2020

Executive Function Deficit as a Precursor to Memory Impairments in hApoE4 Transgenic Rats

Kaitlin McManus

Executive Function Deficit as a Precursor to Memory Impairments in hApoE4 Transgenic Rats

Kaitlin McManus

Department of Psychology, Colby College

Honors Thesis

May 18, 2020

Abstract

The hApoE4 allele is one of the strongest genetic risk factors for Alzheimer's disease (AD). It underlies amyloid-β deposits and neurofibrillary tangles, the two hallmarks associated with AD pathology, and is subsequently associated with AD symptomology. Despite its importance, no rat animal studies to date use hApoE4 knock-ins. In addition to this deficit in the field of AD literature, the vast majority of AD studies focus on memory, even though executive function deficits may precede memory impairments in AD, and may be a predictor of AD development. Thus, the present study addressed these gaps in AD research by investigating the behavioral and physiological changes induced in rat models by an hApoE4 knock-in. hApoE4 rats experienced heightened anxiety compared to their wildtype counterparts, with male rats showing greater anxiety than females. hApoE4 rats had reduced motor capabilities, with males being disproportionately affected compared to females. Limited spatial learning and memory deficits were found at this time, but hApoE4 rats exemplified regressive behaviors in the probe trial of the spatial learning and memory task, indicating impairment in cognitive flexibility at this age. hApoE4 rats had impaired attentional set shifting abilities, with robust effects visualized in male hApoE4 rats. There was no difference in corticosterone levels between rats. Female hApoE4 rats were more sensitive to the hApoE4 knock-in compared to males, with more than half of the female hApoE4 rats failing to finish all measures due to the lethality of the knock-in. These results indicate that the hApoE4 knock-in effectively induced AD-like pathology in rat animal models, potentially to a lethal point in females. Executive function deficits appeared to precede spatial learning and memory deficits, indicating that future clinical interventions for AD should focus on identification of executive dysfunction.

Executive Function Deficit as a Precursor to Memory Impairments in hApoE4 Transgenic Rats

Alzheimer's disease (AD) is the most common form of dementia worldwide, accounting for 50-60% of all dementia cases (Blennow, de Leon, & Zetterberg, 2006). AD disproportionately affects the older adult population (Liu, Kanekiyo, Xu, & Bu, 2013) as most cases are seen in individuals 65 years of age or older (Alzheimer's Association, 2016). In the older adult population, 13% of individuals over the age of 65 have AD, and 45% of individuals over the age of 85 have AD (Alzheimer's Association, 2012). These estimates will continue to grow if interventions for the disease are not developed. Without interventions it is expected that, by 2050, one new case of AD in the United States will develop every 33 seconds (Alzheimer's Association, 2016). Identifying early deficits indicating AD development would allow vigilant intervention to mitigate the debilitating effects of AD.

The most common, stereotypical, deficit in AD is a decline in the ability to remember new and previously known information. This memory loss must affect daily life and could include confusion about time or place, misplacing things, and trouble writing or speaking. Other deficits include issues in planning or problem solving skills, difficulty completing familiar tasks, and increased anxiety, agitation, and sleep disturbances. In advanced stages of AD, people need assistance with basic activities such as dressing, bathing, and eating. They lose the ability to communicate and recognize loved ones, and they require constant care (Alzheimer's Association, 2016). Not only does this affect the patients themselves, but it disproportionately burdens the caregivers as well. Despite the numerous symptoms and high level of debilitation, this disease currently has only two classes of pharmacologic therapy available as treatment (Weller & Budson, 2018). Understanding the pathology of AD could lead to increased treatment interventions.

AD is marked by a vast pathology that underlies these behavioral deficits. It includes selective neuronal loss that is associated with extracellular amyloid deposition and neurofibrillary degeneration (Dickinson, 1997). AD was first described by Alois Alzheimer over 100 years ago when he reported the presence of miliary bodies and dense bundles of fibrils in the brain of his patient (Blennow et al., 2006). Today those entities are referred to as senile (neuritic) plaques and neurofibrillary tangles (NFT),

respectively, and they encompass the histopathology of AD (Dickinson, 1997). The plaques are formed by 40-42-amino acid long amyloid β-peptides (Aβ) derived from an amyloid precursor protein (APP) (Masters et al., 1985; Weller & Budson, 2018). An imbalance between the production of Aβ from APP and the subsequent clearance of Aβ peptides results in the accumulation and aggregation of Aβ (Liu et al., 2013). The aggregated Aβ plaques are a source of neural toxicity because they block synapses and cause neurodegeneration and dementia (Blennow et al., 2006; Hardy & Selkoe, 2002), which leads to the thought that Aβ accumulation is the primary event resulting in AD pathogenesis (Hardy & Selkoe, 2002). There is evidence that neurons synthesizing acetylcholine (ACh) are degenerated in individuals with AD (Selkoe, 2001), possibly because Aβ plaques accumulate in the synaptic clefts of neurons synthesizing ACh and cause their degeneration. ACh is a neurotransmitter involved with memory and learning, and its decreased concentration and function in people with AD provides explanation for why AD is marked by memory impairments (Francis, 2005). In addition to synaptic loss, the degeneration of axons is characteristic of AD (Mandelkow et al., 2003). The neurofibrillary tangles identified in AD pathology (Dickinson, 1997) may cause the dying back of those axons. The tangles are formed by hyperphosphorylated tau peptides (Liu et al., 2013; Weller & Budson, 2018), and the tau protein is a microtubule-associated protein responsible for stabilizing microtubules and facilitating neurite growth (Mandelkow et al., 2002). Hyperphosphorylation of tau causes tau to detach from microtubules and to form NFT. Without tau, microtubules decay and impede axonal transport (Mandelkow et al., 2002), thereby causing axonal degeneration. Together, Aβ plaques and tau tangles are the two hallmarks of AD. Evaluation and diagnosis of AD involves the detection of Aβ plaques and hyperphosphorylated tau tangles (Weller & Budson, 2018). The reason behind why these Aβ deposits and tau tangles are built up is a focus of much research, because minimizing their existence could, in theory, diminish the debilitating behavioral effects that they cause.

The Aβ deposits and tau tangles disproportionally affect different regions of the brain. Amyloid plaques are found in the limbic brain regions, which include the hippocampus and amygdala (La Ferla &

Oddo, 2005), leading to memory and anxiety impairments. Interestingly, human patients with AD have low plaque formation in the hippocampus, but transgenic rodent models have a lot of Aβ deposits in their hippocampal region (Shin et al., 2008). In humans it is the neurofibrillary tangles that are most heavily formed in the hippocampal region (2008), explaining the deficits in memory impairment. Aβ deposits are most significant in the posterior cingulate cortex and frontal cortex (2008), explaining the deficits in emotion, decision making, and cognitive flexibility. Together, the regional placement of Aβ deposits and tau tangles cause the behavioral deficits seen in AD symptomology.

The reason for the occurrence of Aβ deposits and tau tangles is a large field of research. One factor potentially causing their presence is the level of glucocorticoid hormones, which is elevated during dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis (Green et al., 2006). Glucocorticoid increases appear early in AD pathology and increase cortisol levels, which are associated with stress, memory, and cognitive impairments. Glucocorticoids increase Aβ formation and augment tau tangles (2006), thereby causing the observed memory and cognitive impairments.

Another contributing factor for the occurrence of Aβ deposits and tau tangles is genetic risk. Polymorphic alleles of apoliprotein E (*APOE* gene; ApoE protein) are widely recognized as a predisposition for the development of AD in individuals in their 60s and 70s (Selkoe, 2001). *APOE* has three polymorphic alleles, ε2, ε3, and ε4, which have worldwide frequencies of 8.4%, 77.9%, and 13.7%, respectively (Farrer et al., 1997). Genetic analysis identified that the ε4 allele (ApoE4) specifically is a major risk factor for AD (Corder et al., 1993; Strittmatter et al., 1993). Inheritance of one ε4 allele increases the likelihood of developing AD by three times, but inheritance of two ε4 alleles increases the risk of AD development by 15 times (Farrer et al., 1997; Selkoe, 2001). ApoE4 may be a determinant of the Aβ deposits and tau tangles marking AD, as ApoE immunoreactivity occurs in amyloid deposits and neurofibrillary tangles of AD brains (Namba et al., 1991). ApoE4 is associated with increased Aβ deposition in the brain (Morris et al., 2010), impaired Aβ clearance (Jiang et al., 2008), and increased tau phosphorylation (Harris et al., 2003). The relation between ApoE4 and the hallmarks of AD makes the

ApoE4 allele a significant area of study in AD research. ApoE4 is a genetic risk factor for AD pathology, which ultimately causes the debilitating behavioral deficits of AD.

Understanding the underlying pathology and the ways in which different factors make it more likely for an individual to develop AD requires the kind of experimental control and duration that is only achieved with an animal model. Glucocorticoid levels underlying AD pathology studied in mouse animal models found that corticosterone, the glucocorticoid hormone in rodents, is increased in mice overexpressing APP (Green et al., 2006). Studies using transgenic mice expressing human ApoE4 (hApoE4) found that these animals had features of AD pathology, including impaired spatial memory and learning (Raber et al., 2000) and reduced long-term potentiation (Trommer et al., 2004). Mice models overexpressing APP instead of having an hApoE4 knock-in had increased amyloid deposits (Games et al., 1995). Few studies have utilized rat animal models to study AD pathology, despite the fact that transgenic rat models more accurately represent human disease than mice (Do Carmo & Cuello, 2013) and that rats have biological sex differences similar to those of humans (Ma et al., 2019). The AD studies using rat models that do exist genetically modified their rats to overexpress APP in order to investigate AD pathology (Benedikz et al., 2009). Shockingly, no studies to this date have used transgenic hApoE4 knock-in rats to study AD pathology. The present study attempted to address that limitation by performing a novel investigation into the behavioral and physiological implications in rat animal models induced by an hApoE4 knock-in. Another concerning observation is that most studies with AD rodent models are focused entirely on hippocampal dependent cognition, which includes memory and spatial processing (Levit et al., 2019), but Aβ deposits and tau tangles affect other regions including the amygdala, frontal cortex, and posterior cingulate cortex (Shine et al., 2008). Emotion dysregulation, appearing as anxiety and inhibition in AD, may occur from the impairment of the amygdala and posterior cingulate cortex. In rodents, anxiety can be tested in the open field and elevated plus maze. hApoE4 mice consistently have heightened anxiety (Raber et al., 2000), and in humans, female AD patients are typically more anxious than males (Mielke, 2019). Thus, anxiety is a sex dependent symptom of AD that the present study investigated as a means to confirm the efficacy of the hApoE4 knock-in in rats.

Spatial learning and memory is typically measured using the Morris Water Maze (MWM) because it is sensitive to hippocampal dysfunction (Scearce-Levie, 2010). hApoE4 mice experience deficits in spatial learning and memory retention in the MWM, with female hApoE4 mice facing more spatial memory impairment than males (Grootendorst et al., 2005). While learning and memory deficits may be stereotypical of AD, human studies reveal that executive function dysfunction occurs prior to diagnosis of AD, and that patients with Mild Cognitive Impairment (MCI) with executive dysfunction are more likely to develop AD (2019). Executive function deficits could perhaps precede memory impairments and be early indicators of AD development.

Little animal research into this area exists, but hApoE4 mice experience impaired working memory (Mahley et al., 2006) and transgenic APP rats have impaired behavioral flexibility (Levit et al., 2019), both of which are indicators of executive dysfunction. Executive function is typically tested in operant conditioning chambers or with dry land mazes, but these require food deprivation (Bizon et al., 2012) which is not advised for aged rodent models of AD. Thus, a novel task was designed in the present study to measure executive dysfunction, specifically attentional set shifting, using an elevated plus maze placed into the water maze. By putting a dry land maze into the water maze, food restriction would not be required. Executive function deficits, especially in rat animal models, are a seemingly unexplored area of interest related to AD pathology, and early detection of such deficits could be promising for AD intervention. The present study attempted to address this gap in AD research by investigating the idea that executive function deficits precede spatial learning and memory deficits in hApoE4 rats.

A terrifying notion about AD is that its hallmark Aβ deposits occur as much as 25 years before diagnostic criteria of AD can be met (Bateman et al., 2012). This aligns with the observation that executive function impairments precede memory impairments (Levit et al., 2019), because it indicates that AD pathology occurs much earlier than expected. Studying executive dysfunction instead of memory impairment as a behavioral marker of AD could allow for early detection and subsequent intervention. Additionally, using rat animal models instead of mice to study such behavioral markers allows a more accurate generalization to human AD pathology. The present study investigated the anxiety, memory, and

executive function deficits in hApoE4 knock-in male and female rat models. It was expected that hApoE4 rats would display heightened anxiety, impaired memory, and impaired executive function, and that these deficits would be more intense than normal aging deficits. It was also expected that the executive function deficits experienced by the hApoE4 rats would be more prominent than memory deficits in hApoE4 rats, indicating that executive dysfunction is an earlier indicator of AD development. In addition, it was expected that corticosterone levels would be heightened in hApoE4 rats, indicating another physiological explanation for the behavioral impairments. Lastly, it was predicted that female hApoE4 rats would be more sensitive to the effects of the allele knock-in than male hApoE4 rats, meaning that their deficits would be intensified.

Materials and Methods

Animals and colony conditions

Subjects were male (n=20, 625.64 g) and female (n=20, 351.08 g) Sprague-Dawley rats (Horizon Discovery Lab) that arrived in the colony on postnatal day (PD) 25. Half of the females (n=10) and half of the males (n=10) had a hApoE4 knock-in (hAPoE4 KI). The remaining animals were wildtype. Rats were housed in same-sex pairs in individually-ventilated polycarbonate cages (30 cm x 30 cm x 18 cm) with a wire bar lid (Thoren Caging Systems, Hazelton, PA) lined with corncob bedding. At 14 months of age, male rats outgrew their cages and were placed, in their same-sex pairs, in larger individually-ventilated polycarbonate cages (Thoren #8 Expanded Rat Cage; 30.8 cm x 40.60 cm x 22.23 cm) with a wire bar lid lined with corncob bedding. At 18 months of age, shredded paper bedding (Carefresh paper fiber bedding; Petsmart) was used to line the cages. Rats had access to ad libitum food (Harlan Rat Chow) and tap water. Food and water were replenished every three days; rats were weighed and enriched once a week. The enrichment consisted of 2-3 groups of cage pairs interacting with each other for 20 minutes in a novel play area. Research assistants handled the rats during this time as well. The housing facility was kept on a 12-hour light/dark cycle and all procedures were carried out in the light phase. All animal ethics and procedures were approved by Colby College's Institutional Animal Care and Use Committee.

Experimental Overview

The present research was part of a larger study that entailed behavioral testing of all rats from PD 50 to 120.

Exploratory and Anxiety-Like Behaviors

At 14 months of age, rats underwent assessments for exploratory and anxiety-like behaviors using the open field and elevated plus maze. These tests are conducted by placing a rat in the open field for 5 minutes followed immediately with placement on the elevated plus maze for 5 minutes. Digital video footage of rats' movements in each test were collected with a webcam and summarized with ANY-Maze (Stoelting Co., Wood Dale, IL). An open field and elevated plus maze were used in combination to assess exploratory and anxiety-like behaviors.

Open Field

The open field test consisted of an open-topped wooden box (90 cm x 90 cm with 40 cm high walls) with corncob bedding. Rats were tested individually; an experimenter transported rats from their home cage to the box via a clear plastic carrying container. The experimenter placed rats in the same corner of the box to start, and rats were allowed to freely explore for five minutes. The total distance traveled and the average speed of travel were measured. The time spend in the perimeter zone of the field and the number of entries to the perimeter zone were measured. The latency to enter the center of the field, the time spent in the center of the field, and the number of entries to the center of the field were measured. More time spent in the perimeter and more entries to the perimeter zone was associated with less exploratory and more anxious behavior.

Elevated Plus Maze

The elevated plus maze was constructed of black painted wood and consisted of 4 arms (50 cm long and 4 cm wide). Two of the arms were enclosed by 43 cm high walls and the other two arms were open. The maze stood 54 cm above the ground. The experimenter placed the rat individually in the center of all the arms and allowed it to freely explore for 5 minutes; at the completion of the task the rat was removed, returned to its home cage, and the apparatus was cleaned. The amount of time spent in the closed arms, the number of entries to the closed arms, the amount of time spent in the open arms, and the

number of entries to the open arms were measured. More time spent in the closed arms and more entries to the closed arms were associated with less exploratory behavior and more anxious behavior.

Spatial Learning and Memory

One month following the open field and elevated plus maze tasks, when the rats were 15 months of age, all subjects underwent a spatial learning and memory task in a water maze. The task was designed to measure allocentric spatial reference memory by having rats use mental representations of their surroundings to flexibly navigate the area. The water used consisted of a circular tank (153 cm) filled with 21°C – 23°C water. A stationary escape platform (10 cm) was placed in the northwest quadrant of the pool, about 1 cm below the water surface. Spatial cues surrounded the maze: a large black triangle was painted on the wall above the east side of the pool, music played near the south side of the pool, a black square was painted on the wall near the west side of the pool, and the experimenter acted as a cue near the north side of the pool. 4 platoons of 6 rats and 4 platoons of 4 rats were used. All females completed testing before males were tested. The experimenter transported one platoon at a time from their home cages to holding cells in the room containing the water maze. For each trial, the experimenter placed the rat in the pool with its head facing the wall of the pool. Starting positions for each trial were in a randomized order of either the north, south, east, or west orientation. Rats received 6 trials per day for 4 consecutive days. Each trial was a maximum of 60 seconds long. If the animal did not locate the platform in 60 seconds, the experimenter moved the animal to the platform and positioned it on the platform for 30 seconds. After the third trial on an animal's last day of experimentation, a probe trial was used to assess development of spatial bias. The platform was removed from the pool and the rat swam for 60 seconds. A ceiling-mounted camera visualized the rats movements. Data were analyzed with HVS Imaging. Analysis of spatial learning included measurements of latency to platform, speed traveled, path length, and time spent in the target quadrant. A lower latency, faster speed, shorter path length, and higher amount of time spent in the target quadrant were indicative of more allocentric spatial reference memory.

Attentional Set-Shifting

Three months following the spatial learning and memory task, when the rats were 18 months of age, they underwent an attentional set shifting task to assess their cognitive flexibility level. A plexiglass plus-maze (4 arms at 8 in wide x 24.5 in long x 23 in high) was placed inside a circular pool (153 cm) of 21ºC-23ºC water. The top of the maze arms extended above the surface of the water. 4 plexiglass inserts (8 in x 23 in) were constructed that could attach and detach from the start of the arms of the maze in order to block off arms. A visual cue arm was made from 3 plexiglass inserts, 2 of which matched the length of the arms and one of which matched the width. Black electricians tape was used to make stripes on these inserts. Alligator clips were used to attach these visual cue inserts to the sides of a maze arm as needed. A ceiling-mounted camera visualized the rats movements. Data were analyzed with HVS Imaging. 8 platoons of 4 rats each and 2 platoons of 2 rats each were used to complete this experiment. One platoon completed the task per day; the task lasted for 10 consecutive days. Experimenters transported each platoon from their home cages to holding cages in the room containing the pool. The experimenter placed the rat in one of the four arms in a randomized order of north, south, east, or west orientation. The rat was positioned with its head facing the back wall of the arm. The arm directly across from where the rat started was blocked off. The task consisted of three stages, a response discrimination, a reversal, and a shift to an extradimensional cue.

In order to complete all three stages, animals had to achieve a criterion of 8/10 correct consecutive trials, meaning rats had to correctly turn to find the platform 8/10 consecutive times. After all rats in a platoon reached this criteria, they advanced to the next stage. A rat could attempt a maximum of 40 consecutive trials per stage before the next stage began. A 5 minute break was given after every 10 trials. Each trial lasted 60 seconds; if a rat failed to find the platform in 60 seconds the experimenter moved the rat to the platform and positioned it on the platform for 30 seconds.

A rat successfully completed a trial if it turned in the correct direction. If a rat began moving down the wrong arm but corrected its direction before moving halfway down the incorrect arm, the trial was marked as correct. If not, the trial was marked incorrect. There were three ways a trial could be incorrect: 1) “wrong direction” errors occurred when a rat fully committed to an incorrect arm and moved

past the halfway point of that arm; 2) “starting arm” errors occurred when a rat exited the starting arm and then reversed its direction to go back into the starting arm; 3) “time out” errors occurred when a rat failed to find the platform in 60 seconds. If a rat was successful on less than 75% of trials in a block of 4 trials, this was considered a “perseverative error” in that there was an impairment in ability to acquire a new strategy. If a rat successfully made 75% or more of the trials in a block of 4 trials, but then was successful on less than 75% of trials in a following block of 4 trials, this was considered a “regressive error” in that the rat had formed a new strategy but was unable to maintain it.

Level of cognitive flexibility was measured for each stage by the trials to criterion, average time to platform, and total errors. Total errors were broken down into total “wrong direction”, “start arm”, “time out”, “perseverative”, and “regressive” errors. A score of error types per stage was calculated, with the possibility of making 3 different error types per trial. More trials to criterion, longer time to reach platform, and more errors were indicative of impaired cognitive flexibility.

Response Discrimination Stage

In the first stage, a platform was placed 2 cm below the water surface at the end of either the left or the right arm, respective to where the rat started from. This direction was randomized for every platoon and remained consistent for the entire first stage. The rat had to learn a directional cue to find the platform. The visual cue arms were inserted in either the north, south, east, or west arm regardless of the rat’s starting position and in a randomized order.

Reversal Stage

The second stage was an interdimensional reversal, where the platform was now placed in the opposite direction respective to the starting arm for every platoon. The visual cue arms were again inserted in either the north, south, east, or west arm regardless of the rat’s starting position.

Visual Stage

The third stage was a shift to a visual cue. The platform could be in either the left or right arm respective to the rat’s starting position. The location of the platform was determined by the location of the

visual cue arms, which were randomized to be either to the left or right of the rat's starting position. The rat had to shift from using directional cues to locate the platform to now using visual cues.

Corticosterone Immunoassay and Analysis

One day following the last day of testing for the attentional set shifting task, rats were sacrificed. Rats were anesthetized using isoflurane anesthesia delivered in 1.5% oxygen and then decapitated. Their brains were extracted and post-fixed in 4% paraformaldehyde at 4ºC. Within 2 minutes of sacrifice, roughly 500 µl samples of trunk blood were taken from each rat and centrifuged at 4ºC at 14,000g for 10 minutes. The serum was pipetted from each sample and stored in 5mL microcentrifuge tubes in an ultralow freezer at -800ºC. Arbor Assays Corticosterone Enzyme Immunoassay Kit and Arbor Assays online analysis tool were used to evaluate basal corticosterone in the plasma samples. Higher basal corticosterone levels are associated with accelerated cognitive decline and memory deficits, thus increasing corticosterone was indicative of cognitive impairment linked to AD.

Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics. 2 (Sex: male, female) by 2 (Genotype: wildtype, hApoE4) ANOVAs were used to analyze data from most measures in the open field, elevated plus maze, spatial learning and memory, attentional set shifting, and corticosterone tests. A 2 (Sex: male, female) by 2 (Genotype: wildtype, hApoE4) by 2 (Slice: first, second) mixed factorial ANOVA was used to analyze data from the probe trial of the spatial learning and memory task. 2 (Sex: male, female) by 2 (Genotype: wildtype, hApoE4) ANCOVAs controlling for corticosterone, body weight, or performance on spatial learning and memory tasks were used to analyze spatial learning and memory behaviors and attentional set shifting behaviors. An additional variable of status was made to address the loss of rats during the study. 2 (Sex: male, female) by 2 (Genotype: wildtype, hApoE4) by 2 (Status: finished, stopped) ANOVAs were used to analyze data from the open field, elevated plus maze, spatial learning and memory, and corticosterone tests. Means and standard errors were calculated and are shown in figures. Main effects and interactions between or within factors were reported as statistically

significant, with statistical significance set at 0.05. Non-significant p-values between 0.05 and 0.10 were considered a pattern of interest and were discussed.

Results

Weight

There was a significant main effect of Sex on weight (F [1,36]=314.369, p<.001, partial η^2=.897; see Fig. 1), with female rats weighing significantly less than male rats regardless of genotype. A main effect for Genotype on weight was nearing significance (F [1,36]=3.749, p=.061, partial η^2=.094; see Fig. 1), with hApoE4 rats weighing more than WT rats. The interaction between Sex and Genotype was not statistically significant (p=.507).

Open Field

Anxiety-like activity in the open field was assessed by analyzing time in the center of the open field, distance traveled, and numbers of entries to the center area. There was a significant main effect for Sex on number of entries to the center of the open field (F [1,36]=4.490, p=0.041, partial η^2=.111; see Fig. 2A) and time spent in the center of the open field (F [1,36]=22.824, p<0.001, partial η^2=.388; see Fig. 2B). Male rats entered the center of the open field significantly less times than female rats, and male rats spent significantly less time in the center of the open field than female rats. There were no other significant main effects or interactions (all ps > 0.05).

Elevated Plus Maze

Anxiety-like activity in the elevated plus maze was assessed by analyzing time spent in the closed and open arms, number of entries to the closed arms and open arms, and latency to enter the open arms. There was a significant main effect of Sex on latency to enter the open arms (F [1,36]=4.975, p=0.032, partial η^2=.121; see Fig. 3A) and on number of closed arm entries (F [1,36]=4.254, p=0.046, partial η^2=.106; see Fig. 3B). Males took significantly more time to enter the open arms than female rats, and they entered the closed arms significantly less than female rats. There was a significant main effect of Genotype on time spent in the open arms (F [1,36]=5.109, p=0.030, partial η^2=.124; see Fig. 3C), latency to enter the open arms (F [1,36]=4.516, p=0.041, partial η^2=.111; see Fig. 3A), and number of closed arm

entries (F [1,36]=4.854, p=0.034, partial η^2=.119; see Fig. 3B). WT rats spent significantly more time in the open arms than hApoE4 rats, and they entered the open arms significantly faster than hApoE4 rats. WT rats also spent significantly less time in the closed arms than hApoE4 rats. There were no other significant results (all ps > 0.05).

Spatial Learning and Memory

Spatial learning and memory were assessed by analyzing escape latency, speed traveled, and amount of time spent in the target quadrant of the probe trial. There was a significant main effect for Sex on the latency to find the platform on Day 1 of testing (F [1,36]=4.285, p=0.046, partial η^2=.106; see Fig. 4A), with males having a significantly longer latency to find the platform than females. There was a significant main effect for Sex on the speed rats traveled for the first trial of Day 4 (F [1,36]=7.562, p=0.009, partial η^2=.174; see Fig. 4B), with males traveling faster than females. A main effect for Sex on the speed rats traveled for the first trial of Day 2 was approaching significance (F[1,36]=3.358, p=.075, partial η^2=.085; see Fig. 4B), with males traveling faster than females. A significant main effect for Genotype on latency to the platform on Day 3 (F [1,36]=5.381, p=0.026, partial η^2=.130; see Fig. 4A) was also found, with WT rats finding the platform more slowly than the hApoE4 rats. A significant main effect for Genotype was observed for average speed of travel on the first trial of Day 1 (F [1,36]=15.234, p<0.001, partial η^2=.297; see Fig. 4B), with WT rats traveling faster than hApoE4, and this main effect was approaching significance for average speed on the first trial of Day 4 (F [1,36]=3.166, p=0.084, partial η^2=.081; see Fig. 4C), where again WT rats were traveling faster than hApoE4 rats. A significant Sex x Genotype interaction was observed for the average speed rats traveled on Day 3 (F [1,36]=7.141, p=0.011, partial η^2=.166; see Fig. 4C) and on Day 4 (F [1,36]=4.932, p=0.033, partial η^2=.120; see Fig. 4C): male WT rats traveled significantly faster than male hApoE4 rats on Day 3 (p=0.016) and on Day 4 (p=0.013). A significant Sex x Genotype interaction was also observed for the speed rats traveled on the first trial of Day 4 (F [1,364.154, p=0.049, partial η^2=.103; see Fig. 4B), with male WT traveling slower than male hApoE4 rats and female WT traveling faster than female hApoE4 rats, but these differences

failed to reach significance. There were no additional significant main effects or interaction (all *p*s > 0.05).

A significant Sex x Genotype x Slice interaction was observed for the amount of time spent in the target quadrant (F [1,36]=4.234, p=0.047, partial η^2=.105; see Fig. 5). Male WT rats spent more time in the target quadrant than male hApoE4 rats during the first slice, while male WT rats spent less time in that target quadrant compared to male hApoE4 rats during the second slice, though these differences failed to reach significance. Female WT rats spent less time than hApoE4 rats in the target during this first slice, though it failed to reach significance. In the second slice, female WT rats spent significantly less time than female hApoE4 rats (p=0.027). Additionally, all male WT rats spent significantly less time in the target quadrant in the first slice compared to the second (p=0.029), as did male hApoE4 rats (p<0.001) and female WT rats (p=0.001). Female hApoE4 rats followed this same trend but failed to reach significance (p=0.063). Male hApoE4 rats spent significantly less time in the target quadrant than female hApoE4 rats for both the first (p=.012) and second (p=0.019) slices.

Attentional Set Shifting

Cognitive flexibility was assessed by analyzing the trials to criterion, average time to platform, total errors made, amount of "wrong direction," "start arm," and "time out" errors, error score, perseverative errors, and regressive errors.

Response Discrimination Stage

A main effect for Genotype on the average time to reach the platform in the response discrimination stage was approaching significance (F [1,30]=3.011, p=.093, partial η^2=.091), with WT rats taking more time, on average, than hApoE4 rats to find the platform. A significant Sex x Genotype interaction was found for total errors made (F [1,30]=4.337, p=0.046, partial η^2=.126; see Fig. 6A), with male WT rats making significantly fewer total errors than male hApoE4 rats during the response discrimination stage (p=0.041). A Sex x Genotype interaction for "wrong direction" errors neared significance (F [1,30]=3.493, p=0.071, partial η^2=.104); see Fig. 6B), with male WT rats making significantly less directional errors than male hApoE4 rats (p=0.029). A Sex x Genotype interaction for

total "start arm errors" also approached significance (F [1,30]=.069, p=.106, partial η^2=.106; see Fig. 6D), with male hApoE4 rats making significantly more start arm errors than female hApoE4 rats (p=0.048). Male WT rats appeared to have less "start arm" errors than male hApoE4 rats, but this trend was reversed in females. Another interaction for total score of error types approached significance (F [1,30]=3.001, p=.093, partial η^2=.091), where male WT rats had lower error scores than male hApoE4 rats, but this trend was reversed in female rats. No other interactions or main effects reached significance (all ps > 0.05).

Reversal Stage

There were no significant main effects or interactions for the second reversal stage. (all ps > 0.05).

Visual Stage

There was a significant interaction for average time to reach the platform in the visual cue stage (F [1,23]=5.779, p=0.025, partial η^2=.201; see Fig. 6C), with male WT rats reaching the platform in significantly less time than male hApoE4 rats (p=0.026). Additionally, male hApoE4 rats reached the platform significantly slower than female hApoE4 rats (p=0.047). There were no other significant main effects or interactions observed (all ps > 0.05).

Corticosterone

There was no significant difference in corticosterone levels between WT and hApoE4 rats of either sex (all ps > 0.05; see Fig. 7). A 2 x 2 ANOVA controlling for days passed since a rat's last day of testing confirmed that any variance in corticosterone levels was not influenced by the stress of the set shifting task (all ps > 0.05).

Additional Measures

Corticosterone as a Covariate for Spatial Learning and Memory Measures

When controlling for corticosterone level, there was a significant Sex x Genotype x Slice interaction for the amount of time spent in the target quadrant during the probe trial (F [1,36]=4.234, p=0.038, partial η^2=.131; see. Fig. 8). Male WT rats spent significantly more time in the target quadrant

during the second 30-second slice than the first (p=.035), as did male hApoE4 rats (p<.001), female WT rats (p=.002), and female hApoE4 rats (p=.230). Male WT rats spent more time than male hApoE4 rats in the target quadrant during the first slice and less time in the second slice, but not significantly so. Female WT rats spent significantly less time in the target quadrant compared to female hApoE4 rats during the first 30-second slice (p=.035) and second 30-second slice (p=.001).

Speed in Spatial Learning and Memory Task as a Covariate for Attentional Set Shifting Measures

When controlling for speed on the first trial of day 3 and 4 in the Spatial Learning and Memory task, there was a significant Sex x Genotype interaction for trials to criterion in the response discrimination stage (F[1,30]=4.624, p=0.040, partial η^2=.134; see Fig. 9A), with male WT rats having marginally significantly less trials to criterion than male hApoE4 rats (p=0.055). A significant Sex x Genotype interaction for total errors occurred again in this stage (F[1,30]=4.598, p=0.040, partial η^2=.134). A significant interaction for total wrong direction errors in the response discrimination stage neared significance (F[1,28]=3.933, p=0.057, partial η^2=.123), where male WT rats had significantly less wrong direction errors than male hApoE4 rats (p=0.026). Error score neared significance in this stage as well (F[1,30]=3.775, p=0.060, partial η^2=.112; see Fig. 9B), with male WT rats having a lower error score than male hApoE4 rats (p=0.092). There was also a Sex x Genotype interaction for average time to find the platform in the visual stage (F[1,21]=7.733, p=0.011, partial η^2=.269), with male WT showing a significantly faster average time to platform than male hApoE4 (p=0.012). There were no other significant results (all ps > 0.05).

Amount of Time in Target Quadrant of Probe Trial in Spatial Learning and Memory Task as a Covariate for Attentional Set Shifting Measures

When controlling for amount of time spent in the target quadrant during the first 30-second slice of the probe trial in the Spatial Learning and Memory task, there was a significant Sex x Genotype interaction for total errors in the response discrimination stage (F[1,29]=4.789, p=0.037, partial η^2=.142) with male WT making significantly less total errors than male hApoE4 (p=0.036). There was also a significant interaction for the total "wrong direction" errors in this stage (F[1,29]=4.498, p=0.043, partial

η^2=.134), with male WT making significantly less directional errors than male hApE4 (p=0.021). A Sex x Genotype interaction was approaching significance for the total trials to criterion in this response discrimination stage as well (F[1,29]=2.911, p=.099, partial η^2=.091), where male WT rats had less trials to criterion than male hApoE4, but the reverse trend was occurring in female rats. Again, there was a Sex x Genotype interaction for average time to find the platform in the visual stage (F[1,21]=7.733, p=0.011, partial η^2=.260), with male WT showing a significantly faster average time to platform than male hApoE4 (p=0.011). There were no other significant results for the first slice data (all ps > 0.05).

When controlling for the amount of time spent in the target quadrant during the second 30-second slice, there was again a significant Sex x Genotype interaction for total errors made in the response discrimination stage (F [1,29]=4.602, p=0.040, partial η^2=.137), where male WT rats made less errors than male hApoE4 rats, but not significantly so, and female WT rats made more errors than female hApoE4 rats, but not significantly so. The interaction for total "wrong direction" errors in the response discrimination stage failed to reach significance (p=.061), as did the error score (p=0.081) and the total trials to criterion in that stage (p=.129). Again, there was a Sex x Genotype interaction for average time to find the platform in the visual stage (F[1,21]=7.733, p=0.030, partial η^2=.196), with male WT showing a significantly faster average time to platform than male hApoE4 (p=0.029).

Effect of Status Variable on Spatial Learning and Memory Measures

There was a significant Sex x Genotype x Status interaction for the latency to find the platform on the first trial of day 4 of the spatial learning and memory task (F [1,32]=5.524, p=.025, partial η^2=.147; see Fig. 10A), where male hApoE4 rats that were unable to finish all tasks had a significantly longer latency to find the platform than male hApoE4 rats that were able to finish all tasks (p=.049). The same trend appeared with female hApoE4 rats, but it failed to reach significance. Female WT rats that were unable to finish all tasks had a significantly longer latency to find the platform than female WT rats that were able to finish all tasks (p=.039), and this trend appeared with male WT rats but it failed to reach significance.

There was a significant main effect for Status on the average speed rats traveled on day 1 (F [1,32]=8.111, p=.008, partial η^2=.202; see Fig. 10B), day 2 (F [1,32]=6.982, p=.013, partial η^2=.179; see Fig. 10B), and day 3 of testing (F [1,32]=7.209, p=.011, partial η^2=.184; see Fig. 10B), where rats that were unable to finish all tasks traveled more slowly than rats that finished all tasks. There was also a significant Genotype x Status interaction on the speed traveled on day 1 (F [1,32]=4.386, p=.044, partial η^2=.121; see Fig. 10B), where WT rats that finished all trials traveled significantly faster than hApoE4 rats that finished all trials (p=.018). There were no other significant results (all ps > 0.05).

Discussion

The present study aimed to characterize the behavioral and physiological impacts of the hApoE4 allele in rat animal models. It was the first study to show that rat animal models expressing the hApoE4 allele effectively experience behavioral impairments indicative of Alzheimer's disease. At the advanced stage of 18 months, hApoE4 rats exhibited impaired cognitive flexibility and sex differences. These deficits were preceded by more subtle effects on motor function, spatial learning, and emotional behaviors. Importantly, results were sex dependent, indicating that the hApoE4 allele affects males and females differently, with female rats potentially being affected to a lethal extent. That lethality was possibly predicted by early deficits in motor function and spatial learning and memory, bringing attention to the importance of early diagnosis of AD in human subjects, because early interventions could help alleviate future fatalities.

A primary aim of the study was to asses executive function deficits induced by the hApoE4 knock-in. Aligning with the prediction that the hApoE4 allele would induce deficits in set shifting, male hApoE4 rats made more errors than male wildtype rats in the first response discrimination stage, and male hApoE4 rats took a longer time than their wildtype counterparts to reach the platform in the visual cue stage. Surprisingly, there were no significant differences within the reversal stage, which was predicted to reveal hAPoE4-induced cognitive flexibility impairments. The task design had not been used in aged rats or mice before (Bizon et al., 2012), and it might be the case that all rats, regardless of the hApoE4 knock in, had trouble inhibiting previously learned information in favor of new information because of their age.

The appearance of hApoE4 induced deficits in the visual stage is a promising indicator that hApoE4 rats were more cognitively impaired than wildtype rats, but all rats were likely affected because of their advanced age.

In addition to assessing the effects of the hApoE4 knock-in at an advanced age on executive function and cognitive flexibility, the present study sought to detect other changes in learning and behavior at a less advanced age. Assessments of spatial learning and memory in rats at 14 months of age failed to reveal an impairment in spatial learning and memory function in hApoE4 rats. hApoE4 rats did have reductions in speed, specifically on the last two days of the task, indicating that their speed decreased at the points where healthy rats would have been learning the location of the platform. This same pattern was seen with the speed traveled on the first trials of those last two days. Surprisingly, there was no difference between genotypes in escape latency, so even if hAPoE4 rats were traveling at a slower speed, the escape latency remained consistent with that of the wildtype rats. This may indicate an impairment in motor function, not in spatial learning and memory. Deficits in speed during the spatial learning and memory task usually indicate motor deficits (Scearce-Levie, 2011; Weitzner et al., 2015). Transgenic rodents are also more susceptible to hypothermia in the Morris water maze than wildtype rats (Iivonen et al., 2003), which could have slowed them down, but because the water used was between 21ºC-23ºC, motor impairments are a more likely explanation. Additionally, though most AD research focuses on memory impairments and cognitive deficits (Wagner et al., 2019), motor impairments may even precede clinical symptoms in AD patients (Buchman and Bennett, 2011), and AD patients with motor deficits are more likely to have severe cognitive impairments (Waite et al., 2005). Thus, the reduced speed in hApoE4 rats may indicate motor deficits, revealing nonclinical impairments caused by the hApoE4 allele.

To further understand the rats' behavior on the probe test, we separated the data from the probe trial into two 30-second slices. Previous work indicates that healthy rats will initially go to the learned, target quadrant, but after seeing that the platform is not there, those rats will assume the platform is elsewhere and will go attempt to find it (Kraemer & Randall, 1995). We did see this pattern with all rats

regardless of sex and genotype, thereby replicating those findings. However, male hApoE4 rats trended in the direction of spending less time than their wildtype counterparts in the target quadrant during the first 30 seconds of the trial, and more time in that target quadrant during the second 30 seconds than their wildtype counterparts. This finding exemplifies the idea that, after the initial 30 seconds, healthy rats are able to shift their attention to other quadrants, and search for the platform elsewhere. Cognitively impaired rats may be unable to make that shift, or are slower at doing so, and therefore spend more time in the target quadrant in the second 30 seconds. The female hApoE4 rats showed this impairment in cognitive flexibility compared to their wildtype counterparts as well. Interestingly, female hApoE4 rats spent more time in the target quadrant than their wildtype counterparts for the first 30 seconds of the probe trial, indicating that perhaps their escape latency was faster. This was only significant when controlling for corticosterone, so perhaps a stress response was involved in this difference. This conclusion of an impairment in cognitive flexibility seen in the probe trial is supported by a similar finding with TgAPP21 rats who demonstrated regressive-like behavior during their probe test (Levit et al., 2019). To our knowledge, this type of regressive behavior in the spatial learning and memory task has not been described elsewhere (2019). Thus, the probe trial was predicted to reveal a difference in spatial learning and memory, but instead it appeared to show an impairment of cognitive flexibility.

Because of the robust differences in speed during the spatial learning and memory task as well as the intriguing probe data results, we looked to see if using either variable as a covariate predicted performance on the attentional set shifting task. Controlling for speed brought the total trials to criterion in the response discrimination stage to significance, where male hApoE4 rats took more trials to reach criterion that their wildtype counterparts. Controlling for speed also brought error score near significance, where male hAPoE4 rats had higher error scores than their wildtype counterparts, meaning that male hApoE4 rats made more types of errors than male wildtype rats. Thus, motor impairment may be a predictor for executive function deficits, which is supported by the knowledge that motor deficits in AD patients are associated with more severe cognitive impairments (Waite et al., 2005). Because these differences were seen in the response discrimination task, they may indicate signs of deficits in learning

rather than in cognitive flexibility. Surprisingly, controlling for the amount of time spent in the target quadrant of the probe trial did not bring any additional attentional set shifting measures to significance. It was expected that this robust sign of cognitive impairment from the spatial learning and memory task would predict executive function performance, but this did not occur.

In addition to the investigations into executive function and spatial learning and memory, the present study sought to investigate behavioral changes in emotion. In accordance with our predictions, hApoE4 rats appeared to be less exploratory and more anxious compared to wildtype rats. This conclusion is supported by other studies that found hApoE4 mice had reduced activity in the open field and more anxiety in the elevated plus maze than wildtypes (Raber et al., 2000). Male rats appeared to be more anxious than females, which is surprising given evidence that in transgenic AD rat models, subjects have increased anxiety-like behavior regardless of sex (Pentkowski et al., 2018) and in humans, female AD patients are typically more anxious (Mielke, 2019). Because there was no difference in basal corticosterone level between sexes, it is possible that an external factor was causing the male rats to experience increased anxiety on the day of testing.

The observation that males were more anxious than females and that most significant hApoE4 deficits were seen within the male sex only initially appears to indicate that the hApoE4 allele affected males more than females. However, these differences may be due to the inability of more than half of the female hApoE4 rats to finish all trials in the attentional set shifting task. Only 4 female hApoE4 rats finished all trials, compared to 8 male hApoE4 rats. Thus, the heterogeneity of the sample sizes likely influenced the appearance of significant differences between these two groups. A more likely interpretation of these findings is that the hApoE4 allele affected females more than males, to the point of lethality. This conclusion is supported by evidence that female mice are more sensitive to hApoE4 than male mice (Grootendorst et al., 2005) and that ApoE4-expressing females are more likely to develop AD than males (Leung et al., 2012).

Because the lethality of the hAPoE4 allele in females may have prevented us from collecting significant results in the attentional set shifting task, we counterfactually created a variable of Status to

see how the rats that were unable to finish all trials performed in earlier tasks. Aligning with the theory that the most impaired rats were those that were unable to finish, male and female hAPoE4 rats that were unable to finish all attentional set shifting trials had a longer escape latency in the spatial learning and memory task, meaning they potentially had deficits in learning and memory. Thus, perhaps the rats that were most significantly affected by the hAPoE4 allele, meaning the rats that were unable to finish all attentional set shifting trials, were also the rats whose hApoE4 allele induced learning and memory deficits. Rats that were unable to finish also traveled at a slower speed in the spatial learning and memory task than those that did finish. Additionally, of the rats that finished all trials, those with the hApoE4 allele were still significantly slower than their wildtype counterparts, indicating the robustness of the difference in motor ability between these two groups.

The addition of the Status variable allowed the conclusion that the hApoE4 allele likely induced debilitating behavioral deficits in rats that were unable to finish all tasks, and had they been able to complete the attentional set shifting tasks, there likely would have been more significant results indicating deficits in cognitive flexibility between groups. Future work should complete tests for executive function prior to tests of spatial learning to better investigate the idea that executive function precedes memory and learning deficits in AD. We did not see significant differences in memory and learning deficits between sexes or genotypes, except for in rats that were unable to complete all trials. These rats likely experienced the most debilitating effects of the hApoE3 knock in, while the effects may not have been severe enough to cause memory and learning deficits in the other rats that finished all trials. There was clear evidence of impairments in cognitive flexibility in rats with the hApoE4 knock-in, however, rendering this an area worthy of more research. Most studies of AD using rodent models are focused on hippocampal cognition and not on executive function, centered in the prefrontal cortex (Levit et al., 2019). The present study reveals that 18 month old hAPoE4 rats appear to have more robust executive function deficits than spatial learning and memory deficits. To strengthen this conclusion, perhaps a future study could complete tests for executive function before tests of spatial learning and memory so as to more clearly see if individual executive function impairments predict spatial learning and memory deficits. Perhaps a

broader sample size could be used as well, knowing how potentially lethal the hApoE4 knock-in can be for female rats. Additionally, neurobiological correlates could be investigated to support the behavioral data found here, where future work could perhaps compare structural prefrontal cortex damage in comparison to hippocampal damage in hAPoE4 rats.

Importantly, the present study successfully assessed attentional set shifting using a plus maze in water. Dry land studies of executive function rely on food deprivation, which can be detrimental to aged rats. Thus, our model for measuring cognitive flexibility should be adopted by future studies assessing executive function in aged rats. We also demonstrated that the hApoE4 allele successfully induces AD symptomology in rat animal models. Transgenic rat models more accurately represent human diseases compared to transgenic mice (Do Carmo & Cuellom, 2013), and the success of our animal model supports the notion that future studies should use hApoE4 rats to study AD pathology.

The findings of the present study indicate that the hApoE4 allele induces sex dependent behavioral impairments. hApoE4 rats had increased anxiety, decreased motor function, decreased executive function, and possibly decreased spatial learning and memory in the subjects most severely affected by the knock-in. The lack of more robust spatial learning and memory deficits may indicate that the executive function deficits observed precede the development of spatial learning and memory deficits, and that those deficits do not emerge in rats until later than 18 months of age. Thus, decreased motor function and decreased cognitive flexibility may be two early indicators of AD, even preceding memory deficits, meaning that future clinical interventions of AD could focus on identifying early motor and executive dysfunctions.

References

Alzheimer's Association (2016). 2016 Alzheimer's disease facts and figures. *Alzheimer's & dementia : the journal of the Alzheimer's Association*, *12*(4), 459–509. https://doi.org/10.1016/j.jalz.2016.03.001

Alzheimer's Association (2012). 2012 Alzheimer's disease facts and figures. *Alzheimer's & dementia : the journal of the Alzheimer's Association*, *8*(2), 131–168. https://doi.org/10.1016/j.jalz.2012.02.001

Bateman, R. J., Xiong, C., Benzinger, T. L., Fagan, A. M., Goate, A., Fox, N. C., Marcus, D. S., Cairns, N. J., Xie, X., Blazey, T. M., Holtzman, D. M., Santacruz, A., et al. (2012). Clinical and biomarker changes in dominantly inherited Alzheimer's disease. *New England Journal of Medicine, 367*(9), 795–804. https://doi.org/10.1056/NEJMoa1202753

Benedikz, E., Kloskowska, E., & Winblad, B. (2009). The rat as an animal model of Alzheimer's disease. *Journal of Cellular and Molecular Medicine, 13*(6): 1034 - 1042. https://doi.org/10.1111/j.1582-4934.2009.00781.x

Bizon, J. L., Foster, T. C., Alexander, G. E., & Glisky, E. L. (2012). Characterizing cognitive aging of working memory and executive function in animal models. *Frontiers in aging neuroscience*, *4*, 19. https://doi.org/10.3389/fnagi.2012.00019

Blennow, K., de Leon, M. J., & Zetterberg, H. (2006). Alzheimer's disease. *Seminar*, *368*(9533), 387-403. https://doi.org/10.1016/S0140-6736(06)69113-7

Buchman, A. S. & Bennett, D. A. Loss of motor function in preclinical Alzheimer's disease. *Expert Review of Neurotherapeutics, 11*(5), 665-676. https://doi.org/10.1586/ern.11.57

Corder, E. H., Saunders, A. M., Strittmatter, W. J., Schmechel, D. E., Gaskell, P. C., Small, G. W., Roses, A. D., Haines, J. L., & Pericak-Vance, M. A. (1993). Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. *Science (New York, N.Y.)*, *261*(5123), 921–923. https://doi.org/10.1126/science.8346443

Dickinson D. W. (1997). The pathogenesis of senile plaques. *Journal of neuropathology and experimental neurology*, *56*(4), 321–339. https://doi.org/10.1097/00005072-199704000-00001

Do Carmo, S., & Cuello, A. C. (2013). Modeling Alzheimer's disease in transgenic rats. *Molecular neurodegeneration*, *8*, 37. https://doi.org/10.1186/1750-1326-8-37

Farrer, L. A., Cupples, L. A., Haines, J. L., Hyman, B., Kukull, W. A., Mayeux, R., Myers, R. H., Pericak-Vance, M. A., Risch, N., & van Duijn, C. M. (1997). Effects of age, sex, and ethnicity on the association between apolipoprotein E genotype and Alzheimer disease. A meta-analysis. APOE and Alzheimer Disease Meta Analysis Consortium. *JAMA*, *278*(16), 1349–1356.

Francis P. T. (2005). The interplay of neurotransmitters in Alzheimer's disease. *CNS spectrums*, *10*(11 Suppl 18), 6–9. https://doi.org/10.1017/s1092852900014164

Games, D., Adams, D., Alessandrini, R., Barbour, R., Berthelette, P., Blackwell, C., Carr, T., Clemens, J., Donaldson, T., & Gillespie, F. (1995). Alzheimer-type neuropathology in transgenic mice overexpressing V717F beta-amyloid precursor protein. *Nature*, *373*(6514), 523–527. https://doi.org/10.1038/373523a0

Green, K. N., Billings, L. M., Roozendaal, B., McGaugh, J. L., & LaFerla, F. M. (2006). Glucocorticoids increase amyloid-β and tau pathology in a mouse model of Alzheimer's disease. *The Journal of Neuroscience, 26*(35), 9047-9056. https://doi.org/10.1523/JNEUROSCI.2797-06.2006

Grootendorst, J., Bour, A., Vogel, E., Kelche, C., Sullivan, P. M., Dodart, J. C., Bales, K., & Mathis, C. (2005). Human ApoE targeted replacement mouse lines: h-apoE4 and h-apoE3 mice differ on spatial memory performance and avoidance behavior. *Behavioral Brain Research*. *159*(1), 1-14. https://doi.org/10.1016/j.bbr.2004.09.019.

Hardy, J., & Selkoe, D. J. (2002). The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. *Science, 297*(5580), 353–356. https://doi.org/10.1126/science.1072994

Harris, F. M., Brecht, W. J., Xu, Q., Tesseur, I., Kekonius, L., Wyss-Coray, T., Fish, J. D., Masliah, E., Hopkins, P. C., Scearce-Levie, K., Weisgraber, K. H., Mucke, L., Mahley, R. W., & Huang, Y. (2003). Carboxyl-terminal-truncated apolipoprotein E4 causes Alzheimer's disease-like neurodegeneration and behavioral deficits in transgenic mice. *Proceedings of the National*

Academy of Sciences of the United States of America, *100*(19), 10966–10971. https://doi.org/10.1073/pnas.1434398100

Iivonen, H., Nurminen, L., Harri, M., Tanila, H., & Puoliväli, J. (2003). Hypothermia in mice tested in Morris water maze. *Behavioral Brain Research, 141*(2), 207-213. https://doi.org/10.1016/s0166-4328(02)00369-8

Jiang, Q., Lee, C. Y., Mandrekar, S., Wilkinson, B., Cramer, P., Zelcer, N., Mann, K., Lamb, B., Willson, T. M., Collins, J. L., Richardson, J. C., Smith, J. D., Comery, T. A., Riddell, D., Holtzman, D. M., Tontonoz, P., & Landreth, G. E. (2008). ApoE promotes the proteolytic degradation of Abeta. *Neuron*, *58*(5), 681–693. https://doi.org/10.1016/j.neuron.2008.04.010

Kraemer, P. J. & Randall, C. K. (1995). Spatial learning in preweanling rats trained in a Morris water maze. *Psychobiology, 23*(2), 144-152. https://doi.org/10.3758/BF03327070

La Ferla, F. M. & Oddo, S. (2005). Alzheimer's disease: Abeta, tau and synaptic dysfunction. *Trends in Molecular Medicine, 11*(4), 170-176. https://doi.org/10.1016/j.molmed.2005.02.009

Leung, L., Andrews-Zwilling, Y., Yoon, S. Y., Jain, S., Ring, K., Dai, J., Wang, M. M., Tong, L., Walker, D., & Huang, Y. (2012). Apolipoprotein E4 causes age- and sex-dependent impairments of hilar GABAergic interneurons and learning and memory deficits in mice. *PloS one*, *7*(12), e53569. https://doi.org/10.1371/journal.pone.0053569

Levit, A., Regis, A. M., Gibson, A., Hough, O. H., Maheshwari, S., Agca, Y., Agca, C., Hachinski, V., Allman, B. L., & Whitehead, S. N. (2019). Impaired behavioral flexibility related to white matter microgliosis in the TgAPP21 rat model of Alzheimer disease. *Brain, Behavior, and Immunity*, *80*, 25-34. https://doi.org/10.1016/j.bbi.2019.02.013

Liu, C. C., Kanekiyo, T. Xu, H. & Bu, G. (2013). Apoliprotein E and Alzheimer disease: risk, mechanisms, and therapy. *Nature Reviews Neurology, 9*(2): 106-118. https://doi.org/10.1038/nrneurol.2012.263.

Ma, L., Xu, Y., Wang, G., & Li, R. (2019). What do we know about sex differences in depression: A review of animal models and potential mechanisms. *Progress in Neuro-Psychopharmacology and Biological Psychiatry. 89*(8), 48-56. https://doi.org/10.1016/j.pnpbp.2018.08.026.

Mahley, R., Weisgraber, K., & Huang, Y. (2006). Apolipoprotein E4: A Causative Factor and Therapeutic Target in Neuropathology, including Alzheimer's Disease. *Proceedings of the National Academy of Sciences of the United States of America, 103*(15), 5644-5651. Retrieved from http://www.jstor.org/stable/30050154

Mandelkow, E. M., Stamer, K., Vogel, R., Thies, E., & Mandelkow, E. (2003). Clogging of axons by tau, inhibition of axonal traffic and starvation of synapses. *Neurobiology of aging*, *24*(8), 1079–1085. https://doi.org/10.1016/j.neurobiolaging.2003.04.007

Masters, C. L., Simms, G., Weinman, N. A., Multhaup, G., McDonald, B. L., & Beyreuther, K. (1985). Amyloid plaque core protein in Alzheimer disease and Down syndrome. *Proceedings of the National Academy of Sciences of the United States of America*, *82*(12), 4245–4249. https://doi.org/10.1073/pnas.82.12.4245

Mielke M. M. (2018). Sex and Gender Differences in Alzheimer's Disease Dementia. *The Psychiatric times*, *35*(11), 14–17.

Morris, J. C., Roe, C. M., Xiong, C., Fagan, A. M., Goate, A. M., Holtzman, D. M., & Mintun, M. A. (2010). APOE predicts amyloid-beta but not tau Alzheimer pathology in cognitively normal aging. *Annals of neurology*, *67*(1), 122–131. https://doi.org/10.1002/ana.21843

Namba, Y., Tomonaga, M., Kawasaki, H., Otomo, E., & Ikeda, K. (1991). Apolipoprotein E immunoreactivity in cerebral amyloid deposits and neurofibrillary tangles in Alzheimer's disease and kuru plaque amyloid in Creutzfeldt-Jakob disease. *Brain research*, *541*(1), 163–166. https://doi.org/10.1016/0006-8993(91)91092-f

Pentkowski, N. A., Berkowitz, L. E., Thompson, S. M., Drake, E. N., Olguin, C. R., Clark, B. J. (2018). Anxiety-like behaviors as an early endophenotype in the TgF344-AD rat model of Alzheimer’s

disease. *Neurobiology of Aging, 61,* 169-176. https://doi.org/10.1016/j.neurobiolaging.2017.09.024

Raber, J., Akana, S. F., Bhatnagar, S., Dallman, M. F., Wong, D., & Mucke, L. (2000). Hypothalamic-pituitary-adrenal dysfunction in Apoe(-/-) mice: possible role in behavioral and metabolic alterations. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, *20*(5), 2064–2071. https://doi.org/10.1523/JNEUROSCI.20-05-02064.2000

Raber, J., Wong, D., Yu, G. Q., Buttini, M., Mahley, R. W., Pitas, R. E., & Mucke, L. (2000). Apolipoprotein E and cognitive performance. *Nature*, *404*(6776), 352–354. https://doi.org/10.1038/35006165

Scearce-Levie, K. (2011). Monitoring spatial learning and memory in Alzheimer's disease mouse models using the Morris Water Maze. *Methods in Molecular Biology, 670*, 191-205. https://doi.org/10.1007/978-1-60761-744-0_14.

Selkoe D. J. (2001). Alzheimer's disease: genes, proteins, and therapy. *Physiological reviews*, *81*(2), 741–766. https://doi.org/10.1152/physrev.2001.81.2.741

Shin, J., Lee, S. Y., Kim, A. H., Kim, Y. B., & Cho, S. J. (2008). Multitracer PET imaging of amyloid plaques and neurofibrillary tangles in Alzheimer's disease. *NeuroImage, 43*(2), 236-244. https://doi.org/10.1016/j.neuroimage.2008.07.022

Strittmatter, W. J., Saunders, A. M., Schmechel, D., Pericak-Vance, M., Enghild, J., Salvesen, G. S., & Roses, A. D. (1993). Apolipoprotein E: high-avidity binding to beta-amyloid and increased frequency of type 4 allele in late-onset familial Alzheimer disease. *Proceedings of the National Academy of Sciences of the United States of America*, *90*(5), 1977–1981. https://doi.org/10.1073/pnas.90.5.1977

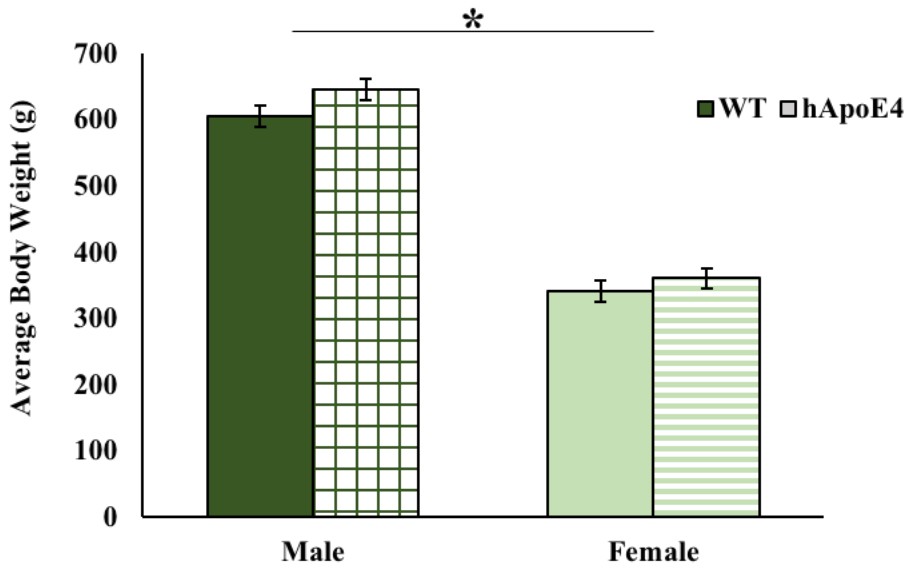
Trommer, B. L., Shah, C., Yun, S. H., Gamkrelidze, G., Pasternak, E. S., Ye, G. L., Sotak, M., Sullivan, P. M., Pasternak, J. F., & LaDu, M. J. (2004). ApoE isoform affects LTP in human targeted replacement mice. *Neuroreport*, *15*(17), 2655–2658. https://doi.org/10.1097/00001756-200412030-00020

Wagner, J. M., Sichler, M. E., Schleicher, E. M., Franke, T. N., Irwin, C., Löw, M. J., Beindorff, N., Bouter, C., Bayer, T. A., & Bouter, Y. (2019). Analysis of Motor Function in the Tg4-42 Mouse Model of Alzheimer's Disease. *Frontiers in behavioral neuroscience*, *13*, 107. https://doi.org/10.3389/fnbeh.2019.00107

Waite, L. M., Grayson, D. A., Piguet, O., Creasey, H., Bennett, H. P., & Broe, G. A. (2005). Gait slowing as a predictor of incident dementia: 6-year longitudinal data from the Sydney Older Persons Study. *Journal of the Neurological Sciences, 229*(230), 89-93. https://doi.org/10.1016/j.jns.2004.11.009

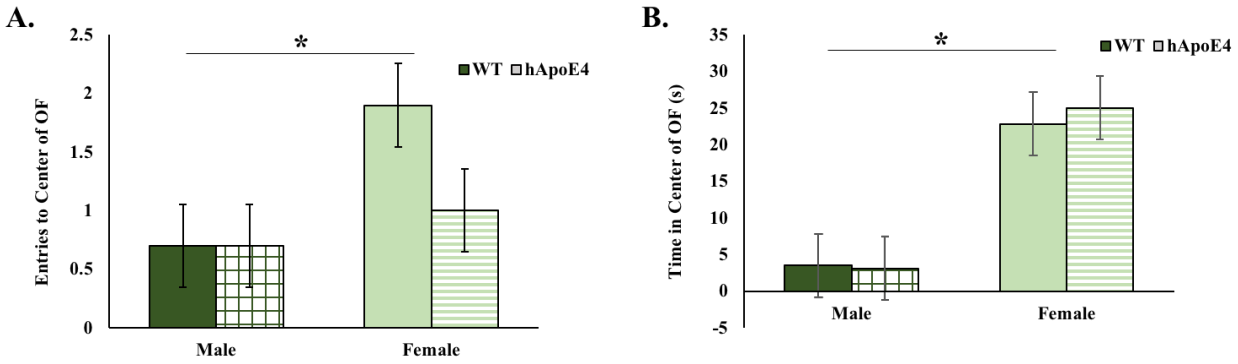
Weller, J. & Budson, A. (2018) Current understanding of Alzheimer's disease diagnosis and treatment. *F1000 Research, 7*: 1161. https://doi.org/10.12688/f1000research.14506.1

Figure 1.



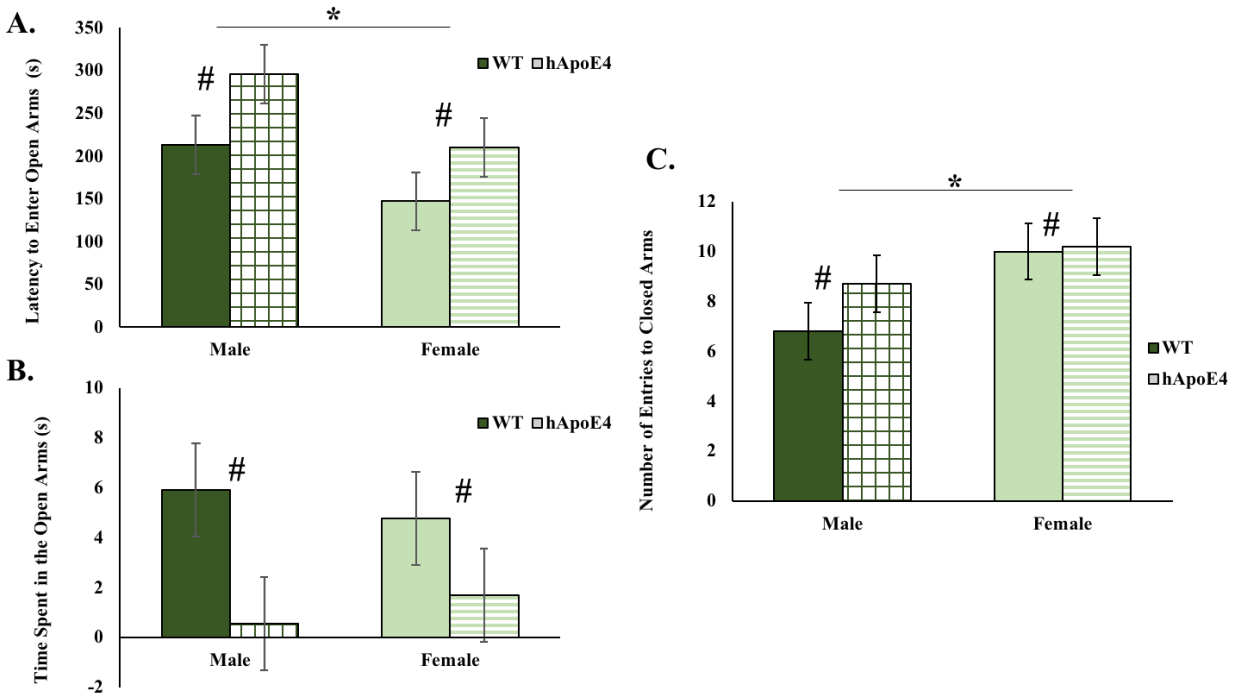
Note. Average body weight of subjects from 18-19 months of age. Male rats weighed significantly more than female rats. * refers to significant difference in sex, $p < 0.05$, n = 10 per group.

Figure 2.



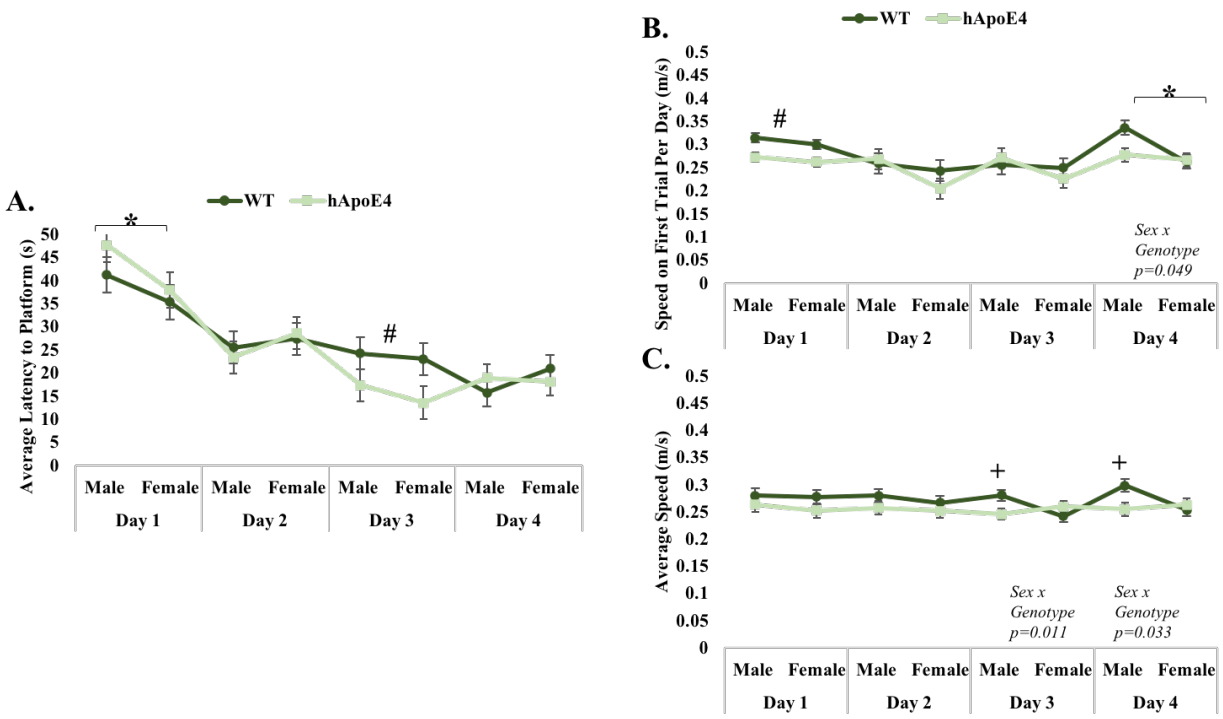
Note. Number of entries to and time in the center of the open field. (A) Male rats entered the center of the open field significantly less times than female rats. (B) Male rats spent significantly less time in the center of the open field than female rats. * refers to significant difference in sex, $p < 0.05$, n = 10 per group.

Figure 3.



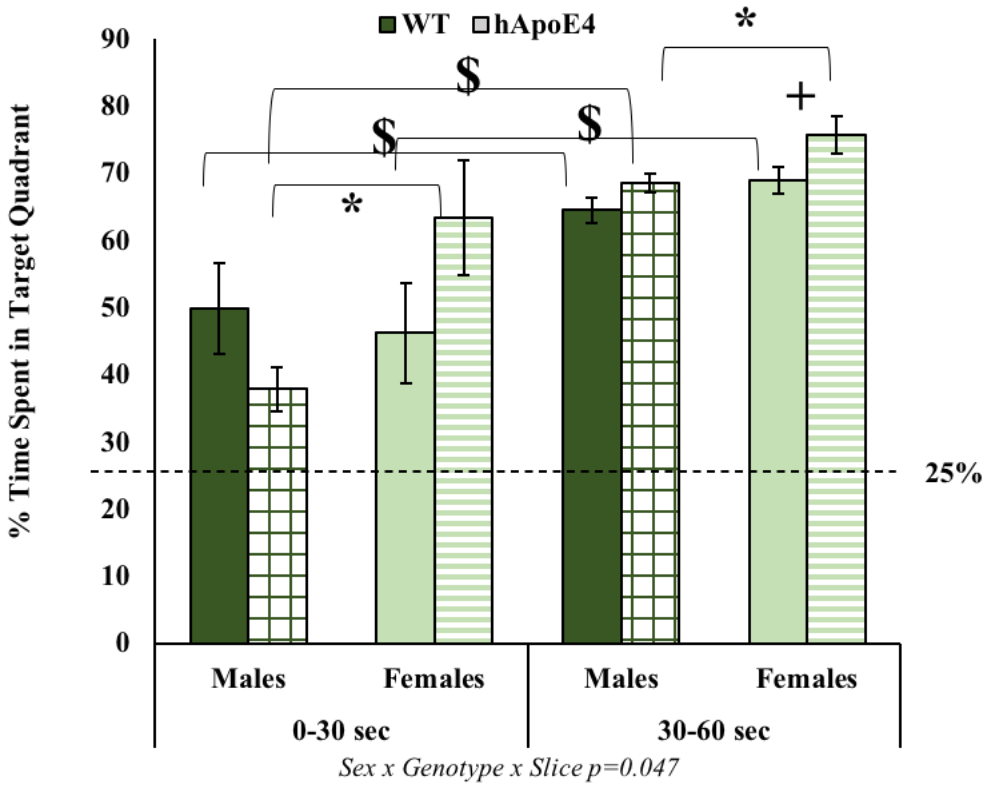
Note. Latency to and time spent in the open arms and number of entries to the closed arms of the elevated plus maze. (A) Males took significantly more time to enter the open arms than female rats and wildtype rats entered the open arms significantly faster than hApoE4 rats. (B) Wildtype rats spent significantly more time in the open arms than hApoE4 rats. (C) Males entered the closed arms significantly less times than females. Wildtype rats entered the closed arms significantly less times than hApoE4 rats. * refers to significant difference in sex regardless of genotype; # refers to significant difference in genotype regardless of sex, $p < 0.05$, n = 10 per group.

Figure 4.



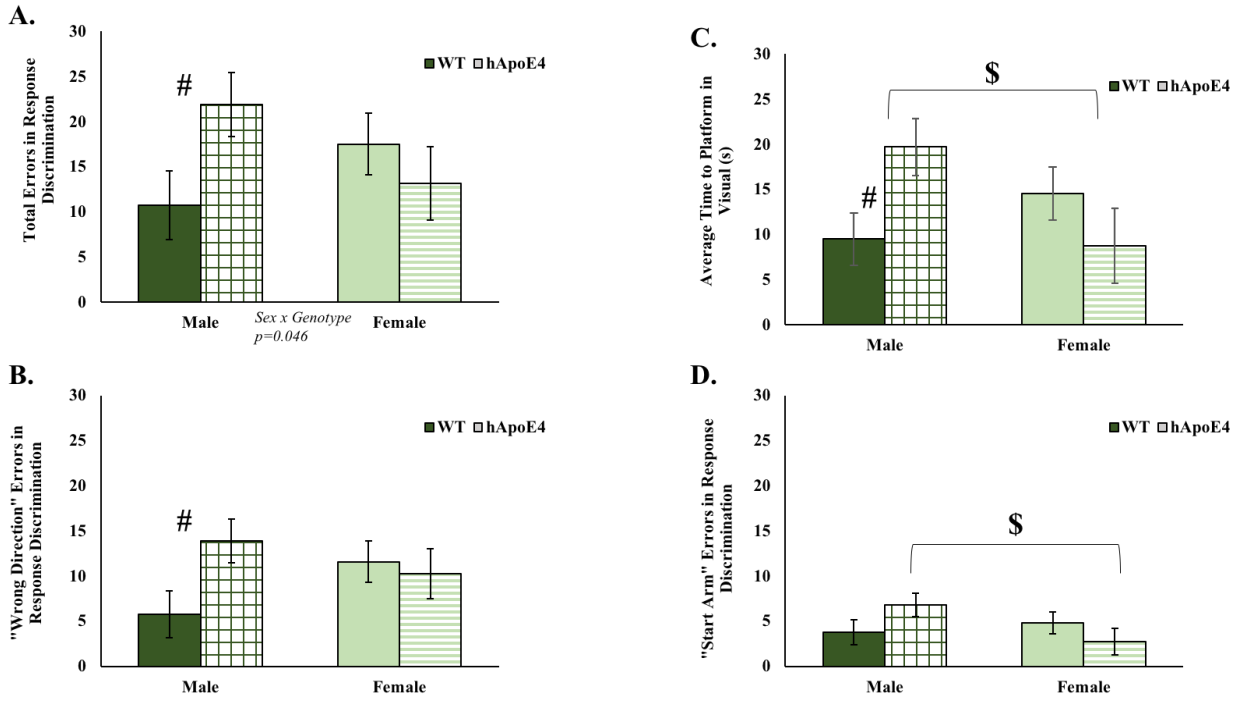
Note. Escape latency, speed on the first trial per day, and average speed per day during the spatial learning and memory task. (A) Males had a significantly longer escape latency than females on Day 1 of testing. WT rats had a significantly longer escape latency than hApoE4 rats on day 3. (B) Males were significantly faster than females on the first trial of Day 4. Wildtype rats traveled significantly faster than hApoE4 rats on the first trial of Day 1.(C) Male wildtype rats traveled significantly faster than male hApoE4 rats on Day 3 and on Day 4. * refers to significant difference in sex regardless of genotype; # refers to significant difference in genotype regardless of sex, + refers to a significant difference in genotype within one sex, $p < 0.05$, n = 10 per group.

Figure 5.



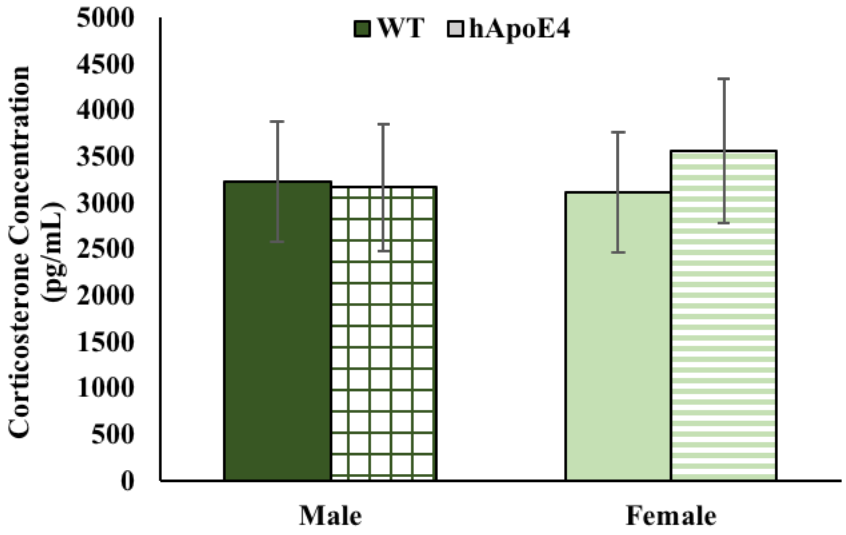
Note. Percent of time spent in the target quadrant of the probe trial in the spatial learning and memory task. Female wildtype rats spent significantly less time than female hApoE4 rats in the second 30-second slice. All male wildtype rats spent significantly less time in the target quadrant in the first slice compared to the second, as did male hApoE4 rats and female wildtype rats. Male hApoE4 rats spent significantly less time in the target quadrant than female hApoE4 rats for both the first and second slices. * refers to significant difference in sex regardless of genotype, $ refers to significant difference in sex within a genotype; + refers to significant difference in genotype within a sex, $p < 0.05$, n = 10 per group.

Figure 6.



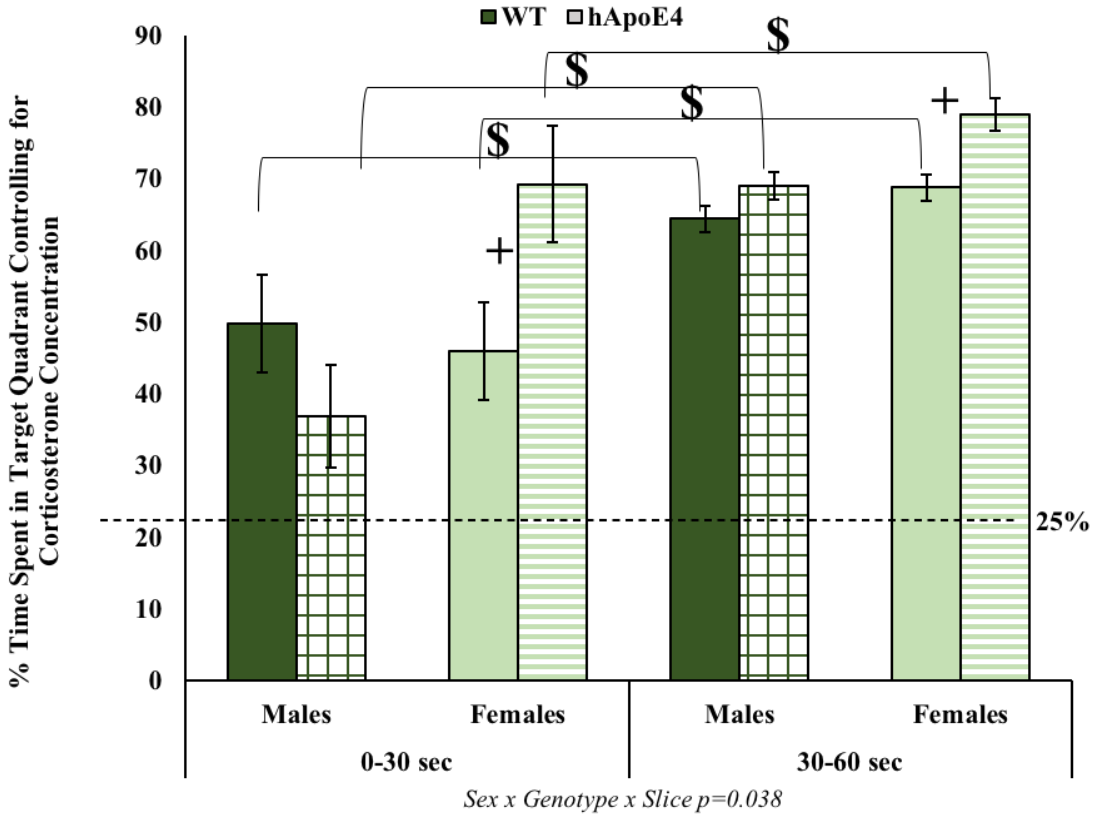
Note. Total errors, "wrong direction" errors, and "start arm" errors in the response discrimination task and average time to platform in the visual task. (A) Male wildtype rats made significantly fewer errors than male hApoE4 rats. (B) Male wildtype rats made significantly fewer "wrong direction" errors than male hApoE4 rats. (C) Male wildtype rats reached the platform significantly slower than male hApoE4 rats. Male hApoE4 rats reached the platform significantly slower than female hApoE4 rats. (D) Male hApoE4 rats made significantly more start arm errors than female hApoE4 rats. * refers to significant difference in sex regardless of genotype; # refers to significant difference in genotype regardless of sex, $ refers to significant difference in sex within a genotype, $p < 0.05$, Response Discrimination: n = 8 male wildtype, n = 9 male hApoE4, n = 10 female wildtype, n = 7 female hApoE4. Visual: n = 8 male wildtype, n = 7 male hApoE4, n = 8 female wildtype, n = 4 female hApoE4

Figure 7.



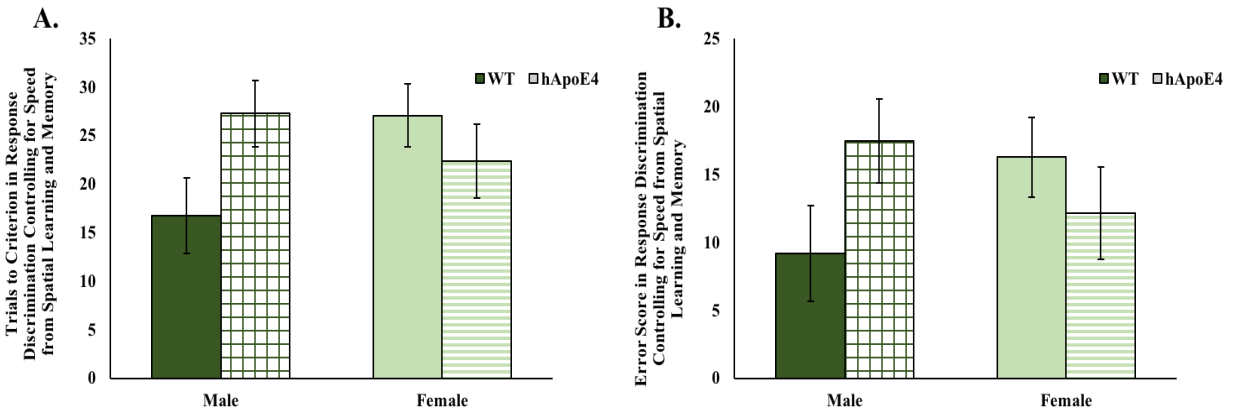
Note. Concentration of corticosterone. There was no significant differences, $p < 0.05$, n = 10 male wildtype, n = 9 male hApoE4, n = 10 female wildtype, n = 7 female hApoE4

Figure 8.



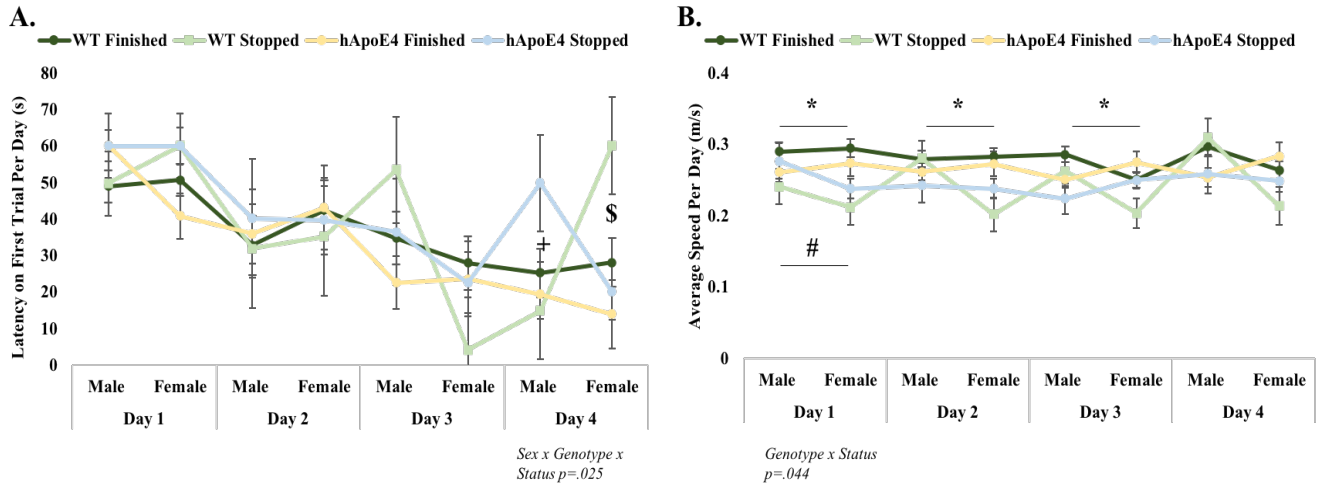
Note. Percent of time spent in the target quadrant with corticosterone concentration as a covariate. Male wildtype rats spent significantly more time in the target quadrant during the second 30-second slice compared to the first. Male hApoE4 rats also spent significantly more time in the target quadrant during the second 30-second slice than the first, as did female wildtype rats and female hApoE4 rats. Female wildtype rats spent significantly less time in the target quadrant compared to female hApoE4 rats in both slices. $ refers to significant difference in sex within a genotype; + refers to significant difference in genotype within a sex, $p < 0.05$, n = 10 male wildtype, n = 9 female hApoE4, n = 10 female wildtype, n = 7 female hApoE4

Figure 9.



Note. Trials to criterion and errors score in the response discrimination stage with speed from the spatial learning and memory task as a covariate. (A) There were no significant differences. (B) There were no significant differences, $p < 0.05$, n = 9 male wildtype, n = 9 male hApoE4, n = 10 female wildtype, n = 8 female hApoE4

Figure 10.



Note. Latency to the first trial per day and average speed per day of the spatial learning and memory task with Status as an independent variable. (A) Male hApoE4 rats that were unable to finish all tasks had a significantly longer escape latency than male hApoE4 rats that were able to finish all tasks on day 4. Female wildtype rats that were unable to finish all tasks had a significantly longer escape latency than female wildtype rats that were able to finish all tasks on day 4. (B) Rats that were unable to finish all tasks traveled more slowly than rats that finished all tasks on days 1, 2, and 3. Wildtype rats that finished all trials traveled significantly faster than hApoE4 rats that finished all trials. * refers to a significant difference in status regardless of sex or status, # refers to significant difference in genotype regardless of sex or status, + refers to significant difference in status within sex and status, $p < 0.05$, n = 2 male wildtype stopped, n = 8 male wildtype finished, n = 2 male hApoE4 stopped, n = 8 male hApoE4 finished, n = 2 female wildtype stopped, n = 8 female wildtype finished, n = 6 female hApoE4 stopped, n = 4 female hApoE4 finished