The starmaze: a new paradigm to characterize multiple spatial navigation strategies

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Abstract

Spatial orientation disorders constitute a major problem in aged subjects. Among the different cognitive strategies used for spatial navigation, the most complex ones seem to be primarily deteriorated during ageing. Being able to dissociate the different cognitive strategies might help us in the early detection of age-related brain dysfunctions. In order to study multiple spatial navigation strategies, a new task was designed and named 'starmaze'. This paradigm was first developed for animal experiments and then adapted to humans using virtual reality. In both cases, up to three different strategies of navigation can be dissociated. The starmaze is composed of a central pentagonal ring from which five alleys radiate. A hidden goal has to be found. To locate the target, the subject can use either distal cues (allocentric strategy), or proximal cues located on the inner walls (guidance strategy), or a sequence of body movements leading to the goal (egocentric strategy). Four versions of the task can be used: *(i)* the multiple strategies, *(ii)* the allocentric, *(iii)* the egocentric, and *(iv)* the guidance version. In the multiple strategies version, the task has two components: the first assesses the learning capability of the subject; the second permits the identification of the strategy spontaneously used by the navigator to solve the task (multiple strategies can be employed: allocentric, egocentric and guidance). The allocentric version of the starmaze requires the subject to learn to reach the goal from different starting points and therefore necessitates a spatial representation of the environment. In the egocentric and guidance versions of the task there are no distal extra-maze cues (the apparatus is surrounded by black uncued curtains); subjects need to either perform a left-right-left movement sequence or to use intra-maze cues (e.g. to first approach a chessboard-like, then a black, and finally a white wall) to reach the target from different departure points. Data acquisition is performed by means of a video recording system and/or a tracking software. A set of parameters are measured to characterize the spatial behavior of the subject quantitatively (for example the distance travelled during each trial or the number of visited alleys). Data processing is automated via a MATLAB batch program developed in our laboratory.

Keywords

Spatial navigation – starmaze – allocentric strategy sequential guidance – sequential egocentric –

1 Introduction

Spatial navigation is a crucial function for many animal species including humans and is primarily deteriorated during aging. Spatial navigation toward an invisible goal involves the ability of a navigating animal or human to first acquire spatial knowledge (e.g. spatio-temporal relations between environmental cues) and to organize it properly. This requires the integration of multimodal sensory signals into a coherent representation. Second, the animal or the human needs to employ this spatial knowledge to adapt its motor behavior to the specific context in which the navigation takes place. Determining and maintaining a trajectory from one place to another calls upon multiple concurrent processes and demands the ability of the subject to adapt a goal-directed strategy to the complexity of the task. Multiple strategies can be employed, including route-based strategies such as learning a sequence of self-movements (egocentric strategy) or a sequence of visual stimuli (guidance) and map-based (allocentric) strategy (Arleo and Rondi-Reig, in press). The 'starmaze' task was designed in order to characterize the different navigation strategies used during a spatial behavior.

2 The starmaze apparatus

2.1 A five-branch maze

The starmaze consists of five alleys forming a central pentagonal ring and five alleys radiating from the vertices of this pentagonal ring (Figure 1). The entire maze is inscribed in a circle (diameter 204 cm) and all the alleys are filled with water made opaque with an inert non-toxic product (Accuscan OP 301). To solve the task, subjects have to swim to a platform hidden below the water surface.

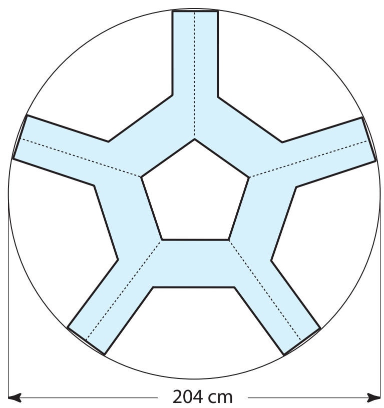

***Figure 1**. Top view of the starmaze apparatus. The light blue regions are the alleys in which the subject has to navigate.*

2.2 Experimental protocols

We have developed four versions of the starmaze task.

***(i)* The 'multiple strategies' version**

This version was designed to permit the identification of the learning strategy spontaneously used by each animal.

In order to achieve this characterization, this multiple strategies version of the starmaze task has two components. The first, called *training test*, lasts twenty days and assesses the animal learning abilities. The second component is called the *probe test* and is designed to identify the strategy used by an animal during the training part of the task. Every five days, one probe test is inserted between two training trials.

In order to learn and then perform the optimal trajectory to the goal (see Figure 2), animals can either use distal visual cues (represented in the figure by the white crosses, the black circle and the black and white stripes), or follow a sequence of intra-maze cues (chessboard-like, black, and white painted walls), or use a sequence of self-movements (turning left, right and then left). We called these three strategies allocentric, sequential guidance, and sequential egocentric, respectively.

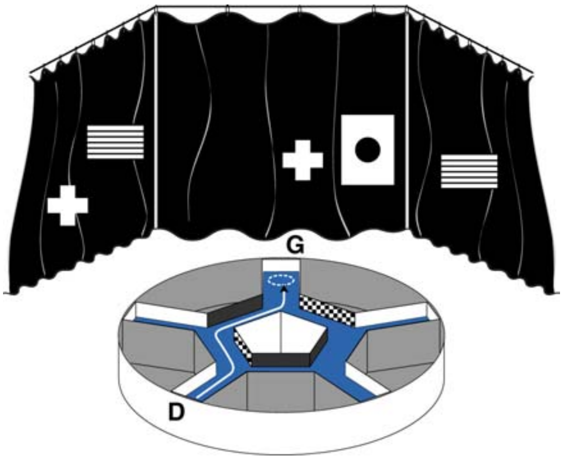

Figure 2. *The 'multiple strategies' protocol: the first component or training part. D is the departure location, G is the goal consisting of an immerged (and therefore invisible) platform (dotted circle). The arrow represents the optimal trajectory performed by the animal after learning. The alleys forming the central pentagonal ring have either black or chessboard-like walls, whereas the radial alleys have white walls. The maze is placed inside a black square curtain with distal visual cues attached on the curtains (crosses, circle, black and white stripes).*

The probe test has been designed to identify which strategy is used by each animal once it has learned to perform the optimal trajectory from the departure point D to the goal G. This probe test relies upon the assumption that an animal occasionally placed at a different departure point will continue to employ the same cues it used during training. This working hypothesis has been already successfully adopted in the cross-maze protocol developed by Barnes, Nadel and Konig (1980). The departure point of the probe test is chosen in order to dissociate the three kind of strategies described previously. This is possible when the D1 departure point is used (see Figure 3). If the animal used the distal visual cues when trained from the departure D, he will continue to use them when placed at the departure location D1 and therefore it will reach the same goal G. On the other hand, if the animal used the proximal visual cues, he will follow the sequence of chessboard-like, black and then white walls and it will arrive at the goal G1. Similarly, if the animal used the idiothetic cues during training, (i.e. it learned to turn left, then right, and then left), it will arrive to the goal G2 goal. During the probe test, three platforms are placed at locations G, G1, G2, corresponding to the three different navigation strategies. Most animals navigate without hesitation to the goals G, G1, or G2, allowing us to characterize their strategy during the learning component. In a few cases, the trajectory followed by the animal can be different from the three described above; we conclude that the animal demonstrates 'no clear strategy' during the probe test.

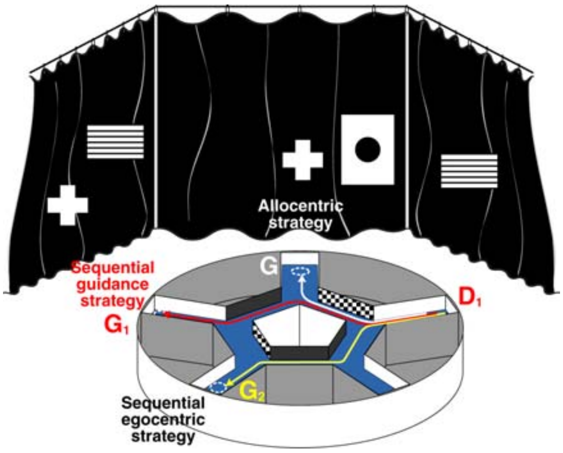

Figure 3. *The 'multiple strategies' protocol: the second component or probe test. As described in the text above, the new departure point is fixed (D1) and G, G1 and G2 goals correspond to allocentric, sequential guidance and sequential egocentric strategies, respectively.*

(ii) **The allocentric version**

This version was designed in order to evaluate the ability of animals to learn a spatial navigation task using exclusively the distal visual cues. Proximal intra-maze cues are removed and each animal is placed in a randomly-selected alley (D1, D2, D3, D4) not containing the platform (Figure 4). In order to learn this task and use the optimal trajectory from each departure points, animals need to encode an allocentric representation of the environment. Similar to the Morris water maze (Morris et al., 1982), solving this version of the 'starmaze' task implies spatial learning capabilities to find a hidden platform from different starting points. However, here animals are constrained to swim in alleys that guide their movements, which permits a detailed analysis of the animals' trajectories.

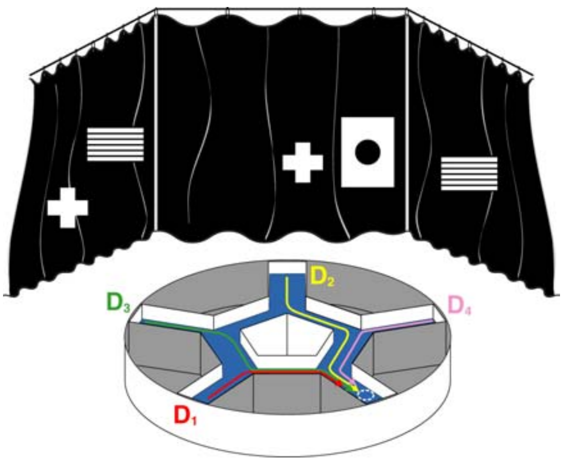

Figure 4. *The allocentric version of the starmaze. D1, D2, D3 and D4 represent the four possible departure locations randomly selected during the training period. White arrows represent the four optimal trajectories.*

***(iii, iv)* The guidance and egocentric versions**

In these two versions extra-maze cues are removed and a circular black curtain is placed around the maze. During the guidance version (Figure 5) the same sequence of proximal cues is rewarded from different starting points. Each departure location D_i is associated to a specific goal location G_i requiring the animal to follow chessboard-like, then black, and finally white walls to solve the task. In the egocentric version of the task (Figure 6), intra-maze cues are also removed (note that all inner walls are white). During learning, the same sequence of movements is rewarded while varying the departure points. Each starting location D_i is associated to a specific goal G_i requiring the animal to turn left, right and then left. These strategies organized in sequence can be tested thanks to the existence of five alleys radiated from a pentagonal ring.

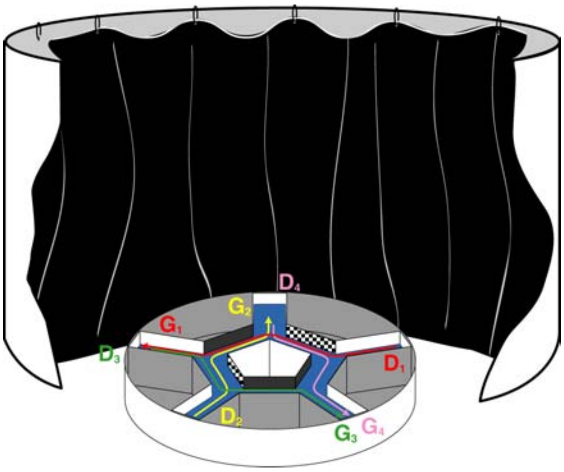

Figure 5. *The guidance version of the starmaze.*

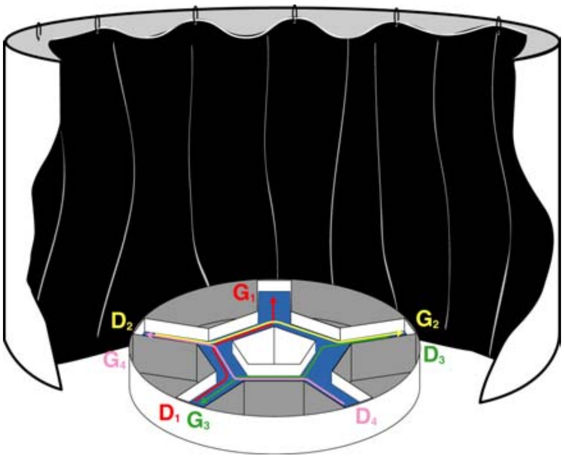

Figure 6. *The egocentric version of the starmaze.*

3 Data acquisition and analysis

We used a videotracking software (SMART@: Spontaneous Motor Activity Recording and Tracking, v2.0) designed for monitoring the behavioral activity of small animals in real time. This software provides a graphic editor suitable to define the zones in which we decide to measure the different behavioral parameters (Figure 7). Based on the defined zones, the tracking software generates a MS Excel file containing the position of the animal (the X-Y cartesian coordinates of the center of mass of the image representing the animal, sampled every 200 ms) over time. The alley in which the animal swims (Z variable) and the mouse number (subject) are also reported in this file (see Figure 8).

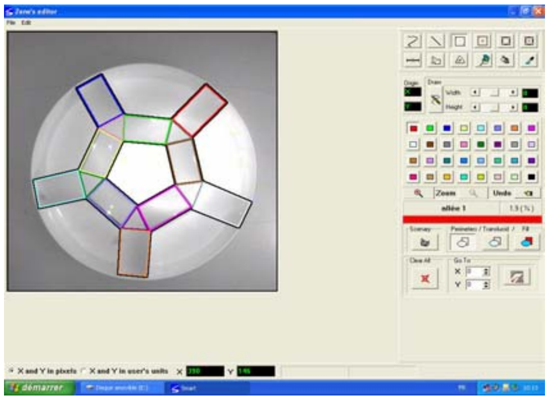

Figure 7. *Snapshot of the computer screen displaying the different zones we defined inside the starmaze (left part). The right part shows the different tools of the graphic editor available with this software.*

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From	00:00:00	to	00:00:08			
Sample	T (sec)	X	Y	Z. Nu	Z. Name	Subject
1	0	152.05	68.033	1	allée 1	89
2	0.2	149.18	73.361	1	allée 1	89
3	0.4	147.53	77.869	1	allée 1	89
4	0.6	145.07	81.967	1	allée 1	89
5	0.8	142.19	84.426	11	inter 1	89
6	1	137.67	87.705	11	inter 1	89
7	1.2	132.74	92.213	10	allée 10	89
8	1.4	128.22	95.902	10	allée 10	89
9	1.6	126.58	97.541	10	allée 10	89
10	1.8	127.4	98.77	10	allée 10	89
11	2	129.04	100	10	allée 10	89
12	2.2	131.51	102.46	10	allée 10	89
13	2.4	134.38	106.15	10	allée 10	89
14	2.6	136.85	110.66	10	allée 10	89
15	2.8	138.9	115.57	10	allée 10	89
16	3	139.73	119.67	10	allée 10	89
17	3.2	137.26	123.36	15	inter 9	89
18	3.4	134.38	125.41	15	inter 9	89
19	3.6	131.51	125.82	8	allée 8	89
20	3.8	130.27	127.05	8	allée 8	89
21	4	128.63	129.1	8	allée 8	89
22	4.2	128.22	133.61	8	allée 8	89
23	4.4	127.81	137.7	8	allée 8	89
24	4.6	126.16	141.39	8	allée 8	89
25	4.8	123.7	145.9	8	allée 8	89
26	5	121.23	150.41	8	allée 8	89
27	5.2	119.59	153.28	8	allée 8	89

Figure 8. *Example of MS Excel file provided by the SMART@ software. The alley in which the animal swims (Z. name) and the mouse number (subject) are reported in this file (see Figure 7).*

The data provided by the tracking system are employed for the further analysis of specific behavioral parameters to quantify the navigation performance of the subjects. A MATLAB batch program has been developed to process all the collected data automatically. The program goes through all trials (e.g. for example 25 animals, 4 trials per day for each animal, and 10 days result in 10^3 trials) and for each trial it computes: *(i)* the time needed to reach the target (termed escape latency); *(ii)* the average speed of the animal; *(iii)* the mean and cumulative distance between the animal and the target; *(iv)* the total distance traveled by the animal; *(v)* the mean and cumulative egocentric angle between the direction of motion of the animal and the direction to the platform; *(vi)* the amount of time spent in each region of the maze; *(vii)* the number of arms visited by the animal before reaching the goal (see Figure 9). Then, the program calculates the mean values of each behavioral parameter by averaging over all the day trials for each subject and/or for each group of subjects (e.g. control and mutant mice). The results are saved as MS

Excel files, one for each behavioral parameter, and used to perform the statistical analysis (e.g. t- and Anova tests) by means of the Statview 5.0 commercial software.

Parameters measured / Methods	Manually	Tracking software	MATLAB batch program
Latency to reach the goal	++	++	++
Number of arms visited	+	+	++
Sequence of arms visited	+	+	++
Mean animal speed	-	++	++
Total distance travelled	-	++	++
Mean distance between animal and goal	-	-	++
Mean angle between animal heading and goal	-	+	++
Time spent in different areas	-	+	++

Figure 9. *The principal behavioral parameters and their observation with different acquisition methods. Symbols: '-' not possible; '+' possible but not automatic; '++' very easy or automatically executed.*

The MATLAB program can also be employed to build three-dimensional (3d) representations of the trajectories followed by the animals at different learning stages (Figure 10). These plots provide a qualitative measure of the ability of a subject (or a group of subjects) to perform optimal goal-oriented behavior. To build these 3d plots, the MATLAB program samples spatial locations by means of a uniform grid (resolution 30x30, each grid cell corresponding to a 5x5 cm area). Then, each cell is given a value representing the time spent by the subject in that region (this values is normalized relative to the duration of the trial, then averaged over many trials and eventually over all the subjects of a same group).

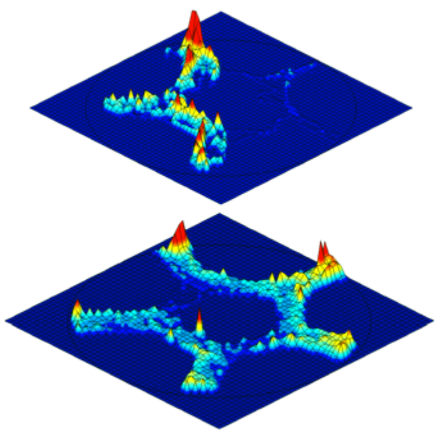

Figure 10. *Two samples of 3d plots providing a qualitative description of the animal behavior when solving the starmaze task. Example of an optimal (top) and a non optimal (bottom) trajectory during navigation.*

5 Conclusion

The design starmaze task took inspiration from the combination of the Morris water maze (Morris et al., 1982) and the cross-maze (Barnes et al., 1980; O'Keefe and Conway, 1980; Packard and Mc Gaugh, 1996; see White and McDonald for review). Similar to the Morris water maze, the complexity of the task requires a subject to develop a map-based (allocentric) strategy. As for the cross-maze, alternative strategies can be used and identified such the egocentric and the guidance strategies. Furthermore, this new task adds the possibility to test route-based strategies such as sequential guidance and sequential egocentric procedures combined with a possible map-based strategy. Therefore, the starmaze allows us to study the ability of a subject to use one of these complex strategies spontaneously. Note that sequential egocentric and sequential guidance as defined in the starmaze refer to route-based strategy as they require a sequential organization of the information. This is different from target approaching or stimulus-triggered response as defined in the cross-maze. Such a difference makes it possible to investigate the distinct neural and cellular bases mediating simple versus sequentially-organized strategies. Finally, the starmaze gives also the possibility to test the subject's navigation capability when it is forced to use a specific strategy.

Multiple applications of the starmaze can be imagined. We are currently employing it to study the neural bases of spatial navigation by using a behavioral genetic approach with conditional mutagenesis models (Rondi-Reig et al., 2004; Burguiere et al., 2004), and to develop a test for early detection of age-related dysfunctions (Petit et al., 2005).

Acknowledgments

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