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The detrimental effects of *APOE4* on risk for Alzheimer's disease may result from altered dendritic spine density, synaptic proteins, and estrogen receptor alpha

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ABSTRACT

Women carriers of *APOE4*, the greatest genetic risk factor for late-onset Alzheimer's disease (AD), are at highest risk of developing AD, yet factors underlying interactions between *APOE4* and sex are not well characterized. Here, we examined how sex and *APOE3* or *APOE4* genotypes modulate object and spatial memory, dendritic spine density and branching, and protein expression in 6-month-old male and female E3FAD and E4FAD mice ($APOE^{+/+}$/5xFAD$^{+/-}$). *APOE4* negatively impacted object recognition and spatial memory, with male E3FADs exhibiting the best memory across 2 object-based tasks. In both sexes, *APOE4* reduced basal dendritic spine density in the medial prefrontal cortex and dorsal hippocampus. *APOE4* reduced dorsal hippocampal levels of PDS-95, synaptophysin, and phospho-CREB, yet increased levels of ERα. E4FAD females exhibited strikingly increased GFAP levels, in addition to the lowest levels of PSD-95 and pCREB. Overall, our results suggest that *APOE4* negatively impacts object memory, dendritic spine density, and levels of hippocampal synaptic proteins and ERα. However, the general lack of sex differences or sex by genotype interactions suggests that the sex-specific effects of *APOE4* on AD risk may be related to factors unexplored in the present study.

1. Introduction

Alzheimer's disease (AD) is the most common form of dementia, yet its cause remains unclear and no effective treatments exist. The high incidence of AD, coupled with its devastating health and economic impacts, highlight the urgent need for continued research into the etiology of this disease (Ernst and Hay, 1994; Rice et al., 1993). *APOE4* genotype is the primary genetic risk factor for late onset AD, contributing up to 15-fold greater risk of AD than the more common *APOE3* genotype (Bertram, 2009; Roses, 1996; Ward et al., 2012). Additionally, *APOE4* genotype acts synergistically with female sex, another major risk factor (Andersen et al., 1999; Launer et al., 1999; Nebel et al., 2018), to further increase risk for AD in women (Altmann et al., 2014; Bretsky et al., 1999; Farrer et al., 1997; Kim et al., 2009). This combination of *APOE4* and female sex contributes to magnified memory deficits, a behavioral hallmark of AD (Mortensen and Høgh, 2001). Understanding why *APOE4* genotype is particularly detrimental to female carriers, and the mechanisms underlying exacerbated memory dysfunction in these individuals, is of central importance to developing new approaches for AD prevention and treatment.

Synaptic dysfunction is a principal component of AD pathology that underlies AD-associated cognitive decline (DeKosky and Scheff, 1990; LaFerla and Oddo, 2005). AD patients experience both pre- and post-synaptic protein loss, particularly in the neocortex and hippocampus, brain regions selectively vulnerable to both AD pathology and neuronal loss (Masliah et al., 2001; Reddy et al., 2005). Increased synaptic protein loss has been linked to *APOE4*

Abbreviations: 4EBP1, eukaryotic translation initiation factor 4E binding protein 1; AD, Alzheimer's disease; ANOVA, analysis of variance; CREB, cyclicAMP response-element binding protein; EFAD, $APOE^{+/+}$/5xFAD$^{+/-}$; ERα, estrogen receptor alpha; ERK, extracellular signal-regulated kinase; GFAP, glial fibrillary acidic protein; Iba1, Ionized calcium binding adaptor molecule 1; JNK, c-Jun N-terminal kinase; mPFC, medial prefrontal cortex; MWM, Morris water maze; OF, open field; OP, object placement; OR, object recognition; PI3K, phosphatidylinositide 3-kinase; PSD95, post-synaptic density 95; Tg, transgenic; sec, seconds; veh, vehicle.

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genotype (Tannenberg et al., 2006), and decreased dendritic spine density in *APOE4*+ individuals mirrors similar decreases in synaptic proteins (Dumanis et al., 2009; Ji et al., 2003). Because excitatory signaling is thought to be localized to dendritic spines, AD-related reductions in dendritic arborization and spine density likely substantially diminish excitatory synaptic plasticity and impair cognition (Herms and Dorostkar, 2016). However, few studies have directly compared the influences of sex and *APOE4* genotype in combination on synaptic protein levels and spine morphology. Indeed, the influence of sex alone on synaptic protein levels and spine morphology in AD patients and mouse models remains unclear, although existing studies suggest synaptic integrity is reduced in females (Jiao et al., 2016; Rijpma et al., 2013). Thus, whether the negative impact of *APOE4* on synaptic integrity and dendritic spine density is exacerbated by female sex remains unclear.

Mitogen associated protein kinase (MAPK) signaling through phosphorylated extracellular signal-regulated kinase (pERK) facilitates hippocampus-dependent learning and memory (Atkins et al., 1998; Selcher et al., 1999), making it an appealing target for cognitive therapeutics targeting memory dysfunction. Moreover, MAPK signaling interfaces with many other signaling cascades such as phosphatidylinositol 3-kinase (PI3K) and c-Jun N-terminal kinase (JNK) within the hippocampus to enhance memory consolidation in both males and females (Koss and Frick, 2017; Sherrin et al., 2011). Previous work indicates that MAPK signaling is differentially affected by *APOE* genotype (Salomon-Zimri et al., 2019) and by sex (Koss et al., 2018). Elucidating whether these memory-related signaling pathways are modulated by *APOE* genotype and sex may yield important insights into the pathogenesis of memory dysfunction in AD.

In addition to aberrant synapse and cell signaling, neuroinflammation has long been observed to be an early symptom in AD pathology, driven by both astrogliosis and microgliosis (Heneka et al., 2015). Importantly, female mice have significantly higher numbers of both astrocytes and microglia relative to male mice, and this cell population increases with age (Mouton et al., 2002). Moreover, microglia-specific gene expression differs markedly by sex in the aged mouse hippocampus (Mangold et al., 2017). Yet, as with synaptic integrity and intracellular signaling, little is known about how glial activation in the AD brain is modulated by female sex and *APOE4* genotype in concert.

The present study was designed to interrogate the mechanisms underlying aberrant cognitive decline linked to both *APOE4* genotype and female sex in the well-characterized EFAD mouse model of Alzheimer's disease (Liu et al., 2015; Tai et al., 2017; Youmans et al., 2012). EFAD-transgenic (Tg) mice ($APOE^{+/+}$/5xFAD$^{+/-}$) express 5 familial AD (FAD) mutations (5xFAD) and human *APOE3* (E3FAD) or *APOE4* (E4FAD), making them an ideal model for investigating the relative contribution of sex in an AD-like phenotype, as well as the differences conferred by either *APOE3* or *APOE4* genotypes (Youmans et al., 2012). To examine potential influences of sex and *APOE* genotype against a background of AD-like pathology, male and female E3FAD and E4FAD mice were tested in object recognition (OR), object placement (OP), and Morris Water Maze (MWM) tasks to assess the relative and synergistic contributions of sex and *APOE3* or *APOE4* genotypes to memory processes. As aberrant cell signaling is implicated in AD, we examined how sex, *APOE3*, or *APOE4* genotype modulate memory-related cell signaling and membrane proteins in the dorsal hippocampus, given the vulnerability of this memory-linked brain region to AD pathology (Hyman et al., 1984). Hippocampal astroglial and microglial proteins were also examined given evidence that glial reactivity is linked to AD (Meda et al., 2001). Because AD results in a substantial loss of excitatory synapses in the hippocampus and medial prefrontal cortex (mPFC; (Masliah et al., 2006; Selkoe, 2003), we also quantified pyramidal neuron dendritic spine density and branching in the CA1 region of the dorsal hippocampus and the prelimbic/infralimbic region of the mPFC. We hypothesized that E4FAD mice would exhibit impaired memory, reduced hippocampal levels of synaptic, membrane, and cell-signaling proteins, increased hippocampal levels of glial proteins, and decreased dendritic spine density and branching in the dorsal hippocampus and medial prefrontal cortex. Given the increased prevalence of AD in women *APOE4* carriers and the extensive AD pathology evident in female E4FAD mice (Farrer et al., 1997; Hohman et al., 2018), we expected that female E4FAD mice would exhibit the greatest memory impairments, followed by E4FAD males, E3FAD females, and finally E3FAD males. The results suggest that E3FAD males are the most resistant to cognitive decline, and support a negative impact of *APOE4* alone, but not in concert with sex, on memory, synaptic proteins, and dendritic spine density.

2. Materials and methods

2.1. Subjects

Male and female EFAD ($APOE^{+/+}$/5xFAD$^{+/-}$) mice co-express 5 familial AD mutations (APP K670N/M671L + I716V + V717I and PS1 M146L + L286V) under control of the neuron-specific mouse Thy-1 promoter, and are homozygous for human *APOE3* or *APOE4* (Youmans et al., 2012). EFAD mice were bred, weaned, and genotyped at the University of Illinois Chicago (UIC; Animal use protocol 17-066) before shipment to the University of Wisconsin-Milwaukee (UWM) at 2 months of age, where they were aged to 6 months before the start of behavioral testing (animal use protocol 19-20-03). At both UIC and UWM, mice were housed in groups of up to 5 per cage and maintained on a 12-hour light/dark cycle with *ad libitum* access to food and water. Mice were received from UIC and behaviorally tested in 2 separate cohorts, whereas brain analyses for all mice were conducted at the same time. Procedures followed the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* and were approved by the UIC Animal Care Committee and UWM Institutional Animal Care and Use Committee.

2.2. General experimental design

At 6 months of age, male and female mice were tested in a battery of commonly used hippocampus-dependent memory tasks including OR, OP, and the MWM (Fig. 1). The order of OR and OP testing was counter-balanced across groups, such that half of the mice underwent OR first, whereas the other half underwent OP first to eliminate any order-of-testing effects. Mice were then tested in the MWM. Two weeks after the completion of all behavioral testing, brains were extracted and hemisected for Golgi analysis and Western blotting experiments. The dorsal hippocampus was immediately dissected from the whole brain or from the left hemisphere and frozen at -80°C for Western blotting (n = 9–16/group), and right hemispheres were collected for Golgi staining and morphological analysis (n = 5–7/group). These sample sizes are sufficiently powered to detect between group differences.

2.3. Behavioral testing

2.3.1. Object recognition and Object placement

To determine whether sex works differentially or synergistically with *APOE3* or *APOE4* genotype to influence memory for a previously seen object or location, object recognition and spatial memory were tested in male and female E3FAD and E4FAD mice

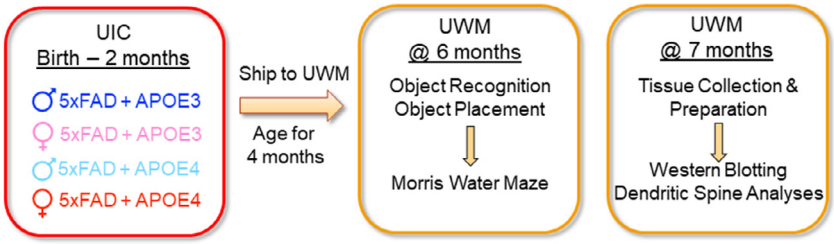

Fig. 1. Experimental timeline. Mice were bred at the University of Illinois Chicago (UIC) and were then sent to the University of Wisconsin-Milwaukee (UWM) at 8 weeks of age, where they were aged for 4 months prior to the start of testing. At 6 months of age, mice were tested in the object recognition and object placement tasks, followed by Morris water maze testing. At 7 months of age, brain tissue was collected and analyzed.

(n = 12–16/group) using the OR and OP tasks, as described previously (Boulware et al., 2013; Fernandez et al., 2008; Fortress et al., 2013b; Kim et al., 2016; Koss et al., 2018; Taxier et al., 2019; Tuscher et al., 2016b). Briefly, mice were handled for 30 seconds /day for 3 days and then habituated to the empty testing arena for 5 minutes/day for 2 consecutive days. On the training day, mice were returned to the empty arena for 2 minutes, and then were removed to a holding cage, during which time 2 identical objects were placed 5 cm from the upper left and right corners of the arena. Upon their return to the arena, mice freely interacted with the objects until they accumulated 30 seconds of object exploration (or until 20 minutes elapsed). Mice were then returned to their home cage for 24 hours or 4 hours to await testing in OR and OP, respectively; wild-type mice exhibit intact memory for the identity (in OR) and location (in OP) of objects at these timepoints (Boulware et al., 2013; Fortress et al., 2013b; Kim et al., 2016).

During OR testing, one familiar training object was replaced with a novel object. Mice again freely interacted with the objects until they accumulated 30 seconds of object exploration. Mice that remember the familiar training object spend more time than chance (15 seconds) with the novel object during testing. During OP testing, one training object was moved to a new location (bottom left or right corner). Mice that remember the location of the training objects spend more time than chance (15 seconds) with the moved object during testing. Time spent with the objects and time to accumulate 30 seconds were recorded using ANYmaze automated tracking software (San Diego Instruments).

2.3.2. Morris water maze

Spatial memory was also tested using the MWM, as described previously (Benoit et al., 2015; Harburger et al., 2007). Data were recorded using ANYmaze software. A 4-trial shaping procedure was conducted one day prior to testing to acclimate the mice to escaping onto a 10 × 10 cm platform. Spatial memory was then tested in 5 consecutive daily sessions consisting of 6 trials/day. The first 5 trials were hidden platform trials during which the escape platform was located 1.5 cm below the water's surface. Mice were allowed 60 seconds to find the platform. The sixth trial was a 60 seconds probe trial in which memory was tested in the absence of the platform, which was lowered for the first 30 seconds, then raised and available for escape for the remainder of the trial to discourage extinction of escape behavior. The platform remained in the same place throughout testing and the intertrial interval was 10–20 minutes. During hidden platform trials, swim time (sec), swim distance to the platform location (cm), and swim speed (cm/sec) were recorded using ANYmaze. During probe trials, the % time in the correct quadrant (containing the platform), time to first entry into the correct quadrant (s), average distance from the platform (cm), and # of platform crossings during the first 30 seconds of the probe trial were measured.

One day after spatial testing, mice were tested in a cued version of the task in which the escape platform was raised above the surface of the water and made visible with red tape. Because memory is not necessary to locate the platform, this task assesses motivation, visual ability, and swimming ability. Six trials/day were conducted for 3 consecutive days during which the platform location changed for each trial. Swim time, swim distance, and swim speed were recorded.

2.4. Western blotting

Western blotting was used to determine the extent to which sex and *APOE* genotype alter the expression of several categories of proteins in the dorsal hippocampus 5 minutes after training with novel objects. This brain region is of particular interest due to abundant evidence that integrity of hippocampal function is compromised in AD, and previous work using the EFAD model suggesting that sex and *APOE* genotype may modulate protein expression within this brain region (Liu et al., 2015).

Western blotting was conducted as described previously (Boulware et al., 2013; Fernandez et al., 2008; Fortress et al., 2013b; Kim et al., 2016; Koss et al., 2018; Taxier et al., 2019; Zhao et al., 2012). Tissue samples were suspended 1:25 w/v in hypotonic lysis buffer and homogenized. Homogenates were electrophoresed on 10% TGX (Tris-Glycine eXtended) stain-free precast gels (Bio-Rad) and transferred to polyvinylidene fluoride membranes; protein transfer was verified using a ChemiDoc MP gel imager (Bio-Rad). After blocking in 5%–8% milk, membranes were incubated overnight at 4°C primary antibodies (Supplemental Table 1). The following day, blots were incubated at room temperature with the appropriate secondary antibody (Supplemental Table 1). Blots were developed using Clarity Max chemiluminescent substrate (Bio-Rad) and protein expression detected using a ChemiDoc MP gel imager. Densitometry was performed using Image Lab software (Bio-Rad Image Lab v 5.2). Phosphorylated proteins were normalized to their respective total proteins. The remaining blots were stripped and reprobed for β-Actin (#4967, 1:1000, Cell Signaling Technology) for protein normalization. Data were expressed as average volume intensity as a percentage compared to male E3FADs.

2.5. Golgi impregnation and analyses

Left hemispheres (n = 5–7/group) were collected for Golgi impregnation, which was performed as described previously (Frankfurt et al., 2011; Kim et al., 2019; Tuscher et al., 2016a) using the Rapid Golgi Stain Kit (FD Neuro Technologies). Tissue was sliced into 100 μm sections and mounted on gelatin-coated microscope slides, which were coded so that the individual counting spines was blind to treatment.

Secondary basal dendrites and tertiary apical dendrites were counted from pyramidal neurons in the dorsal hippocampal CA1

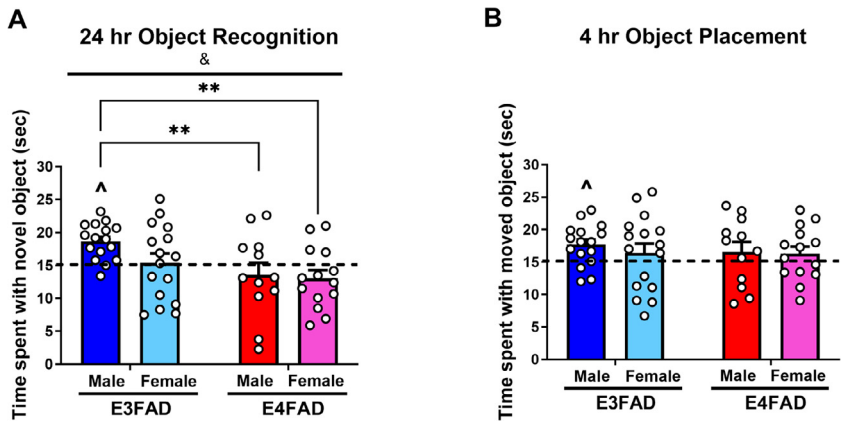

Fig. 2. All mice but male E3FADs displayed impaired object recognition and spatial memory formation. Only male E3FADs exhibited intact memory for the identity (A) and location (B) of the training objects. Male E3FADs spent significantly more time than chance (dashed line at 15 sec; $^{\wedge}p < 0.05$) with the novel (A) and moved (B) objects during testing. E3FADs spent more time than E4FADs with the novel object ($^{\&}p < 0.05$ = main effect of genotype), and male E3FADs spent significantly more time than E4FADs of either sex with the novel object ($^{**}p < 0.01$). Bars represent mean ± standard error of the mean (SEM).

and layer II/III of the mPFC under an Olympus BX51WI microscope (100x with oil) using NeuroLucida (v 11.08, MBF Bioscience). Selected dendrites were between 10 and 20 μm in length and 0.5–1.3 μm thick. Neurons selected for analysis were required to have well impregnated cell bodies and dendrites, and had to be clearly distinguishable from adjacent cells. Two dendritic segments/neuron and 6 cells/region were included in the analysis. Spine density was calculated as the number of spines/10 μm dendrite.

Dendritic spines were identified according to 3 categories based on shape: mushroom, stubby, or thin (Harris et al., 1992; Kim et al., 2019). Mushroom spines had head diameters at least twice the size of their neck diameters, whereas stubby spines had neck diameters relatively equal to the total length of the spine. Thin spines had head diameters less than or equal to their neck diameters.

CA1 dendritic complexity was quantified using Sholl analysis under an Olympus BX51WI microscope (100x with oil). Cell bodies, apical dendrites, and basal dendrites were traced using NeuroLucida. Neurons selected for tracing had to be present within the middle thickness of the section, be fully impregnated, and have at least 3 primary basilar dendrites, each of which needed to branch at least once. Concentric spheres were used to count the number of intersections made by dendritic branches at successive 10 μm steps from the cell body. The number of intersections made by dendritic branches at increasing diameters from the soma served as a measure of dendritic complexity (Sholl, 1953). Sholl analysis was attempted in the mPFC but was not completed because Golgi stain penetrance was not as robust as in CA1, and therefore was not reflective of full branching complexity.

2.6. Statistical analyses

Statistical analyses were performed using GraphPad Prism 9. For OR and OP, one-sample *t* tests were used to determine whether the time spent with each object differed from chance (15 seconds), which assesses within-group learning. Between-group differences were assessed using 2-way ANOVAs with sex and genotype as between-subject variables. Similar 2-way ANOVAs were used to analyze Western blot and dendritic spine density data. For the MWM, 2-way repeated measures ANOVAs, with sex and genotype as between-subject variables, and session as the repeated measure, were conducted to evaluate within and between-group differences in learning across days. For Sholl analysis, 2-way repeated measures ANOVAs, with sex and genotype as between-subject variables and distance from the cell soma as the repeated measure, were conducted to evaluate within and between-group differences in dendritic complexity. Significant main effects were followed by planned comparisons where appropriate, given our *a priori* hypothesis that deficits would be greatest in female E4FAD mice, followed by E4FAD males, E3FAD females, and then E3FAD males. Statistical significance was set at $p < 0.05$ for all statistical tests, and trends were determined by $p < 0.10$.

3. Results

3.1. Object recognition and object placement

3.1.1. Object recognition

Male E3FADs spent significantly more time than chance (15 seconds) with the novel object during testing (Fig. 2A; $t_{(15)} = 5.35$, $p < 0.0001$; n = 12–16/group), indicating intact memory for the identity of the training objects. By contrast, female E3FADs and E4FADs of both sexes did not spend significantly more time than chance with the novel object during testing, suggesting impaired object recognition memory among all but the male E3FAD group. E3FADs spent more time with the novel object during testing than E4FADs ($F_{(1, 55)} = 5.82$, $p = 0.02$), an effect driven by male E3FADs, which spent more time with the novel object than E4FADs of both sexes ($p = 0.009$ vs. E4FAD males, $p = 0.003$ vs. E4FAD females). No other main effects or interactions were significant. Time to accumulate 30 seconds of object exploration did not differ among male E3FADs (M = 461.68, SEM = 51.0), female E3FADs (M = 443.89, SEM = 49.44), male E4FADs (M = 508.63, SEM = 73.06), or female E4FADs (M = 545.76, SEM = 74.39), suggesting that total activity or motivation to explore objects was not impacted by sex or *APOE* genotype.

3.1.2. Object placement

Similar results were observed for OP. Male E3FADs spent significantly more time than chance with the moved object during testing (Fig. 2B; $t_{(15)} = 3.360$, $p = 0.0043$; n = 12–16/group), suggesting intact memory for the location of the training objects. As in OR, female E3FADs and E4FADs of both sexes did not spend more time than chance with the moved object during testing. Neither the main effects nor interaction were significant for OP. Again, time to accumulate 30 seconds of object exploration did not differ among male E3FADs (M = 407.66, SEM = 33.53), female E3FADs (M = 363.61, SEM = 37.63), male E4FADs (M = 436.12, SEM = 31.24), or female E4FADs (M = 426.46, SEM = 53.6).

Together, within-group *t*-tests for OR and OP indicated that only male E3FADs exhibited intact memory for previously learned objects or locations, and suggest both a detrimental effect of *APOE4* genotype on object recognition and spatial memory, as well as an interesting sex difference favoring males within the *APOE3* genotype (Table 2).

3.2. Morris water maze

All ANOVA statistics for each MWM measure are presented in Table 1 (n = 14–16/group for all measures). The sections below summarize the main effects and interactions, and present results of planned comparisons where appropriate.

Two male E4FADs and 2 female E4FADs were excluded from all water maze analyses because their average swim speed was greater than 2 standard deviations away from their respective group's average swim speed in visible platform trials. All 4 excluded mice also spent time immobile (i.e., floating) during the task, suggesting that they were not motivated to search for the hidden platform.

3.2.1. Spatial learning trials

Mice of both genotypes could learn the location of the hidden platform, as indicated by significant main effects of session (Table 1) for swim time (Fig. 3A), cumulative swim distance (Fig. 3B), and swim speed (Fig. 3C). The main effect of genotype was significant for swim time and swim speed, such that E3FADs swam faster and located the platform in less time than E4FADs. The main effect of genotype was not significant for swim distance, suggesting that the genotype effect in swim time may be due primarily to differences in swim speed. The main effect of sex was significant only for swim speed, in which females swam faster than males.

Sex and genotype effects varied across sessions, as indicated by a significant session x sex x genotype effect for swim time and session x genotype effect for swim distance in the first trial. Indeed, by sessions 3 (halfway through testing) and 5 (end of testing), E3FADs of both sexes reached the platform in less time than during session 1 ($p < 0.05$), whereas E4FADs of both sexes did not (Fig. 3A), suggesting that E3FADs of both sexes learned the platform location more rapidly than E4FADs. In addition, the swim times of E3FAD males were significantly faster than E4FAD males in session 4 ($p = 0.02$). Male E4FADs also had a significantly and unusually higher spatial swim times than other groups during session 4, potentially driven by their slower swim speed.

Overall, these data suggest minimal effects of sex or genotype on learning-related parameters (e.g., spatial swim time or spatial swim distance) associated with Morris water maze task acquisition.

3.2.2. Probe trials

During the first 30 seconds of each daily probe trial, the platform was submerged and inaccessible for escape. As in the spatial learning trials, significant session effects for the percent time spent in the target quadrant (quadrant time; Supplemental Fig. 1A), average distance to the platform (distance to platform; Supplemental Fig. 1B), time taken to first enter the location of the platform (time to first entry; Supplemental Fig. 1C), and platform crossings (Supplemental Fig. 1D) indicated that all groups learned the location of the hidden platform. Effects of sex were limited to a single sex x session interaction for distance to the platform and sex x genotype interactions present in all measures but distance to the platform (although there was a weak trend of $p = 0.097$). Effects of genotype also varied by session, as indicated by session x genotype interactions for quadrant time and distance to the platform. Perfor-

Table 1
ANOVA statistics for Morris Water Maze variables

Task	Variable	Sex	Genotype	Sex x Genotype	Session	Session x Sex	Session x Genotype	Session x Sex x Genotype
Spatial	Swim time	$F(1,54) = 0.53$	$F(1,54) = 22.42$, $p < 0.0001$	$F(1,54) = 0.08$	$F(3.48,187.9) = 23.51$, $p < 0.0001$	$F(4,216) = 1.39$	$F(4,216) = 0.23$	$F(4,216) = 2.71$, $p = 0.03$
	Swim distance	$F(1,54) = 0.24$	$F(1,54) = 0.42$	$F(1,54) = 0.20$	$F(3.19,172.2) = 61.3$, $p < 0.0001$	$F(4,216) = 1.25$	$F(4,216) = 0.09$	$F(4,216) = 1.78$
	Swim speed	$F(1,54) = 4.38$, $p = 0.04$	$F(1,54) = 34.72$, $p < 0.0001$	$F(1,54) = 0.017$	$F(3.21,173.1) = 21.54$, $p < 0.0001$	$F(4,216) = 0.7$	$F(4,216) = 2.01$	$F(4,216) = 0.47$
	Quadrant time	$F(1,53) = 2.78$	$F(1,53) = 0.0007$	$F(1,53) = 4.16$, $p = 0.046$	$F(3.46,183.2) = 23.64$, $p < 0.0001$	$F(4,212) = 2.05$	$F(4,212) = 3.48$, $p = 0.009$	$F(4,212) = 1.25$
	Avg distance	$F(1,54) = 1.42$	$F(1,54) = 0.33$	$F(1,54) = 2.85$, $p = 0.1$	$F(3.36,181.2) = 36.22$, $p < 0.0001$	$F(4,216) = 2.4$, $p = 0.05$	$F(4,216) = 3.1$, $p = 0.02$	$F(4,216) = 1.47$
	Time to 1st entry	$F(1,54) = 0.58$	$F(1,54) = 2.26$	$F(1,54) = 4.7$, $p = 0.03$	$F(3.643,196.7) = 4.07$, $p = 0.005$	$F(4,216) = 1.19$	$F(4,216) = 0.45$	$F(4,216) = 1.12$
	Platform crossings	$F(1,54) = 0.29$	$F(1,54) = 1.98$	$F(1,54) = 5.46$, $p = 0.02$	$F(3.74, 201.9) = 4.089$, $p = 0.004$	$F(4,216) = 0.25$	$F(4,216) = 0.42$	$F(4,216) = 0.89$
Cued	Swim time	$F(1,54) = 0.003$	$F(1,54) = 0.78$	$F(1,54) = 3.56$, $p = 0.065$	$F(1.69,90.96) = 56.98$, $p < 0.0001$	$F(2,108) = 0.15$	$F(2,108) = 0.36$	$F(2,108) = 0.23$
	Swim distance	$F(1,53) = 6.408e\text{-}005$	$F(1,53) = 0.03$	$F(1,53) = 1.96$	$F(1.65,87.54) = 6.01$, $p = 0.006$	$F(2,106) = 0.06$	$F(2,106) = 0.69$	$F(2,106) = 0.46$
	Swim speed	$F(1,54) = 0.52$	$F(1,54) = 0.68$	$F(1,54) = 0.27$	$F(1.46,78.90) = 19.97$, $p < 0.0001$	$F(2,108) = 3.45$, $p = 0.04$	$F(2,108) = 0.95$	$F(2,108) = 0.06$

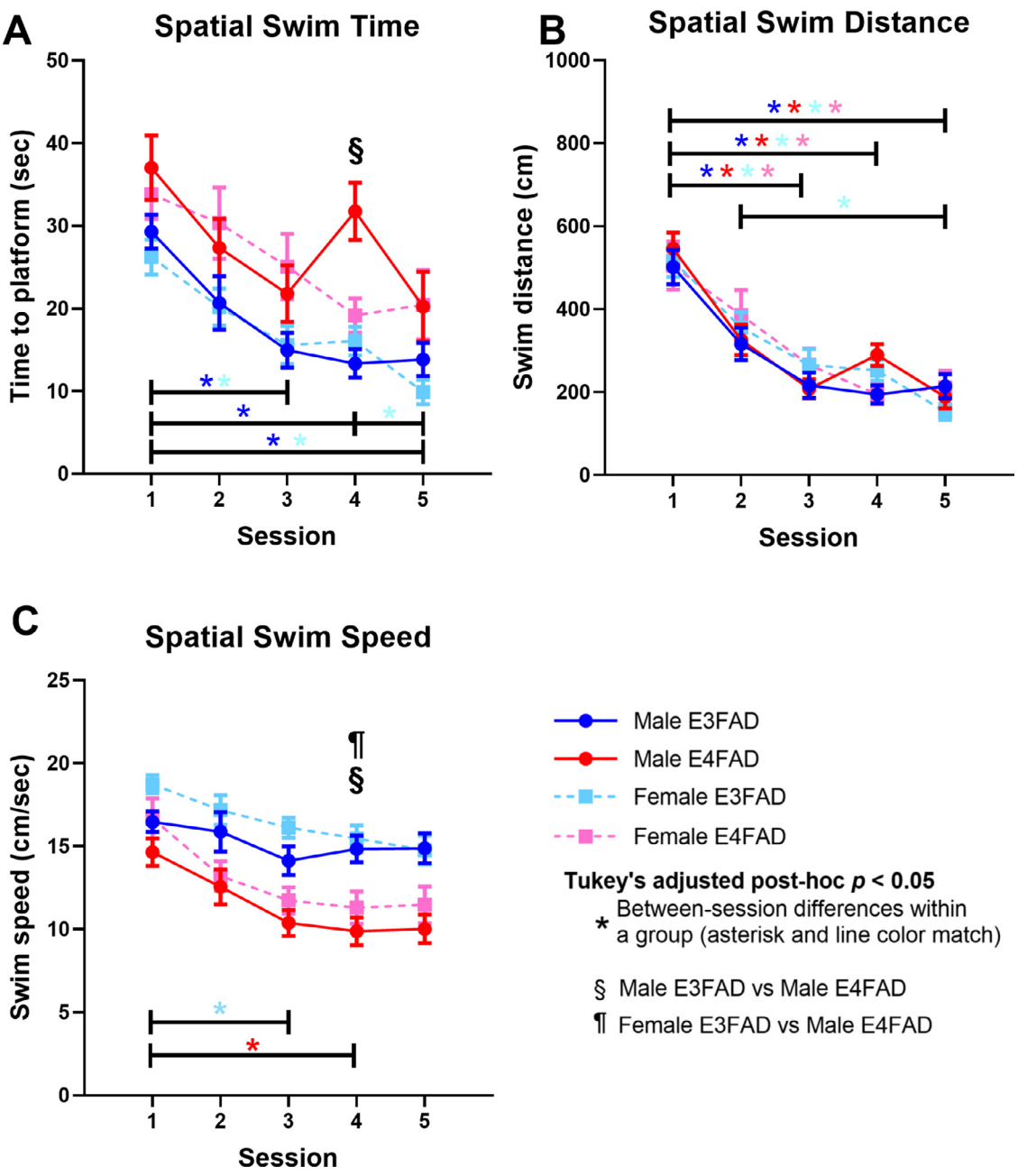

Fig. 3. All groups learned the platform location in the hidden platform trials of the Morris Water Maze. Spatial swim time (A), swim distance (B), and swim speed (C) decreased across sessions in all groups. Differences between sessions within a group are indicated with asterisks that match the group's line/symbol color (*$p < 0.05$; dark blue = male E3FAD, light blue = female E3FAD, red = male E4FAD, pink = female E4FAD). Between-group differences are indicated above the session during which they occurred (§ = male E3FAD vs male E4FAD; ¶ = female E3FAD vs male E4FAD). Symbols represent the mean ± SEM.

mance in all measures tended to be quite variable, making definitive conclusions about effects of sex and genotype difficult to draw. In general, male E3FADs tended to outperform other groups in sessions 1–3, where they spent more time in the target quadrant, swam shorter distances to the platform, entered the platform area faster, and made more platform crossings than other groups. One notable between-group difference was observed in quadrant time, where male E3FADs spent more time in the target quadrant than female E4FADs during session 1 (Supplemental Fig. 1A; $p < 0.03$). E3FAD males also tended to outperform E4FAD males in sessions 1-3. Although subtle sex and genotype interactions were observed in within-group performance, the overall variability and lack of main effects of sex or genotype on probe trial measures suggests that neither sex nor genotype significantly modulated memory expressed in the probe trials of this task.

3.2.3. Cued trials

All groups learned to find the visible platform, as indicated by main effects of session for cued swim time (Supplemental Fig. 2A), swim distance (Supplemental Fig. 2B), and swim speed (Supplemental Fig. 2C). No effects of genotype were observed in any measure, and the sole sex effect was a sex x session interaction for swim speed, reflecting faster speeds in male E4FADs in sessions 2 and 3 compared to session 1 ($p < 0.001$). These data suggest that EFAD mice of either sex or genotype can learn to locate and swim

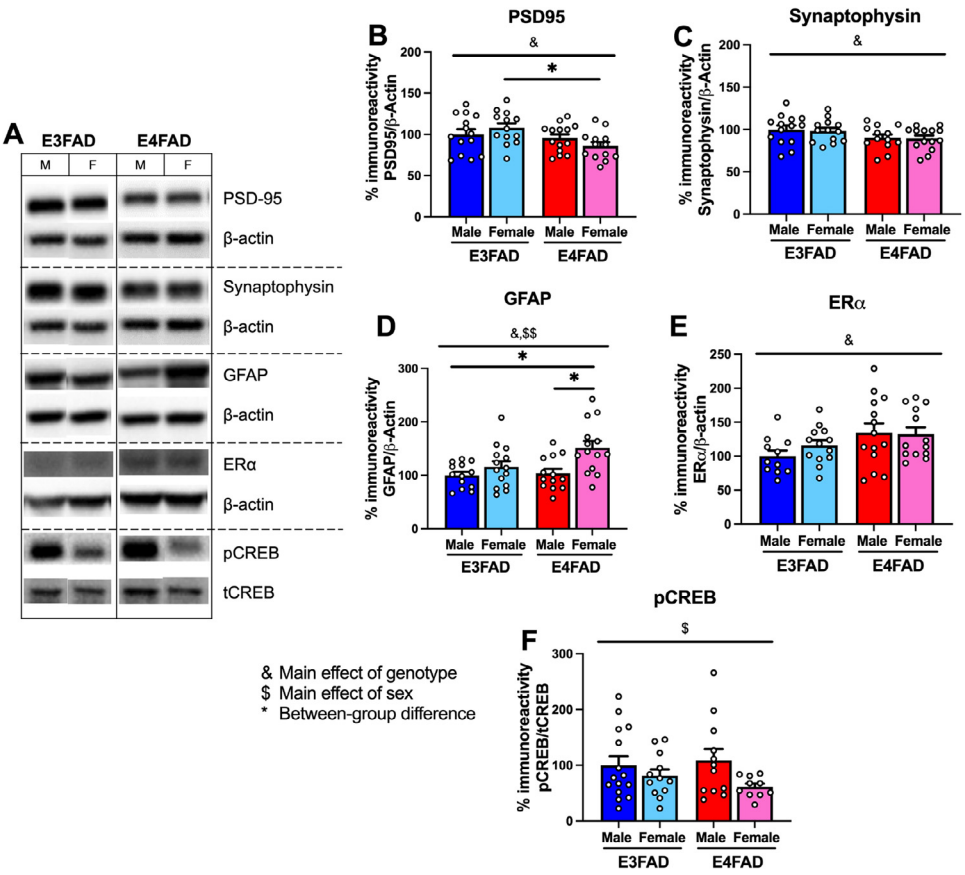

Fig. 4. E4FAD mice, particularly E4FAD females, displayed altered levels of several dorsal hippocampal proteins. (A) Representative blots illustrate relative group differences in protein expression. (B,C) Synaptic proteins PSD95 and synaptophysin were decreased in E4FADs relative to E3FADs ($^{\&}p < 0.05$). (D) GFAP protein expression was increased in female EFADs relative to male EFADs ($^{\$\$}p < 0.01$, main effect of sex) and tended to be higher in E4FADs than in E3FADs ($^{@}p = 0.057$). (E) ERα levels were significantly higher in E4FADs than in E3FADs ($^{\&}p < 0.05$). (F) Levels of pCREB were significantly higher in male EFADs relative to female EFADs ($^{\$}p < 0.05$). $^{*}p < 0.05$ = between-group differences, $^{\&}p < 0.05$ = main effect of genotype, $^{\$}p < 0.05$ = main effect of sex. Bars represent the mean ± SEM.

to a visible platform, indicating no adverse effects of sex or genotype on sensorimotor abilities, motivation, or swimming ability.

3.3. Western blotting

3.3.1. Synaptic proteins

E3FADs of both sexes exhibited higher levels of PSD95 (Fig. 4A; $F_{(1, 50)} = 6.24$, $p = 0.02$) and synaptophysin (Fig. 4B; $F_{(1, 50)} = 5.17$, $p = 0.03$) than E4FADs of both sexes (n = 14/group). PSD95 levels were higher in E3FAD females than E4FAD females ($p = 0.03$). Combined, these data suggest a detrimental effect of *APOE4* genotype on synaptic proteins, perhaps indicating a reduction in synapse or dendritic spine density (Table 2).

3.3.2. Glial proteins

Females had elevated GFAP relative to males (Fig. 4C; $F_{(1, 50)} = 10.01$, $p = 0.003$; n = 13–14/group) and there was a trend for E4FADs to have elevated GFAP relative to E3FADs (Fig. 4C; $F_{(1, 50)} = 3.8$, $p = 0.057$). These effects were driven by elevated levels of GFAP in female E4FADs compared to every other group ($p = 0.004$ vs. male E3FAD; $p = 0.06$ vs. female E3FAD; and $p = 0.008$ vs male E4FAD) and suggest a potential increase in dorsal hippocampal astrocytes or astrocytic activation among female E4FADs (Table 2). In contrast to GFAP, there were no effects of sex or genotype, nor interactions, in levels of Iba1 (Supplemental Table 2; n = 13–14/group), indicating a lack of sex and *APOE* genotype effects on microglial protein expression in the dorsal hippocampus.

3.3.3. Membrane-associated proteins

ERα interacts with mGluR1 at the cell membrane to increase phosphorylation of the 42-kDa isoform of ERK (p42 ERK) and cyclic-AMP response element binding protein (pCREB; Boulware et al., 2005), and increased p42 ERK phosphorylation is necessary for ERα activation to enhance memory in the OR and OP tasks (Boulware et al., 2013). Here, E3FADs exhibited lower ERα levels than E4FADs (Fig. 4D; $F_{(1, 47)} = 5.88$, $p = 0.02$; n = 11–14/group), an effect driven by male E3FADs, whose ERα levels were lower than E4FADs of both sexes ($F_{(1,15)} = 5.67$, $p < 0.03$; $p = 0.03$ vs. male E4FAD; $p = 0.04$ vs. female E4FAD; Table 2). No effects of sex or *APOE* genotype were observed for ERβ protein expression (Supplemental Table 2, n = 13–14/group), indicating that the effect of *APOE* genotype on nuclear estrogen receptor protein expression was specific to ERα. In addition, caveolin-1 protein levels were not affected by sex or genotype (Supplemental Table 2; n = 11–16/group).

3.3.4. Cell-signaling proteins

Males expressed higher pCREB levels than females (Fig. 4E; $F_{(1, 45)} = 4.61$, $p = 0.04$; n = 10–15/group; Table 2). In contrast, no effects of sex or genotype, nor any interactions, were observed for p42 ERK, pPI3K, p4EBP, pcofilin, p46 JNK, or p54 JNK (Supplemental Table 2; n = 9–15/group), suggesting that these phospho proteins are not upregulated in the dorsal hippocampus of EFAD mice 5 minutes after learning.

Table 2
Significant sex and genotype effects

Parameter	Measure	Genotype (E3FAD vs. E4FAD)	Sex (Male vs. Female)
Object Tasks	Object Recognition	**E3FAD > E4FAD** $F(1, 55) = 5.82$, $p = 0.02$	=
	Object Placement	=	=
Protein Expression	PSD95	**E3FAD > E4FAD** $F(1, 50) = 6.24$, $p = 0.02$	=
	Synaptophysin	**E3FAD > E4FAD** $F(1, 50) = 5.17$, $p = 0.03$	=
	GFAP	**E3FAD < E4FAD** $F(1, 50) = 3.8$, $p = 0.057$	**Male < Female** $F(1, 50) = 10.01$, $p = 0.003$
	pCREB	=	**Male > Female** $F(1,45) = 4.611$, $p = 0.037$
	ERα	**E3FAD < E4FAD** $F(1, 47) = 5.88$, $p = 0.02$	=
Spine Density	CA1 Basal	**E3FAD > E4FAD** $F(1, 23) = 8.75$, $p = 0.007$	=
	mPFC Basal	**E3FAD > E4FAD** $F(1, 22) = 31.92$, $p < 0.0001$	=

"=" indicates no significant between-group differences.

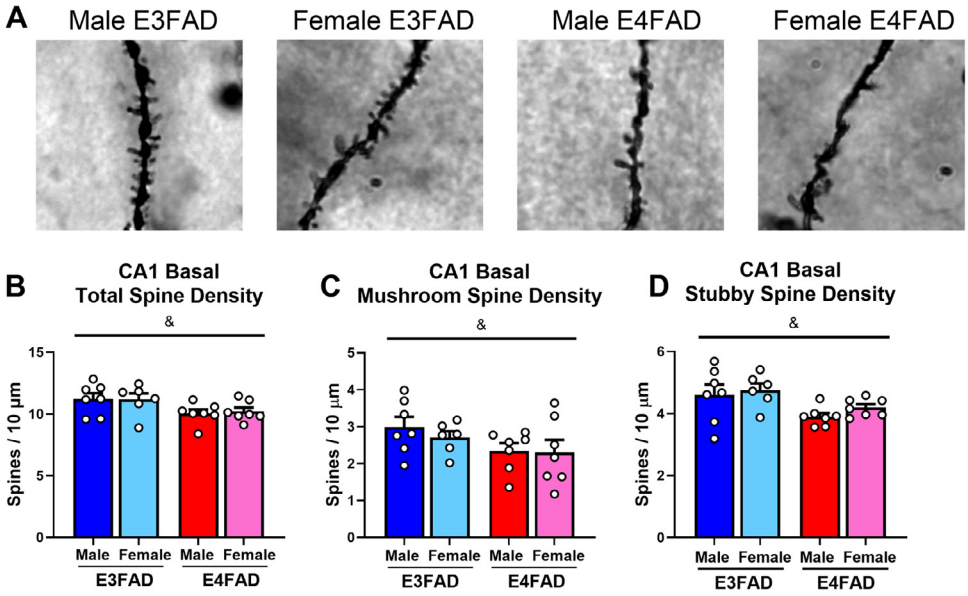

Fig. 5. E4FADs of both sexes exhibited reduced CA1 dendritic spine density. (A) Representative images at 100x of basilar dendritic segments in CA1 illustrate relative differences in spine density. (B) E4FADs exhibited lower total CA1 basal spine density relative to E3FADs ($^{\&}p < 0.05$, main effect of genotype). In particular, E4FADs exhibited reduced numbers of mushroom (C; $^{\&}p = 0.059$) and stubby (D; $^{\&}p < 0.05$) spines relative to E3FADs. Bars represent the mean ± SEM.

3.4. Dendritic analyses

We next examined apical and basal dendritic spine density in hippocampal area CA1 and in the prelimbic/infralimbic area of the mPFC to assess whether sex and *APOE* genotype influence dendritic spine anatomy. Neither sex nor genotype affected the density of apical spines in CA1 or mPFC (Supplemental Table 3). Sex also did not affect basal spines in either brain region. Effects of genotype on basal spines are detailed below.

3.4.1. CA1

Total CA1 basal spines varied significantly by genotype ($F_{(1, 23)} = 8.75$, $p = 0.007$; n = 6–7/group), such that E4FADs had reduced total basal spine density compared to E3FADs (Fig. 5A, B). Differences in total basal spine density were driven by alterations in mushroom spines (Fig. 5C; $F_{(1, 23)} = 3.96$, $p = 0.059$) and stubby spines (Fig. 5D; $F_{(1, 23)} = 8.75$, $p = 0.007$). In both cases, E4FADs exhibited lower density than E3FADs. Stubby spine density was lower in male E4FADs than in female E3FADs ($p = 0.05$). Neither sex nor genotype affected CA1 basal thin spine density (Supplemental Table 3). Combined, these data indicate that *APOE4* genotype contributes to a selective reduction in basilar spine density in the dorsal hippocampus relative to *APOE3* genotype (Table 2). That mushroom and stubby spines were specifically affected suggests that E3FADs have more mature and intermediate spines on basilar dendrites relative to E4FADs.

Sholl analysis was used to evaluate whether sex acts independently or concordantly with *APOE3* or *APOE4* genotypes to alter dendritic branching complexity within the CA1. Dendritic intersections with concentric spheres placed at successive 10 μm intervals from the cell soma were quantified. The pattern of dendritic branching was similar for all groups; dendritic intersections increased in all groups until a distance of approximately 100 μm (apical) or 60 μm (basal) and then declined (Supplemental Fig.

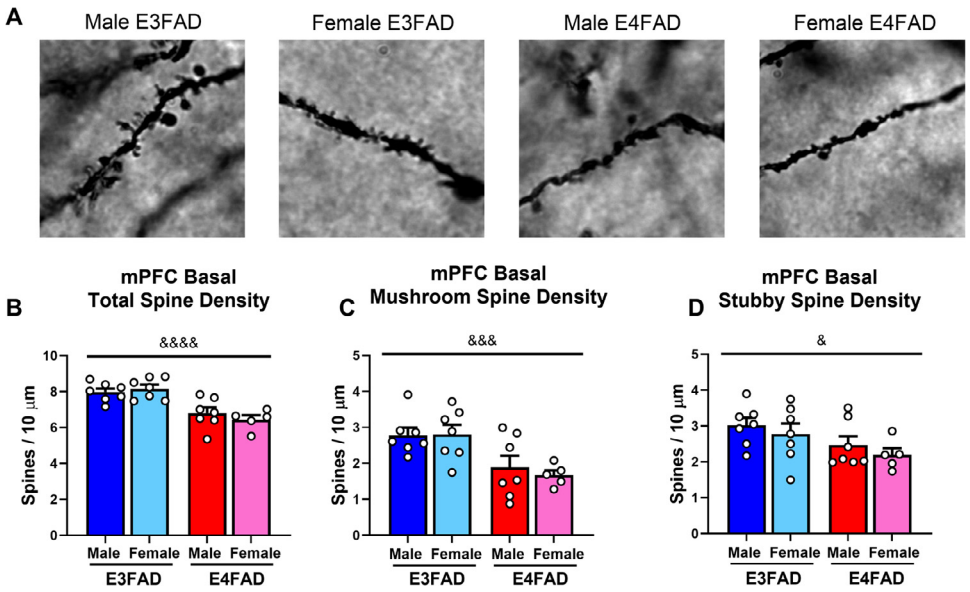

Fig. 6. E4FADs of both sexes exhibited reduced mPFC dendritic spine density. (A) Representative images at 100x of basilar dendritic segments in mPFC illustrate relative differences in spine density. (B) E4FADs exhibited lower total mPFC basal spine density relative to E3FADs ($^{\&}p < 0.05$, main effect of genotype). Notably, E4FADs exhibited reduced numbers of mushroom (C) and stubby (D) spines relative to E3FADs ($^{\&}p < 0.05$). Bars represent the mean ± SEM.

2). Although the main effect of distance from the soma was significant for both apical ($F_{(37,851)} = 240.0$, $p < 0.0001$) and basal ($F_{(2.492, 57.31)} = 350.8$, $p < 0.0001$) dendritic intersections, sex and *APOE* genotype did not influence morphological complexity of dorsal hippocampal CA1 pyramidal neurons.

3.4.2. mPFC

The effects of genotype on dendritic spine density in the mPFC were identical to those observed in CA1 (n = 5–7/group). E4FADs exhibited reduced total mPFC basilar spine density compared to E3FADs (Fig. 6A,B; $F_{(1, 22)} = 31.92$, $p < 0.0001$; Table 2). Total basal spine density was higher in male and female E3FADs than in E4FADs of both sexes ($p = 0.0008$-0.01). As with basilar CA1 spines, the genotype effect in total mPFC basal spine density was driven by decreased mushroom (Fig. 6C; $F_{(1, 22)} = 15.02$, $p = 0.0008$) and stubby (Fig. 6D; $F_{(1, 22)} = 5.12$, $p = 0.03$) spine density in E4FADs relative to E3FADs. Relative to E4FADs of both sexes, basal mushroom spine density was higher in male and female E3FADs ($p = 0.035$–0.04).

4. Discussion

The neural mechanisms underlying the increased risk of AD to women *APOE4* carriers are unclear, thus necessitating a better understanding of how sex and different *APOE* genotypes influence cognition and brain function. Here, we used an EFAD mouse model of AD to examine whether sex modulated differences between *APOE3* and *APOE4* genotypes in mnemonic function, protein expression, and dendritic morphology against the backdrop of AD-like pathology previously reported in this model (Tai et al., 2017; Youmans et al., 2012). We hypothesized that E4FAD mice ($APOE4^{+/+}$/5xFAD$^{+/-}$) would exhibit impaired memory, reduced hippocampal levels of synaptic, membrane, and cell-signaling proteins, increased hippocampal levels of glial proteins, and decreased dendritic spine density and branch complexity in CA1 and mPFC relative to 5xFAD mice expressing two copies of human *APOE3* (E3FAD). We further expected these changes to be most pronounced in female E4FAD mice. The findings suggest that *APOE4* genotype impaired spatial and object recognition memory consolidation in object-based tasks, reduced hippocampal synaptic markers, and decreased dendritic spine density in the CA1 and mPFC relative to *APOE3*, yet increased hippocampal levels of ERα (Fig. 7; Table 2). We observed surprisingly few sex differences (Table 2) or sex x genotype interactions in all measured outcomes, suggesting that the known synergistic effects of *APOE4* and female sex are likely modulated by factors not measured in the present study.

Interestingly, sex differences in object-based memory were observed among E3FADs, but not E4FADs. Specifically, only male E3FAD mice remembered the identity and location of the training objects in the OR and OP tasks, suggesting impaired object recognition and spatial memory consolidation not only in E4FADs of both sexes but also in E3FAD females. However, the sex differences in memory observed within E3FADs were not reflected in any measure of neuronal structure or function, so the underlying mechanisms remain unclear. This preserved brain function in E3FAD females may have allowed them to find the platform in the MWM as well as E3FAD males, as E3FADs of both sexes improved similarly across sessions in this task. Curiously, E3FADs of both sexes had lower dorsal hippocampal ERα expression than E4FADs, suggesting either a potential role for high ERα levels in *APOE4*-induced memory impairments, or a decrease in ERα in E3FADs that serves to maintain memory. Levels of synaptic proteins and the density of mature and developing basal dendritic spines in the CA1 and mPFC were reduced in E4FAD mice of both sexes relative to male and female E3FAD mice, suggesting a detrimental effect of *APOE4* genotype, but not sex, on spine synapses and synaptic plasticity. However, sex did affect levels of the astrocytic protein GFAP and the transcription factor pCREB (Table 2), with females expressing more GFAP and less pCREB. Interestingly, female E4FADs expressed substantially higher levels of GFAP than E4FAD males. These findings suggest increased astrogliosis in E4FAD females. Together, the results indicate limited effects of sex on memory in EFAD mice, with no sex differences observed among E4FADs in either task, and a sex difference (favoring males) only evident among E3FADs in the 2 object tasks. These sex differences were not reflected in any neurobiological measure, although sex differences unrelated to memory were observed in GFAP and pCREB among E4FAD mice.

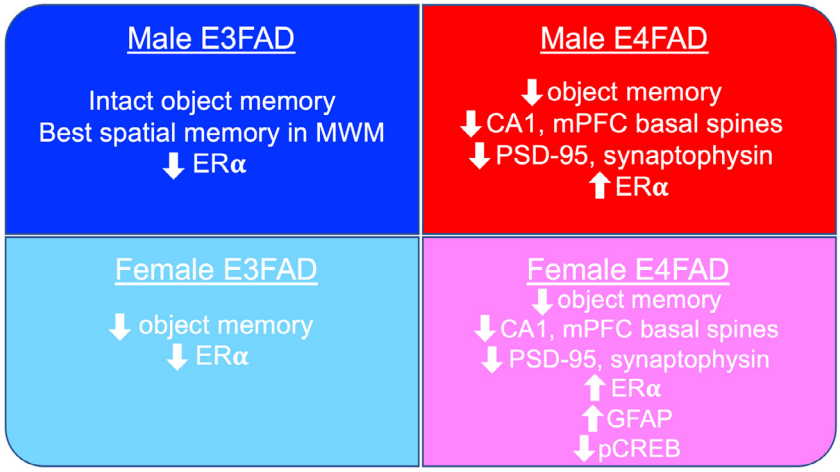

Fig. 7. Summary of the main findings within each of the 4 groups. Results from female E3FADs reflect their inability to demonstrate learning in the object recognition and object placement tasks. Items noted for male E4FADs reflect differences from one or both E3FAD groups. Findings for female E4FADs indicate differences not only from E3FADs but also from male E4FADs in some cases.

In previous work with C57BL/6 mice, ovariectomized females and gonadally-intact or castrated males show intact memory in the OR and OP tasks when tested 24 or 4 hours later, respectively (Fortress et al., 2013a; Kim et al., 2019; Koss et al., 2018; Taxier et al., 2019). Thus, these are delays at which wild-type mice can remember the identities and locations of the training objects. Here, only male E3FAD mice exhibited intact memory in both tasks. The superior object memory of E3FAD males compared to E4FADs of both sexes is consistent with previous reports of reduced pathology in E3FAD males relative to other EFADs (Youmans et al., 2012) and literature suggesting that *APOE3* is less detrimental to memory than *APOE4* in both animal models and human AD patients (Beydoun et al., 2012; Liu et al., 2015). In support, the impaired memory exhibited by male and female E4FADs, as well as female E3FADs, is consistent with previous work showing spatial and object memory impairments in rodent models of AD (Ardiles et al., 2012; Ashe, 2001). Moreover, other AD mouse model studies report exacerbated memory impairment in females relative to males (Schmid et al., 2019; Yang et al., 2018; Yue et al., 2011). Thus, our data showing that female EFADs of both genotypes could not remember the identity and location of previously seen objects are consistent with these findings. Although we expected that all groups but female E4FADs would display intact memory, AD pathology may have been sufficient by 6 months to impair memory in male and female E4FADs and in female E3FADs. E4FAD mice exhibit significant AD pathology and behavioral deficits as early as 4 months, especially relative to E2FAD mice (Liu et al., 2015), so had we tested the mice at an earlier age, we may have captured sex differences in memory within the E4FADs.

In contrast to the object tasks, all mice could learn to find the platform in the MWM task. This is somewhat surprising, as swimming through a large water tank to find a hidden platform seems inherently more complex than exploring objects in an open field. Nevertheless, the lack of impairment among E4FADs is consistent with a previous comparison of 6 month-old female E3FAD and E4FAD mice showing minimal MWM deficits in E4FADs relative to E3FADs (Liu et al., 2015). MWM training is much more extensive than the object tasks and the number and length of training trials per day, in addition to the distinctiveness of room cues during water maze testing, may have greatly aided spatial memory formation in the MWM among mice from all groups. A more challenging training protocol (e.g., fewer trials or fewer cues) may have captured between-group differences.

Because synaptic dysfunction is a key feature in AD (Masliah et al., 2006; Reddy et al., 2005), we investigated whether sex and *APOE* genotype influence expression of synaptic proteins within the dorsal hippocampus of EFAD mice. Our data showing a reduction in PSD95 and synaptophysin levels in E4FADs relative to E3FADs, particularly among females, is consistent with previous work showing reduced hippocampal PSD95 levels in 4-month-old female E4FAD mice relative to E3FAD females (Liu et al., 2015). Our findings are also consistent with clinical data demonstrating that expression of synaptophysin is markedly decreased in AD patients compared to healthy controls (Heinonen et al., 1995), and in *APOE4*-expressing individuals relative to *APOE3* carriers (Love et al., 2006).

The lower levels of hippocampal synaptic proteins observed in E4FADs relative to E3FADs are consistent with the reduced CA1 and mPFC basal dendritic spine density also evident in E4FADs. Previous findings in *APOE*-TR mice indicate a detrimental effect of *APOE4* on cortical, but not hippocampal, spine density across aging (Dumanis et al., 2009), although others using this model have reported an adverse effect of *APOE4* on spine density in the hippocampus (Ji et al., 2003). The decreased mushroom spine density observed in E4FADs suggests that E3FADs have more mature spines than E4FADs, which is consistent with the overall negative effect of *APOE4* genotype on memory processes. Our finding of more stubby spines in E3FADs than E4FADs may reflect ongoing cytoskeletal reorganization in dendritic spines. Previous work in a different mouse model of AD suggests that stubby spines may be more dynamic than other spine types (Spires-Jones et al., 2007). Because spine remodeling is a continual process (Kasai et al., 2010), and our own data present only a snapshot of this process at a single timepoint, we speculate that elevated stubby spine density in E3FADs reflects greater spine plasticity, motility, recycling, and/or remodeling relative to E4FADs.

Moreover, the negative impact of *APOE4* genotype on basal, but not apical, spine density, in the current study suggests the intriguing possibility that *APOE4* genotype does not uniformly modulate spine density, but rather influences distinct sites of synaptic input within both the mPFC and the dorsal hippocampus. Given reports that basal dendrites in mPFC receive input from thalamic projections, and that communication between the dorsal hippocampus

and mPFC may be facilitated via the nucleus reuniens of the thalamus (Hoover and Vertes, 2007), future work should interrogate whether changes in basal dendritic spine density reflect compromised neurocircuitry between the dorsal hippocampus and mPFC in EFAD mice.

In addition to neuronal morphology, we examined expression of glial proteins because both astrocytic and microglial reactivity is closely linked to AD pathology (Meda et al., 2001). Although neither sex nor *APOE* genotype affected microglial Iba-1 expression in the dorsal hippocampus, the astrocytic protein GFAP was elevated in female EFADs relative to male EFADs, and in E4FADs relative to E3FADs. Previous work showed striking differences in astrocytic reactivity between male E4FAD and E3FADs (Tai et al., 2017), with E4FADs having elevated GFAP expression relative to E3FADs. The present genotype effect differs in that it is primarily driven by elevated GFAP in female E4FADs. The sex-specific effect on levels of GFAP, as well as the particularly high GFAP levels in female E4FADs, suggests that both female sex and *APOE4* genotype interact to increase susceptibility to astrogliosis.

In our examination of cell-signaling proteins that are rapidly activated in response to learning, we found that dorsal hippocampal pCREB levels were increased in male EFADs relative to female EFADs. In the hippocampus of AD patients, CREB-mediated transcriptional outcomes are dysregulated (Satoh et al., 2009), and pCREB is reduced relative to age-matched controls (Yamamoto-Sasaki et al., 1999), suggesting that aberrant hippocampal CREB signaling is a feature of AD. Although sex differences in CREB-associated signaling remain poorly characterized in rodent models of normal or pathological aging, previous work with CREB-deficient aging mice showed that aberrant CREB signaling resulted in reduced spatial memory in females compared to males (Hebda-Bauer et al., 2007). In addition, female 3xTg mice exhibited lower pCREB expression relative to male 3xTg mice (Yang et al., 2018). These data are in line with our own, suggesting that reduced pCREB is particularly exacerbated in female E4FADs relative to male E4FADs. We were surprised to find no additional genotype or sex-specific effects on levels of other cell-signaling proteins that are critical for memory processes; however, one training session with novel objects prior to tissue collection may not have been sufficient to drive learning-induced changes in these pathways. Future work should examine multiple time points after a robust learning experience to more definitively determine whether cell-signaling activity is compromised by *APOE4* genotype.

Our finding that dorsal hippocampal ERα levels are increased in E4FADs relative to E3FADs is consistent with previous work showing that ERα levels are increased in the AD brain (Ishunina et al., 2003; Ishunina and Swaab, 2003). Caveolin-1 plays a key role in associating mGluRs with ERα at the cell membrane (Boulware et al., 2013; Razandi et al., 2002), although caveolin-1 protein levels were unaffected by sex or genotype in the present study. Therefore, the increase in ERα levels seen among E4FADs relative to E3FADs suggests that *APOE4* may promote an increase in the cytosolic, rather than membrane, localization of this receptor. Others have posited that estrogens may interact with ERα to increase levels of apolipoprotein E (apoE), particularly in *APOE4* carriers, in a manner that would make such individuals more vulnerable to disease progression (Wang et al., 2006). Although our data lend correlative support to this hypothesis, any differential effect of *APOE* on estrogen therapy remains unclear. Notably, our data indicate that ERβ expression was unaffected by *APOE* genotype, thus implicating ERα as a more promising target for estrogen therapy for *APOE4* carriers.

Collectively, the results from the present study of 6-month-old EFAD mice suggests a substantial influence of *APOE4* genotype, but not sex, on measures of hippocampus-dependent memory, as well as hippocampal spine density and protein expression. Of particular note, *APOE4* genotype had detrimental effects on object memory, CA1 spine density, and dorsal hippocampal levels of synaptic proteins and ERα. E4FAD females additionally exhibited aberrant dorsal hippocampal GFAP levels and CREB phosphorylation. Future studies are critical to determining whether other aspects of neural function can account for the sex and *APOE* genotype interactions observed in humans that increase *APOE4*+ women's vulnerability to AD. Knowing when *APOE4* and sex exert detrimental effects is a key knowledge gap, and the present study is limited by the examination of these variables at a single time point in EFAD mice. Therefore, next steps should include examining whether *APOE4* and sex negatively impact synaptic integrity and memory at an earlier age, and whether the possibility exists to reverse or mitigate the course of neurodegeneration modulated by *APOE4* genotype and sex. Pinpointing the time at which memory deficits emerge may have therapeutic potential in resolving memory decrements and associated neuropathology (Lanfranco et al., 2020; Tai et al., 2014).

Disclosure statement

K.M.F. is a co-founder and the Chief Scientific Officer of Estrigenix Therapeutics, Inc. The other authors have no actual or potential conflicts of interest to declare.

CRediT authorship contribution statement

Lisa R. Taxier: Conceptualization, Methodology, Visualization, Formal analysis, Investigation, Writing – original draft, Writing – review & editing, Visualization, Project administration. **Sarah M. Philippi:** Formal analysis, Investigation, Writing – original draft, Writing – review & editing. **Jason M. York:** Resources, Writing – review & editing. **Mary Jo LaDu:** Conceptualization, Methodology, Resources, Writing – review & editing, Visualization, Funding acquisition. **Karyn M. Frick:** Conceptualization, Methodology, Validation, Writing – original draft, Writing – review & editing, Visualization, Supervision, Project administration, Funding acquisition.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.neurobiolaging.2021.12.006.

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