International Journal of New Technology and Research (IJNTR)
ISSN:2454-4116, Volume-2, Issue-4, April 2016 Pages 13-17

A Novel Universal Virtual Maze for the Evaluation of Spatial Performance-Related Behaviors

Joseph Yanai, Vlad Demartsev, Adi Pinkas

***Abstract*— A Universal Virtual Maze for assessing spatial learning and memory is being presented. The maze is based conceptually on the Morris maze, but bypasses its limitations, which makes it suitable for both "wet" and "dry" situations, for animals of all sizes and free running animals, as well as for wild animals of all sizes in their natural habitats. The test can be applied in three dimensions and can test learning and memory among animals in flight or swimming/diving animals, in a laboratory setting or the wild. All that the virtual maze requires is a video camera and a computer with the appropriate tracking software, a rewarding device, and an alerting device. The efficacy of the maze was tested in mice and human which were tested in species-appropriate virtual mazes. Mice - showed a clear learning curve which was already statistically significant on the 5th testing day and the improvement was increased to 63% after 10 days ($p<0.001$). Human - The subjects easily reached a learning curve. The improvement during the 5 days was 64% ($p<0.01$). They still preformed the task even when the virtual target was moved to a different location. There are preliminary indications that the test could identify ADHD syndrome. Virtual maze offers a test similar to the Morris maze but it is simple and practically suitable for any situation, with no limitations.**

***Index Terms*— Human, Mice, Spatial learning and memory, Universal Virtual Maze**

I. INTRODUCTION

Various procedures were developed for the evaluation of behaviors related to the hippocampus, most notably spatial learning and memory. The archetypal testing devices and procedures used were the Radial Eight-Arm Maze [1] and later the Morris Water Maze [2, 3].These testing devices were first developed for rats but were later adapted for the use in mice [4][5, 6] and other animals. Currently, the Morris maze is more commonly employed than the eight-arm maze test and accordingly the goal of this study is to develop a maze which is based in principle on the Morris maze, but is universal in that it can test a wide variety of animals under wide variety of conditions.

Joseph Yanai, The Ross Laboratory for Studies in Neural Birth Defects, Department of Medical Neurobiology, Institute for Medical Research – Israel-Canada, The Hebrew University-Hadassah Medical School, Box 12272, 91120 Jerusalem, Israel And Department of Pharmacology and Cancer Biology, Duke University Medical Center Durham, NC 27710 USA.

Vlad Demartsev, The Ross Laboratory for Studies in Neural Birth Defects, Department of Medical Neurobiology, The Hebrew University-Hadassah Medical School, Jerusalem, Israel.

Adi Pinkas,The Ross Laboratory for Studies in Neural Birth Defects, Department of Medical Neurobiology, The Hebrew University-Hadassah Medical School, Jerusalem, Israel.

While a significant progress in neurobehavioral research was achieved through the use of the Morris maze, the maze is limited in relation to the size of the experimental animal, the animal's ability to swim, its tendency to avoid deep water and, most pointedly, the lack of a truly equivalent maze for humans.

Consequently, here we present our development of a universal virtual maze which has thus far proved suitable for mice and humans. Mice are placed in a dry Morris maze-like round container and are trained with a sweet-water reward to reach a specific location (a virtual circular target), which is marked only on the computer monitor. Similarly, human test subjects are asked to walk in a specified area and are rewarded when they crossed the virtual target. We believe that this virtual maze can be used on most animals and under a great variety of environments.

II. METHODS

The experiments were videotaped and analyzed using Stoelting's ANY-maze program and the virtual target is marked on the computer monitor with a graphic program.

A. Mice Apparatus

The apparatus wasan adaptation of our mouse Morris maze[6], a galvanized tin circular tank, 87 cm diameter,in its "dry" form (Fig. 1).

The floor was uniformly painted gray to minimize potential inside cues. The only adaptations to converting it to a virtual maze was the addition of a water dispenser and a small 5W blue light, which were installed next to each other on the wall of the maze 7 cm and 12 cm above the floor, respectively. This constituted the rewarding center which was activated when appropriate by the observer. The water dispenser was made to dispense 50 µl of sweetened water (10% sugar).

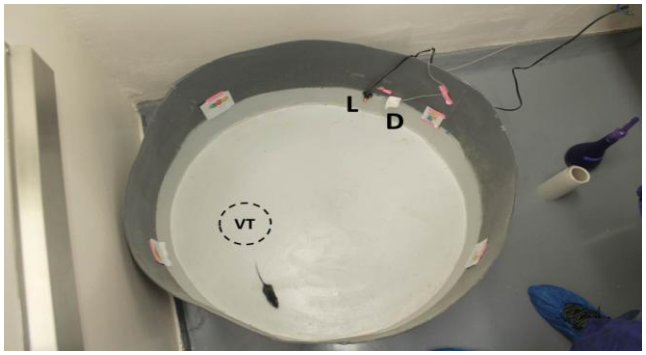


Fig. 1:Virtual maze for mice, **D.** Device for water rewarding **L.** Signaling light **VT.** Virtual target (visible only on the computer monitor).

B. Subjects and Procedure

Fifty day old male F1 C57BL/6 X DBA/1were employed (N=8).

The water scheduling and the test lasted for four weeks, which included the two day breaks between phases, described below.

Week 1 – Water Scheduling: The mice were put on a 7 day water scheduling regimen that consisted of daily access to water for 30min. This scheduling is different than deprivation because the mice, once adapted to it, consumed almost the normal daily amount of water during this drinking period. The scheduled access to drinking was continued throughout the next three weeks, immediately after the daily testing, and during the two day breaks as well.

Week 2 – Training to Associate the Light Signal with the Water Reward: At the same time as the water scheduling of Week 1, the mice were placed in the maze individually at a point by the wall, 20 cm away from the water dispenser. The mouse was allowed to walk about and as soon as it was at least 30 cm from the water dispenser the light was turned on and the reward was released. The light was turned off after the mouse drank the sweetened water, concluding the first trial. When the mouse subsequently walked 30 cm away from the feeding center the light was turned on and the reward was released again. There were 10 trials per day for 5 days. After the testing period the mice were given a 2 day break.

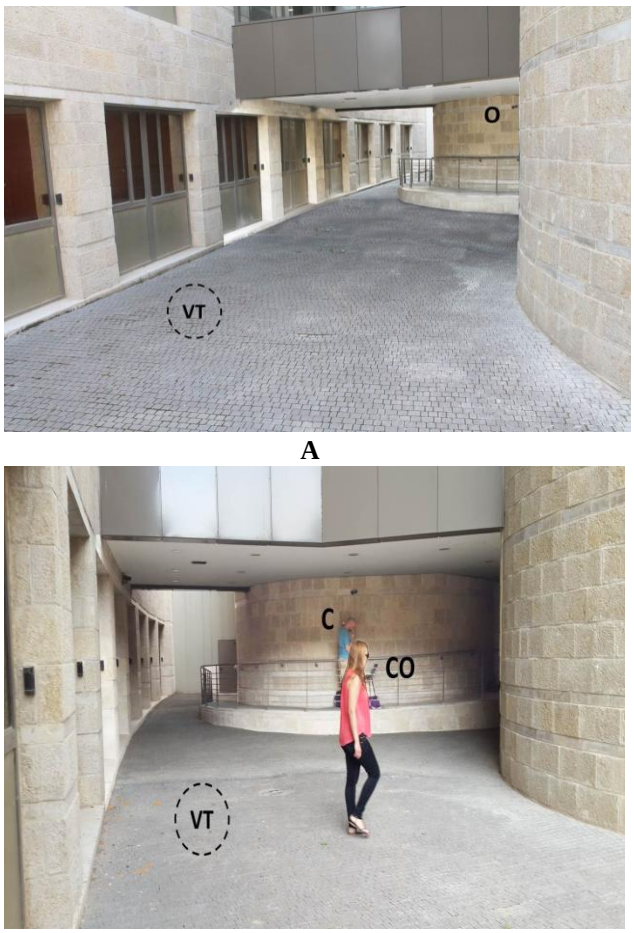
Week 3 – Testing Performance in the Virtual Maze: The virtual target was activated on the computer monitor. At the regular timein the day of behavioral testing, the mice were placed in the maze individually at a point by the wall, 20 cm away from the water dispenser. The mouse was allowed to walk about and as soon as it entered the 20 cm virtual target the light was turned on and the reward released. The light was turned off after the mouse drank the sweetened water ending the first trial. When the mouse subsequently entered the virtual target the light was turned on and the reward was released again. There were 10 trials per day for 5 days. After the testing period the mice were given a 2 day break.

Week 4 – Testing Performance in the Virtual Maze: Same as Week 3.

The following parameters were recorded: During training to associate the light signal with the water reward (week 2):Time to get to the water dispenser after the light is turned on. During testing performance in the virtual maze(Weeks 3 and 4): Time from the beginning of the trial until the mouse enters the virtual target.

C. Human Apparatus (Fig. 2)

The virtual maze is an irregular area, bordered on three sides by walls of buildings with and without low windows, and by a concrete fence on the fourth side. There were small sections of the borders that were not walled, and the subjects are instructed not to cross them. Naturally, the length and the width of the maze vary but on average the length is 13 m and the width 6.5 m. The virtual target(1 m) is determined at a certain point in the virtual maze (VT). An effort is made to keep the virtual maze clean and as unmarked as possible. A small plate with 10 coins of 5 Israeli Shekels (approximately $1.25) is placed on a surface by the observer(Fig. 2.)



A

B

Fig. 2:Virtual maze for human: **A.** General view of the maze: **O.** Observer's post **VT.** Virtual target (visible only on the computer monitor).**B.** Detailed view of the virtual maze: **C**. The observer on the observer's post with the video camera above. **CO.** The computer on the left and the place for the plate with the reward coins below **VT.** Virtual target (visible only on the computer monitor). The tested subject is seen in the Virtual Maze.

The experiment is repeated for five days with 10 daily trials. On the fourth day of testing the virtual target is switched to another location in the maze and remained there for the fifth day as well. The experiment is videotaped and analyzed using Stoelting's ANY-maze program and the virtual target is marked on the computer monitor with a graphic program.

D. Subjects and Procedure:

Eight students, 3 females and 5 males, ages 20- 25, are the subject of the study. Before testing they are introduced to the testing area (virtual maze) at one specific point by the observer and are instructed (see below) to walk around in the enclosed area until they hear a whistle. When they cross the virtual target, the whistle is sound and they are immediately rewarded with a coin. A reversal test was conducted on the fourth day, meaning that the virtual target was moved to a different point in the maze area and remained there until the end of the test, day 5.

Accordingly, the subjects are instructed as follows:

1) The purpose of this test is to measure a certain behavior which will be disclosed only at the end of the study.

2) You are now in in a marked area, walk about continuously. When you hear the whistle, go to the table which is by the observer and the computer, and take a coin from a pile on a small plate, put it in your pocket, it is yours.

3) Rest for 10 seconds and then repeat step 2) until there are no more coins (10 trials).

At the end of the fifth day of testing, the subjects are interviewed and are asked the following questions:

1) In your opinion, what were you expected to do?

2) What cues did you use to reach your expected goals? Minimal, possible marks on the floor or the cues out of the test area such as walls, windows or those more distant?

3) This question expands on the previous one. Imagine that the surface of the virtual maze was covered with a 10cm deep pool of water, made opaque by milk or white pigment. In your best judgment, would you still be able to reach the virtual target?

III. RESULTS

A. Mice

All mice became accustomed to the water scheduling and were drinking the daily amount within less than the 30 minutes allowed (week 1).

During the training to associate the light signal with the sweetened water reward (week 2, the virtual target was not involved at this phase), the mice showed a statistically significant learning curve in that they progressively needed less time to reach the water dispenser after the light was turned on (Fig. 3). The curve was very similar that of our Morris water maze learning curve and they improved during the five days by 74% (regression analysis, $p<0.001$).

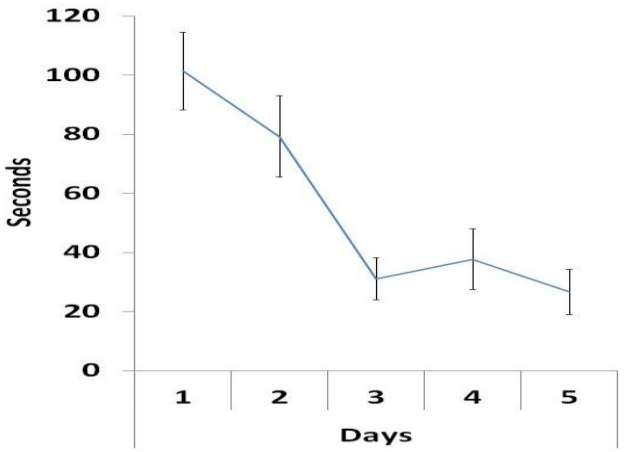


Fig. 3:Pre-training sessions in mice - learning to associate light signal with the sweetened water reward (week 2). The time it took the mice to reach the water reward after the signaling light was turned on. Each daily point represents 10 mice - each having 10 trials. The learning curve shows a 74% improvement during the five testing days ($p<0.001$).

During the 10 days testing for reaching the virtual target in the maze (combining week 3 and 4, Fig. 4), the mice showed a statistically significant learning curve in that they progressively needed less time to enter the virtual target. The improvement on the 5th testing day was of 42% ($p<0.01$, regression analysis) and 63% after 10 testing days ($p<0.001$).

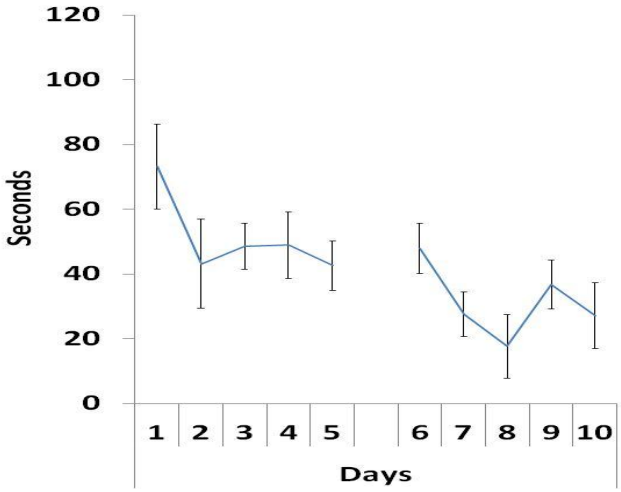


Fig. 4: Virtual maze test for mice. The time it took the mice to reach the Virtual Target (VT) during the 10 days of Virtual Maze testing (weeks 3 & 4). Each point in the curve represents 8 trials. The learning curve shown is significant ($p<0.01$, regression analysis). The improvement on the 5th testing day was of 42% ($p<0.01$) and 63% after 10 testing days ($p<0.01$, $n=8$).

B. Human

The subjects showed a statistically significant learning curve in that they progressively needed less time to enter the virtual target (Fig. 5). The improvement during the 5 days was 64% ($p<00.1$, regression analysis).The reversal which was conducted on day four brought about only a small setback in the performance which was not statistically significant and was corrected during the 10 trials of that day. Apparently, all realized that the virtual target had been moved and acted accordingly.

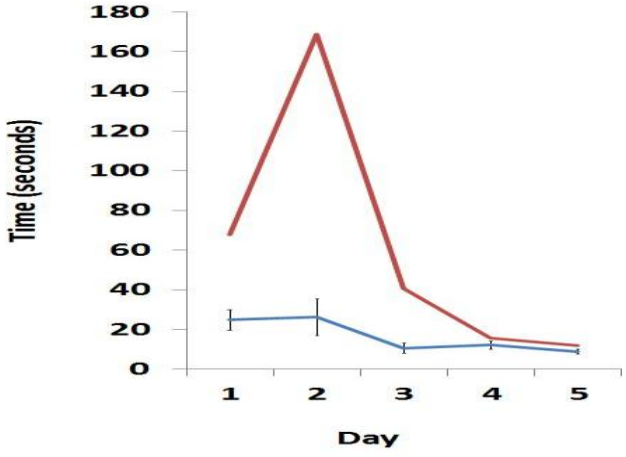


Fig. 5: Virtual maze test for human.(A) The time it took the subjects to reach the Virtual Target (VT) during the 5 days of Virtual Maze testing (bottom, blue curve). Each point in the curve represents 10 trials. The learning curve shown is significant (64% reduction, $p<0.01$, regression analysis, $n=7$). The reversal on day 4 induced only a small setback which was not statistically significant.(B) A similar curve for the ADHD subject (upper, maroon curve): although this subject shows a

great gap when compared to the other subjects, its performance reached the level of the others on days 4 and 5.

After the study had ended one individual was revealed accidentally as having ADHD. The learning curve of this individual is presented separately (Fig. 1B) and although this curve is distinctly different than the results of the other subjects, learning was improved along the experimental days and this subject had similar result to the rest of the group by the last two days of the experiment.

The interview (described in METHODS) at the end of the experiment revealed the following:1) after gaining experience in the maze all subjects realized that they have to reach a virtual point in order to be rewarded. They also realized that the virtual target was moved on the fourth day. Most subjects assumed additional factors were required, such as getting to the virtual point through a certain path or walking a certain distance before getting to the virtual point. 2) Among the 8 subjects tested, 63% used outside cues, and all but one additionally tried to make use of the minimal, subtlecues that they could find within the maze.3) All of the subjects were sure that covering the maze with opaque water would not prevent them from identifying the virtual target and receiving the full reward although 3 of them believed that it would take them longer to do so.

IV. DISCUSSION

In the present study we have developed a universal virtual maze which appeared effective both in humans and in non-human mammals (mice) and has the potential for assessing learning and memory similar to the Morris water maze, but in almost unlimited conditions. The mazes for the mice and humans appear similar in principle, yet further studies are needed to confirm to which extent they indeed measure the same cognitive faculties.

The Morris maze, although an effective test, has several inherent limitations which the presented virtual maze overcomes: 1. the need to use an enclosed pool of water limits the size of the animals being tested, which explains why it is used only for small rodents. 2. The Morris maze is not effective for aquatic (e.g. fish) or semi-aquatic (e.g. ducks, other waterfowl, seals, beavers) species that have no motivation to "escape" from the water. 3. The test is not suitable for large and/or unenclosed spaces and can't be conducted on freely moving/wild animals. 4. The test is limited to two dimensions and is not suitable for swimming and diving or flying animals.

Our Virtual Maze as presented here has bypassed these limitations. The maze can be used for "wet" or "dry" situations and for animals of all sizes, from mice to elephants in the dry form, and from zebra fish and waterfowl to whales in the wet form. Learning can be tested on free running animals, like certain farm animals or fowl, as well as on wild animals of all sizes in their natural habitats. In fact, this test can be three dimensional and can test learning and memory among animals in flight or swimming/diving, in a laboratory setting or in the wild. Importantly, as is shown in the present study, this test has proven effective for humans as well.

All that the Virtual Maze requires is a video camera and a computer with the appropriate tracking software, a rewarding device located in the experimental area which releases an appropriate reward (such as food, water, money etc.), and an alerting device which is usually located by the rewarding device

Despite all efforts, subjects could make use of some subtle cues inside the maze such as small cracks in the floor or some dirt marks that still remained. Indeed, some of the subjects participating in this experiment made use of these markings, but as the interviews showed, they managed without a problem mainly with outside cues. Furthermore, all subjects believed that they could manage even if the floor of the maze would be covered with opaque water. Similarly, since an effort was made to keep the floor uniform in the mouse virtual maze, and based on what is known from the Morris maze literature[3], it is safe to assume that outside cues were the major guide in the mice navigation toward the virtual target.It should be mentioned that being helped with intra-maze is legitimate in the Morris maze literature[7].

A hypothetical example to illustrate the capability of the virtual maze is that of a free bird residing in a certain area that could be trained to visit a virtual target located at any height within the territory of the bird. Again, a simple rewarding device should be installed in the area, as well as a video camera that encompasses the area defined as the "maze". The experimenter and the computer software could be situated at any distance from the maze with remote control of the feeding device and a wireless receiver for the camera.

Virtual mazes for humans are now being developed but are based on a virtual maze on the computer monitor where the human subjects see and approach the target with a joystick. In these mazes the subject does not have to physically walk to the target. Comparing our approach with this should be relevant. The limitations of this maze are its lack of universality and suitability for other animals.

As is shown in RESULTS, although completely unintentional, we found out after the end of the study that one individual was diagnosed with ADHD. The Virtual Maze clearly and unequivocally distinguished this subject and although it is too early to reach a conclusion because this is a case of n=1, the potential that this test could very well identify behavioral syndromes in human, should be further investigated.

Based on experience acquired in the present studies, recommendations for improvement of the protocols can now be composed: **Human** – the surface of the virtual maze area should be made as uniform as possible, and continuously cleaned to prevent any surface marks.

Whereas discomfort to the experimental subject is often unavoidable in research, it is important to note that in the test designed here, this aspect was minimized. In human, the test is actually enjoyable. In the case of the mouse, scheduling drinking still allows drinking the normal daily amount, and it induces much less discomfort than the escape from drowning that the animal experiences in the equivalent Morris water maze.

V. CONCLUSION

A Universal Virtual Maze is being presented based on the Morris Water Maze. Its advantages were discussed above. Briefly, 1) The Maze can run "wet" or "dry", 2) in almost any territorial conditions, 3) without the experimental subject's knowledge, 4) requires very basic hardware, and 5) it works very well on human. It is expected to identify certain behavioral disorders as was already indicated in the present study by very preliminary data.

ACKNOWLEDGMENT

Supported by a grant from Yissum Research Development Company of the Hebrew University of Jerusalem.

V. REFERENCES

[1] Olton, D.S. and R.J. Samuelson, Rememberance of places passed: Spatial memory in rats. Journal of Experimental Psychology, 1976. 2: p. 97-115.
[2] Morris, R., Developments of a water-maze procedure for studying spatial learning in the rat. J Neurosci Methods, 1984. 11(1): p. 47-60.
[3] Morris, R.G.M., Spatial localization does not require the presence of local cuesLearning and Motivation, 1981. 12(2): p. 239-260.
[4] Pick, C.G. and J. Yanai, Eight arm maze for mice. Int J Neurosci, 1983. 21(1-2): p. 63-6.
[5] Upchurch, M. and J.M. Wehner, Differences between inbred strains of mice in Morris water maze performance. Behav Genet, 1988. 18(1): p. 55-68.
[6] Rogel-Fuchs, Y., et al., Hippocampal cholinergic alterations and related behavioral deficits after early exposure to phenobarbital. Brain Res Bull, 1992. 29(1): p. 1-6.
[7] Hamilton, D.A., et al., The relative influence of place and direction in the Morris water task. J Exp Psychol Anim Behav Process, 2008. 34(1): p. 31-53.

Joseph Yanaireceived his PhD in biology/Behavioral Genetics at the University of Colorado at Boulder, Colorado USA. Currently he is a Professor and Director of The Ross Laboratory for Studies in Neural Birth Defects at the Department of Medical Neurobiology, the Hebrew University-Hadassah Medical School, Israel and an Adjunct Professor at the Department of Pharmacology and Cancer Biology**,** Duke Medical Center, North Carolina, USA.

Adi Pinkasreceived his PhD in Neurobiology at the Hebrew University-Hadassah Medical School, Israel. Currently he is a Postdoctoral Research Fellow at theDepartment of Molecular pharmacology, Albert Einstein College of Medicine, NY, USA.

Vlad Demartsevis a PhD student at the department of Zoology, Tel -Aviv University, Israel.