

Novel object recognition is not affected by age despite age-related brain changes

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ABSTRACT

Age-related memory impairments show a progressive decline across lifespan. Studies have demonstrated equivocal results in biological and behavioral outcomes of aging. Thus, in the present study we examined the novel object recognition task at a delay period that has been shown to be impaired in aged rats of two different strains. Moreover, we used a strain of rats, Fisher 344XBrown Norway, which have published age-related biological changes in the brain. Young (10 months old) and aged (28 months old) rats were tested on a standard novel object recognition task with a 50 minutes delay period. The data showed that young and aged rats in the strain we used performed equally well on the novel object recognition task and that both young and old rats demonstrated a right-handed side preference for the novel object. Our data suggested that novel object recognition is not impaired in aged rats although both young and old rats have a demonstrated side preference. Thus, it may be that genetic differences across strains contribute to the equivocal results in behavior, and genetic variance likely influences the course of cognitive aging.

Keywords: Novel Object Recognition; Aging; Learning; Memory; Side Preference

1. INTRODUCTION

Progressive memory decline is a hallmark of the aging process and some impairments are mediated by the medial temporal lobe including the hippocampus [1]. The

neurobiological changes in the aging brain underlying these cognitive alterations have been investigated. It is now well documented that significant cell loss in the structures of the medial temporal lobe does not occur with age and thus, does not contribute to the cognitive changes with age [2-6]. Changes in the number of synapses, which are highly labile structures, are responsive to microenvironmental changes in the brain and result in a continual refinement of neuronal circuitry [7,8], may underlie these cognitive changes. The current literature has been equivocal as to the direction of change in synapses with age. Some studies have found that synapses decline with age [6,9] and some found no change in the number of synapses [4,5]. Thus, it is very likely that subtle changes in the function of synapses contribute to cognitive declines with age.

Changes in the synaptic composition are thought to alter the functional properties of synapses, *i.e.* synaptic plasticity, and contribute to the declines in cognitive abilities. The data about the composition of synapses also are equivocal with respect to age-related changes. Some studies have found changes in key synaptic proteins such as glutamate receptors that underlie synaptic plasticity like long-term potentiation [4-6]; some have found no age-related differences [10], and some have found both [11]. Moreover, it has been demonstrated that synaptic function changes with age [12]. The results demonstrated that long-term potentiation, (LTP) potentiation decreases with age, as well as the NMDA-mediated field excitatory post-synaptic potential (fEPSP), but no change occurs in the AMPA-mediated fEPSP. Interestingly, Barnes *et al.* found age-related decreases in both the AMPA-and NMDA-mediated fEPSPs and no differences in LTP [13].

While the biological changes that contribute to mem-

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ory declines may be equivocal in direction, cognitive impairments, which are observed in aged humans, are also seen in rodent models. These impairments can occur across different cognitive domains but one that is very robust is a deficit in spatial learning and memory. Behaviorally aged rats show age-related impairments on a task such as the Morris water maze task, which indicates a spatial learning and memory deficit [2,10,14-16]. Another test used to evaluate cognitive changes is the novel object recognition task, which is based on the measurement of how much time the animal spends with the novel object versus familiar object. This task takes advantage of the fact that rats have a tendency to search for novel objects rather than the familiar ones [17-19], and the effect of aging on this task again has shown equivocal results [19-23].

The previously stated behavioral and synaptic data suggest that there are age-related decrements but there is some disagreement as to the direction of the alterations. One reason for these differences may be due to the fact that different strains of rats were used. A study by Markowska and Savonenko demonstrated age-related decrements in Morris water maze behavior but an intervention such as caloric restriction only alleviated the deficit in the Fischer 344XBrown Norway strain not the parental strain [24]. Thus, in the present study we examined the novel object recognition task at a delay period that has been shown to be impaired in aged rats of two different strains [19,23]. Moreover, we used a strain of rats, F344XBrown Norway, which have published age-related biological changes in the brain [4-6,15,25]. Our data suggest that novel object recognition is not impaired in aged rats although both young and old rats have a demonstrated side preference for the location of the novel object. Thus, it may conclude that genetic differences across strains contribute to the differences in behavior and genetic variance likely influences the course of cognitive aging.

2. MATERIALS AND METHODS

2.1. Subjects

A total of 12 young (10 months old) and 12 aged (28 months old) Fisher 344XBrown Norway (F344XBN) male rats were used in this study. The rats were housed individually in standard laboratory cages and maintained 12 hour light/dark cycle in standard temperature. During the experiment the rats had access to food and water *ad libitum* and were fed a standard NIH diet. Procedures were taken to minimize the welfare impact on subjects, including choice of sample sizes and predetermined rules for intervention. The animal protocol for this study conforms to the National Institute of Health guidelines for

the care and use of laboratory animals and was approved by the Animal Care and Use Committee of Wake Forest University Health Sciences.

2.2. Apparatus & Stimuli

A rectangular open box made of plastic with a floor (31 cm × 24 cm) and 45.5 cm walls was used in the experiment. Two distinct objects and their copies were used as stimuli. The objects were placed on Velcro strips in order to prevent displacement of objects. Sample and testing phases were videotaped in order to have a complete archive of experiment. A tripod with camera was angled so that the entire arena and objects became fully visible.

2.3. Procedure

Novel Object Recognition Task

The present experiment consists of three phases: Habituation, Sample and Testing. In the habituation phase, rats were habituated to the testing apparatus for 10 minutes per day for consecutive three days.

In the fourth day, the sample phase, in which animals were introduced to the objects, took place. In the sample session, two identical objects were placed in the box (see Figure 1(a)). Rats were allowed to explore objects for 8 minutes and after 2 minutes the next rat was placed in the

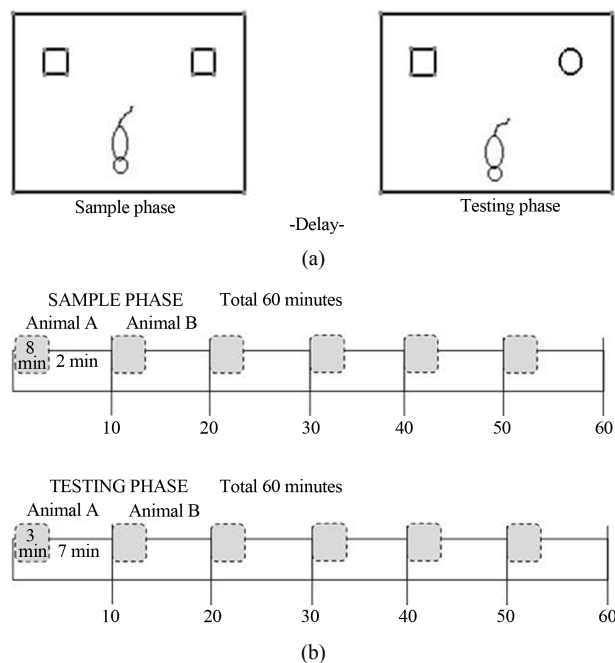


Figure 1. Representation of the novel object recognition task. In the sample phase, the rat is permitted to explore two similar objects located in the arena. In the testing phase, the rat is permitted to explore one familiar object that was the same as the objects in the sample phase and one novel object. The experimenter measures the time spent exploring the novel versus familiar objects.

box (**Figure 1(b)**). The same procedure continued until 6 rats were finished. The entire phase for 6 rats lasted exactly 60 minutes (**Figure 1(b)**).

Immediately after the sample phase, these 6 rats were tested in the testing phase, in the same order with the sample phase. Thus, the delay between the sample and testing phase became approximately 50 minutes for each rat. This delay period was chosen due to the feasibility of handling a cohort of six rats and due to the fact that some studies in the literature indicate that there are age-related decrements in object recognition memory with approximately one hour delay period [19,23]. In the testing phase, there were two different objects, one familiar object from sample phase and one novel object that the rat was never encountered (see **Figure 1(a)**). Rats were allowed to explore objects for 3 minutes and then removed (**Figure 1(b)**). After 7 minutes, next rat was placed in the box and this procedure continued until 6 rats were finished (**Figure 1(b)**). The entire session was completed in 60 minutes (**Figure 1(b)**). After the sample and testing phase had been completed for the first 6 rats, the same procedure was applied for the next six rats until testing of all rats had been completed. In all phases, after each rat was removed from the box, the box was sterilized with alcohol. Sample and novel objects and their locations were counterbalanced across animals.

2.4. Data and Statistical Analysis

The main scoring criterion was exploration of the object. Exploration was determined as time spent with the nose touching object, nose sniffing/directing to object within approximately 1 cm. A novel object preference ratio was calculated as a ratio of the amount of time spent exploring novel object over the total time spent exploring both object and this ratio was used for the statistical analysis. A two-tailed unpaired t-test was used to examine whether mean novel object preference ratios for young and old rats were significantly differ from each other. A two-tailed paired t-test was used to detect within animal differences for side preferences. Significance was set a $P < 0.05$.

3. RESULTS

3.1. Sample Phase

Exploration behaviors of old and young rats were examined in order to observe whether exploration behavior is affected by aging. **Figure 2** shows mean amount of time spent exploring two identical objects in the sample phase for young and old rats. It can be seen that there is only a slight difference between old and young rats. A t-test revealed that this difference is not statistically significant (t-test: $t_{17} = 0.77$, $P > 0.05$). This indicates that age does not significantly affect exploration beha-

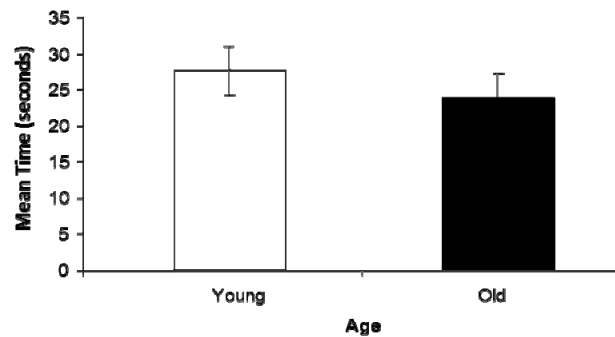


Figure 2. Aging does not affect time spent exploring objects. The mean amount of time spent exploring objects in the sample phase for young and old rats is shown. The data demonstrate that there is no significant difference in the time spent exploring the same objects in the sample phase. Errors bars represent standard error of the mean.

vior.

3.2. Testing Phase

Figure 3 shows mean novel object preference ratio for young and old rats during the testing phase when one familiar object and one novel object were presented to rats. It seems that old rats show slightly higher novel object preference than young rats. However, a two-sample t-test revealed that the difference between old and young rats is not statistically significant (t-test: $t_{17} = 0.53$, $P > 0.05$). Thus, age does not have an effect on preference for novel versus familiar objects or in other words it does not affect novel object recognition.

Interestingly we observe that our rats had a preference for objects on one side of the testing area versus the other. **Figure 4** shows mean novel object exploration ratio for young and old rats across the left and right position of the novel object during the testing phase when one familiar object and one novel object were presented to the rats. It seems that both young and old rats show higher novel object preference when the novel object is on the right position as compared to the left. A paired t-test confirmed these findings as statistically significant (t-test: $t_{17} = 2.41$, $P = 0.026$). Moreover, the difference between young and old rats in the total time spent on the left half is not significantly different nor is the total time spent on the right half (t-test: $t_{17} = 0.85$, $P > 0.05$). These results show that there is no effect of age on the side preference behavior.

In addition to these analyses, stress-related behaviors including grooming, freezing, and defecation were analyzed to determine if there were any differences between old and young rats. In other words, the effect of age on the stress-related behaviors was examined. Our results demonstrated that there were no statistically significant differences in any of these behaviors (all P-values >

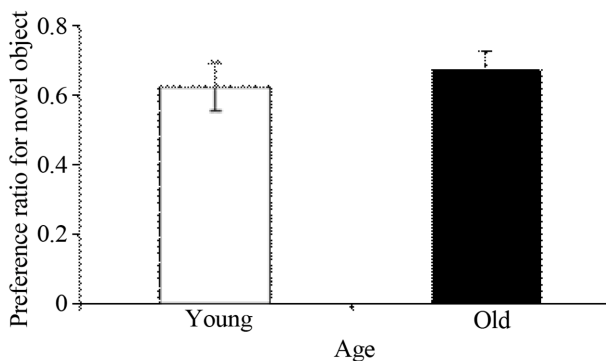


Figure 3. Aging does not affect novel object recognition. The mean novel object preference ratio for young and old rats during the testing phase when one familiar object and one novel object were presented to rats is shown. The data demonstrate that both young and aged rats show the same preference for the novel over the familiar object. Error bars represent standard error of the mean.

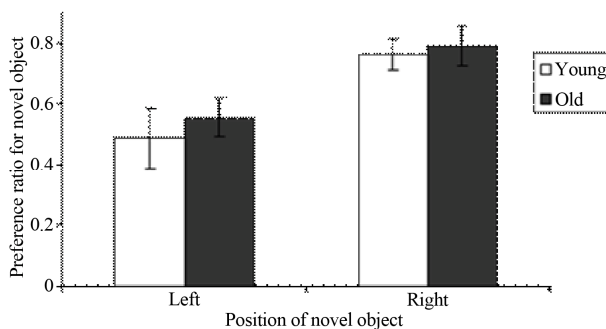


Figure 4. Young and aged rats show a side preference for exploring novel objects. The mean novel object preference ratio for young and old rats across the left and right position of novel object during the testing phase when one familiar object and one novel object were presented to rats is shown. Both young and aged rats prefer the right side versus the left. Error bars represent standard error of the mean.

0.05). Thus, none of the observed differences or lack thereof can be due to the aged animals being more stressed in the behavioral set-up.

4. DISCUSSION

The present study aimed to determine the effects of age on novel object recognition. We chose a strain of rats with known age-related biological changes in the brain [4-6,12,25] and a delay period for the novel object recognition task based on studies in which there are age-related deficits in the novel object recognition task [19,26]. Surprisingly our results demonstrated that there was no difference in young and aged rats in the time spent exploring the novel object. Thus, despite biological changes in the aged brain, it appears that novel object recognition is spared with aging. While no age-related deficits were observed in novel object recognition, we

did observe that both young and aged rats increased their exploration of novel objects located on the right side of the apparatus versus the left.

Our prediction was that we would observe an age-related deficit in novel object recognition. While it was surprising that there was no deficit, it was not unusual as the effects of age on novel object recognition have been equivocal in whether or not age-related differences have been observed [19-23,26,27]. The question then remains what could be contributing to these different results.

The first difference among the majority of these studies could be due to the experimental design. One possibility is the length of the delay period (50 minutes) that was chosen. In some studies age-related deficits were observed with approximately a one hour delay period [19,23] and in some with a 24-hour delay [20,21], while in some no deficits were found [22].

Another theory is that the studies that used a similar delay period to the one that we chose used different strains of rats than those in the current study. It is well known that many strains of rats and mice can show diverse behavioral outcomes, which is thought to be due to their underlying genetic differences [28-31]. A study by Markowska and Savonenko demonstrated age-related decrements in Morris water maze behavior but an intervention such as caloric restriction only alleviated the deficit in the Fischer 344XBrown Norway strain not the parental strain [24]. Thus, it is very possible that the lack of an effect of age on novel object recognition could be due to genetic differences. Current studies are ongoing in the laboratory to parse out this question.

In the current study only male rats were used. It has been documented in the literature that there are sex differences in the response of rodents in an object recognition memory task. Females with lower estrogen levels are similar to males and females with higher estrogen levels are better than males and females with low estrogen [28,32]. Thus, there appears to be a relationship between estrogen levels and behavior during an object recognition memory task. Future studies need to be directed at examining the interactions between hormone levels, aging, and behavior on memory tasks like object recognition memory.

Lastly, this F344XBrown Norway strain has well-documented age-related brain changes [4-6,12,25]. Thus, interestingly, this novel object recognition memory is preserved in spite of these biological differences with age. Future studies need to be directed at determining whether there is some compensation that prevents this cognitive decline in the face of these brain alterations.

In the current study in addition to analyzing the exploration ratio in the novel object recognition, we also asked whether the rats showed any preference for one side of the apparatus. We were prompted to ask this

question since a study by Güven *et al.* showed a distribution of paw preference in rats in food-reaching test [33]. In this study, they found that in a population of 144 rats, 72.7% were right handed, 19.7% were left handed and 7.6% were ambidextrous. Based on the results from this study taken together with our results demonstrating that our rats have a right side preference, we can conclude that our population of rats is likely right handed. Moreover, these results demonstrate the importance for having a design such that the distribution of the novel object is counterbalanced across the left and right hand sides, which was done in the current experiment. Finally, there was no effect of age on the side preference suggesting that these preferences, like in humans, remains throughout life.

Lastly, we observed no effect of age on any stress-related behavior such as grooming, freezing or defecation. Thus, our animals were very well habituated to the apparatus and the experimenter. The effect that we found on side preference then is not likely due to any environmental stressor. In addition, it could be suggested that the animals may have object preferences, *i.e.* one of the objects might attract the animal more than the other. In our study all objects were counterbalanced across the trials, and therefore, our animals would not be affected by any object preference.

5. CONCLUSION

In the present study, we showed that age does not influence novel object recognition and that animals show a right side preference. This lack of an effect of age on novel object recognition may be due to variation in genetic strains or delay periods. As a result, future studies need to have direct strain comparisons. Furthermore, this result may highlight the contribution of genetic variability to distinct aging patterns of individuals.

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