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Los Angeles

**Female-Specific Effects on Age-Related Spatial Learning Decline in
Songbirds**

**A thesis submitted in partial satisfaction
of the requirements for the degree Master of Science
in Physiological Science**

by

Saritha Kosarussavadi

2015

ABSTRACT OF THE THESIS

Female-Specific Effects on Age-Related Spatial Learning Decline in Songbirds

by

Saritha Kosarussavadi

Master of Science in Physiological Science

University of California, Los Angeles, 2015

Professor Barnett Schlinger, Chair

Spatial cognitive decline is a known hallmark for age-related deterioration in learning and memory, as neurobiological changes occur in the hippocampus with advancing age. Sexually dimorphic spatial abilities have also been consistently demonstrated in humans and other mammalian studies. Despite their extended lifespan and adaptations to aging, little is known about avian age-related cognition and physiology. In this experiment, we used zebra finches (*Taeniopygia guttata*) to investigate the effects of aging and sex on spatial cognition. We found a sex difference favoring spatial cognitive advantage in adult male zebra finches such that males outperformed females in learning to acquire correct food locations in a 4-arm maze. This phenomenon, however, was absent in young subjects. Moreover, only old female but not male zebra finches exhibited the expected age-related decline in learning spatial task, suggesting a potential underlying benefit of male aging and

spatial ability. In addition to proposing parallels between the avian and mammalian literature on spatial cognition, this study is the first to concurrently examine both sex- and age-dependent influence on avian spatial learning and memory.

The thesis of Saritha Kosarussavadi is approved.

Aaron Blaisdell

David Glanzman

Barnett Schlinger, Committee Chair

University of California, Los Angeles

2015

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Introduction

Aging poses as a major risk factor in numerous health issues ranging from cardiovascular to neurodegenerative diseases. Despite advances in science and medicine, there is a limited knowledge about the mechanism and effects of aging on functions and physiological systems. With one of the most adverse effects of old age being cognitive decline, normal age-related memory impairment and memory loss as a result of neurodegenerative diseases emerge as an increasing area of interest (Burns and Iliffe, 2009; Larson et al., 2013).

In the central nervous system, normal aging and age-related neuropathologies are associated with structural, biochemical, and functional alterations (Burke and Barnes, 2006; Lister and Barnes, 2009). In particular, the brain region involved in learning and memory—the hippocampus—is vulnerable. For instance, aged human subjects as well as aged animal models, such as rats, display impaired performance in memory tasks including those involving spatial navigation (Winocur and Moscovitch, 1990; Gazova et al., 2012). Specifically, the mode of spatial learning, which utilizes reference or “place” strategies are diminished in aged animals (Barnes et al., 1980; Barnes, 1987; Gallagher and Pellemounter, 1988; Frick et al., 1995; Rodgers et al., 2012). Morphologically, the aging brain is accompanied by various changes in synaptic connectivity such as loss of dendritic spines in the hippocampus (Scheibel, 1979), decrease in the adult neural stem cells and hippocampal neurogenesis (van Praag et al., 2005). It has also been demonstrated that cognitive decline in aging is linked to changes in gene expression observed after memory tasks such as upregulation in lipid metabolism gene for Apolipoprotein D (Blalock et al., 2003) and in immediate early genes (Lanahan et al.,

1997). Moreover, electrophysiological studies show age-correlated shifts in hippocampal place cells dynamic and Ca^{2+} homeostasis (Barnes et al., 1997; Foster and Norris, 1997). Putting all these together, hippocampal-dependent functions such as memory formation, memory consolidation, context and spatial memory (O'Keefe and Dostrovsky, 1971; Morris et al., 1982) are at increased susceptibility with advanced age.

In spite of their relatively high metabolic rates, body temperature, and blood glucose—factors that contribute to decrease in lifespan, birds have physiological advantages that allow them to live up to three times longer than mammals of equivalent sizes (Austad, 1997; Holmes et al., 2001). Because of their relatively long lifespan and successful adaptations to aging compared to other laboratory rodents, avian species are seen as promising study models in resisting senescence.

Many songbird species (Order Passeriformes) possess exceptional spatial memory capabilities. Some can memorize and recall thousands of sites where they have cached food (Gould-Beierle and Kamil, 1999; Salwiczek et al., 2010). In an experimentally controlled setting, zebra finches (*Taeniopygia guttata*) exhibit the ability to effectively learn and recall food locations in a modified version of the Morris water maze and other types of spatial assays (Sanford and Clayton, 2008; Mayer et al., 2010). Importantly, these tasks have been shown to require use of the hippocampus (Patel et al., 1997; Watanabe and Bischof, 2004; Bischof et al., 2006; Mayer et al., 2010), a structure functionally homologous to those of mammals (Bingman, 1992; Colombo and Broadbent, 2000). While age-related deficits in spatial memory have been demonstrated in a non-songbird species, the homing pigeon (Coppola et al., 2015), there has generally been minimal aging studies that employ avian models and none on songbirds.

To determine if spatial memory function declines with age in songbirds, we have adapted a previous protocol (Rensel et al., 2013) to test spatial learning and retention in young and old zebra finches of both sexes. This particular paradigm demanded the animals to use place-learning strategies where birds are required to use allocentric cues in order to locate a food reward. The behavioral assay was designed to control the amount of learning per trial and thus, was sensitive in detecting both sex and age differences in spatial cognition.

Methods

Subjects

Zebra finches from a colony at the University of California, Los Angeles (UCLA) were used in this experiment. In the first experiment, two sets of adult male (n=4) and female (n = 4) zebra finches between the ages of 150-350 days were used to assess memory after a retention time of either 5- or 10-days (total n = 16). In a second experiment, birds of both sexes (n = 5/sex) between the ages of 110-135 days were tested as a “young” group, while aged birds (n= 6 males; n=5 females) between the ages of 1440-2510 days were used in the “old” group. Birds were housed in a communal aviary under a 14:10 light-dark cycle, provided with *ad libitum* food, water, grits, cuttlebone and egg supplement. All experimental protocols were done in compliance with the UCLA Chancellor’s Animal Research Committee.

Behavioral Acclimation

All birds were initially acclimated to separation and to eating from experimental food cups (22 X 22 X 20 mm) by being held within individual holding

cages inside their home vivarium. The acclimation period is especially crucial as zebra finches are normally housed with a flock of 20-30 other birds. Each animal received one food cup per day with *ad libitum* grits, water, and cuttlebone. During the next two days, the animal was food restricted each day from 5:00pm-8:00am where the food cup was removed from the holding cage, followed by a 3-hour period for acclimation to the test maze. The plus-maze was previously used by our lab to assess spatial memory capability in this species (Rensel et al., 2013) which consists of with a Plexiglas frame surrounded by 1-mm mesh, permitting external visual cues of the testing room (3.35 X 3 m). External visual cues remained constant throughout all acclimation and testing sessions. To encourage maze exploration and food foraging behavior, finch seeds were scattered at the end of each maze arm. However, to avoid the animals associating a particular maze arm with food, experimental food and water cups were only placed in the center of the maze. Animals were then returned to their holding cage following the acclimation session, where filled food cups were returned to the cage an hour after and the animals were allowed to eat until the food restriction time.

Acquisition Session

After this four-day period of acclimation, the animals began their behavioral assay with overnight food restriction (5:00pm-8:00am) prior to each test day. All food-restricted subjects were closely monitored to avoid insufficient or excessive food deprivation and the food-restriction period was adjusted (+/- 2-3 hours) accordingly. A bird was considered appropriate for the assay when it immediately responded to placement of an empty food cup.

One constant reward arm was designated for each animal to be used throughout all sessions (arm 1, 2, 3, or 4). For each testing group, the reward arm assignment was also counter-balanced. The reward arm contained the experimental food cup with accessible seeds, while the others contained identical but inaccessible food cups with transparent tops.

Each animal went through one 30-minute behavioral session per day. One behavioral session was capped at ten trials. However, the animal was given a 10-minute extension if it failed to complete ten trials, with the requirement of finishing at least six trials by the end of the 30-minute mark. For each trial, the animal was placed at the end of a randomized starting arm and was allowed to make one move into an arm, either straight, or by making a left or right turn. The arm was considered chosen when the animal's whole body entered the arm. After the animal reached its selected arm, it was allowed to eat the seeds or explore the arm for 10 seconds, or before the bird began to exit the arm, at which timer, the room light was turned off and, while fully dark, the animal was removed and placed in a new starting arm. After each session, the animal was transported back to its holding cage. To avoid creating an association between food availability in the holding cage and the session completion, which could potentially decrease maze performance, the food cup was replaced an hour after completion of the session.

Behavioral Criteria

The number of correct choices out of the total number of trials completed within the session was used to calculate the percent (%) accuracy. Criterion was reached when the subject received 80% accuracy or higher on two consecutive sessions (i.e. days). Thus, the animal would continue to perform the sessions on each

day until it met criterion. Omitted trials included lack of performance (no movement in the maze after five minutes) and haphazard flights when the trial light was illuminated; omissions were not taken into account as part of the total number of trials in each session.

Retrieval Session

After the animal reached criterion, it was returned to its holding cage with the same diet protocol during the cage acclimation but without the normal food restriction schedule as the testing period. For the 10-day retrieval group, animals were given a 9-day rest period followed by a retrieval session on the tenth day. During the rest days, the animals were handled for three minutes per day during the last four days before the retrieval day. Similarly, the 5-day retrieval group animals were given a 4-day rest period where the animals were handled during all those days and tested on the fifth day. The young and aged animal groups employed the 5-day retrieval group protocol.

Tissue Collection

Brain tissues were collected immediately after the animals finished the retrieval session. Prior to rapid decapitation, animals were anesthetized with isoflurane. Both sides of the hippocampi were dissected utilizing stereotaxic coordinates provided in the zebra finch atlas (Nixdorf-Bergweiler and Bischof, 2007). The dissected hippocampal tissues were directly frozen on a foil plate in a container with dry ice and stored in the -80°C freezer for molecular analysis.

Statistical Analysis

All statistical analyses were performed using SPSS (v20; Chicago, IL, USA). The percent accuracy was calculated as the number of correct responses out of the total number of trials completed per session (maximum = 10), excluding the number of omitted trials. Omitted trials were not added to the total number of trials used to calculate the percent accuracy, as the animals failed to perform the task. However, the number of omissions as well as completed trials per session stood as task motivation indices. The criterion for all groups required the animals to achieve at least 80% accuracy on two consecutive acquisition sessions before proceeding to the rest period. For the first experiment, analysis of days to criterion and trials to criterion was completed using individual sample t-tests. Repeated measure ANOVA was used to analyze accuracy across the first three days—the minimum number of days any bird took to reach criterion. For the age study, days and trials to criterion of young and old, male and female zebra finches were analyzed with factorial ANOVA. Differences in accuracy across training days were analyzed using repeated measures ANOVA. For both studies, Mann-Whitney and Kruskal-Wallis tests were used to compare retrieval session accuracy, number of trials completed, and number of omissions, as the distribution of the data did not conform to parametric assumptions.

Results

Adult zebra finch retention group study

There was no significant effect of sex on the number of days ($t_{14} = -1.80$, $p = 0.09$) and number of trials ($t_{14} = -2.01$, $p = 0.064$) the birds took to reach the learning criterion (Figure 2). To assess accuracy, we examined the percent accuracy across

the first three days of training, as those were the minimum number of days completed by any one animal before reaching criterion. We observed a significant increase in accuracy ($F_{2,28} = 27.54$, $p < 0.001$; Figure 3), which was demonstrative of the animals' ability to learn the task in this early stage. Additionally, we observed a significant effect of sex ($F_{1,14} = 5.55$, $p = 0.03$), though no sex by day interaction ($F_{2,28} = 0.04$, $p = 0.96$). Here, female zebra finches achieved significantly lower accuracy across those three days (Figure 3). To eliminate the possibility of motivational differences between each animal's performances, we analyzed the total number of completed trials as well as omitted trials during the first three days of training. Male and female zebra finches did not differ in the number of omitted trials ($U = 24$, $p = 0.39$; Figure 4) or in the number of trials completed on the first three days ($U = 26.5$, $p = 0.57$; Figure 4).

After animals reached criterion, we divided the birds into two retention delay groups: 5-day group (male, $n = 4$ and female, $n = 4$) and 10-day group (male, $n = 4$ and female, $n = 4$) and examined their retrieval session performance. Although males and females did not differ in the number of omitted trials on the retention test day ($U = 20$, $p = 0.23$; Figure 5), males did complete fewer trials than females on this test ($U = 8.5$, $p = 0.01$; Figure 5). However, there was no effect of group on accuracy on the retrieval test ($\chi^2_3 = 3.07$, $p = 0.38$; Figure 6).

Zebra finch age study

As there were no effects on retrieval session accuracy in Exp. 1 between a 5- or 10-day retention delay groups, we chose a 5-day delay period to assess effect of age on recall. Analysis on days to criterion revealed no effect of sex ($F_{1,17} = 2.68$, $p =$

0.12; Figure 7), age ($F_{1,17} = 1.23$, $p = 0.28$; Figure 7), and sex by age interaction ($F_{1,17} = 1.48$, $p = 0.24$; Figure 7). Similarly, on the number of trials to criterion, there was no effect of sex ($F_{1,17} = 1.64$, $p = 0.22$; Figure 7), age ($F_{1,17} = 0.24$, $p = 0.63$; Figure 7), and sex by age interaction ($F_{1,17} = 0.61$, $p = 0.45$; Figure 7). Next, we examined accuracy across the first two days of training, as this was the minimum number of training days completed by any animal in this group. We detected a significant day by sex by age interaction ($F_{1,17} = 7.63$, $p = 0.01$; Figure 8). To explore this interaction further, accuracy data for males and females was first analyzed separately.

Importantly, although females obtained a significant interaction between day and age ($F_{1,8} = 14.09$, $p < 0.01$; Figure 8, B-D), for males, there was neither a day by age interaction ($F_{1,9} = 1.58$, $p = 0.24$) nor a main effect of age ($F_{1,9} = 0.393$, $p = 0.546$; Figure 8, A-C). Specifically, when we examined females across individual days we found that young females outperformed old females on the first, but not the second day (day 1: $t_8 = 2.31$, $p = 0.05$; day 2: $t_8 = 0.71$, $p = 0.5$; Figure 8, D). When examined by age, rather than by sex, a significant interaction between day and sex was found for old ($F_{1,9} = 9.08$, $p = 0.02$, Figure 8, A), but not for young birds ($F_{1,8} = 2.2$, $p = 0.18$; Figure 8, B). There was also no main effect of sex for young birds ($F_{1,8} = 2.2$, $p = 0.18$; Figure 8, B). Although old birds display a day by sex interaction on accuracy across the first two days, old males and old females did not differ on either day 1 accuracy ($t_9 = 1.04$, $p = 0.32$, Figure 8, A) or day 2 accuracy ($t_9 = 0.96$, $p = 0.36$; Figure 8, A). Males and females did not differ in the number of omitted trials ($U = 40.5$, $p = 0.29$), or the number of trials completed ($U = 55$, $p = 1$) on the first two days (Figure 9).

Comparing males and females, including both young and old birds on the retrieval day, there was also no difference in the number of trials ($U = 50.5$, $p = 0.74$) and omitted trials ($U = 45.5$, $p = 0.487$), indicating that there were no motivational

differences between animals (Figure 10). Similarly, when we separated birds by age, males and females still did not differ in the number of trials completed on retention session (old: $U = 11$, $p = 0.46$; young: $U = 11.5$, $p = 0.81$; Figure 10) and omissions (old: $U = 13.5$, $p = 0.79$; young: $U = 9.5$, $p = 0.55$; Figure 10). On the retention session accuracy, there was also no effect of group ($\chi^2_3 = 1.782$, $p = 0.619$; Figure 11). When the ages of old male and females were compared, there was a trend towards males being older than females ($t_9 = 2.4$, $p = 0.06$, Figure 12), while young males and females did not differ in age at test time (Figure 12).

Discussion

This current experiment demonstrated the influence of aging on spatial learning in zebra finches, revealing a striking interaction between age and the female sex. Specifically, this study reported an age-related spatial cognitive decline that was accelerated in female zebra finches. Though studies have explored spatial cognition in birds (Watanabe and Bischof, 2004; Bischof et al., 2006; Mayer et al., 2010; Salwiczek et al., 2010; Bailey et al., 2013; Rensel et al., 2013; Coppola et al., 2015), this is, to my knowledge, the first instance where sex and age effects on spatial learning were shown in an avian model.

Despite a growing body of work focusing on the effects of aging on cognition, most studies are done on laboratory rats and mice, which are relatively short-lived mammalian models. Deviating from the general positive correlation between homeotherm body size and longevity, most avian species have life spans almost three times longer than mammals of similar sizes (Holmes and Austad, 1995; Holmes et al., 2001; Holmes and Ottinger, 2003). For instance, a mouse may only

live up to 4 years, while a zebra finch of comparable size can live up to 9 years (Holmes and Ottinger, 2003). Though birds are susceptible to the same diseases that afflict mammals, their aging rates and age-related physiological decline rates appear to be relatively lower (Holmes and Austad, 1995; Holmes et al., 2001; Holmes and Ottinger, 2003; Munshi-South and Wilkinson, 2010). Therefore, birds such as zebra finches arise as an excellent longevity model that can offer an insight on the mechanisms of senescence and successful aging. In this study, I examined spatial cognition in young adults just after sexual maturation. In that zebra finches live up to 9 years, the birds that were over 1400 days at the start of the experiment were considered old.

The assay developed in this experiment exploited zebra finch ground-feeding/foraging behavior and required the birds to use spatial reference or place memory. That is, in order to find the correct food location, birds were required to apply external spatial cues relative to their randomized starting positions. This learning strategy is distinct from the response or cued spatial learning where animals only learn a response (e.g. always turning left or right) or a previously marked goal (e.g. always going to the cup with the red flag), respectively to obtain reward (Gallagher and Pellemounter, 1988). However, it is important to note that certain extra-maze cues available in this experiment could have been employed by animals using a cued learning strategy. Although extra-maze cues were arranged in ways that discouraged the use of direct cues (e.g. wall cues were not placed directly above the end of the reward arm), it was difficult to rule out such strategy. Only future experiments that test cued spatial learning will be able to confirm if this current paradigm truly required the animals to use reference memory.

Nonetheless, by testing this protocol on adult male and female zebra finches, my experimental results disclosed a parallel to those of the human and non-human mammalian literature, which demonstrated sex differences in spatial cognitive abilities. Similar to many human and rodent studies (Frick et al., 1995; Voyer et al., 1995; Astur et al., 1998; Markowska, 1999; Jones et al., 2003), this data showed male zebra finches consistently perform at higher percent accuracy across the initial days of acquiring the spatial task compared to their female counterparts. Though zebra finch females were eventually able to reach criterion and were able to perform equally well on the retention session, they did so at a slower rate (Figure 3 and Figure 6). In addition to the slower learning rate in females, there was a trend towards those adult birds taking more days and trials to reach criterion (Figure 2). Given that there were no differences in motivational indices (the number of completed trials and omissions), this sex effect was not confounded by non-mnemonic factors such as differences in activity or physical state. Furthermore, other studies also provided evidence that males and females tend to rely on different spatial strategies to solve a task (Kanit et al., 2000; Tropp and Markus, 2001). Therefore, the initial poor performance of females might have been a reflection of those birds utilizing cues and spatial strategies that were not appropriate during the beginning of the training.

Sexually dimorphic spatial abilities have been well documented in mammalian species, where males reliably outperform females (Frick et al., 1995; Voyer et al., 1995; Astur et al., 1998; Markowska, 1999; Jones et al., 2003). This study, perhaps, suggested that sexual dimorphism in spatial cognition could be generalized not only among mammals but also across birds. This result, therefore, added to a body of work, which points to a possible evolutionarily conserved

disadvantage of the female sex regarding aging and spatial learning. Studies investigating factors influencing sex differences in spatial abilities were often led to organizational and activational effects of sex hormones (Williams et al., 1990; Sherry and Hampson, 1997; Isgor and Sengelaub, 1998; Neave et al., 1999). Therefore, it would be of great interest for future studies in avian spatial cognition to consider hormonal impacts on sex and age.

In the second experiment, I assessed a possible age-related difference in the same behavioral paradigm in old and young animals. In young birds, the present study indicated a lack of sex difference—a result that is consistent with previous rodent experiments in young Long-Evans rats (Bucci et al., 1995; Warren and Juraska, 1997) and Fisher-344 rats (Markowska, 1999). A few studies have reported that sex differences on spatial task performance emerge post-puberty (Krasnoff and Weston, 1976; Kanit et al., 2000). Because our males and females were held separately, our birds had not yet engaged in nesting, a factor that could have influenced our results.

We did find that old female zebra finches performed significantly worse on the first day of training compared to young females in percent accuracy (Figure 8, C-D). These data suggest the existence of age-related deficits in reference learning that depend on the sex of the zebra finch.

By examining the acquisition results from exp. 1 and 2, an interesting pattern between aging and sex in spatial learning appeared: 1) young male and female subjects were not different (Figure 8, B), 2) adult males outperformed adult females (Figure 3) and 3) old females performed poorly compared to young females (Figure 8, D). Though the results reveal potentially fascinating pattern of spatial learning across age and sex, it was unclear as to when the development of this

particular cognitive decline occurred in these female zebra finches. One observation worth noting is that the males used in the aging study tended to be slightly older than the females (Figure 12). In that we observed no decline in spatial learning, these males suggest some potential neuroprotection present to withstand aging and spatial learning debility. While a few studies have looked at possible hippocampal substrates of spatial memory in the songbird brain through pharmaceutical manipulation experiments (Bailey et al., 2013; Rensel et al., 2013), there is no known literature that integrates *both* age- and sex-related spatial learning during basal, non-drug manipulated condition.

Many bird species possess incredible abilities to recall food locations and cached sites (Gould-Beierle and Kamil, 1999; Mayer et al., 2010; Salwiczek et al., 2010). Nevertheless, there has been a lack of knowledge in avian spatial retrieval with regards to sex- and age-related differences. In addition to place learning, the current experiment tested the recall abilities of zebra finches in two retention delay period of 4 and 9 days. Despite the sex and age effects observed in the acquisitional phase of the spatial task, there were no significant differences in the retrieval session accuracy between the sexes and age groups (Figure 6 and 11). Notably, more than half of the total subjects performed over 80% accuracy during the retention session. This absence of sex and age differences in spatial memory recall could point to several interpretations. For one, the retrieval paradigm designed in this study might not have been sensitive or challenging enough to detect sex or age effects. Secondly, the brain areas that were responsible for spatial retrieval were perhaps not sexually dimorphic, not affected by age, or not affected in these age groups. Regardless, the overall results from the spatial acquisition data in both experiments

still extend the evidence that spatial learning is susceptible to aging—particularly in females.

Combining the data from exp. 1 and 2, these findings provided an important insight into a songbird's spatial capability in the context of sex and aging. This study supports the idea of a possible difference in neural substrates that are sex- and age-dependent in the hippocampus and perhaps, other brain regions used during spatial learning and memory. These fundamental differences then can contribute to the variability in different schemes of neural processing used during spatial tasks between old and young, male and female zebra finches. Thus, the physiological basis behind these differences—whether it is in neuromorphology or neurochemistry—requires further exploration.

Figure 1: Experimental Timeline.

Zebra finches acclimated to the holding cage during the first two days. The maze habituation continued for approximately 3 hours each day for the next two days. Animals acquired the task until they reached criterion (n = the amount of days the animals take to reach criterion). Rest periods were assigned according to the animals' experimental groups: 4 and 9 days for the 5- and 10-day retention delay groups, respectively. Animals were tested on recall of food location during the retrieval session after the last rest day.

Figure 2: No difference in days and trials to criterion in adult zebra finches.

Criterion was reached when the animals obtained 80% accuracy on two consecutive days. There was no significant difference in the number of days (left panel) and trials (right panel) to criterion between adult male and female zebra finches. Male, n = 8; female, n = 8.

Figure 3: Male zebra finches outperformed females in percent accuracy across the first three days of acquisition.

Percent (%) accuracy was calculated by the number of correct responses divided by the completed trials during each session. It took a minimum of 3 days for a zebra finch to reach criterion. Percent accuracy significantly increased in male and female zebra finches across the first 3 days of training. The dotted line indicates performance at chance where percent accuracy is below 0.33. *p = 0.03. Male, n = 8; female, n = 8.

Figure 4: Males and females did not differ in the number of trials and omissions during the first three days of training.

Completed and omitted trials were indices of motivation. Completed trials were capped at 10 trials per session. Lack of performance and haphazard flights were considered omissions. A zebra finch took a minimum of 3 days to reach criterion. The number of trials (right panel) and omissions (left panel) per session across the first three days of acquiring the task did not differ between the sexes. Male, $n = 8$; female, $n = 8$.

Figure 5: Males completed less trials but not omissions than females during recall.

Zebra finches were tested on food location recall after 4 or 9 days of rest, depending on retention delay groups (5-day or 10-day) after criterion. Completed trials and omissions were motivational indices. Completed trials were capped at 10 trials per session. Lack of performance and haphazard flights were considered omissions. Male zebra finches completed significantly less trials than females during the retrieval (RT) session, $*p = 0.01$ (right panel). Omitted trials (left panel) did not differ between groups. Five-day retention delay group: male, $n = 4$; female, $n = 4$. Ten-day retention delay group: male, $n = 4$; female, $n = 4$.

Figure 6: No effect on the retrieval (RT) session percent accuracy.

Zebra finches were tested on food location recall after 4 or 9 days of rest, depending on retention delay groups (5-day or 10-day) after reaching criterion. Percent (%) accuracy was calculated by the number of correct responses divided by the completed trials during the session. Zebra finch groups did not vary in retrieval % accuracy.

Five-day retention delay group: male, n = 4; female, n = 4. Ten-day retention delay group: male, n = 4; female, n = 4.

Figure 7: No difference in the number of days and trials to criterion in the age study groups.

Animals reached criterion when they obtained 80% accuracy on two consecutive days. The amount of days (left panel) and trials (right panel) the animals took to reach criterion did not differ between the sex and age groups. Old: male, n = 6 and female, n = 5; Young: male = 5 and female, n = 5.

Figure 8: Day by sex by age interaction in percent accuracy.

Percent (%) accuracy was calculated by the number of correct responses divided by the completed trials during each session. It took a minimum of 2 days for a zebra finch to reach criterion. **A-B:** Aged zebra finches exhibited a day by sex interaction but males and females did not differ on percent accuracy. There was no significant interaction in the young group. **C-D:** There was a significant interaction between day and age in females. Young female zebra finches outperformed old females on the first day of training, *p = 0.02. Male zebra finches did not differ in percent accuracy. The dotted line indicates performance at chance as the percent accuracy is below 0.33. Old: male, n = 6 and female, n = 5; Young: male = 5 and female, n = 5.

Figure 9: No difference in acquisition day trials and omissions in the age study groups.

Completed and omitted trials were indices of motivation. Completed trials were capped at 10 trials per session. Lack of performance and haphazard flights were

considered omissions. A zebra finch took a minimum of two days to reach criterion. Omissions (top panel) and trials (lower panel) across the first two days of training did not differ between groups. Old: male, $n = 6$ and female, $n = 5$; Young: male = 5 and female, $n = 5$.

Figure 10: No difference in retrieval day trials and omissions in the age study groups.

Completed and omitted trials were motivational indices. The numbers of completed trials were capped at 10 trials per session. Trials were considered omitted when there was a lack of performance or haphazard flights. Trials (left panel) and omissions (right panel) during the retrieval (RT) session did not vary between the sex and age groups. Old: male, $n = 6$ and female, $n = 5$; Young: male = 5 and female, $n = 5$.

Figure 11: No effect of age or sex on retrieval session accuracy in the age study groups.

Percent (%) accuracy was calculated by the number of correct responses divided by the completed trials during the retrieval (RT) session. There was no difference in % accuracy between young and old, male and female zebra finches. Old: male, $n = 6$ and female, $n = 5$; Young: male = 5 and female, $n = 5$.

Figure 12: Age in days of males and female zebra finches in the age study groups.

Male zebra finches were marginally older than female zebra finches in the aged birds group. Young males and females did not differ in age. Old: male, $n = 6$ and female, $n = 5$; Young: male = 5 and female, $n = 5$.

Figure 1

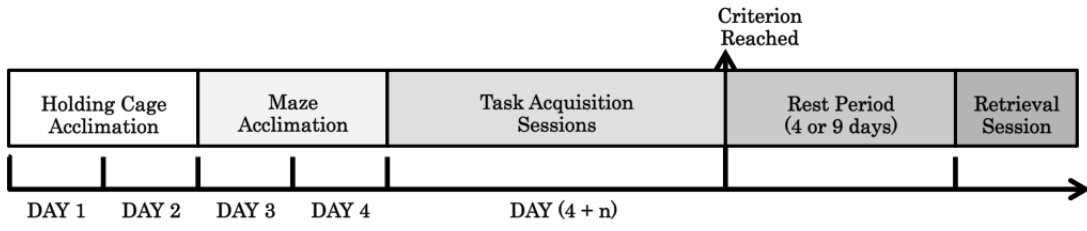


Figure 2

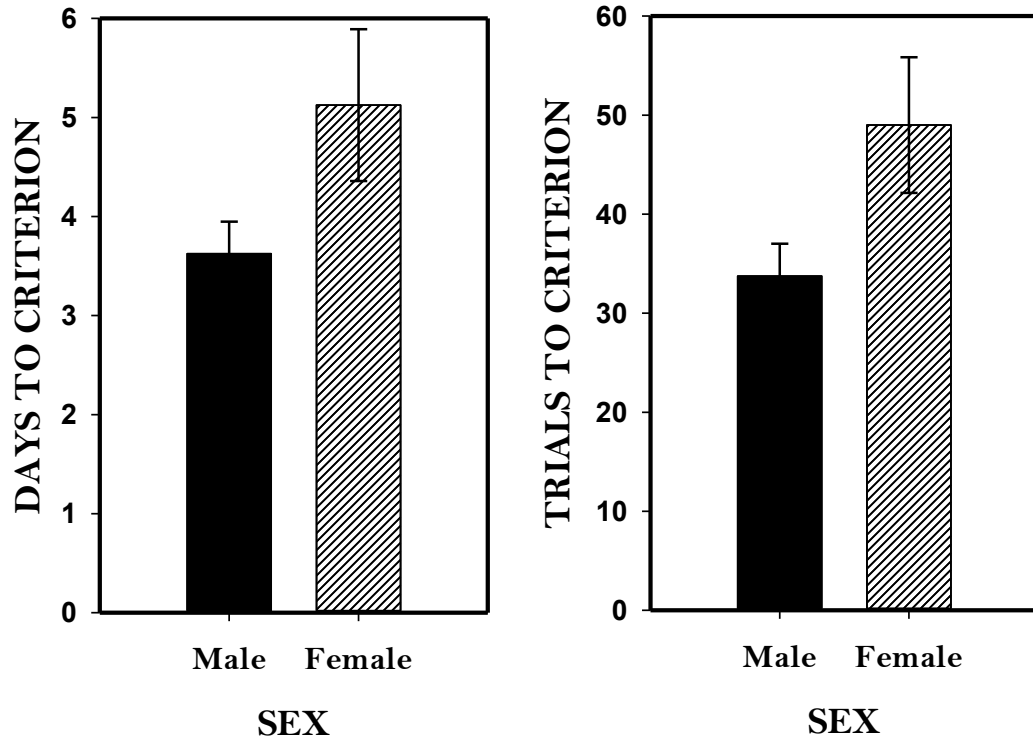


Figure 3

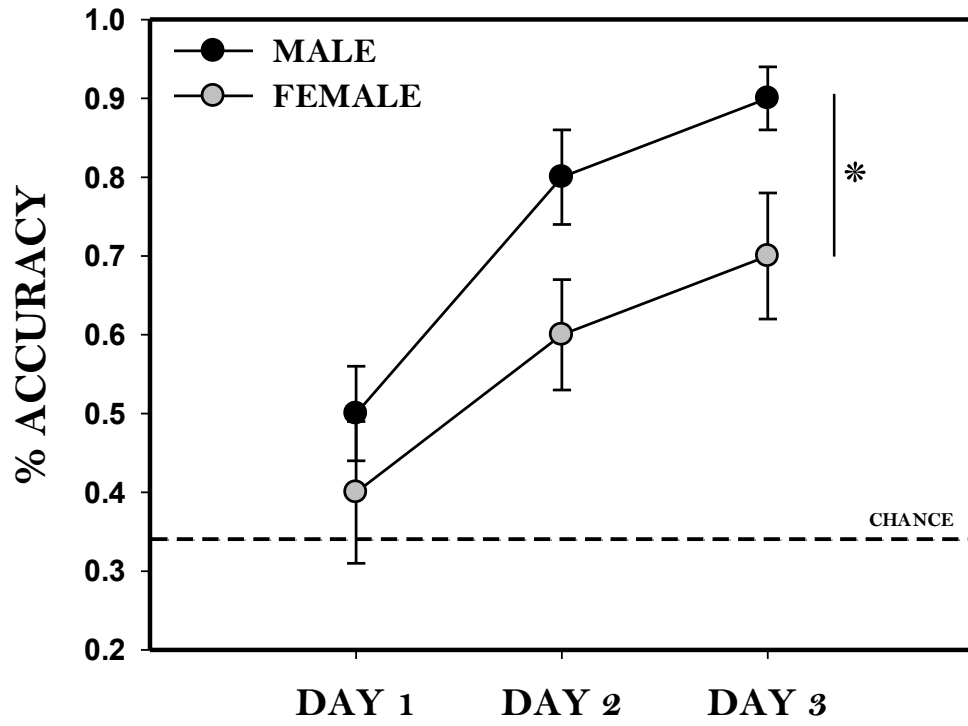


Figure 4

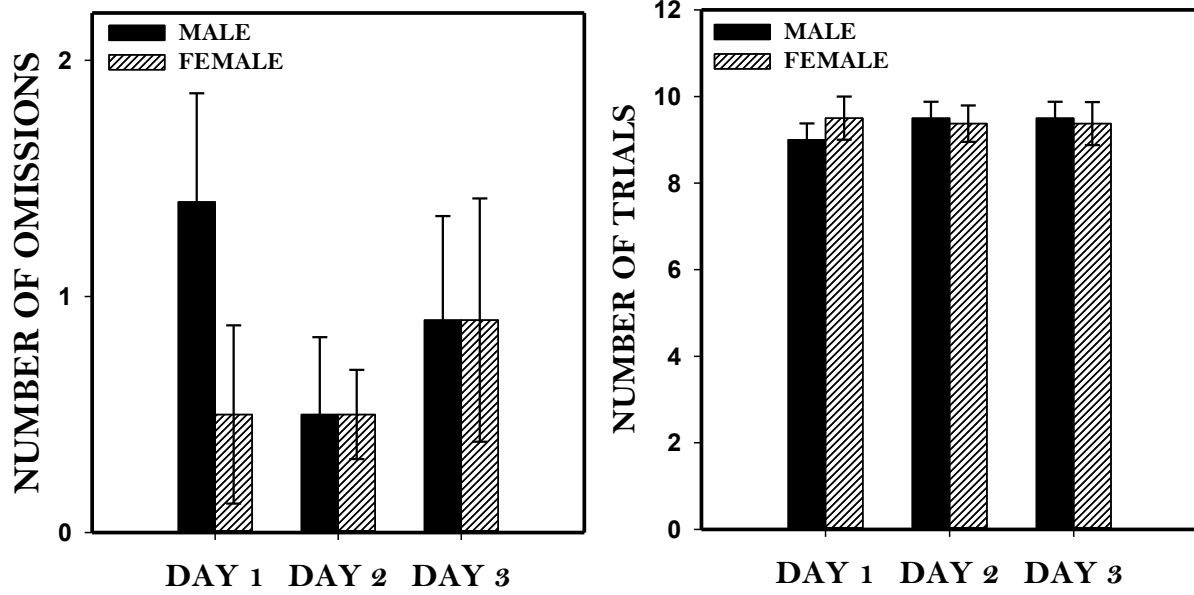


Figure 5

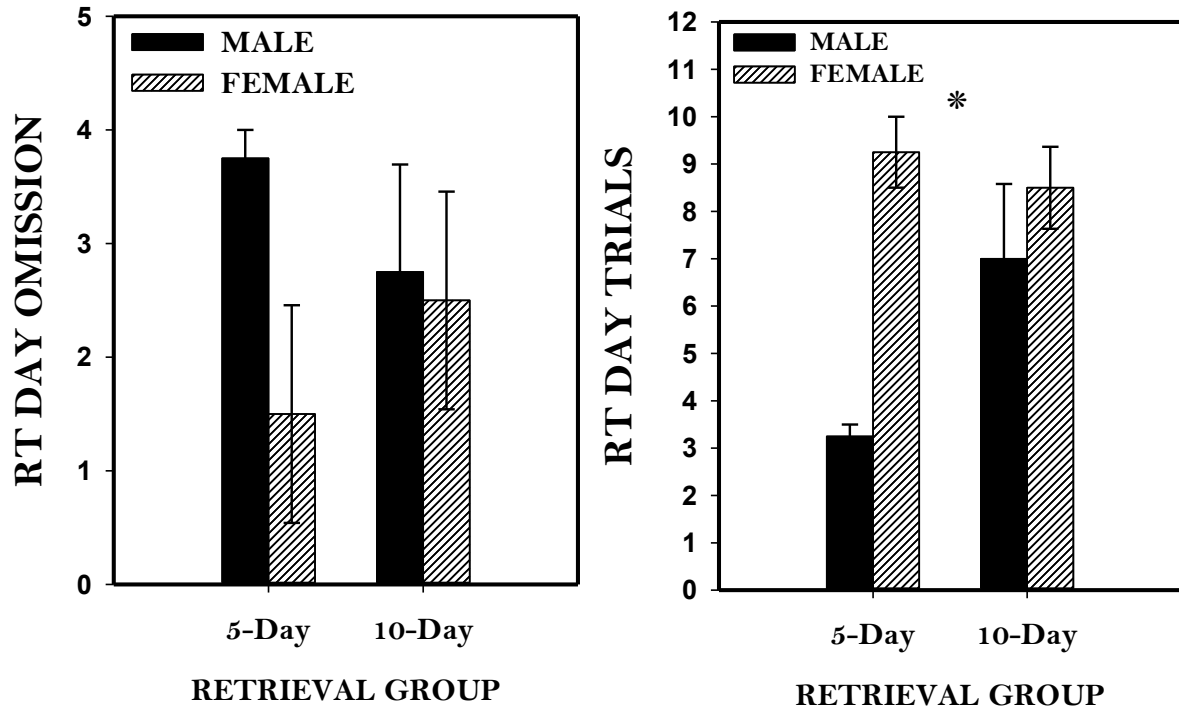


Figure 6

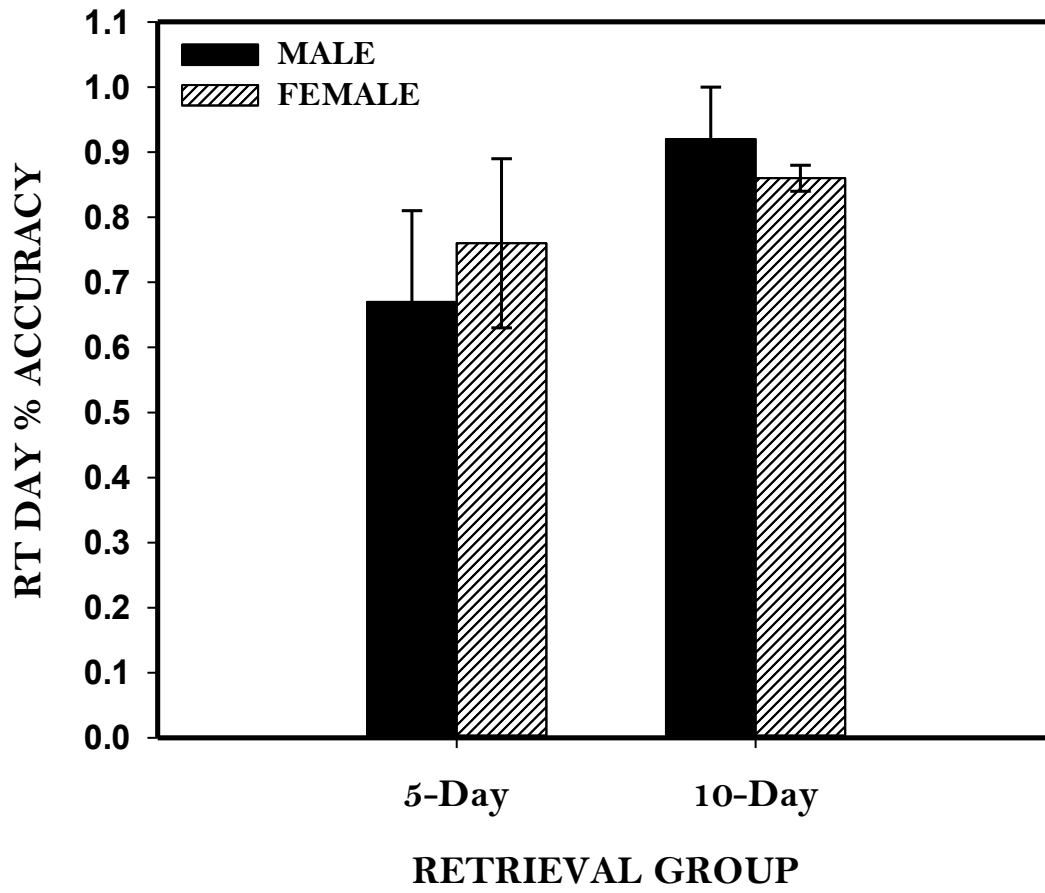


Figure 7

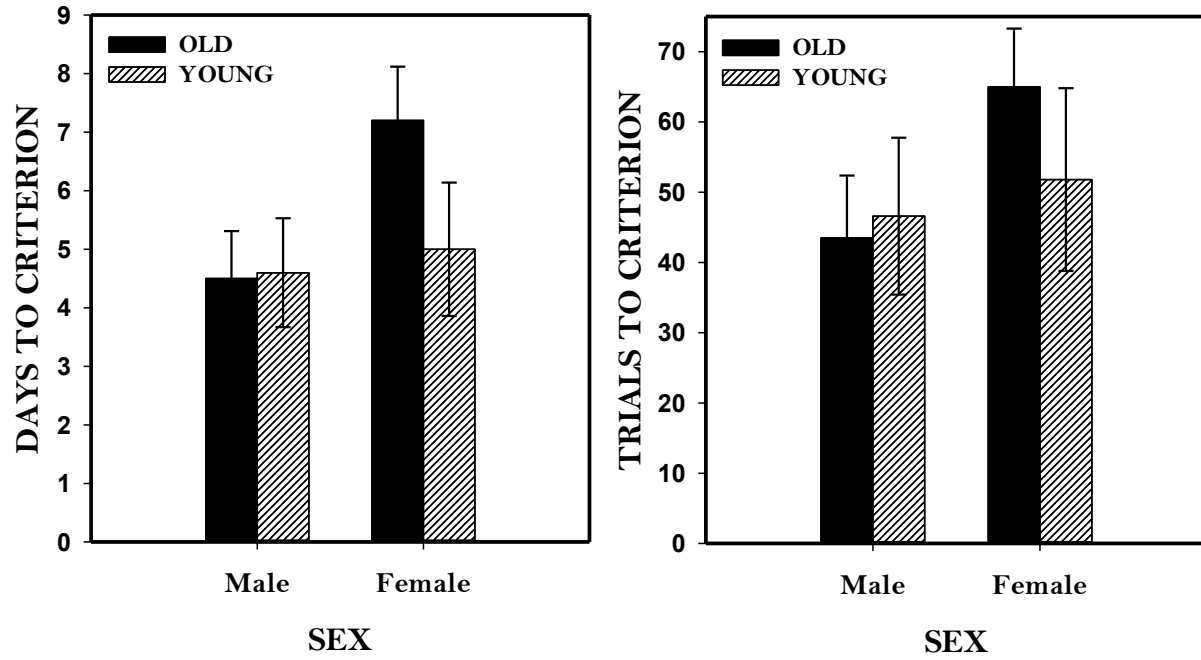


Figure 8

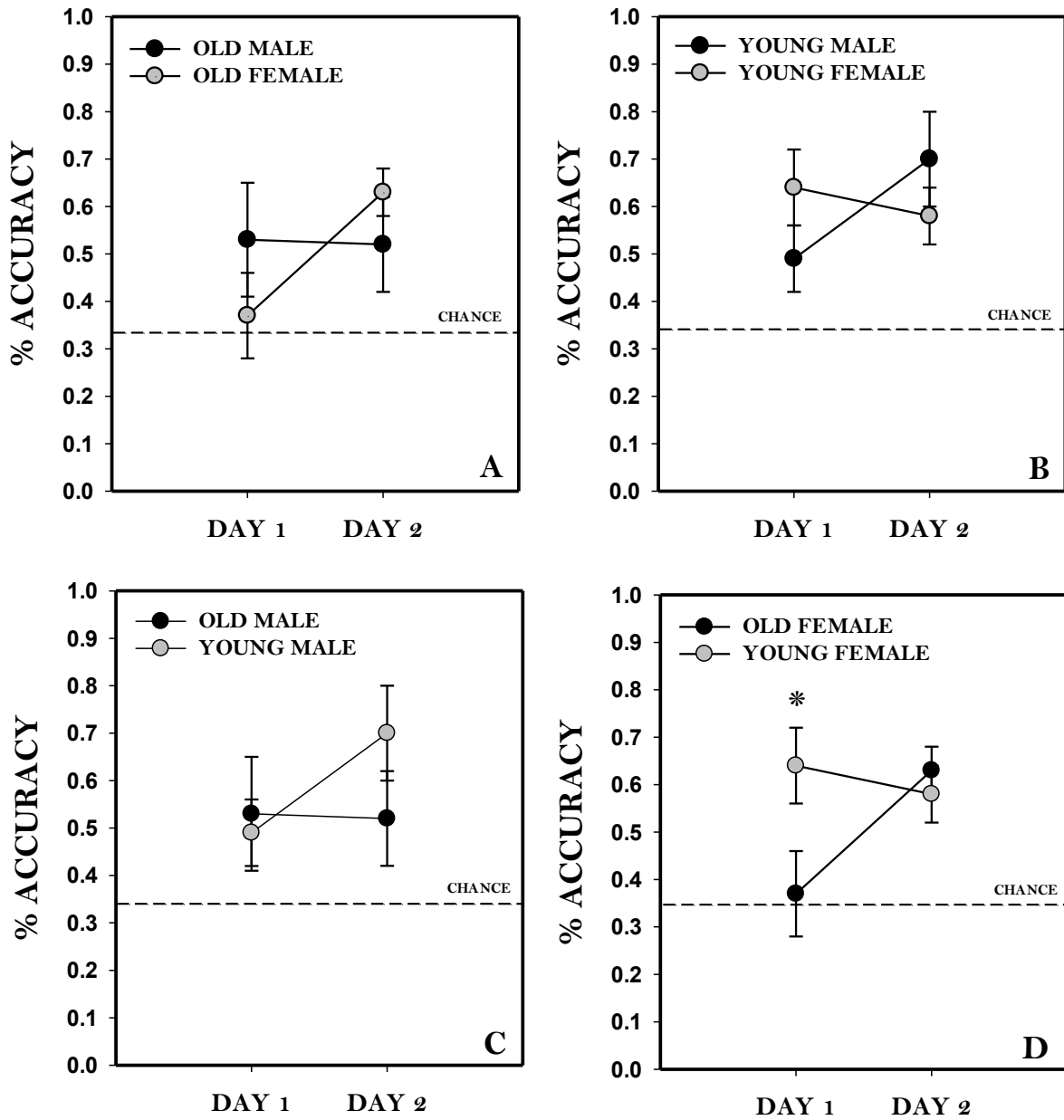


Figure 9

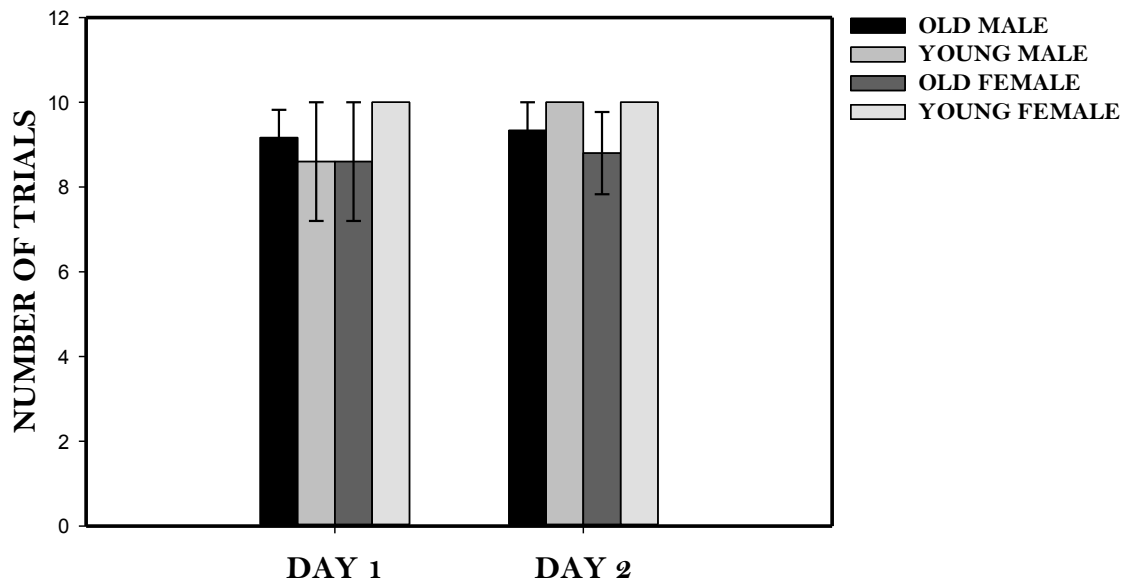
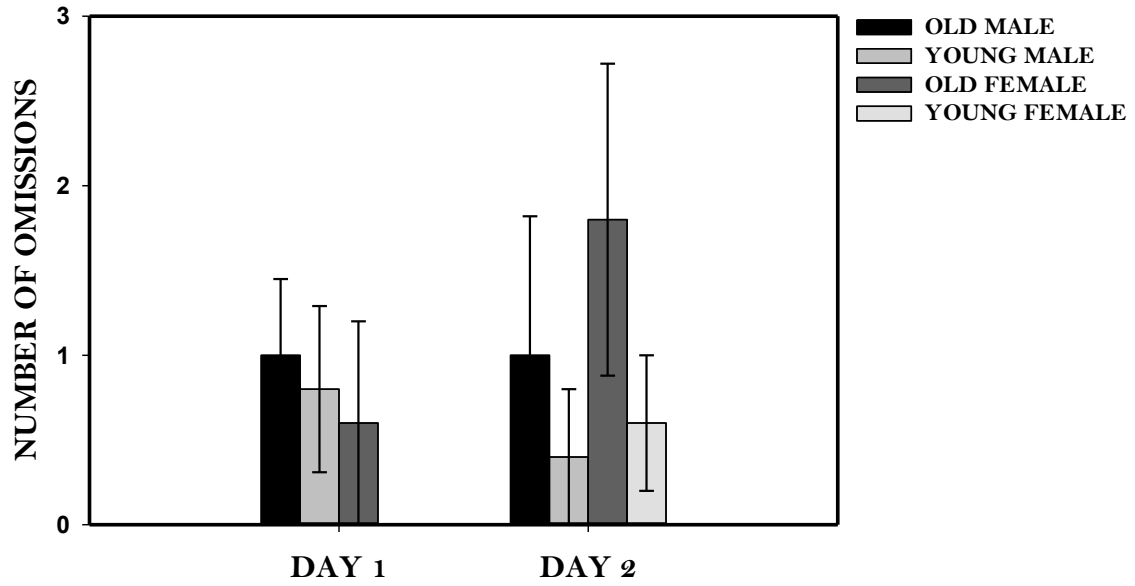


Figure 10

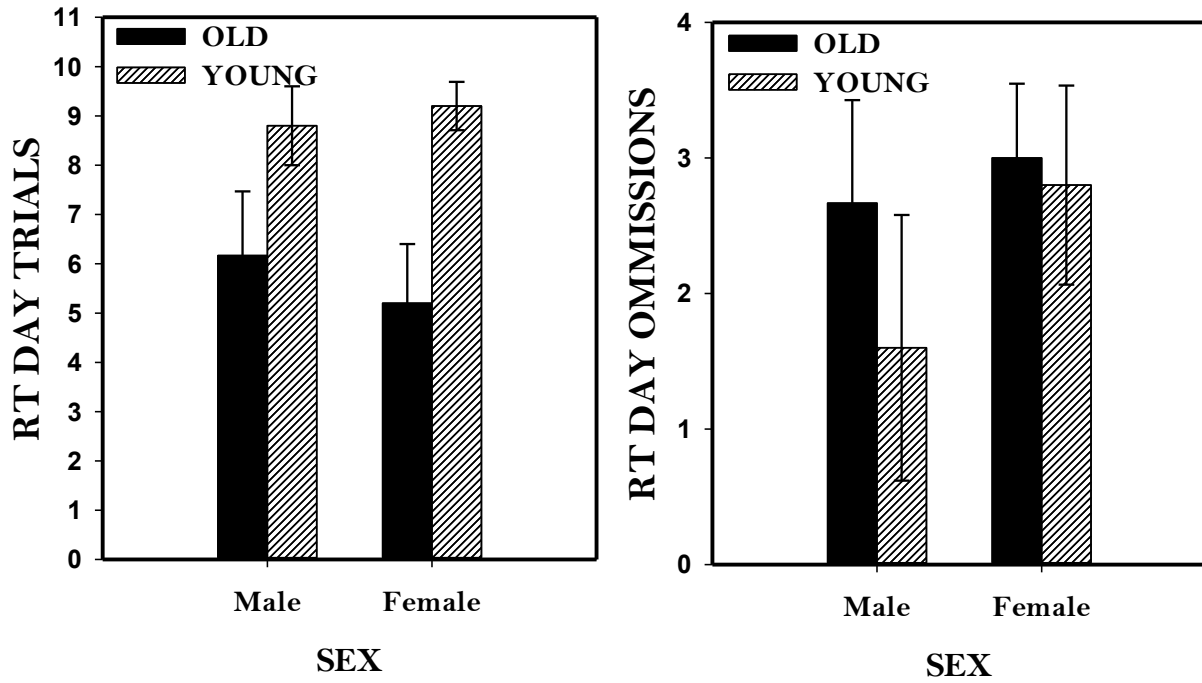


Figure 11

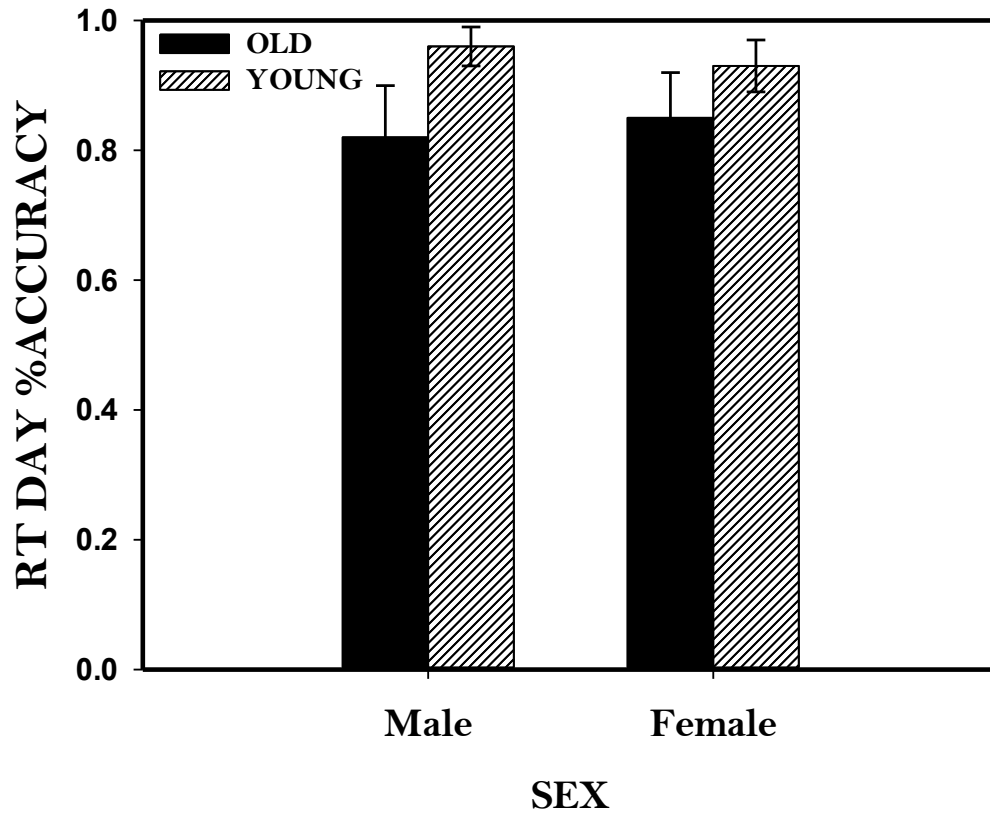
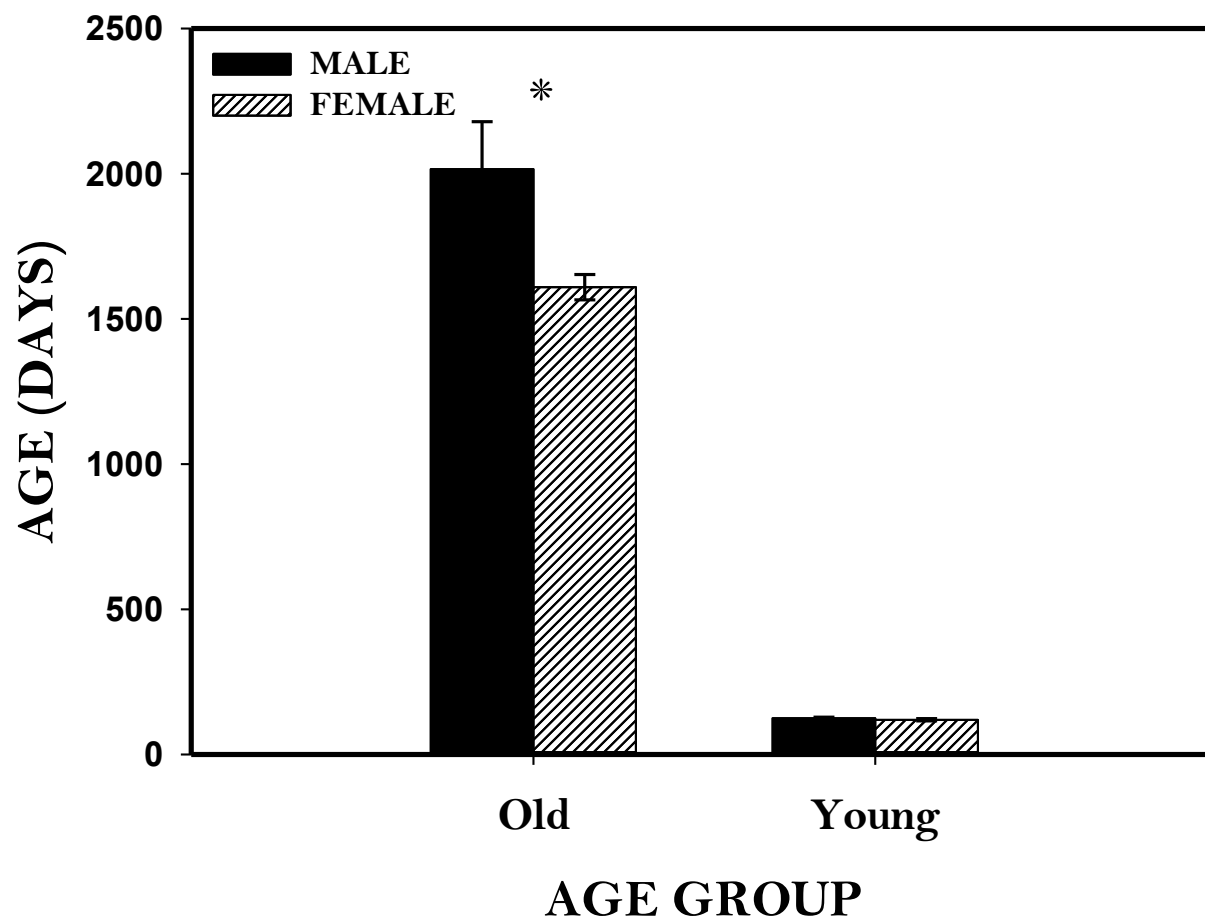


Figure 12



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