MORRIS WATER MAZE – A VERSATILE COGNITIVE TOOL

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Abstract

The Morris water maze is widely used to study spatial memory and learning. Animals are placed in a pool of water that is colored opaque with powdered non-fat milk or non-toxic tempera paint, where they must swim to a hidden escape platform. Because they are in opaque water, the animals cannot see the platform, and cannot rely on scent to find the escape route. Instead, they must rely on external/extra-maze cues. As the animals become more familiar with the task, they are able to find the platform more quickly. Developed by Richard G. Morris in 1984, this paradigm has become one of the "gold standards" of behavioral neuroscience.The purpose of this short article is to briefly describe use and flaws of the Morris water maze as an experimental tool for testing learning and memory.

Key words:
Learning,
Memory,
Cognition,
Acquisition,
Morris water maze

1. INTRODUCTION

Memory is a cognitive process that can be studied throughout life span. Cognitive skills are used to be able to adapt to an ever changing environment. For thousand years spatial memory has contributed to our knowledge and exploration of available resources in our surroundings [1]. The acquisition and retention of a spatial navigation task is examined using a Morris Water Maze [2].

The hippocampal formation plays an important role in memory and learning. The Morris Water Maze (MWM) is a test of spatial learning for rodents that relies on distal cues to navigate from start locations around the perimeter of an open swimming arena to locate a submerged escape platform. Spatial learning is assessed across repeated trials and reference memory is determined by preference for the platform area when the platform is absent [3].

Since its first application in 1981, the Morris water maze has become one of the most frequently used tool for analyzing spatial learning and memory [4]. This task can be altered in numerous ways to investigate working memory, reference memory and task strategy [5, 6].

It has been used widely in investigations of different aspects of learning and memory in rodents and in investigations of the variables that may affect the animal's behaviour in the task [5, 7]. It has also been used as a tool to investigate chemically induced effects on learning and memory [8, 9].

There are numerous other components of the task that do not involve spatial memory: the stress involved with the task, the understanding of the rules of the task (that to "escape", the animal must find a hidden platform, and stay on it in order to be "rescued"), and the understanding that there is a means of escaping the task[10]. Learned helplessness also involves a tank

of water, but the rules (there is no means of escape) are quite different [11]. The three pretraining trials "teach" the animals about these properties of the task. Animals learn that they will be placed into a pool of tepid water and swim around for a minute, but be removed after. They are taught to find the platform (because it is visible) and that staying on it will lead to their "escape" from the maze. And they are taught that the task has an end. Therefore, this hippocampal-independent learning does not confound the analysis of the water maze testing data.

The hippocampus and the cerebral cortex are the key structures of memory formation. Because the hippocampus is especially indispensable in the integration of spatial information, a decline in learning ability may be induced by the deterioration of hippocampal function.

Morris water escape task can test of the hippocampus in learning and memory [12]. Cognitive function is often considered to encompass learning, memory and attention processes. However, no exact definition of either learning or memory is agreed upon. In general, learning is defined as the process by which new information is used to modify subsequent responses, while memory uses processed information to modify subsequent behavior. From a logical standpoint it is also clear that memory is not possible without learning, and learning cannot occur if there is no memory. In addition, because these processes are not directly observable, they cannot be measured directly and thus must be inferred from observed changes in behaviour over time. Measures of cognitive function may also be influenced by sensory, motor, attention and motivational variables [13]. These factors are described as non-associative effects. In the Morris water maze, for example, changes in locomotor ability or visual capacity may affect performance but not as a consequence of an effect on cognitive function. In order to conclude that an agent affects learning and/or memory, non-associative effects must be excluded. In practice, as measurement of learning and memory depends on adequate functioning in other categories, it is only possible to be confident of a specific learning or memory effect if other functions are unimpaired.

Most often, water maze testing occurs across two to four days 3. In this way, acquisition and retention can be assessed. However, in some populations, this is not a viable option. Such is the case when investigating female mammals. Female rodents, humans, primates, etc, all have cyclic changes in steroid hormone levels [14]. These hormones have profound effects on hippocampal-dependent task performance, hippocampal anatomy and hippocampal cell function [15, 16]. Testing across multiple days would be akin to testing a single animal across numerous conditions low estradiol and progesterone, elevated estradiol and progesterone, and intermediate estradiol and progesterone. To eliminate this confound, testing occurs across a single day.

Practically speaking, maze consists of a circular pool (1.2 m in diameter and 0.47 m high) made of white plastic [4]. The pool is filled to a depth of 20 cm with water (24°C-25°C) that is made opaque by the addition of non toxic white paint. An escape platform (10 cm in diameter), made of white plastic with a grooved surface for a better grip, is submerged 0.5 cm under the water level. The animal has to swim until it finds the hidden platform. The animal generally uses cues outside the maze to develop a spatial map of the environment and guide its performance. The pool is divided into four equal quadrants labeled N (north), O (east), S (south) and W (west). Their order of use was randomized daily. The time the mouse needs to find the hidden platform (latency) so that it can stop swimming is recorded. A trial is started by placing the mouse into

the pool close to the rim, facing the wall of the tank into one of the four quadrants. The mice were given four days of training with four 60-seconds training trials per day. During a spatial reference memory (SRM) training, the platform is always placed in the same spatial location of the pool (NE quadrant) throughout the training period in both paradigms. During spatial working memory (SWM) training, the escape platform is placed from the edge of the pool in one of the four possible locations (designated N, S, E and W) [12].

The principle of the test is that rodents can learn to swim, from any starting position, towards a hidden escape platform. They do this using distal extra-maze cues that are remote from the actual place in the pool to which the animal is heading. Therefore, the room containing the tank should have permanently positioned distinctive objects such as posters placed outside of the pool and/or on the walls.

However, many other procedures can be used in which the number and position of extra maze cues, water temperature, platform size and/or its availability can also be varied. The variations in procedure are considered to allow investigation of different aspects of cognitive function. For example, if after training and achievement of stable performance the platform is moved rather than removed between trials on a particular day this is considered to assess short-term spatial memory [17]. In addition, although the water maze is used primarily to study spatial learning, various non-spatial protocols have been developed. In visual discrimination learning the extra maze cues are removed (e.g. by putting curtains around the pool) and the rat has to learn to discriminate between two visually different platforms, one of which will provide an escape and one that will not [18].

A number of indices of performance in the water maze may be used. The simplest measure is escape latency and others include path length (distance travelled by the animal), swimming speed (calculated by dividing path length by the latency), directionality (a measure of the direction of travel a specified distance from the start) and quadrant times (amount of time spent in specified sectors of the pool). Typically, measures are recorded remotely, often with the assistance of a video recorder and image analyser.

Morris water-maze performance involves several components, including concept formation (learning the general rules of the task), attention, working memory, and reference memory, which are not readily distinguishable in a simple form of this paradigm[19,20]. Thus, several variants of the Morris water-maze in an effort to determine whether the rat lines differed in their (1) associative abilities, (2) conceptual abilities, (3) working and reference memory, (4) search strategies, and (5) native attention/distractibility are used.

2. LIMITATIONS AND INTER-PRETATIONAL DIFFICULTIES

Interpretation of results in MWM suffers drawbacks as the performance of subjects in the Morris water maze can be significantly influenced and interfered by a variety of technical as well as procedural variables like dimensions of the pool, the water temperature[17], strain and sex of rats[1,5,21], different schedules of training[22],task parameters[23], age pre-natal stress[24] pre-natal nutritional status[26], post-natal nutritional status[5,27], hormonal status[27], day of oestrus cycle[28], body temperature[29,30] and home care environment[31].

The influence of many variables can be minimized relatively easily as age, sex and strain, environmental conditions and schedules of training will normally be

carefully controlled and can be kept identical for all animals. However, particularly where Morris water maze tests are part of large developmental neurotoxicity studies, it may not be possible to control all potential confounders. For example, maternal stress will typically be greater in high dose animals due to the requirement to have a measurable level of maternal toxicity. In turn, this may lead to maternal neglect and/or reduced nurturing behaviour that may result in undernutrition. Even transient periods of under nutrition can result in changes in learning behaviour [32]. Typically oestrous cyclicity and body temperature are not recorded and tests of visual acuity are crude or absent.

Morris water maze is widely were used to assess learning and memory particularly in neurodegenerative disorders like Alzheimer's disease [33] but neurobehavioral deficits in animals are less directly translatable to humans than many other toxic end-points (e.g. pathological change). This is primarily because behaviours tend to be highly species specific and adapted to the survival needs of the species. Spatial learning is a good example of behaviour that rats and other rodents are particularly good at but is not generally as well developed or used as an end-point to assess cognitive function in humans. Therefore, direct extrapolation of results of a Morris water maze task is probably ill advised. Nevertheless, the underlying functional mechanisms of the brain and their involvement in behaviours are shared across most mammalian species. Therefore, changes in rat behaviour are likely to model effects in humans where the same functions are shared, although the precise behaviours may be different.

3. CONCLUSION

Although Morris water maze is regarded as a valuable tool for assessing spatial learning in the rat. Measures of performance in the Morris water maze are not, however, direct measures of cognitive function. Conclusions about cognitive function can only be drawn after excluding known potential confounders. Even then, it is possible that unrecognised non-associative effects may be influencing the results (e.g. visual acuity, anaemia and response to stress) making a conclusion about cognitive function unreliable. Differences in performance between experimental groups may represent differences in behaviour that are attributable to treatment and may represent changes in basic function. However, changes in performance do not always represent changed cognitive function and should not automatically be considered adverse.

4. REFERENCES

[1] Cimadevilla, J.M., Gonzalez-Pardo, H., Lopez, L., Diaz, F., Cueto, E.G., Moreno, L.M., Arias, J.L., Behavioural Processes. 1999, 46/2,159-171
[2] Kumar, A., Seghal, N., Padi, S. V., Naidu, P. S., Europ J Pharmacol. 2006, 551,58-66
[3] Vorhees, C.V., Williams, M.T., Nat Protocols. 2006, 848-858
[4] Morris, R. G., Learning and Motivation.1981, 12, 239-260
[5] Brandies, R., Brandys, Y., Yehuda, S., International Journal of Neuroscience. 1989, 48, 29-69
[6] Morris, R., J Neurosci Methods.1984, 11, 47-60
[7] McNamara, R., Skelton, R.W.,Brain Research Reviews.1993,18, 33-49
[8] Hass, U., Lund, S. P., Hougaard, K.S., Simonsen, L., Neurotoxicology and Teratology. 1999, 21, 349-357
[9] Hougaard, K. S., Hass, U., Lund, S. P., Simonsen, L., Neurotoxicology and Teratology. 1999,21, 241-50
[10] Hodges, H., Cogn Brain Res.1996, 167-181
[11] Shumake, J., Gonzalez-Lima, F., Behav Cogn Neurosci.2003, 2,198-221
[12] Rebai, Q., Djeblie, N., Advances in Biological Research. 2008,2, 26-33
[13] Tilson, H.A., Cabe, P.A., Spencer, P.S., Experimental and Clinical Neurotoxicity. 1980,22,758-761
[14] Owen, J.A., Am J Clin Nutr.1975,28, 333-338
[15] Warren, S.G., Humphreys, A.G., Juraska, J.M., Greenough, W.T., Brain Res. 1995, 703, 26-30

[16] Warren, S.G., Juraska, J.M., Behav Neurosci. 1997,11, 259-266
[17] Kikusui, T., Tonohiro, T., Kaneko, T., Journal of Veterinary Medical Science. 1999, 61(6),673-81
[18] Robinson, L., Bridge, H., Reidel, G., Behavioural brain research. 2001,119,77-84
[19] Morris, R.G., J Neurosci.1989,9, 3040–3057
[20] Whishaw, I.Q., Auer, R.N., Psychopharmacology.1989, 98,500–507
[21] Lindner, M. D., Neurobiology of Learning and Memory. 1997, 68 (93), 203-20
[22] Kanit, L., Yilmaz, O., Taskiran, D., Balkan, B., Furedy, J., Pogun, S., International Journal Of Neuroscience. 1988, 96, 197-204
[23] Roof, R. L., Stein, D. G., Physiology and Behavior. 1999,68, 81-6
[24] Aleksandrov, A.A., Polyakova, O. N., Batuev, A.S., Neuroscience and Behavioral Physiology. 2001,31/1: 71-74
[25] Hayashi, A., Nagaoka, M., Yamada, K., Ichitani, Y., Miake, Y., Okado, N., Int J Devl Neuroscience. 1998,16,:209-216
[26] Tonkiss, J., Shultz, P.L., Shumsky, J.S., Galler, J.R., Neurotoxicol Teratol. 1997,19, 363-372
[27] Daniel, J. M., Roberts, S. L., Dohanich, G. P., Physiology and Behavior. 1999,66,11-20.
[28] Healy, S. D., Braham, S. R., Braithwaite, V.A., Proceedings of the Royal Society of London Series B: Biological Sciences. 1999,266, 2303-2308
[29] Hunter, A.J., Roberts, F.F., Pharmacol Biochem Behav.1988,30, 519-523
[30] Panakhova, E., Buresova, O., Bures, J., Behav Neural Biol. 1984, 42, 191-196
[31] Tees, R.C., Buhrmann, K., Hanley, J., Developmental Psychobiology,1990,23, 427-439
[32] Bedi, K. S., Physiology and Behavior.1992, 51,1001-1007
[33] Deshmukh,R.,Sharma,V.,Mehan,S.,Sharma ,N.,Bedi,K.L.,European journal of Pharmacology.2009,620,49-56
[34] Yamamoto, K., Hagino, M., Kotaki, H., Iga, T., J. Chromatogr. *B* 1998, *720*, 251 – 255