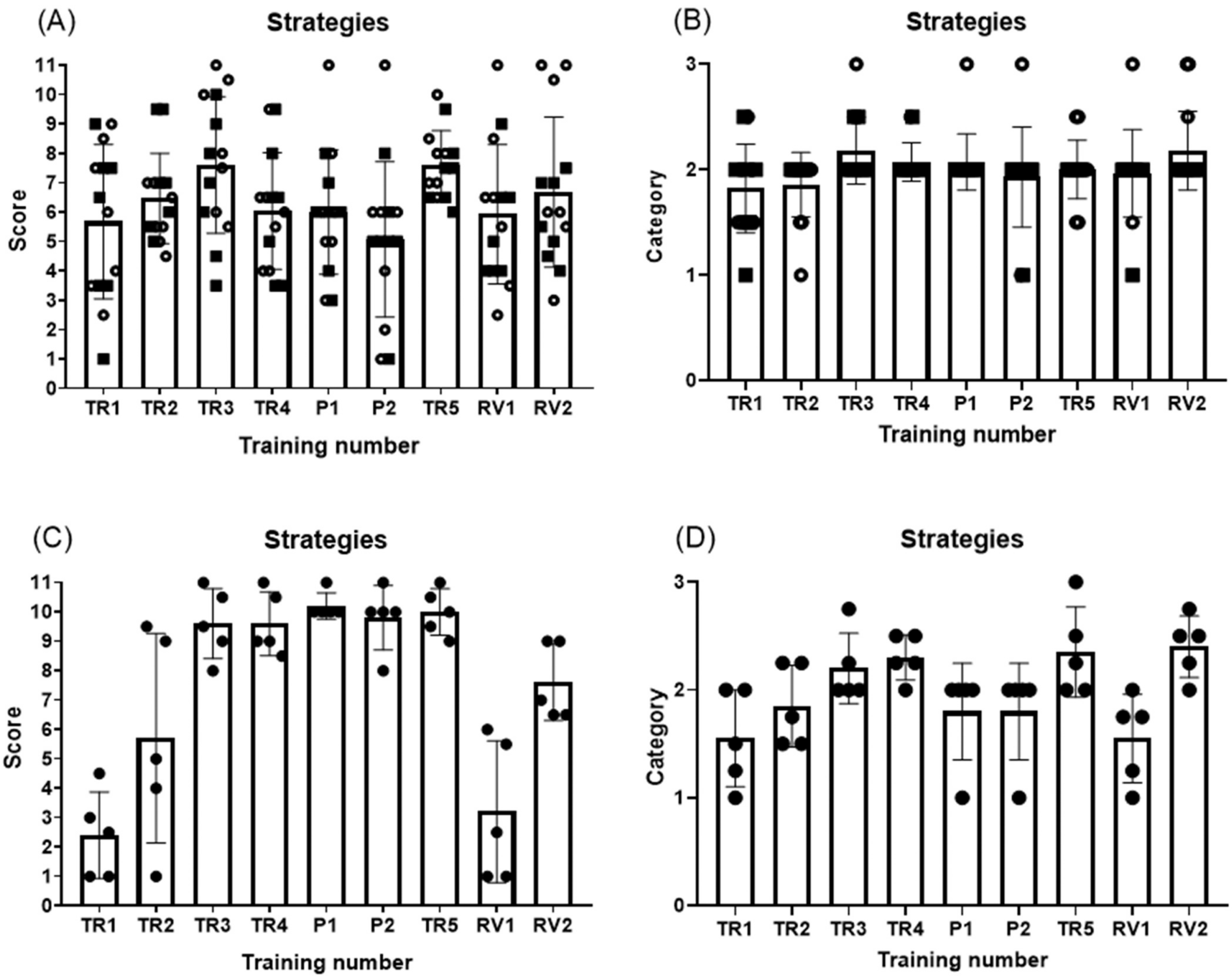

Fig. 7. Comparison of the widely used 3-category system to the 11-point scoring system presented in this manuscript. The first row of graphs (Fig A and B) corresponds to the strategies of intact mice (males= 7 and females= 7). The lower row of graphs (Fig C and D) represents the scores of TBI mice (n = 5) across the training days.

Investigation. **Marie Braun:** Investigation, Formal analysis. **Martin E. Schwab:** Conceptualization, Writing review & editing, Supervision.

Data Availability

Data will be made available on request.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.bbr.2023.114730.

References

[1] S. Sharma, S. Rakoczy, H. Brown-Borg, Assessment of spatial memory in mice, Life Sci. 87 (17–18) (2010) 521–536. Doi: 10.1016/j.lfs.2010.09.004.

[2] C.V. Vorhees, M.T. Williams, Value of water mazes for assessing spatial and egocentric learning and memory in rodent basic research and regulatory studies, Neurotoxicol. Teratol. 45 (2014) 75–90. Doi: 10.1016/j.ntt.2014.07.003.

[3] S.S. Patil, B. Sunyer, H. Höger, G. Lubec, Evaluation of spatial memory of C57BL/6J and CD1 mice in the Barnes maze, the multiple T-maze and in the Morris water maze, Behav. Brain Res. 198 (2009) 58–68. Doi: 10.1016/j.bbr.2008.10.029.

[4] C.V. Vorhees, M.T. Williams, Assessing spatial learning and memory in rodents, ILAR J. 55 (2) (2014) 310–332. Doi: 10.1093/ilar/ilu013.

[5] G. Koopmans, A. Blokland, P. Van Nieuwenhuijzen, J. Prickaerts, Assessment of spatial learning abilities of mice in a new circular maze, Physiol. Behav. 79 (2003) 683–693. Doi: 10.1016/s0031-9384(03)00171-9.

[6] C.A. Barnes, Memory deficits associated with senescence: a neurophysiological and behavioral study in the rat, J. Comp. Physiol. Psychol. 93 (1) (1979) 74–104. Doi: 10.1037/h0077579.

[7] K. Gawel, E. Gibula, M. Marszalek-Grabska, J. Filarowska, J.H. Kotlinska, Assessment of spatial learning and memory in the Barnes maze task in rodents – methodological consideration, Naunyn Schmiede Arch. Pharm. 392 (1) (2019) 1–18. Doi: 10.1007/s00210-018-1589-y.

[8] T. Illouz, R. Madar, E. Okun, A modified Barnes maze for an accurate assessment of spatial learning in mice, J. Neurosci. Methods 334 (2020). Doi: 10.1016/j.jneumeth.2020.108579.

[9] D.I. Briggs, M. Angoa-Pérez, D.M. Kuhn, Prolonged repetitive head trauma induces a singular chronic traumatic encephalopathy- like pathology in white matter despite transient behavioral abnormalities, Am. J. Pathol. 186 (11) (2016) 2869–2886. Doi: 10.1016/j.ajpath.2016.07.013.

[10] A. Biegon, Considering biological sex in traumatic brain injury, Front. Neurol. 12 (2021). Doi: 10.3389/fneur.2021.576366.

[11] R.B. Frost, T.J. Farrer, M. Primosch, D.W. Hedges, Prevalence of traumatic brain injury in the general adult population: a meta-analysis, Neuroepidemiology 40 (3) (2013) 154–159. Doi: 10.1159/000343275.

[12] C.R. Marmarou, R. Prieto, K. Taya, H.F. Young, A. Marmarou, Marmarou Weight Drop Injury Model, Springer Protocols Handbooks, 2009.

[13] I.K. Douglas, S.I. Cohen, M.P. Alexander, Mild traumatic brain injury, Handb. Clin. Neurol. 127 (2015) 131–156, 10.1016/B978-0-444-52892-6.00009-X.

[14] R.A. Laskowski, J.A. Creed, R. Raghupathi, Pathophysiology of Mild TBI: Implications for Altered Signaling Pathways, in: F.H. Kobeissy (Ed.), Brain Neurotrauma: Molecular, Neuropsychological, and Rehabilitation Aspects, CRC Press/Taylor & Francis, 2015.

[15] M.W. Pitts, Barnes maze procedure for spatial learning and memory in mice, Bio Protoc. 8 (5) (2018) 1–11. Doi: 10.21769/BioProtoc.2744.

[16] Y. Van Den Herrewegen, L. Denewet, A. Buckinx, G. Albertini, A. Van Eeckhaut, I. Smolders, D. De Bundel, The Barnes maze task reveals specific impairment of spatial learning strategy in the intrahippocampal kainic acid model for temporal lobe epilepsy, Neurochem. Res. 44 (3) (2019) 600–608. Doi: 10.1007/s11064-018-2610-z.

[17] T. Illouz, R. Madar, C. Clague, K.J. Griffioen, Y. Louzoun, E. Okun, Unbiased classification of spatial strategies in the Barnes maze, Bioinformatics 32 (21) (2016) 3314–3320. Doi: 10.1093/bioinformatics/btw376.

[18] F.E. Harrison, R.S. Reiserer, A.J. Tomarken, M.P. McDonald, Spatial and nonspatial escape strategies in the Barnes maze, Learn. Mem. 13 (6) (2006) 809–819. Doi: 10.1101/lm.334306.

[19] T.P. O'Leary, R.E. Brown, The effects of apparatus design and test procedure on learning and memory performance of C57BL/6J mice on the Barnes maze, J. Neurosci. Methods 203 (2) (2012) 315–324. Doi: 10.1016/j.jneumeth.2011.09.027.

[20] B.L. Tucker, T.J. McCabe, Measuring anxiety-like behaviors in rodent models of traumatic brain injury, Front. Behav. Neurosci. 15 (2021) 1–16. Doi: 10.3389/fnbeh.2021.682935.

[21] D.L. Meyer, D.R. Davies, L.J. Barr, P. Manzerra, L.G. Foster, Mild traumatic brain injury in the rat alters neuronal number in the limbic system and increases conditioned fear and anxiety-like behaviors, Exp. Neurol. 235 (2) (2012) 574–587. Doi: 10.1016/j.expneurol.2012.03.012.

[22] L.E. Moore, L. Terryberry-Spohr, A.D. Hope, Mild traumatic brain injury and anxiety sequelae: a review of the literature, Brain Inj. 20 (2) (2006) 117–132. Doi: 10.1080/02699050500443558.

[23] G. Riedel, L. Robinson, B. Crouch, Spatial learning and flexibility in 129S2/SvHsd and C57BL/6J mouse strains using different variants of the Barnes maze, Behav. Pharmacol. 29 (8) (2018) 688–700. Doi: 10.1097/FBP.0000000000000433.

[24] T.P. O'Leary, V. Savoie, R.E. Brown, Learning, memory, and search strategies of inbred mouse strains with different visual abilities in the Barnes maze, Behav. Brain Res. 216 (2) (2011) 531–542, https://doi.org/10.1016/j.bbr.2010.08.030.

[25] T.P. O'Leary, R.E. Brown, Optimization of apparatus design and behavioral measures for the assessment of visuo-spatial learning and memory of mice on the Barnes maze, Learn. Mem. 20 (2) (2013) 85–96, https://doi.org/10.1101/lm.028076.112.