The Radial Arm Maze (RAM) for the Evaluation of Working and Reference Memory Deficits in the Diurnal Rodent *Octodon degus*

E. Tarragon[1], L. Lopez[1], F. Ros-Bernal[2], J.E. Yuste[1], V. Ortiz-Cullera[1], E. Martin[3], E. Schenker[4], F. Aujard[5], R. Bordet[6], J.C. Richardson[7], M.T. Herrero[1]

[1]*Clinical & Experimental Neuroscience (NiCE) and Centro de Investigación Biomédica en Red sobre Enfermedades Neurodegenerativas (CIBERNED), School of Medicine, University of Murcia, Spain. ertarr@um.es*

[2]*University Jaume I of Castellon;* [3]*University of Albacete;* [4]*Institut de Recherche Servier*

[5]*Centre National de la Recherche Scientifique, UMR;* [6] *University Lille Nord de France*

[7] *GlaxoSmithKline R&D China, U.K. Group*

Spatial memory is highly relevant in biology because it is related with both individual and species survival. Among behavioural tests, one of the most suitable devices for measuring spatial learning and memory is the radial arm maze (RAM) [1,2]. Briefly, RAM consists of eight horizontal arms (57x11 cm) placed radially around a central platform above the floor. Automated doors (20 cm high) are located at the entrance of each arm (Fig. 1). Experimental subjects are placed on a central platform from which they have to collect hidden baits placed at the end of the arms.

The standard version of the RAM animals are habituated to the environment, placed on the central platform and allowed to explore the maze for 15 min per day. Reinforcers (or baits) are scattered on the arms. On the last day of habituation (day 3), the amount of reinforcer is reduced to half, and the session ends when all eight arms have been visited. Following habituation, the animals are trained one session per day for eight consecutive days. One piece of reinforcer is placed at the end of each arm in a well that hides the food from sight, and the animal is allowed to freely explore the maze. Each session lasts until (a) all eight arms have been entered (consider enter an arm when the whole body, except the tail, is inside the arm), (b) 10 min passed since the start of the test, or (c) 2 min passed since the animal's last arm entrance [3,4]. Arm entries are recorded for later analysis. To prevent odour cues, the maze must be wiped clean between animals. The variables commonly used for the analysis of the performance are (a) the number of errors in each session (entering an arm that has been visited previously counted as an error) and the total number of errors across eight sessions, (b) the number of correct choices in the first eight arm entries of each session, (c) the location of the first error in each session, (d) the number of adjacent arm entries in each session, (e) the time taken to visit each arm (total time to complete the session divided by the total number of arm entries), and (f) the number of sessions to reach the criterion of one error or less, averaged over four consecutive days of training [3-5]

There is extensive evidence that attending to the visual cues located outside the apparatus is one of the elements that subjects use to avoid re-entering the different arms in RAM. Furthermore, at least in rats, a correct

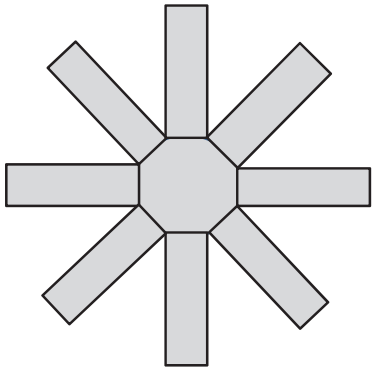

Figure 1. Schematic diagram of the eight-arm radial maze.

performance of the task seems to depend primarily on extra-maze cues [6]. On contrary, olfactory cues were rather related with the improvement of choice accuracy in reduced-visibility situations [7]. Avoiding revisits has been directly related to the amount of surrounding environment cues available, as well as to the viewing duration [8] Moreover, when extra-maze cues predicted the reinforced arms, rats performed almost perfectly on the RAM, whereas following intra-maze cues (such as odour) push performance outcome not better than chance [9]. In this sense, it seems that external cues apparently control choice behaviour when they are easily accessible [10]. Under these circumstances, each visit to an arm may be regarded as a go-no-go discrimination based on extra-maze cues [10]. Finally, it was assumed that when extra-maze cues are limited a representation of spatial locations rather than intra-maze cues might be used when navigating in the radial maze [11].

Modifications of the initial procedure permitted the distinction of spatial working memory errors (double entries into baited places) *versus* spatial reference memory errors (entering never baited arms) [12]. This version of the RAM aims to test working and reference memory at the same time. In their version of the task, only four maze arms are baited [13]. The same maze arms are baited each day and, across sessions, the rats learn to ignore the remaining four arms, which never contain reward. This is the reference memory component of the task, and entry into a never-baited arm is considered a reference memory error. Within a training session, re-entry into one of the four baited arms would be considered a working memory error.

Traditionally, the most extensively studied species in the RAM is the rat. However, a number of experiments have recently demonstrated that another rodent, *Octodon degu* (*O. degu*), is also a valid model for testing reference and working memory in this paradigm.

The experiments in our laboratory show that O. degu are able to learn the task following the traditional protocol for reference memory evaluation, as well as the combined protocol for assessing both working and reference memory (unpublished data). Moreover, animals show a clear learning curve in several variables registered, such as latency to the reinforced arms, total time to complete the essay, the number of errors committed within session, and the number of errors at the end of the training. Furthermore, it has also proved to be sensitive to the temporary impairment in memory caused by sleep deprivation. In this sense, this model results not only valid as a memory testing in normal states, but also in discriminating possible deficits in memory following different protocols for memory impairment.

Conclusions

RAM is a consolidated paradigm for the evaluation of memory. Despite the fact that the most extended model used in this kind of experiments is the rat, RAM has also demonstrated its validity across other animal species (including humans). Over the years, different versions and variations of the RAM have developed, all of them proved as solid as the traditional regarding memory assessment. Accordingly, animal models have also augmented. O. degu is a rodent that has been lately discovered as a valid experimental animal to test memory impairment associated with dementia. In this sense, evaluating the performance of this recent model using RAM results very useful to understand multiple cognitive and behavioural components of memory testing. Moreover, the validity demonstrated with this paradigm opens numerous possibilities within the field of memory and learning studies, especially those regarding cognitive impairment, which in last term will contribute to a better knowledge of these processes.

The research leading to these results has received support from the Innovative Medicines Initiative Joint Undertaking under grant agreement No 115009, resources of which are composed of financial contribution from the European Union's Seventh Framework Programme (FP7/2007-2013) and EFPIA's in kind contribution (www.imi.europa.eu).

References

1. Olton, D.S., Samuelson, R.J. (1976). Remembrance of placed passed: spatial memory in rats. *Journal of Experimental Psychology: Animal Behavior Proceedings* **2**, 97-116.

2. Olton, D.S. (1978). Characteristics of spatial memory. In: Hulse S.H., Fowler H, Honig W.K. (Eds.), *Cognitive processes in animal behavior*, Hillsdale, NJ: Lawrence Erlbaum Associates, 341-73.

3. Liu, P., Bilkey, D.K. (2002). The effects of NMDA lesions centered on the postrhinal cortex on spatial memory tasks in the rat. *Behavioral Neuroscience* **116**, 860-73.

4. Liu, P., Rushaidhi, M., Collie, N.D., Leong, M.T., Zhang, H. (2008). Behavioral effects of intracerebroventricular microinfusion of agmatine in adult rats. *Behavioral Neuroscience* **122,** 557-569.

5. Liu, P., Bilkey, D.K. (1999). The effect of excitotoxic lesions centered on the perirhinal cortex in two versions of the radial arm maze task. *Behavioral Neuroscience* **113,** 672-682.

6. Suzuki, S.W., Augerinos, G., Black, B.A. (1980). Stimulus control of spatial behavior on the eight-arm maze in rats. *Learning and Motivation* **11**, 1-18.

7. Lavenex, P., Schenk, F. (1995). Integration of olfactory information in a spatial representation enabling accurate arm choice in the radial arm maze. *Learning and Motivation* **2**, 299-319.

8. Mazmanian, D.S., Roberts, W.A. (1983). Spatial memory in rats under restricted viewing conditions. *Learning and Motivation* **14**, 123-139.

9. Olton, D.S, Collison, C. (1979). Intramaze cues and `odor trials' fail to direct choice behavior on an elevated maze. *Animal Learning and Behavior* **7**, 221-223.

10. Babb, S.J., Crystal, J.D. (2003). Spatial navigation on the radial maze with trial-unique intramaze cues and restricted extramaze cues. *Behavioral Processes* **64**, 103-111.

11. Brown, M.F. (1992). Does a cognitive map guide choices in the radial-arm maze? *Journal of Experimental Psychology: Animal Behavioral Processes* **18**, 56-66.

12. Jarrard, L.E. (1978). Selective hippocampal lesions: differential effects on performance by rats of a spatial task with preoperative versus postoperative training. *Journal of Comparative and Physiological Psychology* **92**, 1119-1127.

13. Jarrard, L.E. (1983). Selective hippocampal lesions and behavior: effects of kainic acid lesions on performance of place and cue tasks. *Behavioral Neuroscience* **97,** 873-889.