Spatial Learning Characteristics of Transgenic Mice as Revealed by Detailed Video-Analysis Using EthoVision

Valeria Melis[1,2], Gernot Riedel[1,2], Charles R. Harrington[1,2]

[1]*Institute of Medical Sciences, University of Aberdeen, Aberdeen, United Kingdom. v.melis@abdn.ac.uk*

[2]*TauRx Therapeutics Ltd, Aberdeen, United Kingdom.*

The water maze (WM) test was first described in the early 1980s [1] and, currently, is one of the most widely used tasks in behavioural neuroscience to explore spatial memory in rodents. Methodological variations of the WM task have been developed and are being used by researchers in many different applications for instance, to test the impact of aging, to investigate various neurodegenerative diseases or for the screening of novel therapeutic drugs [2]. In order to take full advantage of the assessment of rodent models by means of the WM test, consideration of the behavioural paradigms employed is critical in investigating the phenotype under scrutiny. In addition, common proxies used to quantify cognitive abilities of animals are not always satisfactory and complete descriptors of behavioural anomalies. Standard performance measures include: path length (i.e. the length of the path swam from the release point to the platform) and latency (i.e. the time the animal takes to find the platform) as indices of learning achieved during training. Learning is usually defined by shorter latencies and by decreased path length. Furthermore, swimming velocity, as measure of genotype-related effects on motor activity and thigmotaxis, defined as the proportion of time spent swimming along the edge of the pool which is an index of animal's anxiety, are considered.

Nowadays, several reliable video tracking systems (e.g. EthoVision, Noldus Information Technology; AnyMaze, Ugo Basile; HomeCageScan, Clever Systems; etc.) for automated behavioural research are available. Although such software programs have the benefit of measuring a wide range of quantitative performance parameters, very basic indices are preferred to more complicated and time-consuming quantifications, resulting in an oversimplified description of the behaviour. For example, animals may use different search strategies; an animal that swims close to the platform during training may display the same latency or path length to the platform as animals that use a circular strategy, but do not show direct search toward the platform area [3]. As a consequence, investigation of path shape parameters would be helpful, for example, in discriminating between groups of animals that show otherwise no differences according to the usual parameters. Path shape or strategy may be determined as a) "cumulative distance to platform" (or "proximity") [4] which is the distance (cm) between the position of the animal and the platform location measured several times per second (the closer the animal swims to the platform, the shorter the cumulative distance); b) "turn angle" (degrees), that is the change in swimming direction; c) "angular velocity" (degrees/s) which indicates the change of direction of movement of the animal in the maze per time unit and d) "meander" (or " tortuosity") (degrees/cm) which is the amount of turning per distance unit.

The aim of this study was to provide a more detailed analysis of the water maze performance of a transgenic mouse model of Alzheimer's disease overexpressing mutated tau in brain, termed Line 1 [5], using EthoVision 3.1 (Noldus Information Technology, Wageningen, The Netherlands). Two different WM paradigms were used to investigate cognitive functions in 5-month old Line 1 and age-matched wild-type mice, namely the standard reference memory (wild-type: n = 10; Line 1: n = 13) and the problem-solving (wild-type: n = 10; Line 1: n = 12) WM tasks. The former requires the incremental learning of a constant platform location over multiple days of training [6]. By contrast, in the spatial problem-solving task, which was adapted from a protocol elaborated by Chen and co-workers [7], an initial visual pre-training is followed by a training to a hidden platform until a criterion is met (problem), then the platform location is changed and animals are trained until the same criterion of the next problem is met [8]. Mice were housed in groups of up to 10 until the beginning of the study and continuing through the completion of behavioural testing. Animals were 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 in strict accordance with the UK Home Office regulations outlined in the Animals (Scientific Procedures) Act 1986.

Path length, latency, swim speed and thigmotaxis were measured for both paradigms. In addition, the percentage of time the animal spent within the quadrant where the platform was located during training, as an index of spatial memory, was recorded for the standard reference memory WM task whereas the number of trials required to reach criterion for each problem was calculated as a measure of the animal's learning capacity for the problem-solving WM paradigm. Standard parameters revealed a learning impairment for transgenic mice in both paradigms along with an increased swimming speed and a greater level of anxiety. For the standard reference memory WM, the learning impairment was also confirmed by the decreased proximity to the target platform location (see Figure 1). The analysis of the number of trials to reach criterion in the problem solving task highlighted a learning deficit for Line 1 mice in achieving the first problem only. On the other hand, the proximity parameter revealed a more robust phenotype for transgenic animals in achieving all the three problems (see Figure 2). This parameter reflects the searching pattern of the animal and a goal-directed searching strategy that centers on the area of the platform location results in a shorter cumulative distance as compared with random patterns. Therefore, the longer cumulative distance recorded for Line 1 mice suggests a less efficient searching strategy in the maze.

Furthermore, both paradigms revealed a decreased turn angle, angular velocity and path tortuosity for Line 1 mice when compared to wild-type animals. This may suggest either a different search strategy or a reduced exploratory response toward the environment. In support of this latter view is the observation that Line 1 mice exhibited increased level of anxiety measured as thigmotaxis. However, a further manual categorization of search strategy may help in the analysis of performance since it provides a qualitative description of complex explorative behavior.

In conclusion, the present study revealed that the rarely used shape parameters along with the traditional measures allow a more refined and comprehensive analysis of water maze behaviour.

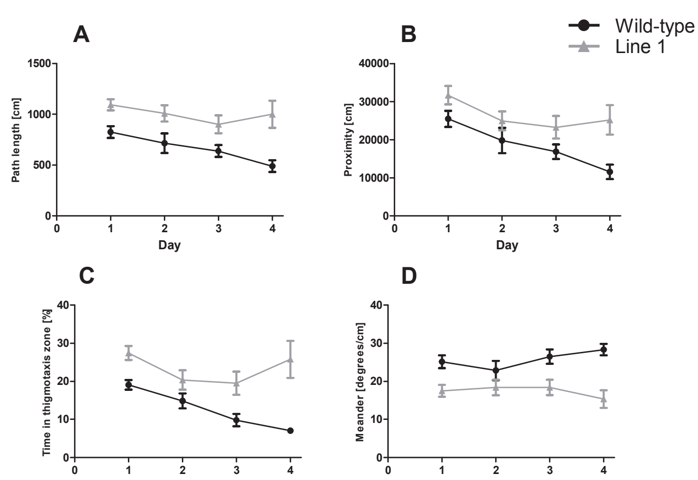

Figure 1. Spatial learning deficits in Line 1 mice in the standard reference water maze paradigm. Swim path length (A): Line 1 exhibited an inferior performance compared to wild-type mice [$F(1,63) = 16.25$; $p = 0.0006$]. Proximity (B): Learning deficit in transgenic animals was also confirmed by the decreased proximity to the target platform location [$F(1,63) = 8.01$; $p = 0.01$]. Thigmotaxis (C): Line 1 mice presented with greater level of anxiety [$F(1,63) = 13.23$; $p = 0.001$]. Meander (D): the swim path tortuosity was significantly reduced for Line 1 mice [$F(1,63) = 24.88$; $p < 0.0001$]. Data were analysed with repeated measures two-way ANOVA with genotype and day as independent variables. For all comparisons, a 95% confidence level ($p < 0.05$) was set for the differences to be considered as significant. Values are expressed as a daily mean (± S.E.).

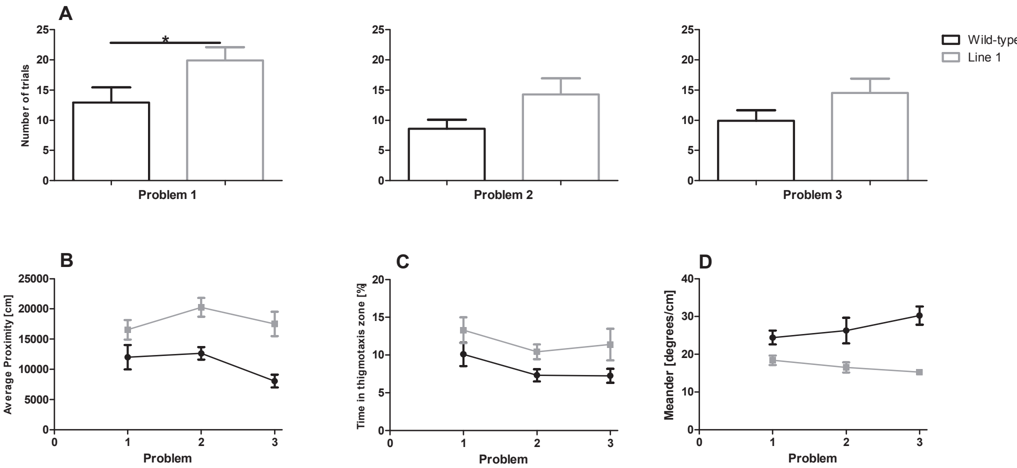

Figure 2. Spatial learning deficits in Line 1 mice in the problem-solving water maze paradigm. Number of trials to problems (A): Line 1 needed longer training to attain the first problem (Student t-test: $t = 2.1$; $p = 0.04$); no reliable difference was obtained in reaching criterion for the other two problems (p's > 0.05). * indicate statistically significant difference ($p < 0.05$). Proximity (B): Transgenic mice presented with greater proximity in achieving criterion of all problems [two-way ANOVA: $F(1,38) = 22.36$; $p = 0.0001$]. Thigmotaxis (C): Similarly to results obtained in the standard reference water maze task, Line 1 mice spent more time swimming along the edge of the pool [two-way ANOVA: $F(1,38) = 5.57$; $p = 0.02$]. Meander (D): Wild-type mice presented with a greater amount of turning per distance unit [two-way ANOVA: $F(1,38) = 33.57$; $p < 0.0001$]. For all comparisons, a 95% confidence level ($p < 0.05$) was set for the differences to be considered as significant. All values are expressed as a mean (± S.E.).

References

1. Morris, R.G., Garrud, P., Rawlins, J.N., O'Keefe, J. (1982). Place navigation impaired in rats with hippocampal lesions. *Nature* **297**(5868), 681-683.
2. D'Hooge, R., De Deyn, P.P. (2001). Applications of the Morris water maze in the study of learning and memory. Brain Research. *Brain Research Reviews* **36**(1), 60-90.
3. Schenk, E., Morris, R.G. Dissociation between components of spatial memory in rats after recovery from the effects of retrohippocampal lesions. *Experimental Brain Research* **58**, 11-28.
4. Gallagher, M., Burwell, R., Burchinal, M. (1993). Severity of spatial impairment in aging: Development of a learning index for performance in the Morris water maze. *Behavioral Neuroscience* **107**, 618-626.
5. Zabke, C., Dietze, S., Stamer, K., Rickard, J.E., Harrington, C.R., Theuring, F., Seng, K.M., Wischik, C.M. (2008) P1-054. Early and advanced stages of Tau aggregation in transgenic mouse models. *Alzheimer's and Dementia* **4**, T221-T222.
6. Deiana, S., Harrington, C.R., Wischik, C.M., Riedel, G. (2009). Methylthioninium chloride reverses cognitive deficits induced by scopolamine: Comparison with rivastigmine. *Psychopharmacology* **202**(1-3), 53-65.
7. Chen, G., Chen, K. S., Knox, J., Inglis, J., Bernard, A., Martin, S.J., *et al*. (2000). A learning deficit related to age and beta-amyloid plaques in a mouse model of Alzheimer's disease. *Nature* **408**(6815), 975-979.
8. Melis, V., Deiana, S., Zabke, C., Stamer, K., Harrington, C.R., Riedel, G., Theuring, F., Seng, K.M. & Wischik, C.M. (2008) P2-383. Reversal of spatial problem-solving cognitive deficit in a transgenic mouse line overexpressing the repeat domain of Tau by treatment with the Tau aggregation inhibitor methylthioninium chloride. *Alzheimer's and Dementia* **4**, T485.