# A 3D SPATIAL NAVIGATION TASK FOR ASSESSING MEMORY IN RODENTS

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**Summary:** In the present report we describe a 3-D maze spatial navigation task for rats based on a modification of an eight-arm radial maze. The arms radiating from a central platform can be presented in a horizontal plane either raised 10 cm above the level of a central platform or lowered 10 cm below. Memory of visited and non-visited arms can be guided by distinct internal cues (patterns) that are presented on panels set at the end of each arm. Rats are trained in three different maze configurations (eight session each) with arms set lowered, flattened or raised relative to a central platform. A food pellet is placed at the end of each arm and rats are allowed to make eight arm choices only, in each testing session. In this task rats perform better when moving uphill to raised arms than when moving on flattened arms or when moving downhill to lowered arms. It is likely that spatial navigation on raised arms is based on visual cues that were highly visible from the central platform. This result can be accounted for by the position of the rats' eyes that are placed laterally on the sides of their heads.

**Key words:** vision, recognition, retrieval, hippocampus, ethology, transgenic, knockout, mice

**Introduction:** The spatial maze tasks proved valuable tools for assessing effects of drugs, lesions and other manipulations in rodents on behavior and cognition (1, 3-4, 7-12, 14, 16). These mazes are presented in a horizontal flat plane while in a real word space is unevenly shaped. We navigate in a 3D environment while planning our direction to a specific place from underground or a valley, from the height of a tower or just from a street. It is unlikely that spatial navigation in the city streets resembles to spatial navigation from the top of a tower or a tall building, or from an underground or a valley. From the top of a tower we need only to recognize familiar landmarks (e.g. buildings and monuments) while from a valley or underground we need to bring back from memory a representation of hidden places and locations. When moving in a street we may need, either or both, recognition of landmarks and a spatial representation of the city. Rodents would behave in the same way in the wild. Rats and mice navigate in an uneven landscape where the location of foods is scattered in different locations either in underground tunnels or in open fields. As in human, it is unlikely that spatial navigation in a horizontal plane resembles to spatial navigation when moving downhill from a raised platform or moving uphill from a lowered platform. This hypothesis will be examined in the experiment described in the present paper. Rats are trained in a 3D spatial maze based on a modification of an eight-arm radial maze. Several aspects of behavior will be examined to account for performance of rats in this novel model.

**Materials and Methods:**

Subjects: The study used 6 naive male rats (270-300g) of the pigmented Lister Hooded strain. Throughout the period of the experiment the animals were housed individually under diurnal conditions (12 hr light / 12 hr dark), all testing occurring at a regular time during the light period. During testing on the radial arm maze rats were deprived of food so that their body maintained 80-85% of the initial body weight. All rats were on ad lib water throughout.

**Apparatus:** It consists of eight arms radiating from a central platform (Fig. 1). Each arm (51 x 11.2 cm) is made from two segments, extended from an octagonal shaped central hub (30 cm in diameter) and can be manipulated independently. The first segment of an arm (15.2 x 11.2 cm) directly attached to the central platform can be tilted (max. 90º) and constitutes a ramp that allow access to the second segment (35 x 11.2 cm) of the arm which is presented horizontally either at the same, below or above the level of a central platform. Sidewalls, about 1 cm high, extended the length of each arm. The arms of the maze are raised or lowered by 10 cm above or below the level of the central platform which is elevated 55 cm from the floor. The maze was placed in a sound attenuated room with a masking noise of 70 dbs above the human thresholds. The end of each arm was extended with panels of identical size (20.2 x 11.2 cm). These panels are used for holding intra-maze cues made of distinctive pattern drawings designed on plastic adhesive material and attached to a PVC board (18 x 11.2 cm). A small cup (2.5 cm wide) was placed at the end of each arm. The maze is made from gray PVC (5 mm thick).

The maze was totally surrounded with a heavy green-light colored curtain. A dim light and an infrared camera were centered 1.85 cm above the central platform.

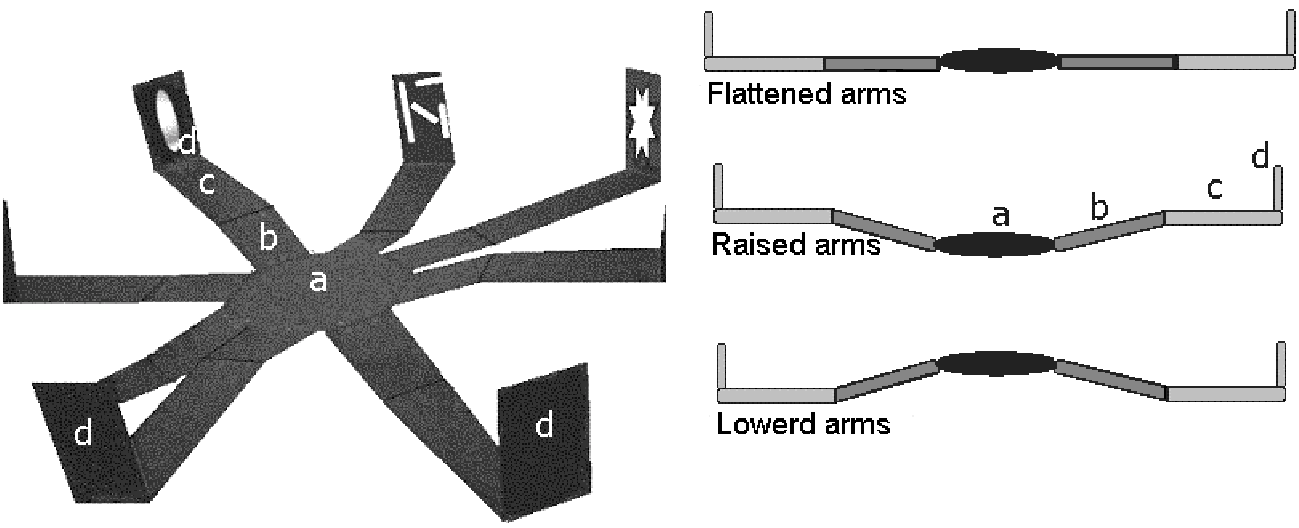


**Figure 1:** Graphic representation of the 3D maze. (a) Central platform, (b) Ramp for access to arms of the maze, (c) Horizontal arm, (d) Panel for displaying visual stimuli

**Training:** Rats were tested in three testing conditions that were randomly alternated between sessions (24 sessions in total). There were sessions with all arms presented at the level of the central platform, sessions with all arms raised above the level of the central platform and, sessions with all arms lowered below the level of the central platform. In order to change the configurations of the maze, the central platform is raised or lowered by 10 cm. A session lasted until an animal made eight choices. There was only one session a day. The cups at the end of each arm contained one reward pellet (45mg P.J. Noyes Company Inc., UK). A session is observed through a video monitor and observations were recorded through a computer program. One hour after the completion of the session animals had a free access to 5g of biscuit.

**Measurements and statistical analysis:** Five measures were considered: *1) Mean number of correct choices:* a correct choice is defined as an entrance to an arm of the maze that has not been visited yet; *2) Mean number of correct choices before the first error:* a first error is defined as the first time when a rat re-visits an arm of the maze that has been already visited earlier in the session; *3) Average duration of sessions:* records of the time required for a rat to make eight choices per session; *4) Mean latency to first choice and mean latency to first choice relative to the whole session duration:* records of the first entry of a rat in an arm and, the proportion of the latency period on the whole duration of a session; *5) Mean latency to first error and mean latency to first error relative to the whole session duration:* records of first time entry of a rat in an arm that has been already visited within a session and, the proportion of the latency period on the whole duration of a session; *6) Mean visit duration to correct choices and mean visit duration relative to the whole session duration:* records of the cumulated time spent in the correct arm and, the proportion of the latency period on the whole duration of a session; *7) Mean visit duration to incorrect choices and mean duration relative to the whole session duration:* records of the cumulated time spent in the incorrect arm and, the proportion of the latency period on the whole duration of a session.
The 3D maze is run manually. This method provides no control over the parameters of the task, specially the latency to entries and duration of visits. These are subjects to individual variations because animals make their choices of the arms of the maze at their own pace. For this reason we examined the mean arm entry latencies/session duration ratio and the mean arm visits/session duration ratio that would compensate for the effects of individual variations.
Repeated measures ANOVA test and Student-Newman-Keuls post-hoc comparisons are performed on each measurement. The threshold level for significance is $p \leq 0.05$.

**Results:**

*1. Mean number of correct choices (Fig. 2):* Performance of rats is significantly different between positions ($F_{2,10}= 52.44$, $p<0.001$) and between sessions ($F_{7,35}= 8.39$, $p<0.001$) but there is no significant interaction between sessions and positions ($F_{14,70}= 1.25$, $p>0.10$).

Student-Newman-Keuls post hoc comparisons demonstrate significant differences between performances in the three configurations of the maze ($p<0.01$). Performance in raised arms is significantly better than in flat and lowered arms and, performance in flat arms is better than in lowered arms. There are also significant differences between sessions 5-8 compared to sessions 1-2, and between 7-8 compared to sessions 3-4 ($p<0.01$).

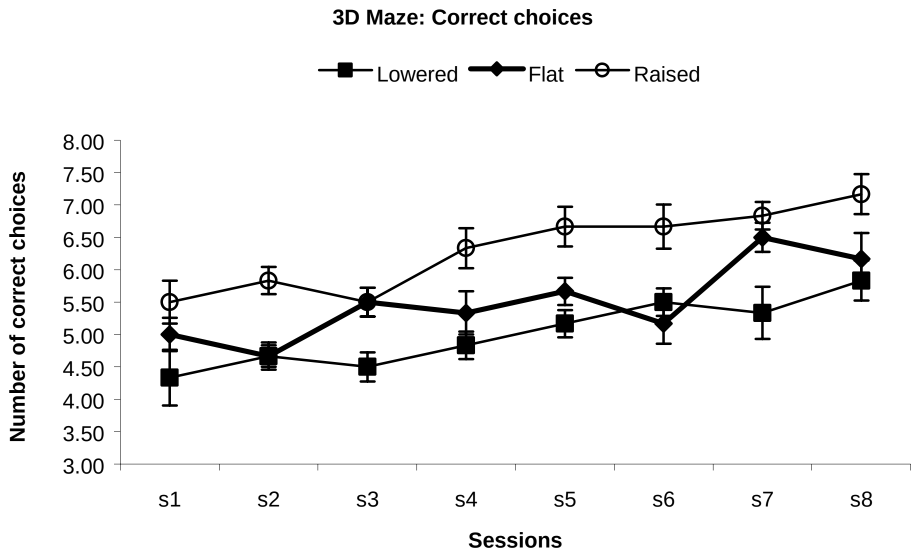


**Figure 2.** Mean number of correct choices made by rats in each session (±s.e.m.) in the condition where the arms of the maze are lowered, flattened or raised relative to level of the central platform.

*2. Mean number of correct choices before the first error (Fig. 3):* Performance of rats is significantly different between positions ($F_{2,10}= 4.54$, $p<0.04$) and between sessions ($F_{7,35}= 5.79$, $p<0.001$) but there is no significant interaction between sessions and positions ($F_{14,70}= 1.40$, $p>0.10$).

Student-Newman-Keuls post hoc comparisons demonstrate that the number of correct choices before first error is higher in raised arms than in lowered arms ($p<0.01$). There are also significant differences between sessions 4-8 compared to sessions 1, and between 7-8 compared to sessions 2 ($p<0.01$).

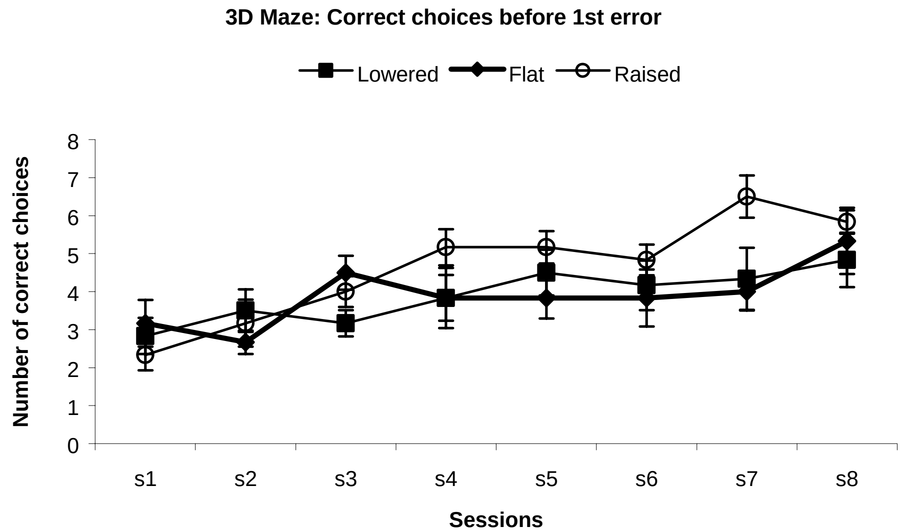


**Figure 3.** Mean number of correct choices made by rats before first error in each session (±s.e.m.) in the condition where the arms of the maze are lowered, flattened or raised relative to level of the central platform.

*3. Average duration of sessions (Fig. 4):* The time spent by rats in each session is not significant between positions (F2,10= 0.42, p>0.10) and between sessions (F7,35= 1.78, p>0.10). There is also no significant interaction between sessions and positions (F14,70= 0.68, p>0.10).

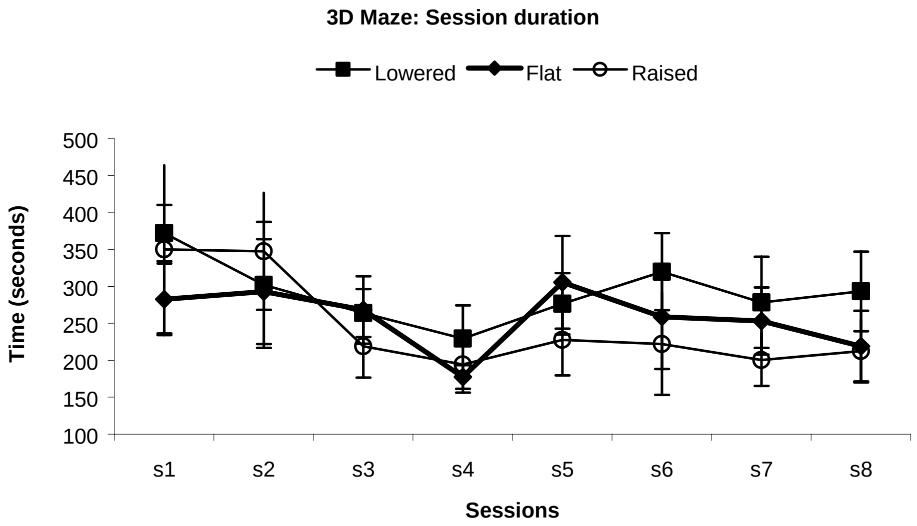


**Figure 4.** Mean time spent by rats per session (±s.e.m.) in the condition where the arms of the maze are lowered, flattened or raised relative to level of the central platform.

*4. Mean latency to first choice (MLFC) and mean latency to first choice/session duration ratio (MLFCR) (Fig. 5):* There are no significant differences between positions on both MLFC and MLFCR (F2,10= 0.41, p>0.10 and F2,10= 0.45, p>0.10, respectively). There are, however, significant differences between sessions (F7,35= 2.86, p<0.02 and F7,35= 3.21, p<0.001, respectively) but no interaction (F14,70= 0.62, p>0.10 and F14,70= 0.70, p>0.10, respectively).

Student-Newman-Keuls post hoc comparisons demonstrate that MLFC and MLFCR measures are significantly higher in the first session than in any other session (p<0.01).

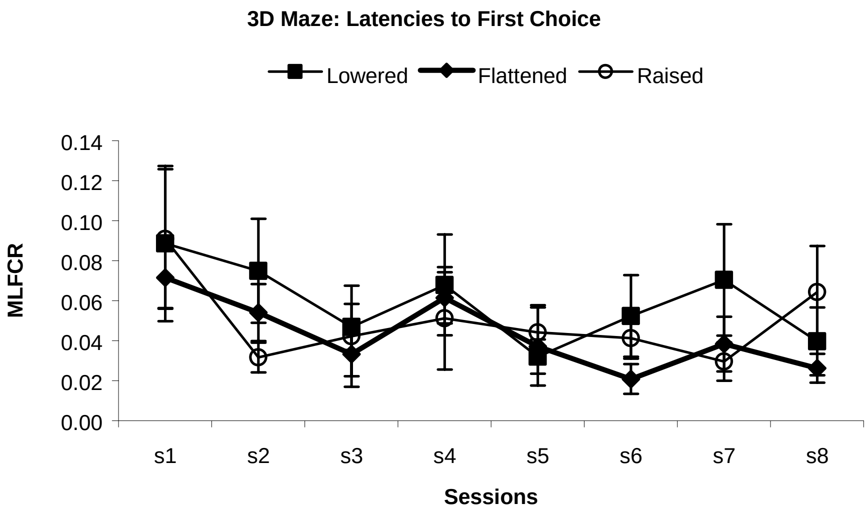


**Figure 5.** Mean latency to first arm visit/session duration ratio (±s.e.m.) in the condition where the arms of the maze are lowered, flattened or raised relative to level of the central platform.

*5. Mean latency to first error (MLFE) and mean latency to first error/session duration ratio (MLFER) (Fig. 6):* There are no significant differences between positions on both MLFE and MLFER ($F_{2,10}= 0.48$, $p>0.10$ and $F_{2,10}= 3.13$, $p<0.09$, respectively). There are, however, significant differences between sessions on MLFER ($F_{7,35}= 3.19$, $p<0.01$) but not on MLFE ($F_{7,35}= 0.42$, $p<0.01$). The interaction between positions and sessions is significant on the MLFER ($F_{14,70}= 2.16$, $p<0.02$) but not on MLFE ($F_{14,70}= 0.36$, $p>0.10$) .

Student-Newman-Keuls post hoc comparisons demonstrate that MLFER is significantly lower in the first two sessions than in session 8 ($p<0.01$).

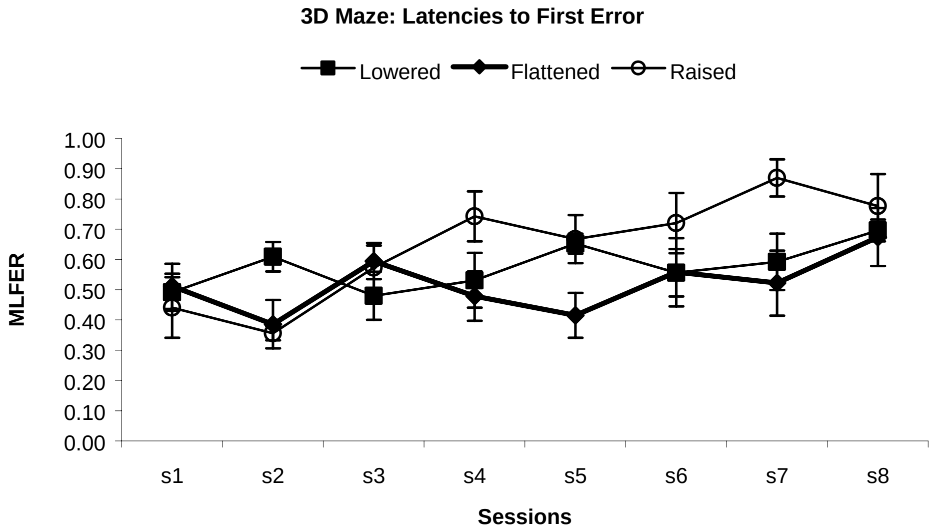


**Figure 6.** Mean latency to first error/session duration ratio (±s.e.m.) in the condition where the arms of the maze are lowered, flattened or raised relative to level of the central platform.

*6. Mean visit duration to correct choices (MVDCC) and mean visit duration to correct choices/session duration ratio (MVDCCR) (Fig. 7):* There are no significant differences between positions ($F_{2,10}= 0.37$, $p>0.10$ and $F_{2,10}= 1.11$, $p>0.10$, respectively), no significant differences between sessions ($F_{7,35}= 1.82$, $p>0.10$ and $F_{7,35}= 1.98$, $p<0.09$, respectively) and no interaction between sessions and positions ($F_{14,70}= 1.54$, $p>0.10$ and $F_{14,70}= 1.75$, $p<0.07$, respectively) on both MVDCC and MVDCCR.

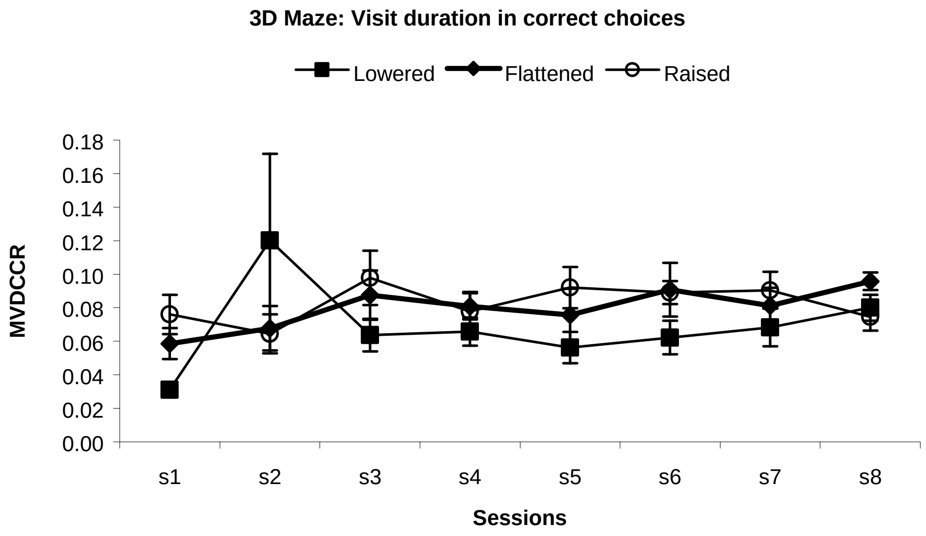


**Figure 7:** Mean visit duration to correct choices/session duration ratio (±s.e.m.) in the condition where the arms of the maze are lowered, flattened or raised relative to level of the central platform.

*7. Mean visit duration to incorrect choices (MVDIC) and mean visit duration to incorrect choices/session duration ratio (MVDICR) (Fig. 8):* There are no significant differences between positions ($F_{2,10}$= 0.33, $p>0.10$ and $F_{2,10}$= 0.56, $p>0.10$, respectively), no significant differences between sessions ($F_{7,35}$= 1.82, $p>0.10$ and $F_{7,35}$= 2.12, $p<0.07$, respectively) and no interaction between sessions and positions ($F_{14,70}$= 1.54, $p>0.10$ and $F_{14,70}$= 0.89, $p>0.10$, respectively) on both MVDIC and MVDICR.

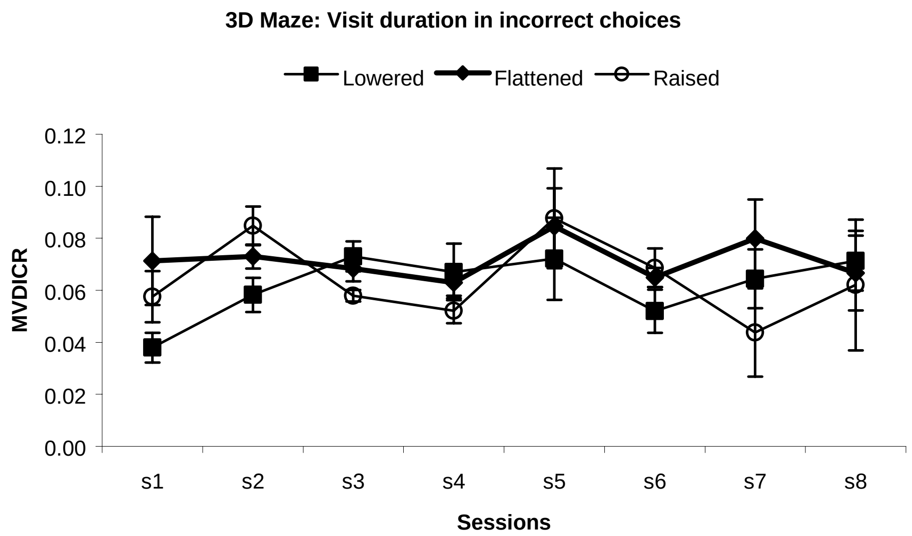


**Figure 8.** Mean visit duration to incorrect choices/session duration ratio (±s.e.m.) in the condition where the arms of the maze are lowered, flattened or raised relative to level of the central platform.

**Discussion:** The experiment described in the present paper demonstrates that rats perform easily a 3D navigation task and that performance is better when moving uphill from a lowered surface than when moving on a flat surface or downhill from a raised surface. Rats made less errors and more correct choices before first error when navigating uphill to raised arms than when moving forward on flattened arms or moving downhill to lowered arms. In this task, animals have no difficulty in moving on the ramps that gives access to the arms of the maze that are either raised or lowered. There is no difference in performance of rats between the different configurations of the maze on the measures of latency to first arm choice and duration of visits.

This preliminary result is confirmed in other studies conducted in our lab with different strains of mice (Ennaceur, Michalikova, Bontempi, in preparation). In these studies, mice performed comparably in the three configurations of the maze when the visual stimuli occupied the lower part of the display panels (e.g. the cues were hardly visible from the central platform). In the present study, the visual stimuli occupied the full surface of the display panels; they were entirely visible to rats either from the central platform or when they engaged on the ramps that give access to the arms of the maze.

Inversely to what is expected in human and primates, navigation uphill from a lowered surface is much better than navigation downhill from a raised surface in rats. To account for this result we consider that performance of rats on raised arms is facilitated by the immediate and constant visibility, from the central platform, of the cues at the end of each arm. Rats have their eyes placed laterally on the sides of their heads (2). Because of this, it is unlikely that they can see anything below their snouts. This evidence seems trivial though in many behavioral studies the visual stimuli are displayed straight in front or below the body of the animals (10, 11, 15). In one study for example (15) the surface of an eight-arm radial maze was covered with distinct cardboards on which inserts from different materials were glued and covered with a transparent plastic coating. This plastic coating was meant to eliminate the preference shown by mice in a preliminary experiment for inserts with specific tactile features. In the view of these authors, the arms of the maze can be discriminated and recognized then from their visual features only. Unfortunately, they didn't consider if mice could see the visual cues displayed under their paws. In this study the authors recognize that mice performed with very poor choice accuracy and suspect that their "mice failed to identify the visual patterns signaling the reward" (see 15, p. 396).

The visibility of visual cues on a horizontal flat surface cannot be entirely excluded; it is likely to be limited to aspects of luminance and hue of the stimuli. A careful observation and examination of the behavior of rodents towards visual stimuli placed at different angles of vision are necessary. A rat or a mouse can approach an arm with one side of its head tilted lightly in the direction of the floor and can see part of the visual cues --it would see only the distal parts. We didn't notice such behavior but we observed at different occasions, in a standard radial-maze and a T-maze, rats or mice standing on their hind paws at the entrance of the arms and apparently scrutinizing the surrounding environment. There are many evidence showing that rats use vision in spatial navigation, object recognition and light or pattern discrimination but we don’t know yet to what extent their visual system is efficient in encoding visual stimuli. Apparently their vision is not as poor as it was first thought. The good performance of our rats in navigating towards raised arms in the 3D maze is certainly due to the visibility of the visual cues and to their constant availability during the test. After each choice a rat has only to recognize which arm was already visited and which one is remaining. This is apparently less demanding than when the choices (from a raised platform) are based on a retrieval of a spatial representation of the maze or the arrangement of the cues after each visit to an arm of the maze.

In several tasks where object or visual recognition is involved it is important to raise the stimulus display at least above the level of the body of the animal. At a short distance, rat or mice can see only the upper part of an object displayed in front of them. Object recognition requires that an object is a bit taller than the height of the animal or that it is raised above the height of the animal. In addition it is worth considering the distance between the animal and the stimulus display (5-6). Because of the position of the eyes on the head, rodents have some binocular capability which is limited to distant objects. They have, however, panoramic vision that gives them a wide uninterrupted field of view (2). When the stimuli are displayed at a distance from the position of the animal, the comparison between visual displays is made possible by the simultaneous visibility of the cues and, the incidence of impulsive responses on memory performance is reduced.

Most current radial maze apparatus use Perspex Plexiglas on each side of the arm of the maze. Depending on their height and whether or not they cover the entire length of the arms or just the entrance of the arms, rats and mice can be prevented from seeing the visual cues surrounding the maze. In the conditions where the arms are partially or totally enclosed, animals might be able to see separately the cues that are raised at the end of an arm but it is unlikely that they can see through the Perspex layers that separate the arms of the maze. In the present 3D apparatus sidewalls of about 1 cm high extended the length of each arm, rats have unobstructed view to all arms of the maze and to the cues that were attached to these arms.

In our experiment we could not control the scent marking because rats were removed from the maze after making eight arm visits. The issue of scent marking in the radial maze has been examined in several experiments and discussed in many publications but did not report any valid solution.

One would argue that a maze with open arms is anxiogenic just like the open arms of a plus-maze. Anxiety in our conditions, however, can be detected easily by the measure of latency to entries in the arms following drug, lesion and genetic or other manipulations. Furthermore, anxiety is not always evident in a plus maze as it is affected by factors independent of the maze apparatus (9). In addition, it cannot be excluded that enclosed arms made from Perspex Plexiglas do offer attenuated light intensity that is much more preferred than an intense direct light on open arms.

In our laboratory we are investigating the validity and sensitivity of the 3D maze. Present results are very encouraging as the 3D maze proved very sensitive to strain differences in mice (Ennaceur, Michalikova, Bontempi, in preparation) and to lesions of the hippocampus (Ennaceur, Michalikova, Cho, Bontempi, in preparation). This novel spatial maze apparatus can provide some insights on the way rats and mice navigate in their real world (13). It can be very useful for better characterization of the behavioral profile of animals following lesions, drugs or genetic manipulations.

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