

# A Computer-Based Application for Rapid Unbiased Classification of Swim Paths in the Morris Water Maze

Kurt R. Stover, Timothy P. O'Leary, Richard E. Brown

*Department of Psychology and Neuroscience, Dalhousie University, Halifax, Canada. kurt.stover@dal.ca*

## Introduction

The Morris water maze (MWM) is a commonly used apparatus for measuring visuo-spatial reference or working memory in rodents. In the reference memory task in our lab, mice are trained to locate a submerged platform in a circular pool filled with opaque water by using distal extra-maze visual cues. Learning in the MWM is typically assessed with measures of performance including latency and distance travelled to reach the platform, as well as swim speed and thigmotaxis (the amount of time spent swimming near the walls of the pool). To assess memory, probe trials are used in which the platform are removed and the amount of time mice spend in the quadrant of the pool where the platform was located and the frequency that they swim across the location of the platform is measured. Visual ability and motivation to escape are assessed in training trials with a visible escape platform.

Although latency and swim distance are the most commonly used measures of learning in the MWM it is not possible to determine whether or not mice are using visuo-spatial cues based on these measures of performance [1]. Furthermore, latency and distance may not always have sufficient sensitivity to detect impairments in learning, because mice that use non-spatial strategies may not differ in performance from those that use visuo-spatial strategies. As mice learn the location of the escape platform in the MWM they typically display a progression of search strategies with an increasing use of visuo-spatial cues [2,3]. On the first day of training, mice often begin with a random search strategy and then, as training continues, progress to non-spatial procedural strategies, such as swimming in a circle around the wall a fixed distance from the edge of the pool, then to strategies using extra-maze cues, such as scanning around the general area of the platform. As they learn to use the extra-maze cues, mice begin to swim from the release point directly to the platform. Mice may also swim in small circles, looping around the maze, engage in thigmotaxis, or float, which may be a result of non-cognitive factors including impaired motor abilities, stress, or hypothermia [4,5].

Latency and distance measures are not able to discriminate between search strategies in the MWM [6]. Therefore, using traditional measures of MWM performance without analyzing search strategy use may result in incorrect conclusions drawn about visuo-spatial ability. Although search path analysis is important for the assessment of learning in the MWM, it is rarely carried out. This may be due to the increased time and labor required for these analyses, a perceived lack of objectivity of search path analysis, or the potential for experimenter bias in search path analysis. While these limitations can be avoided using data-driven classification with relatively sophisticated multivariate statistical techniques, such as discriminate function analysis, this requires a large number of behavioural measures to be recorded [6], has not yet been validated across different laboratories, and may not be suitable for high-throughput behavioural experiments.

In order to efficiently categorize MWM swim paths we developed a simple computer program called SwimPath to display the image of a swim path and allow the user to indicate which search strategy was used. SwimPath can be used in conjunction with commercially available tracking systems. The SwimPath program ensures that the scorer is blind to the identity of the mouse and trial number, and allows responses to be recorded electronically and exported into a database. To assess the usefulness of this program in detecting strain differences, we analyzed the swim paths of male and female mice of five inbred strains which have different levels of visual acuity [7]. We also recorded latency and distance to the platform, swim speed, and thigmotaxis, and correlated these traditional measures of performance with search strategy use in the MWM. To determine the relationship between search strategy and memory we correlated search strategy use on day six of training with performance in the probe trial.

## Methods

### Mice

Seventy-four mice, with approximately equal numbers of males and females from each of five strains were used: A/J (5F, 4M) and BALB/cByJ (5F, 6M), which are albino; C3H/HeJ (4F, 7M) which have retinal degeneration (RD) [8]; BTBR T+ tf/tf (11F, 12M), which have normal vision but are used as a model of autism; and C57BL/6J (10F, 10M), which have normal vision. The mice ranged from 2 – 5 months of age. Mice were housed in same sex groups of two to four mice per age and had free access to food and water. The BTBR T+ tf/tf mice were provided by Dr. Valerie Bolivar (Wadsworth Center, New York State Department of Health), and all other mice were purchased from the Jackson laboratory (Bar Harbor, ME). The protocol for this experiment was approved by the Dalhousie University Committee on Animal Care.

### Morris water maze procedure

Mice were tested in the MWM reference memory task using the procedure described by Wong and Brown [9]. The test lasted 8 days, which included 3 days of acquisition training (4 trials/day), 3 days of reversal training (4 trials/day) (where the platform is moved to the opposite side of the pool), a probe trial with no platform present, and a cued test with a visible flag added to the platform (4 trials). The latency and swim distance to the platform, swim speed, and thigmotaxis were calculated by the tracking program (Watermaze, Actimetrics Inc., Wilmette, IL) which also recorded the swim paths, the time spent in each quadrant and the number of annulus crossings in the probe trial. Strain differences for each behaviour were analyzed using a mixed design ANOVA for acquisition and reversal, and a between subjects ANOVA for the probe trial. Mice were pooled over sex for these analyses.

### Swim path analysis

The swim paths of the mice were classified into nine different types, which were then combined into four strategies: spatial accurate, spatial inaccurate, procedural, and non-cognitive. Spatial accurate strategies consisted of (1) spatial direct (Figure 1A), a straight path leading directly to the escape platform, with no turns greater than 180° or (2) spatial indirect (Figure 1B), a relatively direct path to the location of the escape platform but with some deviation away from the most optimal path. Spatial inaccurate strategies were (1) focal-correct (Figure 1C), swimming towards the quadrant with the escape platform followed by a focused search confined to the area immediately surrounding the platform, and (2) focal-incorrect (Figure 1D), swimming directly to a quadrant of the pool without the escape platform then searching in this area with circling behaviour. Procedural strategies included (1) scanning (Figure 1E), when mice swam selectively in the center area of the pool, (2) looping (Figure 1F), a circular path at a similar distance from the wall that the escape platform was positioned, passing through at least two adjacent quadrants before reaching the escape platform,. The non-cognitive strategies included (1) thigmotaxis (Figure 1G), a path within a 5 cm zone next to the wall of the maze which may include sporadic movement away from the walls into the center of the maze, and (2) random (Figure 1H), when the mouse did not selectively swim in any area of the pool and contained straight movement or wide circular arcs through the center of the pool and (3) circling (Figure 1I), a tight circular path, or a short path with several tight circles. When a path contained features of more than one type they were categorized based on which type occupied the majority of the path. The SwimPath program (coded in Python 3.2, and can be run on any operating system capable of running Python) presented the images to the user in a random order and rotated the image randomly 0, 90, 180, or 270°. Each search strategy was assigned to a key, which was used to classify the images into one of the nine strategies.

The proportion of trials in which mice used each of the four search strategies was calculated for all training days (not the probe trial) and analyzed for strain differences using a Kruskal-Wallis one way analysis of variance by ranks. Post-hoc analyses were conducted using the Mann-Whitney U test. Search strategy use on the last day of training was correlated with measures of performance in the MWM on the last day of training and probe trial performance using the Pearson product-moment correlation coefficient.

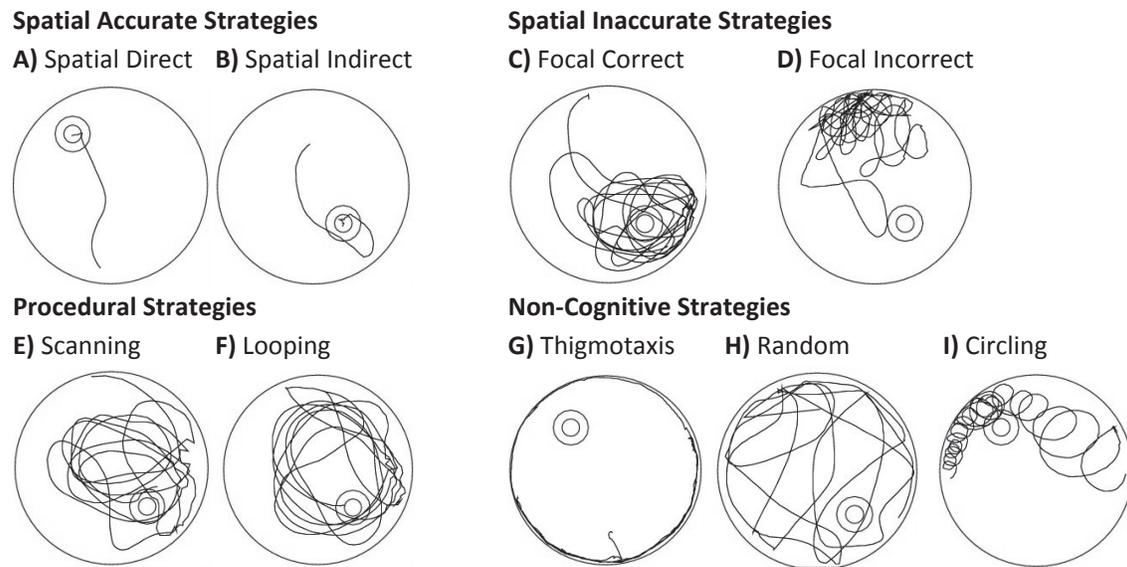


Figure 1. Example swim paths of the three nine types of search strategies which were classified into three categories: Spatial Accurate: A) Spatial Direct and B) Spatial Indirect, Spatial Inaccurate: C) Focal Correct and D) Focal Incorrect, Procedural: E) Scanning and F) Looping, and Non-Cognitive: G)Thigmotaxis, H) Random, and I) Circling.

## Results

### Tracking system measurements

Both pigmented and albino mice performed better than RD mice during acquisition, reversal, probe, and the visible platform trials. Within the albino mice, BALB/cByJ often performed better than A/J mice (Figure 2 A&B). In the probe trial memory test, C57 mice performed the best followed by BTBR, BALB/cByJ, C3H/HeJ, and A/J mice (Figure 2 C&D).

### Swim path analysis

In general all mice began with non-cognitive strategies then shifted to procedural or spatial accurate strategies. Spatial inaccurate strategies were rarely used (Figure 3). There were significant strain differences in search strategy use on all days except spatial accurate and spatial inaccurate on day 1. By day six of training and on the visible platform trials, the C57, BTBR, and BALB/cByJ mice used the spatial accurate search strategies more than the A/J and C3H/HeJ mice (Figure 3A). BALB/cByJ mice used the spatial inaccurate strategies more than other mice during training and during the visible platform trials (Figure 3B). BTBR mice used the procedural strategies more than all other strains on all but day six of training and there were no differences between strains in the visible platform trials (Figure 3C). On day one all strains used the non-cognitive search strategies. The C57, BALB/cByJ, and BTBR mice quickly reduced their use of non-cognitive strategies over days, but A/J and C3H/HeJ did not (Figure 3D). In the visible platform trials the A/J and C3H/HeJ continued to use non-cognitive strategies.

### Correlations between swim paths and tracking system measures of performance

On day 6 of training, use of the spatial accurate strategies was negatively correlated with latency ( $r=-0.884$ ,  $p<0.001$ ,  $n=74$ ) and distance ( $r=-0.858$ ,  $p<0.001$ ,  $n=74$ ) to the platform, and thigmotaxis ( $r=-0.685$ ,  $p<0.001$ ,  $n=74$ ), and positively correlated with two measures of probe trial performance: percentage of time spent in the correct quadrant ( $r=0.623$ ,  $p<0.001$ ,  $n=74$ ), and number of correct annulus crossings ( $r=0.520$ ,  $p<0.001$ ,  $n=74$ ). Use of the spatial inaccurate strategies was not correlated with any measures of MWM performance. Use of the procedural strategies was positively correlated with distance to the platform ( $r=0.312$ ,  $p<0.01$ ,  $n=74$ ) and swim speed ( $r=0.328$ ,  $p<0.01$ ,  $n=74$ ). Use of the non-cognitive strategies was positively correlated with latency

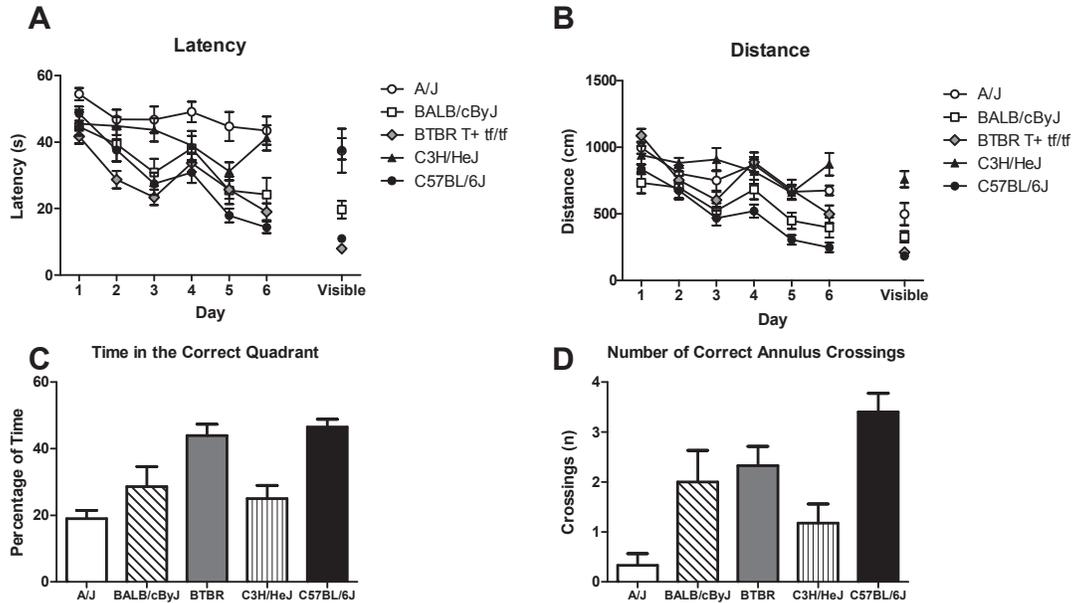


Figure 2. The (A) latency and (B) swim distance to the hidden platform over six acquisition days and the visible platform trials in the MWM. (C) The percentage of time in the correct quadrant and the (D) number of correct annulus crossings during the probe trial in the MWM. A/J and BALB/cByJ are albino, C3H/HeJ have retinal degeneration, BTBR T+ tf/tf have normal vision but are used as a model of autism, and C57BL/6J have normal vision. Error bars represent the standard error of the mean

( $r=0.798$ ,  $p<0.001$ ,  $n=74$ ) and distance ( $r=0.660$ ,  $p<0.001$ ,  $n=74$ ) to the platform, thigmotaxis ( $r=0.862$ ,  $p<0.001$ ,  $n=74$ ), and negatively correlated with two measures of probe trial performance: percentage of time spent in the correct quadrant ( $r=-0.519$ ,  $p<0.001$ ,  $n=74$ ), and number of correct annulus crossings ( $r=-0.404$ ,  $p<0.001$ ,  $n=74$ ).

## Discussion

These results demonstrate that search path classification using our SwimPath program is able to detect differences in learning in the MWM across strains with differing levels of visual ability [10]. Use of the spatial accurate search strategy by C57, BALB/cByJ, and BTBR mice, was correlated with shorter latencies and distance to the platform and better visuo-spatial memory scores in the probe trial. Use of the spatial inaccurate strategy was not correlated with any measures of performance in the MWM, but it was able to distinguish between the BALB/cByJ mice and all other strains, indicating that the search path analysis is measuring an aspect of MWM performance which is not captured by the performance measures typically used in the MWM. The higher use of the procedural strategies in the BTBR mice differentiates them from the BALB/cByJ mice, which have very similar levels of spatial accurate and non-cognitive search strategies. Mice using spatial accurate strategies had lower latencies and shorter distances to reach the escape platform during training and spent more time in the correct quadrant and had more platform crossings in the memory test than mice using non-cognitive strategies. These results demonstrate that increased use of the spatial accurate strategies is correlated with better performance and use of non-cognitive strategies with worse performance in the MWM. Overall we have demonstrated that this method of scoring search strategies is an effective measure of MWM performance which is able to detect differences between strains that traditional measures were unable to detect. The use of search strategy analysis is the only way to determine whether or not mice are actually using a visuo-spatial search strategy to learn in the MWM and this has implications for understanding strain differences in the neural basis of learning and memory in the MWM.

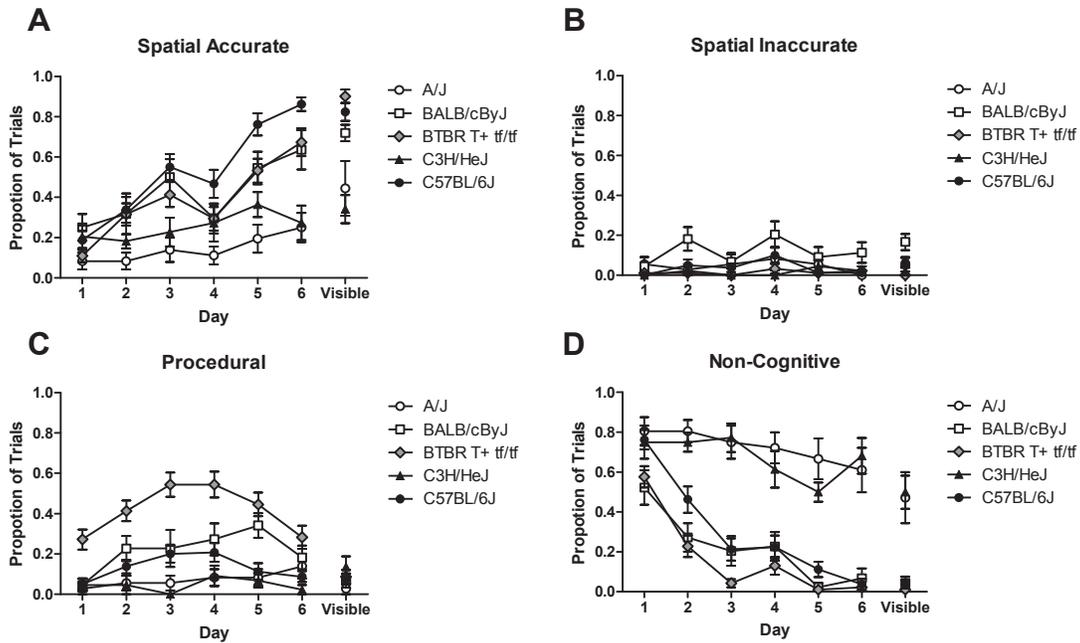


Figure 3. The proportion of usage of (A) spatial accurate, (B) spatial inaccurate, (C) procedural, and (D) non-cognitive search strategies in five strains of mice over six acquisition days and the visible platform trials in the Morris water maze. A/J and BALB/cByJ are albino, C3H/HeJ have retinal degeneration, BTBR T+ tf/tf have normal vision but are used as a model of autism, and C57BL/6J have normal vision. Error bars represent the standard error of the mean.

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