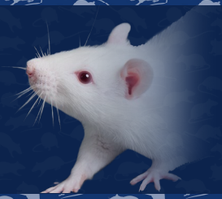

Possible strain differences of the Fischer 344 rat in a temporal order object recognition task

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INTRODUCTION

Object recognition tasks are commonly used to assess learning and memory processes in rodents. Such tasks are extremely useful in the investigation of the function of targeted brain regions without the need for extensive training protocols. The temporal order memory (TOR) task is a simple and efficient test used to assess recognition memory, specifically, the ability to recall *when* an object or event was committed to memory[1]. Previous work has shown that lesions to the medial prefrontal cortex (mPFC) significantly disrupt performance on this task in young rats[2,3]. Although the TOR task has been utilized in several studies, there has been little to no research on the effect of age on performance of this task. The purpose of this study was to investigate whether the TOR task is effective in detecting age-related performance differences in a rodent model of healthy aging.

METHODS

Subjects: Male, 12 young (5-12 mo) and 16 old (22-23 mo), and female, 12 young (5-12 mo) and 12 old (22 mo), Fisher 344 (F344) rats.

Apparatus: The behavior arena consists of a 50 x 50 x 30cm (L x W x H) wooden box, painted black. The room is lit by several overhead red lights, providing an intensity of ~19.5lux within the test apparatus.

Exploratory Objects: Objects are made from nonporous materials and are affixed to the floor using re-closable fasteners. Object pairing was determined following the conduct of an object preference test using a separate, naïve cohort of F344 rats to avoid exploration biases based on object interest level.

Procedure: After habituating the rats to handling, they underwent 4 days of testing on the spatial version of the Morris watermaze, followed by 2 days of testing on a cued watermaze task to evaluate visual competency. A food restricted diet is implemented 1 week prior to beginning TOR to encourage exploration and rats were maintained at 80-85% of the animal's *ad libitum* weight for the duration of this task (3 days). Days 1 and 2, individuals are placed in the empty apparatus for a 30 min habituation period. Day 3, each rat undergoes a single trial consisting of three phases: two exploration phases and one test phase. During the 1st exploration phase, the rat is given 4 min to explore a pair of identical objects. Following a 1 hr delay period, the same procedure is followed for the 2nd exploration session, with a different pair of identical objects. After a 2hr retention period, the test phase is administered using the same procedure as the previous phases, except one object from both previous object pairs is presented. Object position during the test phase was counterbalanced between animals to control for potential side biases.

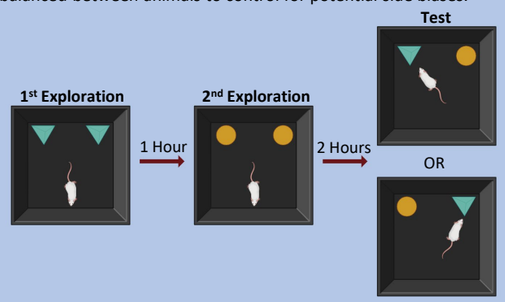

Figure 1. Schematic of the TOR task

ANALYSIS & RESULTS

Video recordings were scored for time spent exploring each object by an individual blind to the order of object presentation. Rats were excluded if they did not explore each object ≥ 5 sec during the exploration phases or if their total exploration time during the test phase was not ≥ 10 sec. Previous studies have shown that the most sensitive period of object recognition occurs within the first 2 minutes of the test phase[3,4]. Therefore, we calculated a discrimination ratio for each rat's exploratory behavior during the first 2 minutes of the test phase using the formula described below:

$$\frac{t_{first\ object} - t_{last\ object}}{t_{total}} = \textit{Discrimination Ratio}$$

Indicates preference for…	
> 0	*First presented object*
= 0	*No preference*
< 0	*Last presented object*

Of the male F344s, 2 young and 7 old rats were excluded and of the females, 1 young and 3 old rats were excluded from analyses due to insufficient exploration times. These exclusions resulted in final groups of male n_{young} = 10, n_{old} = 9; and female n_{young} = 11, n_{old} = 9. To examine if individual groups had discriminated between the objects, we used one-sample two-tailed *t* tests. Group comparisons of discrimination ratios and total test exploration (in seconds) used two-way ANOVA followed by *post hoc* Tukey tests. All statistical analyses were completed at a significance level of α = 0.05.

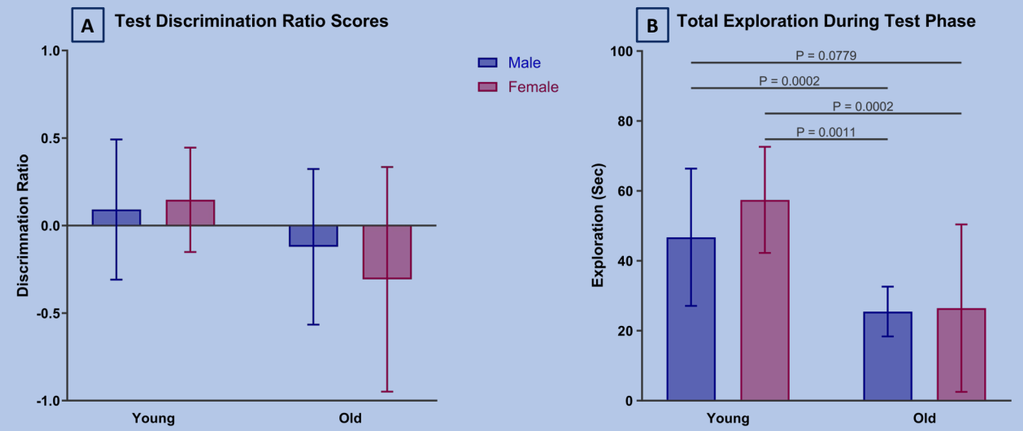

Figure 2. A) Object discrimination in the first 2 minutes of the test phase. Shown are the average discrimination ratios for each group (±SD). The results do not indicate a significant temporal preference for either sex or age. **B)** Total time rats exhibited exploratory behavior for either object during the during the 4-minute test phase. Shown are the average exploration times for each group (±SD) and *P* values from Tukey's multiple comparisons tests. Results indicate the young rats exhibited more exploratory behavior than the old rats.

Discrimination ratios were not found to differ between sexes (μ_{male} = -0.018, μ_{female} = -0.074, $\mu_{male\text{-}female}$ = 0.056, 95% C.I. -0.237 to 0.350; $F_{1,36}$ = 0.15, *P* = 0.70). Discrimination ratio scores for young rats (μ_{young} = 0.122) averaged 0.336 greater than (95% C.I. 0.042 to 0.631) discrimination ratio scores for old rats (μ_{old} = -0.214; $F_{1,36}$ = 5.37, *P* = 0.026). However, *post hoc* analyses did not reveal differences among any group comparisons. No groups were found to exhibit preferential exploration for one object over the other during the test phase, indicating an inability to discriminate between the first and last presented objects (see Table 1). Total test phase exploration did not differ between sexes (μ_{male} = 35.99 sec, μ_{female} = 42.19 sec, $\mu_{male\text{-}female}$ = -6.201 sec, 95% C.I. -17.560 to 5.159 sec; $F_{1,36}$ = 1.23, *P* = 0.28). Total test exploration of young rats (μ_{young} = 52.20 sec) averaged 26.22 sec more than old rats (μ_{old} = 25.98 sec; $F_{1,36}$ = 21.79, *P* < 0.0001). *Post hoc* analyses supported significant differences in amount of exploration among all young and old group comparisons (see Figure 2B for *P* values).

	Young		Old	
	Male *n* = 10	Female *n* = 11	Male *n* = 9	Female *n* = 9
Mean	0.092	0.147	-0.121	-0.307
Standard Deviation	0.401	0.298	0.444	0.642
t value	0.72	1.64	0.82	1.44
Degrees of Freedom	9	10	8	8
p value	0.49	0.13	0.44	0.19

Table 1. *t* test results for discrimination ratios as an indicator of object preference during the test phase.

DISCUSSION

Despite multiple reiterations and adjustments to the TOR protocol, we have been unable to successfully replicate results that others have published using this task with young animals of different strains. Rather than exhibiting a preference for the object presented in the first exploration phase, we found that, on average, none of our F344 rats were able to discriminate between the temporal order of the presented objects. In fact, the results obtained here are more similar to reports in rats with a variety of lesions (bilateral hippocampal, perirhinal, and medial prefrontal cortex lesion groups)[3,5]. Because our F344 rats were hesitant to explore the objects, often remaining stationary in the corner of the behavior arena, we implemented diet restriction as a method to increase exploration. This manipulation did increase exploratory behavior, but they still did not exhibit the temporal preference expected. The strain most often used on this task have been Long-Evans rats[1,2,3]. Rodent strain-related differences in behavior have been observed and reported in other tasks, but there are currently no direct comparisons between Fisher 344 and other rat strains on object recognition tasks[4,6,7]. Additionally, the previous studies using the TOR task have used much younger animals and thus, another possibility is that the differences between studies are a result of this age variable[1,2,3,5]. These results suggest that the TOR task will not be effective in assessing temporal order memory using the mature, adult, F344 rat model.

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1. Ennaceur & Delacour, Behav. Brain Res., 1988, 31:47
2. Mitchell & Laiacona , Behav . Brain Res., 1998, 97:107
3. Barker et al., J. Neurosci ., 2007, 27:2948
4. Dix & Aggleton, Behav. Brain Res., 1999, 99:191
5. Barker & Warburton, J. Neurosci., 2011, 31:10721
6. Van Der Staay & Blokland, Physio. & Behav., 1996, 60:97
7. Van Goethem et al., Behav. Brain Res., 2012. 232:323