Data and text mining

Unbiased classification of spatial strategies in the Barnes maze

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Abstract

Motivation: Spatial learning is one of the most widely studied cognitive domains in neuroscience. The Morris water maze and the Barnes maze are the most commonly used techniques to assess spatial learning and memory in rodents. Despite the fact that these tasks are well-validated paradigms for testing spatial learning abilities, manual categorization of performance into behavioral strategies is subject to individual interpretation, and thus to bias. We have previously described an unbiased machine-learning algorithm to classify spatial strategies in the Morris water maze.

Results: Here, we offer a support vector machine—based, automated, Barnes-maze unbiased strategy (BUNS) classification algorithm, as well as a cognitive score scale that can be used for memory acquisition, reversal training and probe trials. The BUNS algorithm can greatly benefit Barnes maze users as it provides a standardized method of strategy classification and cognitive scoring scale, which cannot be derived from typical Barnes maze data analysis.


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Supplementary information: Supplementary data are available at Bioinformatics online.

1 Introduction

While the molecular and genetic tools applied in neuroscience are becoming ever more sophisticated, the most robust tasks that assess cognitive spatial memory have been largely unchanged since the early 1980’s. The Morris water maze (MWM), described by Morris (1984), and the Barnes maze, described by Barnes (1979), are the most widely used paradigms for assessing spatial learning in rodents (Vorhees and Williams, 2006). We have recently described the MUST-C (Morris water maze Unbiased Strategy Classification) algorithm, which rapidly classifies spatial swim strategies in the MWM and provides an unbiased assessment of the cognitive capacity of rodents (Illouz et al., 2015). Similar to the MWM, the convenient and robust nature of the Barnes maze has facilitated the investigation of spatial learning in both control and disease states. For example, assessing spatial learning using the Barnes maze has important implications for many pathologies in which learning is impaired such as aging (Krause et al., 2008), Alzheimer's disease (Puzzo et al., 2014), neurodevelopmental disorders, mental retardation, pharmacological assays to assess drug efficiency and more.

The Barnes maze also has advantages in assessing improvements in spatial capacity, such as following enriched environment (Bonaccorsi et al., 2013), caloric restriction (Ma et al., 2014) or exercise (Inoue et al., 2015). In contrast to the MWM paradigm, the Barnes maze is a dry maze, reducing water-related stress and enables the use of in vivo devices such as optogenetics. However, assessment
of performance in the Barnes maze is susceptible to experimenter bias, calling for a more objective method of data analysis.

The difference between the MWM and Barnes maze tasks, often results in different sets of navigation strategies utilized by rodents (Illoz et al., 2015). As a consequence, a novel classifier dedicated to the Barnes maze is required.

In the Barnes maze, rodents are trained to find an escape box hidden under the surface of a circular table by relying on extra-maze cues (O'Leary and Brown, 2012). The high illumination used in this task (1350 lux), causes anxiety in the mice, providing the motivation to find the hidden chamber. Performance in this task relies on hippocampal place cells, validating its use as a task for spatial learning (Bimonte-Nelson, 2015). The efficiency in which mice perform this task is then quantified using specific parameters such as the latency to reach the escape chamber, total distance traveled, mean moving speed and path efficiency (calculated as the distance between the first and last locations divided by the total distance). Additional attributes are typically used to control for the animal’s behavior, such as immobility and mean speed. Both cognitive and non-cognitive traits are evident during spatial navigation, including changes in mean speed, path efficiency, duration and body rotations. Mice typically exhibit specific navigation strategies when tested in the Barnes maze, reflecting the extent of their spatial acquisition of the environment and thus provide information about the integrity of hippocampus-dependent functions and cognitive state. Based on narrower previous strategy definitions (direct, serial and mixed) (Harrison et al., 2006) as well as our empirical data, we have defined six main navigation strategies for the Barnes maze.

This broader range of spatial strategies is described from highly spatial to non-spatial strategies: (i) direct, reflects the optimal acquisition of the target’s location, as the animal efficiently identifies its location and the target’s location and thus uses the shortest trajectory possible; (ii) corrected, reflects a slightly lower efficiency, as the animal makes a minor correction in the trajectory toward the target, and often follows re-angulation; (iii) long-correction consist of a major correction in the trajectory, as the animal navigates to a distal region of the environment, re-angulates and makes a direct movement toward the target; (iv) focused search reflects a localized scanning of the region surrounding the target; (v) serial search reflects a systematic non-spatial method of enhancing the probability of finding the target, as the animal travels from one hole to the adjacent hole in a serial manner; and (vi) random search reflects no acquisition of the target location, as the animal utilizes a non-systematic method of enhancing the probability of finding the target by traveling randomly in the environment. The specific combination of strategies employed by mice in the Barnes maze are considered representative of the level of cognitive resources being used for the task (Bimonte-Nelson, 2015). However, despite thorough assessment of trajectory parameters, non-spatial navigation strategies can increase in efficiency over time, resulting in a decreased latency to reach the escape box. Moreover, simple quantification of latency, mean speed, distance and other parameters describing performance in the Barnes maze often result in only a partial understanding of mouse behavior and cognitive capacity. In order to avoid such erroneous or incomplete interpretation of behavioral data in the Barnes maze, manual assessments of mouse behavior are often required. Manual assessments of spatial strategies are time-consuming, and different experimental conditions alter searching attributes used in the apparatus, hindering cross-experiment analysis of mouse behavior. Moreover, manual assessments are prone to experimenter bias during analysis. Thus, manual assessment fails to reliably detect important aspects of cognitive spatial behavior in the Barnes maze, due to reduced sensitivity to nuances, bias and environmental factors.

For these reasons, we constructed the Barnes maze unbiased strategy (BUNS) classification tool, an automatic classification algorithm, freely available for the analysis of mouse behavior. This tool uses generic features of the mouse path to enable unbiased analysis of spatial learning strategies, and presents a cognitive scoring scale based on the spatial learning strategy used by the mice. We have compared performance of mice in the Barnes maze in order to validate and assess the sensitivity of our algorithm. Our data demonstrate that the BUNS algorithm provides unbiased analysis of Barnes maze behavioral data and extends the capacity of researchers to understand the mechanisms of spatial learning and memory in rodents.

2 Methods

Animals. Congenic 8-week-old male mice (n = 15) from a C57bl/6 genetic background were purchased from Jackson Laboratories (Bar Harbor, ME). Animals were housed in a reversed 12:12 h cycle to ensure cognitive assessment during the animals’ activity period. Animal care and experimental procedures followed Bar Ilan University guidelines and were approved by the Bar Ilan University Animal Care and Use Committee.

Barnes maze. A circular table 105 cm high with a diameter of 92 cm was used. Eighteen holes are located at the perimeter of the table at equal distances, each with a diameter of 5 cm. One hole only (the target hole) leads to an escape chamber in which the animal can hide. Illumination was measured at the center of the table and maintained at 1350 lux in order to encourage the animal’s motivation to search for the target hole. During the habituation phase, which lasted 1 day, the animal was placed in a cylinder at the center of the maze. Five seconds later, the cylinder was removed and the mouse was allowed to explore the environment for 120 s. Mice that found the target hole were able to enter the escape chamber; mice that did not find it within this period of time were placed back in the cylinder, now located above the target hole. In this phase mice were given one trial only.

Four visual cues were presented on the walls surrounding the Barnes table (Supplementary Fig. S1). In the spatial acquisition phase, mice were given 120 s per trial to find the target hole. Mice that did not find it were placed at the target hole area until they located it. Mice were divided into groups of 4. Each animal was given 2, 3 or 4 trials (separate experiments) with an inter-trial interval of 10 min. Thus, all four mice completed a trial before the next trial began. This procedure was repeated daily until no significant improvement in performance was identified. Following spatial acquisition, a probe test was conducted with closed holes and no escape chamber. Animals were given a single 60 s trial to explore the environment. Following the probe test, the target hole and escape chamber were moved 180° from the original target location. Similar to the spatial acquisition phase, mice were given four non-sequential trials, 120 s each, to find the new escape chamber. This procedure was repeated daily until no significant improvement in latency was identified. Latency to reach the escape chamber, total distance travelled and mean speed were automatically calculated by the video tracking system (ANY-maze, Stoelting Co, Wood Dale, IL).

Algorithm

Support vector machine. Support vector machine (SVM) (Boser et al., 1992; Vapnik, 1998) is a supervised learning classification
technique. It has a strong theoretical basis and has been proven to be successful in numerous classification tasks from handwritten digit recognition to bioinformatics (Osuna et al., 1997; Tong and Koller, 2002). Linear SVM separates a binary-labeled training data set by a hyper plane (decision boundary) that maximizes the margin between the two classes. A test set point is labeled according to its location in the feature space, relative to the decision boundary. The kernel function used in this study is the radial basis function. We used the MATLAB SVM function (The MathWorks, Inc., Natick, MA) in this classifier.

Our Training set consists of over 600 labeled trials, which include six possible strategies. Input is generated by video tracking software (ANY-maze, Stoelting Co, Wood Dale, IL) and contains a computer-graphic x, y coordinate of the animal’s center-point or head location according to the user’s preference. Animal coordinates are converted into Cartesian coordinates to extract a set of 15 factors characterizing different aspects of the animal trajectory (Supplementary Table S1), such as trial duration, total distance, absolute angles and path efficiency. Variables were chosen to characterize the physical properties of the different strategies (time, distance and speed) as well as the spatial performance of the animal (path efficiency, number of circles, distance from perimeter). All data were normalized using the Z-score method (see Supplementary Methods).

Prediction accuracy. Prediction accuracy of the BUNS algorithm was compared to 962 trials that had been manually classified by researchers using confusion matrices.

Multi-class classification. In order to categorize data into multiple classes, we performed a set of hierarchically ordered binary decisions to separate the data into one of several subclasses. To build the suitable tree we first performed an unsupervised binary k-mean clustering. This analysis was conducted with 1–5 hierarchical layers (2–16 clusters, Fig. 2F). A separation index was calculated for each branch as the percentage of trials belonging to a single strategy divided by the sum of percentages of all other strategies in this branch. The strategy’s location was determined according to the maximal separation index obtained (Fig. 2F). Based on this unsupervised dendrogram we have designed a similar tree for the SVM-analysis (Fig. 2G). As demonstrated, related strategies are neighboring branches, for example, direct and corrected are both highly cognitive strategies and therefore located at the same branch. However, they are also based on the strategy’s actual features. For example, although long correction is theoretically related to correction, it was located near focused search due to its high distance. At each decision junction, the relevant features were selected in the same manner. In the first layers, more general features, such as trial length, were used to separate the data into short or long trials. At deeper layers, each decision was made by a different set of finer features relevant to the theoretical basis and actual characteristics of the junction, such as number of sequential hole visitations to separate serial from random.

Cognitive score. We provide a quantification method for performance in the Barnes maze, as each training trial was scored with a numerical value in a non-arbitrary manner. To do so we have applied the well-known unsupervised machine learning method of k-means clustering with possible 1–5 layers. The optimal tree was then chosen according to the highest homogeneity of its branches. Next, we scaled each strategy according to its location in the dendrogram, that is, the number of junctions from the optimal direct strategy (scored 1, Fig. 2G): random = 0, serial = 0.25, focused search = 0.5, long correction =0.5, corrected = 0.75 and direct = 1. Statistical analysis. All statistical methods can be found in Supplementary information.

3 Results
Spatial learning in the Barnes maze. We developed an SVM-based BUNS classifier algorithm for the classification of spatial strategies in the Barnes maze. In order to assess whether the algorithm detects changes in the navigation strategy of mice, we used BUNS to analyze raw data from three Barnes maze experiments conducted with 2, 3, or 4 non-consecutive trials per day (n = 15 per group). Mice tested with three and four trials per day exhibited a significant reduction in latency to reach the escape box ranging from 72.5 ± 4.1 s and 78.3 ± 6.8 s, respectively, on the first day, to 18.5 ± 1.2 s, 21.4 ± 2.1 s, respectively, on the last day (P < 0.0001, Fig. 1A). Mice trained with only two trials per day exhibited a milder reduction in latency ranging from 66.62 ± 8 s to 56.8 ± 7.5 s at Day 9 (P < 0.0001, Fig. 1A). The distance traveled was also reduced in mice receiving three or four trials, ranging from 2.95 ± 0.23 m and 3.6 ± 0.4 m covered to 1.15 ± 0.07 m, 1.04 ± 0.1 m on the last trial, respectively (P < 0.0001, Fig. 1B). In contrast, mice trained with only two trials per day did not exhibit a reduction in the distance traveled (2.74 ± 0.4 m to 2.6 ± 0.38 m, P < 0.0001, Fig. 1B). Path efficiency on the last day of training was higher in mice that received three or four trials: 0.48 ± 0.03 and 0.54 ± 0.03 compared with two trials, 0.32 ± 0.04 on the last day of training (P < 0.001, Fig. 1C).

Moreover, mice tested with three and four trials exhibited a significant enhancement in path efficiency, whereas mice tested with two trials did not (P < 0.001, P < 0.0001, P = 0.97, respectively, Fig. 1C). Mean speed increased in mice trained with three and four trials per day, from 0.039 ± 0.002 m/s, 0.042 ± 0.002 m/s to 0.049 ± 0.002 m/s, 0.052 ± 0.002 m/s on the last day of training (P < 0.05, Fig. 1D). However, mean speed did not differ between groups in mice trained with only two trials per day (P = 0.95, Fig. 1D). Thus, three trials per day is the minimum number required for successful spatial learning by the mice in the Barnes maze.

Although the conventional measures of latency, distance, path efficiency and mean speed provide a general quantification of the

Fig. 1. Target acquisition in the Barnes maze is dependent on the number of trials. Mice were trained in the Barnes maze until optimal performance was maintained for two consecutive days (n = 15 per group), measured by latency to reach the target hole. Mice trained with two trials per day exhibited a slower learning curve compared to mice tested with three or four trials per day in the following parameters: (A) latency to reach the target hole, P < 0.001, and a time effect for 3.4 trials-per-day P < 0.0001. (B) total distance traveled, P < 0.001, time effect for 3.4 trials-per-day P < 0.0001 and (C) path efficiency, P < 0.001, time effect for 3.4 trials-per-day P < 0.001 and P < 0.001 accordingly. (D) Mean speed increased significantly over time in mice trained with three or four trials per day (P < 0.05, Fig. 1D) but not with two trials per day (P = 0.95). Two-way RM ANOVA. **** P < 0.0001, *** P < 0.001, * P < 0.05
Spatial strategies in the Barnes maze

Spatial strategies in the Barnes maze reflect the animal's cognitive state during navigation. They are defined by the animal's movement patterns, such as direct, corrected, serial, and random search. These strategies are critical for understanding the animal's cognitive performance and can be used to assess the efficacy of different navigation strategies. For example, latency and distance distinguish between direct and random search (Fig. 2B and C), however they do not sufficiently discriminate between finer differences such as between direct and corrected search, or long correction and focused search, since their distributions overlap in different strategies (Fig. 2B and C). Path efficiency provides a better measure of spatial acquisition, as it reflects the efficacy of the animal's navigation strategy based on its trajectory. However, this variable also fails to provide a fine discrimination between direct and corrected strategies, as their distributions partially overlap in different strategies (Fig. 2D).

Mean speed does not provide any discriminating information, as there is no significant variation between strategies (Fig. 2E). Nevertheless, the animal’s speed does affect the latency to reach the target, and thus provides information about the motor performance of the tested strain. Because the parameters used in the Barnes maze have substantial overlap, manual assessment is often required to accurately differentiate between individual strategies, introducing potential bias in analysis. The BUNS algorithm provides fine discrimination of learning strategies and sets a cognitive scale to enable the researcher to better understand the cognitive state of the subjects.

Spatial strategy classification using the SVM. The BUNS algorithm we employed (Fig. 2F) was composed of five binary decision junctions (Fig. 2G). This architecture was based on an unsupervised tree obtained by performing k-means binary clustering analysis with 1–5 layers of separation (2–16 clusters, Fig. 2F). The optimal tree was determined according to the maximal separation indices (Fig. 2F). X and y coordinates were used to generate 15 parameters (Supplementary Table S1), which were then normalized to Z-score values (see Supplementary Algorithm). These factors were fed to the SVM algorithm, which was previously trained using labeled trials (see Fig. 2A, Algorithm). The prediction accuracy of the BUNS algorithm for experiments using 2, 3 and 4 trials per day was 93%, 90% and 89%, respectively (Fig. 3A). These prediction rates are represented in their respective confusion matrices (Fig. 3B–D). In order to assess the classification bias of human judges and the BUNS algorithm, we have compared the classification results of three independent judges and the BUNS algorithm with previously validated human labels. The bias of the human classification is significantly higher than the machine bias (0.5 ± 0.005, 0.2, respectively, P < 0.05, Supplementary Figs S3 and S4) and the classification standard deviation was nine-times the magnitude of the bias. In addition, the human classification suffers from between-judges differences, as the between-subject standard deviation was 0.53 ± 0.39, the stable machine classification suffers from no such variance (P < 0.0001, Supplementary Figs S3 and S4).

Using this algorithm, we found that mice tested with two trials per day shifted from mostly random search on the first day (69.3% ± 8.4%) to mostly serial search (36% ± 8.7%) on the last day (Fig. 4A, Supplementary Table S2). Mice tested with three trials per day shifted from mostly random (27.2% ± 6.6%) and serial search on Day 1 (29.8% ± 6.8%, Fig. 4B, Supplementary Table S2), to mostly corrected search on Day 6 (33.3% ± 7%, Fig. 4A, Supplementary Table S2). Mice tested with four trials per day shifted from mostly random search on Day 1 (45% ± 7.4%), to mostly direct (23.3% ± 6.3%) and corrected (38.3% ± 7.2%) search on the last day (Fig. 4C, Supplementary Table S2).

The cognitive level of a particular strategy used by mice can be quantified in order to provide a definitive score for spatial cognitive capacity. In order to accomplish this, we attributed a scoring scale for the different navigation strategies based on their relevance to spatial learning (see the Algorithm section, distance between strategies in Fig. 2F), that is, the scaled distance of each strategy in the hierarchical tree from direct swim (Fig. 2G), quantified as number of junctions between them. This analysis indicates that mice tested with two trials per day obtained significantly lower cognitive scores compared with mice receiving four trials (P < 0.05, Fig. 4D). In addition, mice tested with three trials per day obtained significantly...
lower cognitive scores compared with those with four trials ($P < 0.05$, Fig. 4D). This observation is further strengthened when measuring the latency distribution of the mice. While mice trained with three and four trials per day exhibited a shift in the latency distribution of the mice. While mice trained over 120 relevant pre-labeled trials (Fig. 6B). The prediction accuracy of the BUNS algorithm in the probe trial analysis was 97.7% ($P < 0.0001$, Fig. 6D). This prediction accuracy is also represented in a confusion matrix (Fig. 6D). Using this algorithm, we found that during probe trials, the navigation strategy of the mice mostly consisted of focused search (57% ± 12%), serial search (14% ± 8.9%) and serial-random search (29% ± 11%) (Fig. 6E, Supplementary Table S3). To quantify the cognitive capacity of mouse performance, we again assigned a scoring scale for search strategies unique to the probe trials based on their relevance to spatial learning (see the Algorithm section). Mice tested with four trials per day obtained a score of 0.68 ± 0.1 (Fig. 6F).

If spatial information is not reinforced, spatial memory extinction can result (Rossato et al., 2006). To further demonstrate that the SVM algorithm is capable of detecting spatial memory extinction, we conducted an additional reversal task only in mice tested with three trials per day ($n = 15$). Mice exhibited a significant reduction in latency to reach the escape chamber from 70.1 ± 6.4 s to 17.7 ± 1.9 s ($P < 0.0001$, Fig. 7A). This was accompanied by a reduction in the distance travelled from 3.1 ± 0.28 m to 1.05 ± 0.15 m ($P < 0.0001$, Fig. 7B). Similarly, path efficiency also improved.
Further, we have created a cognitive score scale that assesses memory acquisition, reversal training and probe trials.

While performance in spatial learning tasks such as the Barnes maze is typically quantified using factors such as latency and path efficiency, these parameters are limited in their ability to illustrate the complexity of the animal’s spatial cognitive capacity. In addition, since researchers in different laboratories use different experimental setups and animals from different backgrounds, the ability to quantify the various spatial strategies exhibited by the mice enables investigators to directly compare phenotypic data obtained in different apparatuses. Notably, the BUNS tool enables this analysis to be performed in different variants of the Barnes maze, such as during acquisition, probe trials, reversal and memory extinction, which enables one to obtain a complete picture of the spatial cognition phenotype of the mice.

The BUNS tool exhibits sensitivity that exceeds the discrimination obtained by typical parameters such as latency, distance and path efficiency. In fact, when comparing the performance of the mice, which were given three trials per day versus four trials per day, no significant difference was observed in either latency, distance or path efficiency. However, the cognitive score derived from the spatial strategy assessment did reveal a higher cognitive score obtained by mice trained with four trials per day compared with mice trained with three trials per day. Moreover, our findings reveal a limitation in the spatial acquisition of the target hole when animals were given two trials per day. Thus, the BUNS algorithm was able to tease out slight differences in navigation strategies that enabled a differential classification and separation of the mice.

Current methodologies and analysis of the Barnes maze, while effective, still neglect to discriminate between important differences in navigation strategy and cognitive ability in mice. The BUNS tool enables efficient, accurate and objective comparison between mouse strains and experimental conditions that was previously unavailable. Overall, the BUNS algorithm offers the research community a powerful tool in assessing spatial learning in rodents.

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References


4 Discussion

The BUNS algorithm offers an accurate and unbiased determination of the spatial strategies utilized by mice in the Barnes maze, a widely used task for the assessment of cognitive learning in rodents. Our data confirm that the BUNS algorithm can be effectively used to analyze Barnes Maze data in a reduced-bias and stable fashion.


