neuroservices ALLIANCE

OptoPath™

MEMORY DEFICITS

Memory degrades in aging and early phases of the major form of age-related neurodegenerative disease, Alzheimer's disease. The degradation concerns both short-term and long-term memory components. Regarding long-term memory, the two major forms declining in aging are declarative and spatial memory, both of which are characterized by flexible expression. In this context, we propose rodent models that allow evaluating the different memory components degrading in aging, i.e. short-term memory maintenance and updating, as well as long-term memory flexibility, and testing the negative and positive effects of new psychoactive compounds on these functions. These tests are performed using Morris water-maze task in rats.

SAFMEM-PRODUCTS: SAFETY TESTS IN THE CONTEXT OF MEMORY

- **SAFMEM2B : Evaluation of the deleterious effects of new compounds on long term working spatial memory in rodents.**

THERAPMEM-PRODUCTS: THERAPEUTIC TESTS IN THE CONTEXT OF ALZHEIMER DISEASE

- **THERAPMEM 2 : Evaluation of the pro-memory effect of new compounds on long term working spatial memory in rodents.**

SAFMEM-2B- Evaluation of the deleterious effects of new compounds on long term spatial memory in rodents.

Person in charge: Dr. Nora Abrous, PhD

ASSAY INFORMATION

Biological models	Young adult male rats (2-4 months)
Methods	Water maze
Readouts	• Ability to learn • Ability to remember • Behavioural flexibitilty
Standard reference	
Subjects per group	10-12 rats
Turn around time	

BACKGROUND

Some compounds can have adverse effect on memory. Drugs can be tested and screened for their deleterious using young adult rats (also possible in mice).

ASSAY PRINCIPLE

Animals are tested for their abilities to navigate through space. Analysis of the performances across days allows concluding on the property of a compound on long-term memory whereas the analysis of the performances within sessions gives information on the efficacy of the compound on working memory.

The apparatus : Rats will be tested in a Morris water maze (180 cm diameter, 60 cm high) filled with water (21°C) made opaque by addition of milk powder.

Pre-learning phase: Before the start of training, they will be first habituated to the pool by a daily 1 minute session (without any platform) over one or two days to decrease the stress of the procedure.

LEARNING PHASE

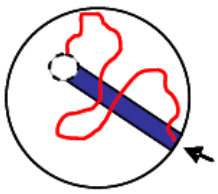
Animals will be tested for their spatial reference memory using variable start position. An escape platform will be hidden 2 cm below the surface of the water in a fixed location 1 of 4 quadrants halfway between the wall and the middle of the pool. During training, animals will be required to locate the submerged platform using distal extra-maze cues. They will be tested for 4 trials per day (90 seconds with an inter-trial interval of 30 seconds and beginning from 3 different start points that varied randomly each day). If an animal does not find the platform, it will be placed upon it at the end of the trial. The number of the testing days will be adjusted according to the behavior of the animals. By experience, animals are trained between 6 and 8 days. The time to reach the platform (latency in seconds) and the length of the swim path (distance in cm) will be measured with a computerized tracking system (Videotrack, Viewpoint).

ABILITY TO REMEMBER

The ability to remember is measured with a *probe test*. The probe test can be performed at different time point after the end of the learning phase. The platform will be removed and the time spent in the target quadrant (over 60 sec) will be measured with the computerized tracking system. Note that a more refine analysis of the performances can be realized using wintrack software: Wishaw's index, cumulative search error and path efficiency index. For all these analyses, all data points in the goal zone, as well as the periods of inactivity are not considered for computation.

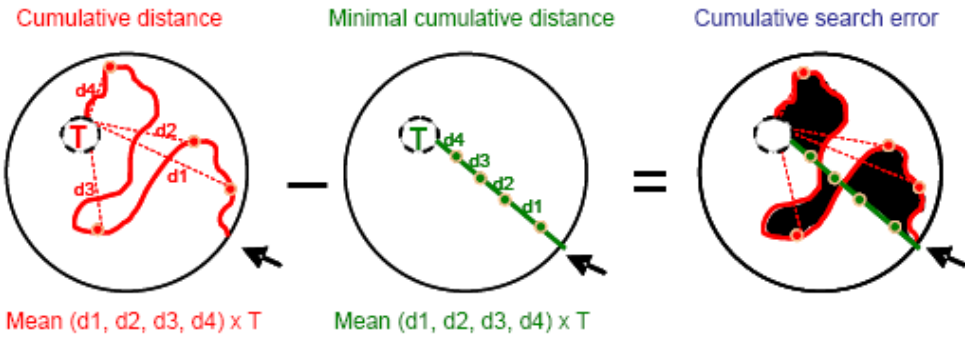
Wishaw's Index

This index corresponds to the percentage of path traveled within a straight corridor connecting the start and the goal.



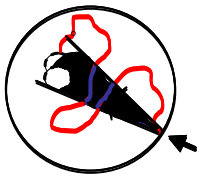
Cumulative search error

The cumulative search error corresponding to the difference between observed cumulative distance and minimal cumulative distance. The cumulative distance to the goal is computed by multiplying the average distance to the goal by the time elapsed until reaching the goal (T); the minimal cumulative distance for an optimal swim path is estimated by dividing the squared airline distance from the release point to the goal by two times the animal's average swim speed.



Path efficiency index

This index corresponds to the percent of path traveled with a deviation by 15° or less of the direction of movement from the direction pointing to the goal.



BEHAVIOURAL FLEXIBILITY

In order to determine the compound of drugs on behavioural flexibility two protocols can be used.

1) Reversal training

If in the first phase of training no deficits are observed, then the location of the hidden platform is moved to another quadrant. Animals are trained as previously described (ii). During this training, animals should learn very quickly the novel location of the platform (2 days).

2) Novel start

If deficits are observed in the first training phase, then the following protocol will be used. During this test animals are also required to locate the hidden platform using distal extra-maze cues. Procedures were similar to the ones used for training with variable start positions (see above ii). However this time the start position will be maintained constant between trials and days of training. Also the hidden platform was localized in a different quadrant than the one used for the variable-start paradigm. When performances reached a criterion of less than 10 sec of escape latency over two successive trials, animals will be tested to locate the hidden platform from a novel start position that had never been used before (1 trial with a 60 seconds cut-off).

REPRESENTATIVE RESULTS

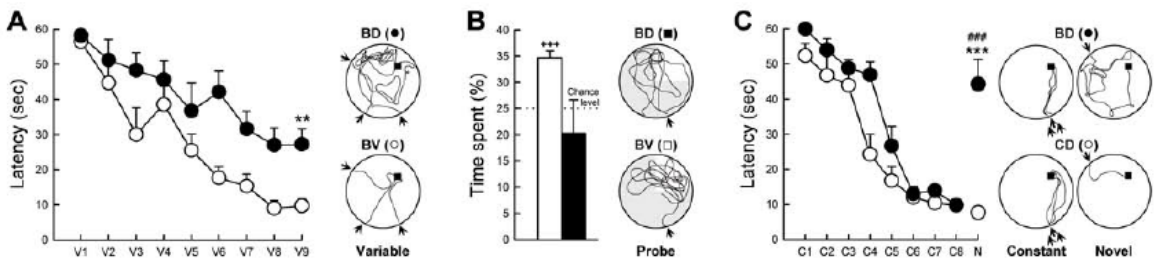


Figure 1: (A) Latency to reach the hidden platform using variable start positions. Right: representative swim paths during the last training day for a bigenic mouse treated with Dox (black, BD) and a bigenic

mouse treated with vehicle (white, BV). (B) Time spent in the target quadrant during the **probe test**. Right: representative swim paths during the probe test. (C) Latency to reach the hidden platform using constant (C1 to C8) or novel (N) start positions. Right: representative swim paths during constant (C8) and novel (N) start position training days. BV= bigenic-vehicle mice; BD= bigenic-Dox mice; CD= control-Dox mice (From Dupret et al., 2009).

BIBLIOGRAPHICAL REFERENCES

Dupret D, Fabre A, Döbrössy MD, Panatier A, Rodríguez JJ, Lamarque S, Lemaire V, Oliet SHR, Piazza PV*, Abrous DN*. Spatial learning depends on both the addition and removal of new hippocampal neurons PLos Biol, 2007, 8:e214. http://www.f1000biology.com/article/id/1090727/ evaluation.

Dupret D*, Revest JM*, Koehl M, Ichas F, De Giorgi F, Costet P, Abrous DN*, Piazza PV*. Spatial relational memory requires hippocampal adult neurogenesis. PLos One, 2008, 3, e1959. Science editors choice May 21, 2008.

*Authors contributed equally to this work.

THERAPMEM-2: EVALUATION OF the cognitive enhancer property of new compounds on long term spatial memory in old rodents.

Person in charge: Dr. Nora Abrous, PhD

ASSAY INFORMATION

Biological models	Male old adult rats (18-24 month-old
Methods	Water maze
Readouts	• Ability to learn • Ability to remember
Standard reference	Depending on the pharmacological features of the tested compound
Subjects per group	10-12 rats
Turn around time	

BACKGROUND

Aging is associated with a decline in cognitive function and the ability of a given compound to be a cognitive enhancer can be assessed in the water maze.

ASSAY PRINCIPLE

Animals are tested for their abilities to navigate through space. Analysis of the performances across days allows concluding on the property of a compound on long-term memory whereas the analysis of the performances within sessions give information on the efficacy of the compound on working memory. Given the existence of individual differences in cognitive aging, old groups are divided into two subgroups: aged unipaired (AU) and aged impaired (AI) rats. According of the design of the experiment, the AU can be used as a control group. It can be also used to verify that the compound does not have deleterious effects. A maximum of 40 old rats can be tested simultaneously.

The apparatus : Rats will be tested in a Morris water maze (180 cm diameter, 60 cm high) filled with water (21°C) made opaque by addition of milk powder.

Prelearning phase : Before the start of training, they will be first habituated to the pool by a daily 1 minute session (without any platform) over one or two days to decrease the stress of the procedure.

Given that some aged rats can be blind, animals are first trained to find a visible in a fixed location in 1 of 4 quadrants halfway between the wall and the middle of the pool. Animals will be tested for 4 trials per day (90 seconds with an inter-trial interval of 30 seconds and beginning from 3 different start points that varied randomly each day) until they learn the task (1 to 3 days depending of the batches). Animals unable to learn the task are excluded from the experiment. This step can be performed before drug treatment.

LEARNING PHASE

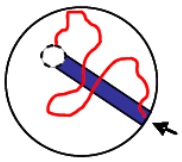
Animals will be tested for their *spatial reference memory*. An escape platform will be hidden 2 cm below the surface of the water in a fixed location in a quadrant different from that used in the previous phase. During training, animals will be required to locate the submerged platform using distal extra-maze cues. They will be tested for 4 trials per day (90 seconds with an inter-trial interval of 30 seconds and beginning from 3 different start points that varied randomly each day). If an animal does not find the platform, it will be placed upon it at the end of the trial. The number of the testing days will be adjusted according to the behavior of the animals. By experience, animals are trained between 10 and 14 days. The time to reach the platform (latency in seconds) and the length of the swim path (distance in cm) will be measured with a computerized tracking system (Videotrack, Viewpoint).

ABILITY TO REMEMBER

The ability to remember is measured with a *probe test*. The probe test can be performed at different time point after the end of the learning phase. The platform will be removed and the time spent in the target quadrant (over 60 sec) will be measured with the computerized tracking system. Note that a more refine analysis of the performances can be realized using wintrack software: Wishaw's index, cumulative search error and path efficiency index. For all these analyses, all data points in the goal zone, as well as the periods of inactivity are not considered for computation.

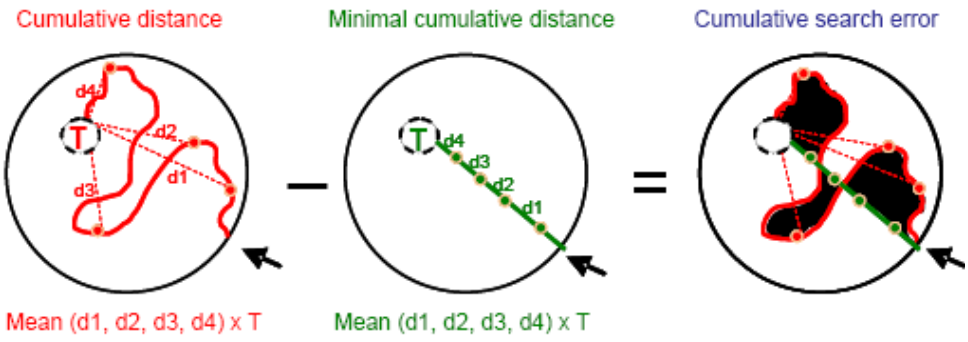
Wishaw's Index

This index corresponds to the percentage of path traveled within a straight corridor connecting the start and the goal.



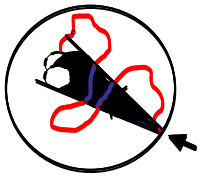
Cumulative search error

The cumulative search error corresponding to the difference between observed cumulative distance and minimal cumulative distance. The cumulative distance to the goal is computed by multiplying the average distance to the goal by the time elapsed until reaching the goal (T); the minimal cumulative distance for an optimal swim path is estimated by dividing the squared airline distance from the release point to the goal by two times the animal's average swim speed.



Path efficiency index:

This index corresponds to the percent of path traveled with a deviation by 15° or less of the direction of movement from the direction pointing to the goal.



REPRESENTATIVE RESULTS

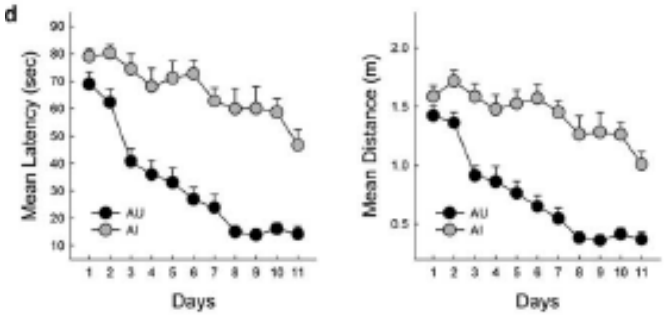


Figure 1: Example of inter-individual differences in learning in aged rats (From Drapeau et al., 2007). On the left panel are shown the latencies to escape onto the hidden platform and on the right panel the distance travelled to find the hidden platform.

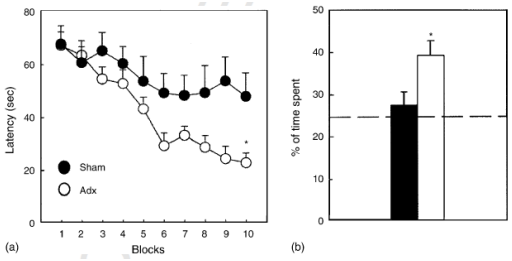


Figure 2: Influence of adrenalectomy (in white) on learning (left) and remembering (right panel) (From Montaron et al., 2006).

BIBLIOGRAPHICAL REFERENCES

Drapeau E, Mayo W, Aurousseau C, Le Moal M, Piazza PV, Abrous DN. Spatial memory performances of aged rats in the water maze predict levels of hippocampal neurogenesis. *Proc Natl Acad Sci USA* 2003;100:14385–14390.

Drapeau E, Montaron MF, Aguerre S, Abrous DN. Learning-induced survival of new neurons depends on the cognitive status of aged rats. *J Neurosci.* 2007 May 30;27(22):6037-44.

Dupret D, Fabre A, Döbrössy MD, Panatier A, Rodríguez JJ, Lamarque S, Lemaire V, Oliet SHR, Piazza PV*, Abrous DN*. Spatial learning depends on both the addition and removal of new hippocampal neurons PLos Biol, 2007, 8:e214. http://www.f1000biology.com/article/id/1090727/ evaluation.

Montaron MF*, Drapeau E*, Dupret D, Kitchener P, Aurousseau C, Le Moal M, Piazza PV, Abrous DN. Lifelong corticosterone level determines age-related decline in neurogenesis and memory. Neurobiol Aging, 2006, 27:645-654.

*Authors contributed equally to this work.