New approach to analyse navigational search strategies used by mice during a water maze task

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The open-field water maze task is one of the most widely used behavioural tasks to investigate cognition and to assess spatial learning and memory in rodents [1]. The principle of the task relies on the motivation to locate a fixed hidden platform as the only mean of escape from the water. An improvement in performance recorded over trials and days, for example of latency or path length to reach the platform location, reflects learning and is typically expressed as a reduction in the length of the swim path. During recent years, in addition to the analysis of common proxies (path length, swim speed, latency to platform), many researchers have shown a growing interest in the qualitative aspect of learning. Indeed, it is now established that in the water maze the animal develops navigational search pattern based on the use of multiple different strategies [2]. As a consequence, analysis of these strategies would be helpful, for example, in discriminating between groups of animals that show otherwise no differences according to the standard parameters. In light of this consideration, investigation of the type of strategy used to solve the task provides a more refined and comprehensive analysis of spatial learning in the water maze.
Search paths, such as those described by Garthe and colleagues [3], are now categorised into 3 main classes: "*Spatial, Procedural and Random*". The spatial search strategies are commonly classified as "*Directed, Focal* and *Direct"* and the procedural ones as "*Scanning and Chaining*" (see Figure 1). In the literature, most analyses are performed visually through a process of manual scoring. However this method can be time consuming and may lead to biased interpretation of results linked to the subjectivity of the experimenter.

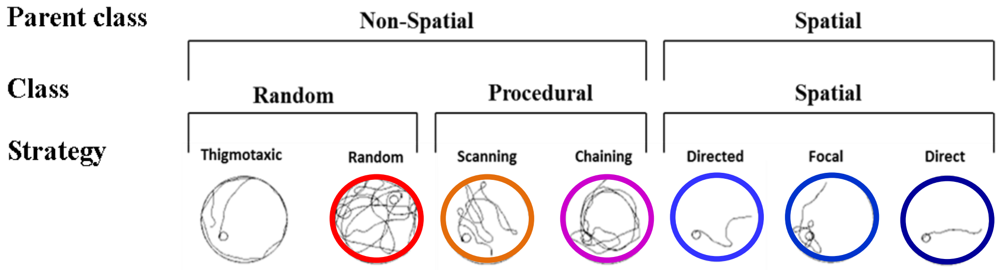

Figure 1: General classification of the search strategies commonly used in the water-maze. Parent classes are defined as *Spatial* and *Non-spatial*. They further dissociate into 3 classes, within the non-spatial are regrouped *Random*: path with no directional preference, *Procedural* defined as an allocentric search and *Spatial* related to a search based on the use of visual landmarks. The individual members in these classes include: *Thigmotaxis*: "Wall hugging", *Random*: total pool surface covered with no directional preference, *Scanning*: scanning of environment confined to the centre of the pool, *Chaining*: circular swimming at a fixed distance of the pool, *Directed*: swim with a directional preference, *Focal*: search close to the platform, *Direct*: direct navigation to the platform. Learning typically progresses through these categories from random searches to direct swims.

The aim of this study was to provide a more detailed analysis of the water maze by means of a new automated strategy analyser developed in our group and to assess its strength in discriminating search strategies relative to manual scoring. The MATLAB script is based on the highly flexible fuzzy logic sorting system, which classifies swim tracks automatically into episodes corresponding to search strategies (see Crouch et al. in this conference for details of methods).
In this experiment, two different transgenic mouse lines, named Line 1 and Line 66 aged 6 and 3 months respectively were trained in the open-field water maze task and search strategies were analysed both visually and automatically. Mice were housed in groups of up to 10 and allowed food and water *ad libitum* and were kept under standard conditions (temperature 20−21°C, 60−65% relative humidity) on a 12 hours light/dark cycle (light on at 7:00 a.m.). Tests took place during the light phase of the cycle. All experiments were conducted with local ethical permission and in strict accordance with UK Home Office regulations outlined in the Animals (Scientific Procedures) Act 1986.

Learning in the water maze was recorded as a progressive lowering of the distance moved to locate the platform, no difference was found for Line 66 and wild-type mice and both groups improved their performance over days

(see Figure 2A). Similarly, the visual manual scoring, as well as the automatic analysis, did not show differences in the pattern of strategies used by the mice during the task. From day 1 to day 4 of training, both analysis systems highlighted a progressive diminution in random searches while increasing the propensity of spatial strategies (see Figures 2B, C). By contrast, Line 1 animals present with impairment in learning the water maze task as indicated by a longer distance moved to reach the platform compared the control mice (see Figure 3A). This deficit was confirmed with both strategy analyses (see Figures 3B, C) revealing a persistent level of random searches along with a lower percentage of spatial strategies on day 4.
Regarding the comparison between the visual and MATLAB script analyses, the automatic scoring matches 92% of the visual analysis in terms of spatial and non-spatial parent classes, but fell to 60% in terms of sub-divisions strategies. This can be seen for example for the search strategies of control animals recorded during the first day of both experiments, where the percentage of scanning reaches up to 70% when visually analysed and less than 10% in F-logic. On the contrary, the level of random and chaining strategies detected automatically is higher than the visual manual scoring. This difference could be explained by a different classification method (see Crouch et al., this conference). Indeed, the experimenter can only base the analysis on the entire track which leads to only one possible choice of strategy. By contrast, F-logic segments the single track into different episodes assigning the appropriate strategy based on the probabilities of occurrence and selecting the strategy with highest probability value.

In light of these results, the use of an F-logic based analysis reduces the subjective characterisation of search strategies attributed with a visual manual scoring and offers more flexibility in the qualitative assessment of phenotyping spatial navigation.

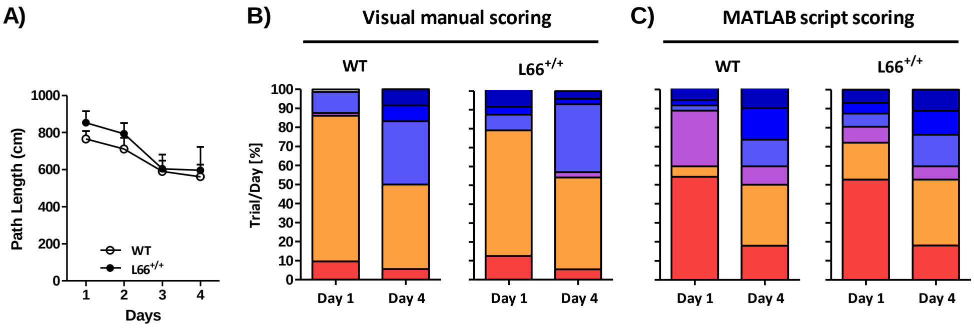

Figure 2: Comparison between visual and automatic analysis regarding the strategy used by transgenic mice ($L66^{+/+}$) and wild type mice (WT) in the water-maze. A) Average path length to reach the platform. Both group improved their performance similarly. B) Percentage of strategy used by $L66^{+/+}$ and WT mice on Day 1 and 4 analysed by means of a visual manual scoring or C) F-logic.

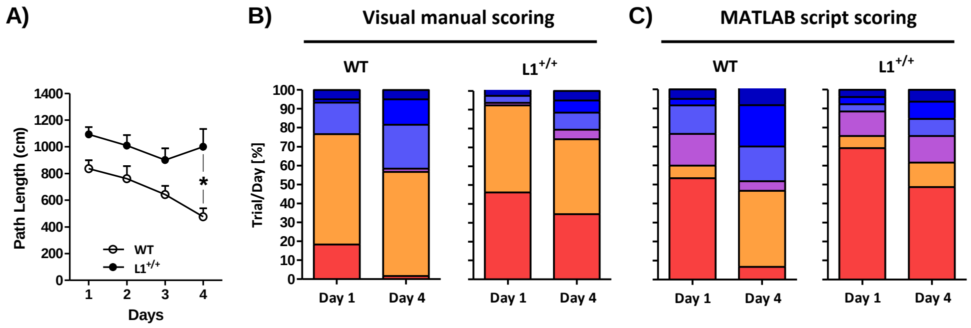

Figure 3: Comparison between visual and automatic analysis regarding the strategy used by transgenic mice ($L1^{+/+}$) and wild type mice (WT) in the water-maze. A) Average path length to reach the platform. Line 1 mice showed slower learning and differed significantly from WT mice on the 4th day. *$P < 0.01$, significantly different from control, Bonferroni test. B) Percentage of strategy used by $L1^{+/+}$ and WT mice on Day 1 and 4 analysed by means of a visual manual scoring or C) F-logic. Note the genotypic and categorisation-related differences.

References:

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