Use of a complex water maze to test cognitive abilities of aged rats

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ABSTRACT

Accurately testing learning and memory performance in rodents is crucial to many researchers in aging and neurodegenerative diseases. A possible issue in testing the same animals multiple times over their lifetime is that they can become trained in the task, negating the discriminating effects and goals of the testing. There are a number of behavioral tasks to test learning and memory. Two commonly used are the Radial Arm water maze (RAWM) and the Morris water maze (MWM). Here we have optimized a RAWM protocol to detect differences and changes in learning and memory performance in aged rats. The RAWM forces the animal to find a submerged platform after exploring hallways (arms) that extend from a central area of the pool. The water maze version is used instead of the dry land version because of the inherent and instinctual motivation of swimming and escaping the swimming pool. Here we have optimized the RAWM for aged rats, to avoid fatigue and stress in the animals, by limiting the number of trials performed in one day. This RAWM protocol is useful in testing learning and spatial memory in aged rats, particularly if these animals have already performed other behavioral testing such as the MWM. MWM results in long lasting memories, so using the MWM a second time may give a false positive result, making the RAWM a robust alternative to be used. Tracking subtle changes in cognition due to aging and neurodegenerative diseases can be difficult; however, the challenge of the RAWM provides a method in separating aged rats based on their cognitive ability. In the future we will rely on this separation to probe the cellular and molecular differences between SL and IL aged animals.

RAWM Methods

Allow animals to acclimate to the room ~30-45 min prior to trials. Water is kept at 25-28C, the platform ~3cm below surface, pictorial cues scattered around perimeter of pool and room.

Training day:

- 6 of 8 arms closed, one has platform (Arm C), one empty.
- Place rat in water in center of circle, allow it to swim to platform.
- Mark error if either >1/2 of its body enters incorrect arm, or if the animal enters the *correct* arm but doesn't swim to the platform.
- If the animal stays in the center or in any particular arm (not on the platform) for >30 seconds, mark an error.
- If after 90 seconds it hasn't found platform guide it to the platform, and allow it to sit ~10-15 seconds. Repeat 3x per animal before continuing to next animal.

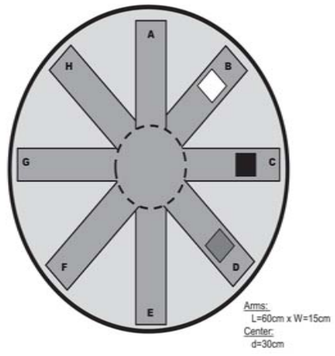
Days 1-3:

- Open all 8 arms of platform.
- Placing animal in outer end of arm, follow Test Schematic for new platform (Arm B) and drop locations (rotating), using the error and time rules from Training Day.

Day 4:

- Move platform to specified arm (Arm D).
- Perform trials in same fashion as days 1-3. In addition to marking the errors from entry into empty arms, tally arm entries of the old platform location arm separately (see Error Recording Spreadsheet figure)

RAWM Design



RAWM Test Schematic

Testing Scheme	Training	Day 1	Day 2	Day 3	Day 4 Reversal
Platform Location	C	B	B	B	D
Drop Location Trial 1	Center	F	G	D	H
Drop Location Trial 2	Center	H	D	H	C
Drop Location Trial 3	Center	D	E	F	G

Separation Criteria

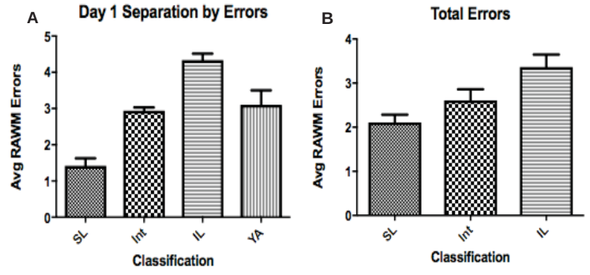
The average of each animal's errors on Day 1 is determined, the aged animals are then compared to the young adults. If the aged animal's average errors on day 1 are *much greater* than the young adult's, they are considered Inferior Learners. If the errors are similar, they are considered Intermediate, and if they are *much less* than the young adult's they are considered Superior Learners. We have found that both the positive and negative changes by individual animals on a particular day or trial do not influence the cohort in total, and we still retain statistical significance in separation shown in multiple parameters of analysis.

Sample Error Recording Spreadsheet

TRIAL	1-REF	1-OLD PLAT (B)	1-FOUND	TIME		2-REF	2-OLD PLAT (B)	2-FOUND	TIME		3-REF	3-OLD PLAT (B)	3-FOUND	TIME
DROP	H	H	H	H		C	C	C	C		G	G	G	G
PLATFORM	D	D	D	D		D	D	D	D		D	D	D	D
Animal														
1														
2														

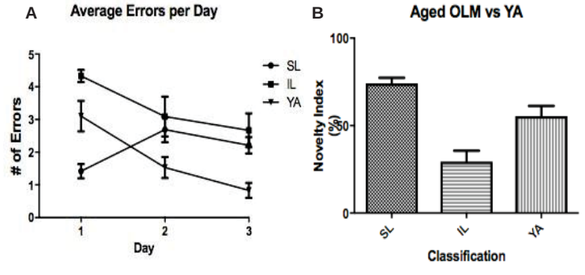
Example spreadsheet used on Reversal day. Reference errors are total # of errors. Old Platform (old plat) are errors where the animal returned to the arm used in days 1-3. (Platform) Found is yes/no. Trial time is also recorded, with a maximum of 90 seconds. The sheet is largely the same for days 1-3, minus the column for Old Platform.

Aged rats can be classified into superior and inferior learners based on behavioral performance in the RAWM



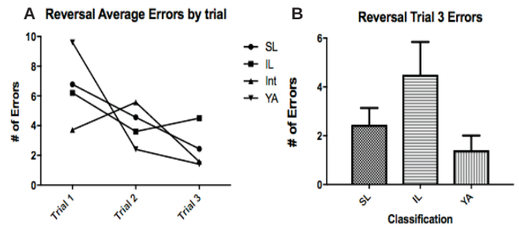
(A) Average error number from Day 1 of testing used to separate the aged animals into the different classifications and **(B)** the separation is then shown in their performance over all 3 testing days. These separation results are significant after using statistical analysis, which determines that our results are not by chance.

Separation of SL and IL based on error number over each day to locate target platform, and correlation of performance on other memory task



(A) The separation of SL and IL on Day 1 is normalized by Day 3, showing that the SL animals initially learn the task much faster, then performance of both groups plateaus over time. **(B)** In the Object Location Memory task there is also a significant separation of the SL/IL groups when compared to the YA animals, showing the consistency of multiple separation methods.

Superior learners show much greater flexibility in adapting to changes in the test



(A) While there is variability in the first reversal trial, performance improves in all groups until the third trial, when the superior learners showed much greater flexibility in adapting to the new task, **(B)** committing ~½ as many errors as the inferior learners.

SUMMARY

- An adapted method of radial-arm water maze can be used to separate aged rats into SL and IL.
- Total errors were significantly different, by ~1/3 between SL and IL.
- On day 1 we found a significant difference in both errors between SL and IL when compared to YA animals, which normalized by day 3.
- The correlation to other memory tests preserves separation into different cognitive abilities, allowing for more options depending on the cohort's prior experiences.
- Reversal testing suggests that SL animals possess greater cognitive flexibility and adaptive learning strategies given a novel choice in a previously recognized task whereas IL animals show significant perseverative behavior in this test.
- These findings will be used to probe the basis of the cellular and molecular differences between the groups of animals.

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