How to measure episodic-like memory in rats and cats: Implications for future research

Ryszard Bobrowicz, Katarzyna Bobrowicz

Faculty of Artes Liberales, University of Warsaw

We aim to introduce some new solutions in the studies involving the subjects who are not able to use spoken language, both animals and pre-verbal infants. Although our research focuses in general on the episodic memory introduced by Tulving in 1972, this paper is to present the case of Wistar rats and domestic cats.

What-where-when

Tulving introduced the term of episodic memory as the ability to recall facts and events awarely. Episodic memory was to record what, where and when happened, but semantic memory was to do this as well. However, the episodic element meant that the individual was able to re-experience the events from their autobiography. The human subjects evinced such an ability in verbal interactions outside the experimental space, which was tough in case of the non-verbal subjects. That is why in 2003 Clayton and Dickinson proposed the term of episodic-like memory for integrated recall of past events in case of what-where-when [2]. In addition, it should be elastic so that the animal may use their memories under the new circumstances. The concept was then introduced into the studies by Eacott and Norman [3], Babb and Crystal [1] and Kart-Teke et al [6]. In 2012 we decided to replicate the last study as it claimed to prove episodic-like memory in rats.

The procedures concerning the "what" aspect include two phases: one training with the exploration of two copies of object A and one test, where the B object is introduced instead of one copy of A. B should be explored longer than A. The "where" aspect includes training and test again: first a copy of A is introduced in certain location and then second copy of A is placed in new location, expected to be explored significantly longer. Finally, the "when" question is answered thanks to two training phases and a test one. In the first training a copy of A and a copy of B are presented, in the second: just A. In the test A and B are presented again and the subject should explore B longer.

Kart-Teke et al.

The study by Kart-Teke et al. integrated these procedures in a way [6]. It included three phases: two training and a test one. Each lasted for 5 minutes and in each four copies were placed in four different locations. All objects had the same material with different shape, size, colour and surface. The object were heavy enough so that the rats could not move them. There were two types of objects chosen randomly for each rat. In the first training the subject was placed in the open field and presented with four copies of object A in four different locations. After the 50-min pause four copies of B were presented in four other locations. After second 50-min pause the test phase started. One copy of A was placed in its previous location and another copy of A was dislocated. Likewise, one B was placed as previously and another was dislocated. The objects were explored if the rat touched it with their nose or legs. The authors expected them to explore the earlier, non-dislocated object significantly longer than the later non-dislocated object and to spent more time exploring the dislocated than the non-dislocated objects. Then they would evince the recall of "where" and "when". These correlations proved significant along with another: the rats also preferred the later non-dislocated objects to the earlier dislocated. Altogether, according to the authors, they indeed seemed to evince episodic-like memory.

Our pilot study

These conclusions inspired us to conduct a pilot study to replicate the procedure with a few changes. First, we introduced an eight-arm maze instead of the open field. Secondly, we introduced three types of wooden objects of different tone, shape and surface which could have been easily moved. Each of three phases included 10 trials of 15 min. They were conducted twice a day: in the morning and in the afternoon. A 6-day pause between the

second training and the test was introduced. We chose randomly a set of three objects: A, B and C for each of the ten Wistar rats: five male and five female. Two copies of A were presented in the first training in two personalized arms, and two copies of B in the second training in two other arms. In the test a copy of A and a copy of B were introduced in the same arms as previously, while one copy of each was dislocated. Also two copies of C were introduced in two out of four remaining arms. In the aspect of "when" we expected that the pair of A objects would be explored significantly longer than the pair of B objects. In the aspect of "what" we assumed that the rats would spent more time exploring the C objects, and in the aspect of "where": more time exploring the dislocated than non-dislocated objects.

Main study

The results failed to prove our expectations. There was no significant relationship neither between the exploration and the object type nor its dislocation. We considered the objects too alike and decided to introduce more distinctive objects: a wave-shaped porcelain white cylinder, a mushroom-shaped metal grey object and a standard wooden light brown cuboid. The trials were shorter and lasted for 10 min, and there was no pause between the training phases and the test phase. We also involved 10 male subjects instead of 4 male and 4 female that participated in the pilot study.

During the testing the maze was positioned 20 cm above the ground to prevent the animals from the escape and, simultaneously, fit the view of the video camera placed above the maze. White light of 20 lx iluminance was introduced due to the shape variation of the objects presented. The videos were watched one by one with no use of any specialized programme and the timing was measured with a stopwatch.

As expected, the rats spent significantly more time exploring the new than the old , both A and B, objects (BCI: 1.86158-7.25381 and 2.46398-7.49567, correspondingly). They also explored C object significantly more often than the familiar objects regardless of their dislocation (BCI: 0.124-0.940). It suggested that the rats did recognize both the "what" and the "when" aspect of the appearing objects. However, we failed to check their awareness of the "where" aspect, as the difference between the stationary A and dislocated A was not significant. It was similar for the stationary B and dislocated B, however in both cases the insignificant tendency was observed. Its weakness was probably caused by the number of the test trials and – consequently – the number of variables. As the stimuli seem to be adequate, more trials, both training and test, should be conducted in the future to prove the hypothesized differences significant.

Systematic observation with cats

We decided to apply our procedure and background to cats with a key change: form the "what" aspect to "who". We have planned pilot observation, where two male cats and a female were introduced into the modified open field covered with a thick blanket in a room with dim light. We used a box with an entrance cut out in the shorter side and connected with a start box with a sliding gate. Two additional exits were made in the farthest ends of the perpendicular walls, both with similar sliding gates. Each cat received twenty four training phases and four test phases, which covered all possible sets of who-where-when. Each phase consisted of two parts – exploration and choice. In first part of the training the animal was allowed by one of the experimenters into the start box for a second. Then the gate went up allowing into the field, where both gates remained open and were explored for thirty seconds. Right after, a one- or fifteen-minute delay was introduced and then the cat was allowed to the field again with one open gate only, either left or right according to the procedure. Once the cat used it to leave the field, the phase was over. We expected that in the test phase they would go first to the closed gate when given the choice and then to the open one. We could observe their behavior thanks to the motion under the blanket. Each set of who-where-when was individualized and random.

The results seem to prove the episodic-like memory in the subjects. The older male responded properly in all four test phases, the younger male in 3 out of 4, and the female failed the test due to her preference for one gate.

Implications for future research

We would like to discuss our methods to apply them to other species if possible. We have also some ideas for their enhancement, which we are willing to discuss during the presentation.

Ethical statement

We conducted our rat studies thanks to the permission of First Warsaw Local Ethics Committee for Animal Experimentation. The permission of the main study received the following number: 369/2012. Our cat observation involved the cats we take care of and was of a play character for them. They were not forced to take part in our project. All studies focused on animal behaviour and neither involved any invasive interaction nor caused any discomfort to the animals.

References

[1] Babb S.J., Crystal J.D. (2005). Discrimination of what, when, and where: Implications for episodic-like memory in rats. *Learning and Motivation*, ***36***, 177 – 189.

[2] Clayton, N. S., Bussey, T. J., Dickinson, A. (2003). Can animals recall the past and plan for the future? *Nature Reviews Neuroscience*, ***4***, 685-691.

[3] Eacott M.J., Norman G. (2004). Integrated Memory for Object, Place, and Context in Rats: A Possible Model of Episodic-Like Memory? *The Journal of Neuroscience*, ***24(8)***, 1948 – 1953.

[4] Gaffan D. (1994). Scene-specific memory for objects: a model of episodic memory impairment in monkeys with fornix transection. *Journal of Cognitive Neuroscience*, ***6***, 305–320.

[5] Hampton R.R., Schwartz B.L. (2004). Episodic memory in nonhumans: what, and where, is when? *Current Opinion in Neurobiology*, ***14***, 192 – 197.

[6] Kart–Teke E., De Souza Silva M.A., Huston J.P., Dere E. (2006). Wistar rats show episodic-like memory for unique experiences. *Neurobiology of Learning and Memory*, ***85***, 173 – 182.

[7] Squire L.R., Zola S.M. (1996). Structure and function of declarative and nondeclarative memory systems. *Proceedings of the National Academy of Sciences*, ***93***, 13515-13522.

[8] Tulving, E. (2002). Episodic Memory: From Mind to Brain. *Annual Review of Psychology*, ***53***, 1-25.