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RECEIVED 20 March 2025

ACCEPTED 22 April 2025

PUBLISHED 14 May 2025

CITATION

Marongiu R, Platholi J, Park L, Yu F,
Sommer G, Woods C, Milner TA and Glass MJ
(2025) Promotion of neuroinflammation in
select hippocampal regions in a mouse model
of perimenopausal Alzheimer's disease.
Front. Mol. Biosci. 12:1597130.
doi: 10.3389/fmolb.2025.1597130

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Promotion of neuroinflammation in select hippocampal regions in a mouse model of perimenopausal Alzheimer's disease

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Introduction: Alzheimer's disease, the most common form of dementia, is characterized by age-dependent amyloid beta (Ab) aggregation and accumulation, neuroinflammation, and cognitive deficits. Significantly, there are prominent sex differences in the risk, onset, progression, and severity of AD, as well as response to therapies, with disease burden disproportionately affecting women. Although menopause onset (i.e., perimenopause) may be a critical transition stage for AD susceptibility in women, the role of early ovarian decline in initial disease pathology, particularly key neuroinflammatory processes, is not well understood.

Methods: To study this, we developed a unique mouse model of perimenopausal AD by combining an accelerated ovarian failure (AOF) model of menopause induced by 4-vinylcyclohexene diepoxide (VCD) with the 5xFAD transgenic AD mouse model. To target early stages of disease progression, 5xFAD females were studied at a young age (~4 months) and at the beginning stage of ovarian failure analogous to human perimenopause (termed "peri-AOF"), and compared to age-matched males. Assessment of neuropathology was performed by immunohistochemical labeling of Ab as well as markers of astrocyte and microglia activity in the hippocampus, a brain region involved in learning and memory that is deleteriously impacted during AD.

Results: Our results show that genotype, AOF, and sex contributed to AD-like pathology. Aggregation of Ab was heightened in female 5xFAD mice and further increased at peri-AOF, with hippocampal subregion specificity. Further, select increases in glial activation also paralleled Ab pathology in distinct hippocampal subregions. However, cognitive function was not affected by peri-AOF.

Discussion: These findings align with the hypothesis that perimenopause constitutes a period of susceptibility for AD pathogenesis in women.

KEYWORDS

amyloid beta, astrocyte, microglia, ovarian failure, pyramidal cell

Introduction

Dementia is a leading contributor to the global burden of disease (Alzheimer's disease facts and figures, 2023) with Alzheimer's disease (AD) constituting approximately 50%–70% of cases (Alzheimer's, 2016). AD is characterized by progressive neurodegeneration and cognitive dysfunction (Arvanitakis et al., 2019; Zhang et al., 2024). A main hallmark of AD neuropathology is the accumulation of parenchymal plaques containing aggregated amyloid-beta ($A\beta$) in the cerebral cortex and the hippocampal formation (Braak and Braak, 1991). In addition to $A\beta$ deposition, AD involves a complex set of related neurodegenerative processes including neuroinflammation (Lista et al., 2024). In AD animal models, neuroinflammation characterized by the activation of reactive astrocytes and microglia (Raz et al., 2016) is one of the earliest pathological manifestations, likely contributing to synaptic and neuronal loss.

Important sex differences in AD are well documented, with women experiencing a disproportionately greater disease burden (Kolahchi et al., 2024). The incidence of AD is at least two-fold higher in women compared to men (Alzheimer's, 2016) with women exhibiting faster disease progression (Pike, 2017) and greater cognitive impairment at comparable stages of AD (Laws et al., 2016). Perimenopause, the transitional phase of irregular gonadal hormonal production and cycling before full menopause, may be a particularly vulnerable period for the onset of mild cognitive impairment and AD. This is supported by evidence that low endogenous estrogen levels are associated with increased AD risk (Gong et al., 2022), and that early or late age at menopause is associated with an elevated or decreased risk for AD, respectively (Liao et al., 2023; Wedatilake et al., 2024; Steventon et al., 2023). Furthermore, there is growing evidence that initiating estrogen replacement soon after menopause may help mitigate dementia development (Mills et al., 2023). Significantly, perimenopause is also associated with declines in brain volume (Mosconi et al., 2018) and increases in $A\beta$ expression (Mosconi et al., 2018; Mosconi et al., 2017). Despite these associations, the mechanisms underlying the heightened perimenopausal risk for AD, particularly those related to neuroinflammation and cognitive decline in the hippocampus, remain unclear.

Animal models may help elucidate the mechanisms driving perimenopausal AD risk. The commonly used 5xFAD transgenic mouse model, which expresses five familial mutations in two AD risk genes, exhibits increased $A\beta$ production and plaque formation that parallels AD pathology (Oakley et al., 2006). Notably, sex differences have been reported in 5xFAD mice, with females showing earlier increases in inflammatory gene expression (Manji et al., 2019), glial markers (Sil et al., 2022; Islam et al., 2024) as well as $A\beta$ levels (Manji et al., 2019; Sil et al., 2022;

Bhattacharya et al., 2014). This neuropathology correlates with worse cognitive performance, in some studies (Manji et al., 2019; Sil et al., 2022; Islam et al., 2024), but not all (Ganesh et al., 2024; O'Leary et al., 2020). Markers of brain inflammation appear as early as 3 months of age, suggesting that sex differences in AD-like pathology emerge at a prodromal stage (Manji et al., 2019). Additionally, female 5xFAD mice show increased hippocampal $A\beta$ and elevated expression of immune-related genes and proteins compared to age-matched males (Oakley et al., 2006; Bundy et al., 2019; Blasco Tavares Pereira Lopes et al., 2022; Poon et al., 2023; Sadleir et al., 2015; Maarouf et al., 2013). These sex differences may be linked to changes in estrogen signaling (Prange-Kiel et al., 2016; Kubota et al., 2016; Quinn et al., 2022). Thus, combining mouse models of AD with perimenopause in females may help to isolate the effects of hormonal changes on AD neuropathology.

The 4-vinylcyclohexene diepoxide (VCD) ovatoxin mimics perimenopause in rodents by producing accelerated ovarian failure (AOF) paralleling the irregular hormone fluctuations seen during human perimenopause (termed “peri-AOF” in rodents) before transitioning to full menopause (Van Kempen et al., 2014; Brooks et al., 2016; Marques-Lopes et al., 2017). This model allows for controlled induction of AOF at various times/ages following sexual maturity in younger animals, reducing the confounding effects of chronological aging, a main AD risk factor (Van Kempen et al., 2014; Brooks et al., 2016; Marques-Lopes et al., 2017). The VCD model has been used to investigate how ovarian failure influences metabolic (Abi-Ghanem et al., 2025; Abi-Ghanem et al., 2024; Gannon et al., 2023), aging (Golub et al., 2008), and cerebrovascular (Blackwell et al., 2022) factors to influence cognitive function, brain plasticity and amyloid pathology in wild-type (WT) mice or in mouse models of cognitive impairment and dementia. However, the impact of early ovarian failure (peri-AOF) on $A\beta$ -related pathology and neuroinflammatory response in 5xFAD mice is unknown.

We investigated whether peri-AOF contributes to hippocampal neuroinflammation in young 5xFAD mice treated with VCD. A granular assessment of $A\beta$ levels and glial markers of neuroinflammation was conducted by analyzing all major hippocampal subfields at rostral and caudal levels. Cognitive performance was assessed using tests of learning and memory. To characterize early AD-like neuropathology, we focused on young (~4 months) 5xFAD mice. Age-matched male mice were also tested to evaluate the effect of biological sex.

Experimental procedures

Animals

Young adult [~2 month-old at the initiation of the experiments (Flurkey and Curren, 2004)] C57BL/6 WT mice (n = 22 females and n = 11 males) and transgenic 5xFAD (C57BL/6 background; n = 22 female and n = 11 male) mice were bred and maintained in a colony at Weill Cornell Medicine (WCM). Breeding pairs of hemizygous 5xFAD mice were obtained from the Jackson Laboratory (Bar Harbor, ME; JAX MMRRC stock#034840). Mice were bred in-house and genotyped prior to experimentation.

Abbreviations: $A\beta$, beta amyloid; AD, Alzheimer's disease; AOF, accelerated ovarian failure; cCA1, caudal CA1; Cen, central hilus; cDG, caudal DG; DG, dentate gyrus; GFAP, glial fibrillary acidic protein; Iba1, ionized calcium binding adapter molecule 1; IFG, infragranular blade; PB, phosphate buffer; PCL, pyramidal cell layer; rCA1, rostral CA1; rDG, rostral DG; SG, supragranular blade; SLM, stratum lacunosum-moleculare; SLu, stratum lucidum; SO, stratum oriens; SR, stratum radiatum; TS, Tris saline; VCD, 4-vinylcyclohexene diepoxide.

5xFAD mice express human APP and PSEN1 transgenes with a total of five AD-linked mutations under the control of the Thy1 promoter. This mouse model exhibits early-onset parenchymal A β aggregation correlated with cognitive deficits (Xu et al., 2014a; Richard et al., 2015). Amyloid deposition begins in the cerebral parenchyma at 2–3 months of age, with little accumulation in the cerebral vasculature, and amyloid plaques are found throughout the hippocampus and cortex by 6 months (Oakley et al., 2006; Xu et al., 2014b). Astrogliosis and microgliosis begin around 2 months, developing in parallel with plaque deposition (Oakley et al., 2006). Mice were housed in groups of three to four animals per cage and maintained on a 12-h light/dark cycle (lights out 18:00 h) with *ad libitum* access to water and rodent chow. At euthanasia, mice weighed 23–32 g. All experiments were approved by the WCM Institutional Animal Care and Use Committees and followed the National Institutes of Health guidelines for the Care and Use of Laboratory Animals guidelines.

AOF model of perimenopause

AOF induction by VCD treatment in mice has been shown to recapitulate the gradual hormonal fluctuations that correspond to peri- and post-menopause in humans (reviewed in (Van Kempen et al., 2011; Marques-Lopes et al., 2018)). The AOF model can be used to separate hormonal effects from aging effects and can be applied to any mouse genotype (Van Kempen et al., 2014; Brooks et al., 2016; Marques-Lopes et al., 2017). Low dose VCD injections selectively deplete ovarian primary follicles without negatively affecting peripheral tissues, kidney, or liver function (Brooks et al., 2016; Haas et al., 2007; Sahambi et al., 2008; Wright et al., 2008). VCD does not directly increase inflammation markers in the brain, including the hippocampus (Van Kempen et al., 2014).

AOF induction

Gonadally intact 53–58-postnatal-day-old female mice received 130 mg/kg VCD (cat. #S453005 Millipore Sigma, St. Louis, MO) in sesame oil (cat. # 8008-74-0 Millipore Sigma) for 5 days per week for 3 weeks (Marques-Lopes et al., 2017; Van Kempen et al., 2011). Control mice received injections of sesame oil only. Prior studies from our lab and others (Van Kempen et al., 2014; Haas et al., 2007; Lohff et al., 2005) established the peri-AOF stage as occurring 58 days after the first VCD injection. At this stage (~3.5 months old), the mice exhibit irregular, prolonged estrous cycles and elevated plasma follicle stimulating hormone (Lohff et al., 2005; Mayer et al., 2004; Harsh et al., 2007). Behavioral assessments of VCD- and oil-treated females, as well as aged-matched males, were initiated when mice were about 4 months of age (the peri-AOF stage of VCD mice). A timeline of the experimental procedures is shown in Figure 1.

Estrous cycle assessment

At euthanasia, vaginal smears (Turner and Bagnara, 1971) were collected to determine the terminal estrous cycle stage via

cytological examination. Estrous cycle phases were classified as proestrus (high estrogen), estrus (declining estrogen), or diestrus (low estrogen and progesterone). Most females were in estrus or diestrus at euthanasia.

Antibodies

4G8: A mouse monoclonal antibody raised against amino acid residues 17–24 of beta-amyloid (4G8, Biolegend Cat. # 800701) was employed. This antibody recognizes abnormally processed isoforms as well as precursor APP forms (manufacturer's instructions), and labels both parenchymal and vascular A β aggregates (Kövari et al., 2013; Alafuzoff et al., 2009; Marazuela et al., 2022; Davis et al., 2004). GFAP: A rabbit polyclonal antibody (Abcam # ab7260; lot # GR20948-21; RRID:AB_305808) raised against full-length human GFAP was used. On Western blot, this antibody recognized 48 kDa and 55 kDa GFAP bands (manufacturer's datasheet). Iba1: A rabbit polyclonal antibody raised against a synthetic peptide corresponding to the C-terminus of Iba1 (#SAR6502; 019-19741 FUJIFILM Wako Pure Chemical Corporation) was employed. The antibody reacts with rat, mouse and human Iba1 and recognizes a 17 kDa band protein on Western blot (manufacturer's datasheet). These antibodies have been used in our prior studies (Milner et al., 2022; Platholi et al., 2023).

Brain fixation and histology

Mouse brains were processed for immunocytochemistry using established procedures in our labs (Milner et al., 2011). Briefly, mice were deeply anesthetized with sodium pentobarbital (150 mg/kg, i. p.), and then perfused with saline. The brains were extracted, bisected sagittally, and the right hemisphere fixed in 4% paraformaldehyde in 0.1 M phosphate buffer (PB, pH 7.4) for 24 h on a shaker (70 rpm) at 4°C. The forebrain containing the hippocampus was sectioned (40 μ m thick) on a vibratome (VT1000X Leica Microsystems, Buffalo Grove, IL) and stored in cryoprotectant (30% sucrose, 30% ethylene glycol in PB) at –20°C until immunocytochemical processing.

For each experiment, one rostral (–2.00 to –2.70 mm from Bregma (Hof et al., 2000)) or caudal (–2.90 to –3.50 mm from Bregma (Hof et al., 2000)) hippocampal section per animal was selected and then punch coded in the cortex. Tissue sections from each experimental group were pooled into single containers to ensure identical reagent exposure (Milner et al., 2011).

Light microscopic immunocytochemistry

Hippocampal sections from each genotype/sex group were processed for 4G8, Iba1 or GFAP (n = 11/group). Sections were rinsed in 0.1 M Tris-saline (TS; pH 7.6), blocked with 0.5% bovine serum albumin (BSA) in TS for 30 min, and incubated in primary antibodies mouse anti-4G8 (1:4000), rabbit anti-GFAP (1:6000) or rabbit anti-Iba-1 (1:4000) diluted in 0.1% Triton-X and 0.1% BSA in TS for 24-h at room temperature followed by 24-h at +4°C.

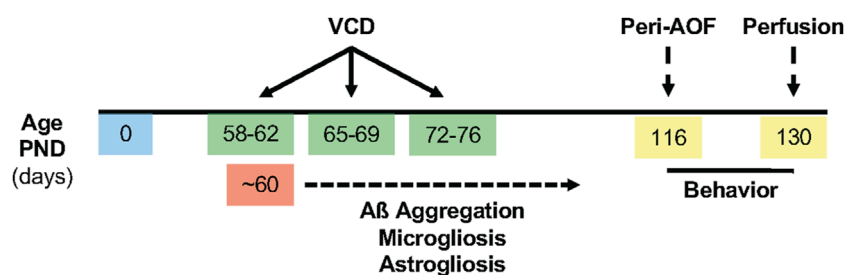


FIGURE 1

Timeline of experimental procedures. VCD (130 mg/kg, i. p.) was injected for 3 weeks, 5 days per week beginning between postnatal day (PND) 53–58. The extracellular deposition of Aβ plaques was expected to begin at ~ PND 60, accompanied by microgliosis and astrogliosis. Behavioral assessments were performed for 2 weeks following the initiation of the peri-AOF phase. Brains were harvested at ~ PND 130.

Next, sections were rinsed in TS and incubated in either biotin-conjugated goat anti-rabbit IgG (for GFAP and Iba1; #111-065-144, Jackson ImmunoResearch Inc., West Grove, PA; RRID:AB_2337965) or goat anti-mouse IgG (for 4G8; # 115-065-166, Jackson ImmunoResearch Inc.; RRID:AB_2338569) in 0.1% BSA and TS. Sections were washed in TS and incubated in Avidin Biotin Complex (ABC; Vectastain Elite kit, Vector Laboratories, Burlingame, CA) at half the manufacturer's recommended dilution for 30-min. After rinsing in TS, the bound peroxidase was visualized by reaction in 3,3'-diaminobenzidine (Sigma-Aldrich, St. Louis, MO) and 0.003% hydrogen peroxide in TS for 6-min (4G8), 3-min (GFAP), or 8-min (Iba1). All primary and secondary antibody incubations were carried out at 145 rpm, whereas rinses were at 90 rpm on a rotator shaker. Sections were mounted in 0.05 MPB onto gelatin-coated glass slides, dehydrated through an ascending series of alcohol to xylene, and coverslipped with DPX (Sigma-Aldrich).

Image acquisition and field densitometry

Quantification for 4G8, Iba1 and GFAP labeling in the hippocampus were performed using previously established densitometric methods (Pierce et al., 2014; Williams and Milner, 2011; Williams et al., 2011). To ensure unbiased data quantification, the analysis was performed by investigators blinded to experimental conditions. Images were acquired using a Nikon Eclipse 80i microscope with a Micropublisher 5.0 digital camera (Q-imaging, BC, Canada) and IP Lab software (Scanalytics IPLab, RRID: SCR_002775). ImageJ64 software (ImageJ, RRID:SCR_003070) was used to measure the pixel density within regions of interest (ROI) in defined hippocampal subregions. ROIs within four subregions of the rostral and caudal hippocampus were selected: 1) CA1: stratum oriens (SO), pyramidal cell layer (PCL), stratum radiatum (SR) and stratum lacunosum-moleculare (SLM); 2) CA2/3a: SO, PCL, near and distal SR; 3) CA3b: SO, PCL, stratum lucidum (SLu) and SR; 4) Dentate gyrus (DG): the supragranular blade (SG), the infragranular blade (IFG) and the central hilus (Cen) and 5) Subiculum (caudal section). Background pixel density from non-labeled regions (e.g., corpus callosum) was subtracted to control for illumination variability and background labeling. Prior studies (Pierce et al., 2014) demonstrated a strong correlation between pixel density and actual transmittance, confirming measurement accuracy.

Behavioral assessments

Mice were tested sequentially over 2 weeks in the Novel Object Recognition, Y maze, and Barnes maze tasks, as described in prior studies (Park et al., 2020; Park et al., 2008). The same investigator conducted all behavioral tests. Testing occurred at the same time each day, with results recorded using ANY-maze (Stoelting Co.). Mice were habituated to the testing room for 2 h daily for 5 days before testing. On testing days, mice were acclimated to the room for 1-h before each session. The order of the Y-maze and Novel Object tests were counterbalanced, with 24-h rest between the tests. Behavioral apparatuses were cleaned with 70% ethanol between trials.

Y-maze

This test was used to assess spatial working memory as previously described (Milner et al., 2011). Mice were placed in a three-arm maze (40 cm long, 9.5 cm high, 4 cm wide) diverging at a 120° from the central point, and allowed to explore two arms for 5 min (training). After 30 min, the previously blocked arm was opened, serving as the novel arm (test trial). The sequence of arm entries (spontaneous alternation) and locomotor activity were recorded for 5 min. A Spontaneous alternation was defined as entries into all the 3 arms on consecutive occasions, and was manually recorded from the recorded videos. The total number of arms entered during the sessions, which reflect locomotor activity, also was recorded. The maximum alternation was subsequently calculated by measuring the total number of arm entries minus 2 and the percentage of alternation was calculated as ((actual alternation/maximum alternation)×100).

Novel object recognition (NOR)

The NOR apparatus (height 30 cm × width 28 cm × length 46 cm) consisted of an open field chamber with dim illumination throughout, and it is used to measure spatial and working memory. On day 1 (habituation phase), each mouse was allowed to explore the empty arena for 5-min. On day 2 (familiarization phase), two identical objects (type A) were placed on the floor of the arena and the mouse was allowed to explore for 5-min followed by a 30-min rest. For the exploration phase, One of the type A objects was replaced with a novel object (type B) and the mouse was allowed to explore for 5-min the familiar and novel objects at the same

time. For each phase, the total distance traveled, the average speed, the total object exploration time, and the time spent exploring each one of the two objects was recorded. A discrimination index for day 2 exploration phase was calculated as percentage time spent exploring the novel object out of the total object exploratory time.

Barnes maze

The apparatus consisted of a maze with a 10 cm cylindrical white start chamber in the middle, multiple hole and one escape hole in the periphery. Mice were trained in the apparatus for four sequential days. Each training day consisted of 4 trials (with 15 min inter-trial intervals) in the following sequence: 1) Adaption period. The mouse was placed in the white start chamber, and a buzzer was switched on for 10-s. Following, the mouse was guided to an escape hole for 15-20-s. The buzzer was turned off and the mouse was allowed to stay in the escape box for 2-min 2) Spatial acquisition period. The mouse was placed in the start chamber and the buzzer was switched on for 10 s. After 10 s, the start chamber was removed, and the mouse was allowed to move around the maze to find an escape hole (maximum 3-min).

Immediately after the mouse entered the escape hole, the buzzer was turned off and the mouse was allowed to stay in the tunnel for 1-min. The average values collected from the spatial acquisition period of all four trials in a given training day is used as datapoint in the figures.

Twenty-four hours after the last training session, mice underwent the probe trial. For this, the mouse was placed in the maze in the white chamber and the buzzer was switched on. After 10-s, the chamber was removed, and the mouse behavior was recorded for 90-s. For each mouse, the latency time, errors, total length traveled to find the escape hole were recorded.

Image adjustments for figures

Images were adjusted first for contrast and sharpness in Adobe Photoshop 9.0 (Adobe Photoshop, RRID:SCR_014199). Next, images were imported into Microsoft PowerPoint, where final adjustments to brightness, sharpness and contrast were achieved. Images of different groups in the same figure were similarly adjusted to same brightness and contrast. Adjustments were made to the entire image, none of which significantly altered the appearance of the initial raw image.

Data analysis

Data are presented as means \pm SEM. Statistical analyses were conducted using Prism 9 software (Graphpad Prism, RRID:SCR_002798) and significance was set at $\alpha < 0.05$. Group comparisons were performed using analysis of variance (ANOVA; one-, two-, three-way) with Tukey or Sidak's post-hoc tests. Two-group comparisons used Student's t-tests. Specific analysis conducted are indicated in figure legends. Graphs were generated in Prism 9 software.

Results

Peri-AOF is associated with increased amyloid fibrils in select regions of the CA1 and CA3, but not in the dentate gyrus, in 5xFAD mice

AD dementia is more prevalent in women and may emerge at the onset of menopause. However, there is limited evidence that A β levels in the hippocampus, which are a hallmark of AD, are influenced by perimenopause. To evaluate whether A β increases in the hippocampus of females at a stage of early ovarian failure, 5xFAD females were treated with VCD, or sesame oil as a control, and A β levels quantified at peri-AOF, a stage corresponding to human perimenopause. To obtain a more granular understanding of perimenopause role on AD pathology, analysis was performed across major hippocampal subregions. Given the small size and complex geometric borders of these hippocampal subregions, ELISA and similar methods requiring precise tissue punches were not feasible. Instead, we employed light microscopic immunohistochemistry.

The density of 4G8, a marker of A β , was examined in CA1, CA3, DG and subicular subregions in rostral and caudal hippocampal sections of the 5xFAD mice (Figures 2A,B). No immunoreactivity was detected in the WT mice. As described below, 4G8 labeling in the 5xFAD mice varied across hippocampal subregions with sublayer specificity.

CA1 and subiculum: In the rostral CA1, dense 4G8-immunoreactivity (ir) was found in the pyramidal cell layer (PCL), with scattered clusters in the other laminae, particularly in the stratum oriens (SO) (Figure 2C–E). In the rostral CA1 PCL, 5xFAD male mice exhibited lower 4G8 labeling than both 5xFAD-oil treated and 5xFAD VCD-treated female mice following post-hoc analysis ($F = 5.080$, $p = 0.013$) (Figure 2F). Similarly, in the stratum radiatum (nSR), 5xFAD males showed lower 4G8 labeling compared to 5xFAD-oil treated female mice ($F = 3.783$, $p = 0.034$) (Figure 2F). Analogously, in the deep stratum radiatum (dSR), the density of 4G8 labeling following post-hoc analysis was lower in 5xFAD male mice compared to 5xFAD oil- and 5xFAD VCD-treated female mice ($F = 4.178$, $p = 0.025$) (Figure 2F). The 4G8 labeling in the caudal CA1 was similar to that seen rostrally. Moreover, scattered 4G8-positive cells were found in the subiculum though no significant effects were found (Supplementary Figures S1A–D).

CA3: Scattered 4G8-labeled cells were found in the PCL of CA3a and CA3b, with labeled clusters dispersed throughout other laminae (Figures 2G–I). No significant difference in 4G8 density was observed in any sublayers of CA3a (Supplementary Figures S1E–H). However, in the CA3b PCL, 5xFAD VCD females showed greater 4G8 labeling than both 5xFAD oil-treated females and 5xFAD male mice following *post hoc* analysis ($F = 5.049$, $p = 0.013$) (Figure 2J). Additionally, in the stratum lucidum (SLu) of CA3b, 5xFAD VCD-treated females showed higher 4G8 density than 5xFAD males ($F = 5.98$, $p = 0.007$; $t(20) = 3.374$, $p = 0.003$) (Figure 2J).

Dentate Gyrus (DG): In both the rostral and caudal DG, 4G8-labeling appeared in clusters throughout all laminae, especially in the hilus. No significant difference in 4G8 density was observed in any DG sublayers (Supplementary Figures S1I–P).

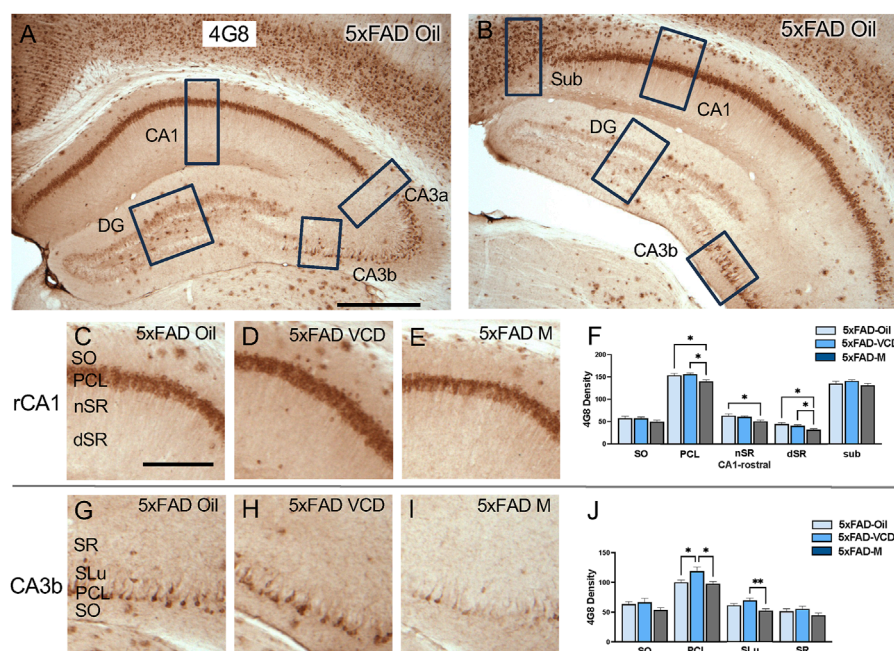


FIGURE 2

4G8 labeling is differentially altered in select regions of the hippocampus of oil and peri-AOF female and male 5xFAD mice. (A,B) Low-magnification photomicrographs of 4G8 labeling in the rostral (A) and caudal (B) hippocampus. Boxes indicate regions of the CA1, CA3a, CA3b, dentate gyrus (DG), and subiculum (Sub) that were sampled. (C–E) Representative photomicrographs showing 4G8 labeling in the rostral CA1 of 5xFAD-oil (C), 5xFAD-VCD (D), and 5xFAD-male mice (E). (F) In the rostral CA1 PCL and dSR, 5xFAD-male mice show significantly less 4G8 labeling than 5xFAD-oil and 5xFAD-VCD female mice. In the rostral CA1 nSR, 5xFAD-male mice show significantly less 4G8 labeling than 5xFAD-oil female mice. (G–I) Representative photomicrographs showing 4G8 labeling in the CA3b of 5xFAD-oil (G), 5xFAD-VCD (H), and 5xFAD-male mice (I). (J) In the CA3b PCL, 5xFAD-VCD female mice show greater 4G8 labeling than both 5xFAD-oil female and 5xFAD-male mice. In the CA3b SLu, 5xFAD-VCD female mice show greater 4G8 labeling than 5xFAD-male mice. * $p < 0.05$; ** $p < 0.01$ by One-way ANOVA with Tukey's post-hoc comparisons. Data are expressed as mean \pm SEM, $n = 11$ animals per experimental group. Scale bars (A,B) = 500 μ m, (C,D,E,G,H,I) = 200 μ m.

Peri-AOF is associated with increased levels of reactive astrocytes in select regions of the rostral CA1, DG, and caudal CA1 of 5xFAD mice

Astrocytes facilitate the removal of A β from the brain parenchyma by mediating efflux into the cerebral vasculature (Liu et al., 2018). Their function is influenced by sex, partly through the actions of estrogen signaling via its estrogen receptors (Santos-Galindo et al., 2011; Chowen and Garcia-Segura, 2021; Lu et al., 2020). Therefore, astrocytes are expected to play an important role in amyloidosis during ovarian failure. However, evidence is limited regarding changes in astrocyte activity across hippocampal subregions in both intact and reproductively compromised females.

To assess astrocyte activation, the density of the astrocytic marker GFAP was examined in CA1, CA3, DG and subicular subregions within rostral and caudal hippocampus (Figures 3A,B) of WT and 5xFAD oil- and VCD-treated female mice, as well as oil-treated male mice. Consistent with our prior studies (Milner et al., 2022; Platholi et al., 2023), GFAP-labeled cells were found throughout all lamina of CA1, CA3 and DG, with fewer GFAP-positive cells in the pyramidal and granule cell layers (Figures 3,4). Representative micrographs of GFAP labeling are shown for rostral CA1 (Figures 3C–H), caudal CA1/subiculum (Figures 3J–O), rostral DG (Figures 4A–F), and caudal DG (Figures 4H–M).

CA1 and subiculum: In the rostral CA1 SO region, there was a significant main effect of genotype ($F_{\text{genotype}} = 18.50$, $p < 0.0001$) and treatment ($F_{\text{treatment}} = 3.770$, $p = 0.029$). Post-hoc analysis showed that the density of GFAP labeling in SO was greater ($p < 0.05$) in 5xFAD mice than their WT counterparts (Figure 3I). In the PCL region, a significant main effect of treatment was found ($F_{\text{treatment}} = 4.822$, $p = 0.011$), with WT-VCD mice showing greater GFAP labeling than WT male mice ($p = 0.048$) (Figure 3I). In the SLM region, a significant main effect of genotype was observed ($F_{\text{genotype}} = 12.20$, $p = 0.0009$), and post-hoc analysis showed that 5xFAD male mice had significantly greater GFAP labeling than WT male mice ($p = 0.006$) (Figure 3I).

In the caudal CA1 SO region, there was a significant main effect of genotype ($F_{\text{genotype}} = 38.26$, $p < 0.0001$). Post-hoc analysis showed increased GFAP labeling in 5xFAD mice compared to WT controls ($p < 0.05$) (Figure 3P). In the PLC region, a main effect of genotype was also observed ($F_{\text{genotype}} = 7.458$, $p = 0.008$). Post-hoc multiple comparisons showed that WT-VCD mice had greater GFAP labeling than WT-oil mice ($p = 0.022$) (Figure 3P). Additionally, 5xFAD-oil mice had significant higher GFAP labeling in the PLC than WT-oil mice ($p = 0.001$) (Figure 3P). In the SR region, of caudal CA1, a significant main effect of genotype was observed ($F_{\text{genotype}} = 9.803$, $p = 0.003$), with increased GFAP labeling in 5xFAD-oil compared to WT-oil mice ($p = 0.005$) (Figure 3P). In the SLM region, there was a significant main effect of genotype ($F_{\text{genotype}} = 12.49$, $p = 0.001$),

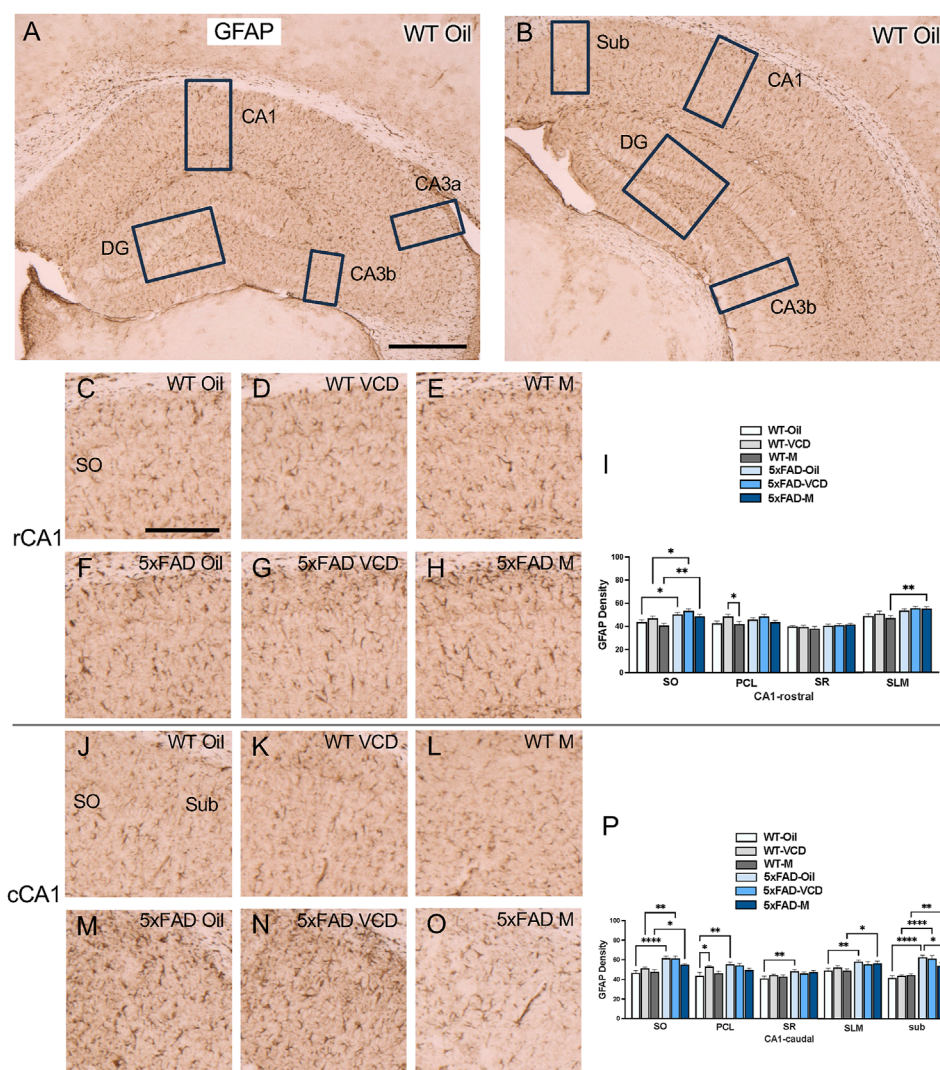


FIGURE 3

Increased GFAP labeling is associated with peri-AOF and/or 5xFAD genotype in select regions of the hippocampus. (A,B) Low-magnification photomicrographs of GFAP labeling in the rostral (A) and caudal (B) hippocampus. Boxes indicate regions of the CA1, CA3a, CA3b, dentate gyrus (DG), and subiculum (Sub) that were sampled. (C–H) Representative photomicrographs showing GFAP labeling in the rostral CA1 of WT-oil (C), WT-VCD (D), WT-male (E), 5xFAD-oil (F), 5xFAD-VCD (G), and 5xFAD-male mice (H). (I) In the rostral CA1 SO, the density of GFAP was increased in 5xFAD mice compared to WT mice, irrespective of sex/AOF treatment. In the PCL, WT-VCD female mice show greater GFAP density than WT-male mice. In the SLM, 5xFAD-male mice show more GFAP labeling than WT-male mice. (J–O) Representative photomicrographs showing GFAP labeling in the caudal CA1 of WT-oil (J), WT-VCD (K), WT-male (L), 5xFAD-oil (M), 5xFAD-VCD (N), and 5xFAD-male mice (O). (P) In the caudal CA1 SO, the density of GFAP was increased in 5xFAD mice compared to WT mice, irrespective of sex/AOF treatment. In the PCL, WT-oil female mice show less GFAP labeling than 5xFAD-oil and WT-VCD female mice. In the SR, 5xFAD-oil female mice show greater GFAP density than WT-oil female mice. In the SLM, both 5xFAD-oil female and 5xFAD-male mice had greater GFAP labeling than WT-oil and WT-male mice, respectively. In the Sub, the density of GFAP was increased in 5xFAD mice compared to WT mice, irrespective of sex/AOF treatment, and 5xFAD-oil female mice showed greater GFAP labeling than 5xFAD-male mice. * $p < 0.05$; ** $p < 0.01$; **** $p < 0.0001$ by two-way ANOVA with Tukey's hoc multiple comparison analysis. Data are expressed as mean \pm SEM, $n = 11$ mice per experimental group. Scale bars (A,B) = 500 μ m, (C,D,E,F,G,H,J,K,L,M,N,O) = 200 μ m.

with 5xFAD-oil mice exhibiting greater GFAP labeling than WT-oil mice ($p = 0.0071$), and 5xFAD-male showing increased GFAP compared to WT-male mice ($p = 0.0243$) (Figure 3P). Similar to other regions of caudal CA1, in the subiculum, a significant main effect of genotype was found ($F_{\text{genotype}} = 61.46$, $p < 0.0001$), with post-hoc comparisons revealing greater GFAP labeling in 5xFAD mice compared to WT controls ($p < 0.05$) (Figure 3P). Additionally, 5xFAD-oil mice had significantly higher GFAP labeling in the subiculum than 5xFAD-male mice ($p = 0.045$) (Figure 3P).

CA3: No significant effect of treatment or genotype on GFAP density was observed in any CA3 subregions (Supplementary Figures S2A–N).

DG: In the rostral DG crest, there was a significant effect of genotype ($F_{\text{genotype}} = 6.914$, $p = 0.011$) on the density of GFAP labeling. Post-hoc analysis revealed significantly greater GFAP labeling in 5xFAD-VCD compared to WT-VCD mice ($p = 0.021$) (Figure 4G). In the hilus, a significant main effect of genotype was observed ($F_{\text{genotype}} = 12.21$, $p = 0.001$), with post-hoc multiple

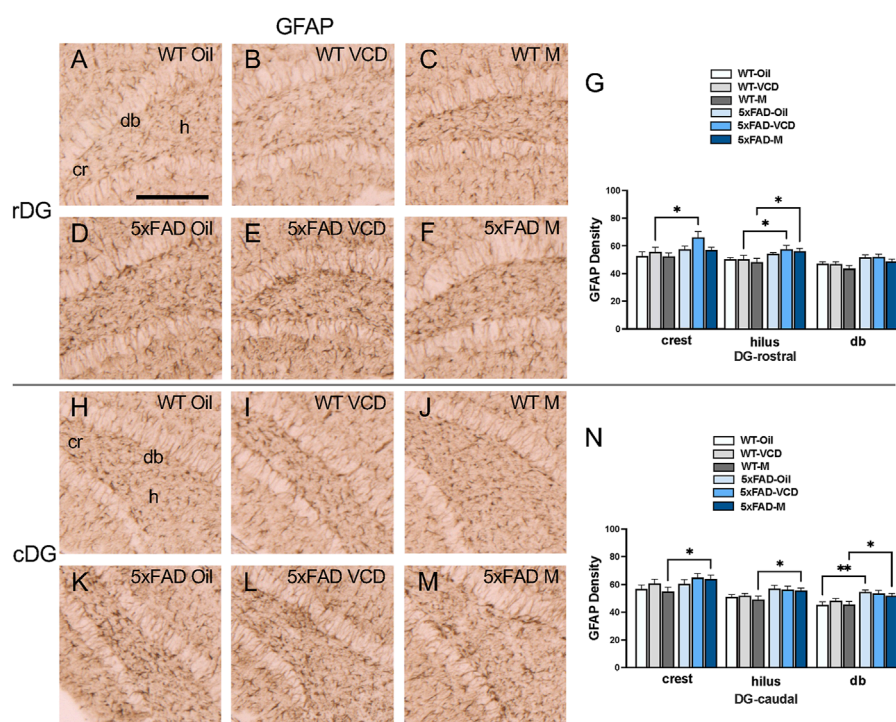


FIGURE 4

Increased GFAP labeling is associated with peri-AOF and/or 5xFAD genotype in select regions of the hippocampus. (A–F) Representative photomicrographs showing GFAP labeling in the rostral DG of WT-oil (A), WT-VCD (B), WT-male (C), 5xFAD-oil (D), 5xFAD-VCD (E), and 5xFAD-male mice (F). (G) In the rostral DG crest, 5xFAD-VCD female mice show increased GFAP labeling compared to WT-VCD female mice. In the hilus, 5xFAD-VCD female and 5xFAD-male mice show more GFAP labeling than WT-VCD female and WT-male mice, respectively. (H–M) Representative photomicrographs showing GFAP labeling in the caudal DG of WT-oil (H), WT-VCD (I), WT-male (J), 5xFAD-oil (K), 5xFAD-VCD (L), and 5xFAD-male mice (M). (N) In the caudal DG crest, hilus, and db, the density of GFAP was increased in 5xFAD-male mice compared to WT-male mice. In the db, GFAP density was also higher in 5xFAD-oil female mice compared to WT-oil female mice. * $p < 0.05$; ** $p < 0.01$ by two-way ANOVA with Tukey's *post hoc* multiple comparison analysis. Data are expressed as mean \pm SEM, $n = 11$ animals per experimental group. Scale bar = 200 μ m.

comparison analysis revealing that 5xFAD-VCD and 5xFAD-male mice had greater GFAP labeling than their WT counterparts ($p < 0.05$) (Figure 4G).

In the caudal DG crest, there was a significant main effect of genotype ($F_{\text{genotype}} = 5.325$, $p = 0.025$), with increased GFAP in 5xFAD-male compared to WT-male mice ($p = 0.034$) (Figure 4N). In the hilus, a main effect of genotype was also found ($F_{\text{genotype}} = 10.18$, $p = 0.002$), with 5xFAD-male mice exhibiting greater GFAP labeling than WT-male mice ($p = 0.036$) (Figure 4N). In the db region of the DG, a main effect of genotype was observed ($F_{\text{genotype}} = 18.47$, $p < 0.0001$), with increased GFAP labeling in 5xFAD-oil compared to WT-oil mice ($p = 0.002$) (Figure 4N). Similarly to the crest and hilus, 5xFAD-male mice showed significantly more GFAP labeling than WT-male mice ($p = 0.031$) in the db (Figure 4N).

These patterns of GFAP labeling suggest that peri-AOF is associated with increased astrocyte activity in select hippocampal regions of the rostral and caudal CA1 and DG.

Increased microglia activation in select regions of the CA1 and DG of 5xFAD mice

Microglia, the brain's resident macrophage, regulate neuroinflammation and cognitive function (Cornell et al., 2022). The

protein Iba1 is constitutively expressed in microglia and upregulated upon activation (Sasaki et al., 2001; Imai et al., 1996), a common feature of aging and neurodegenerative disorders (Prinz et al., 2021). Significantly, microglia activity is influenced by ovarian hormone changes. Ovariectomy increases Iba1 expression in middle-aged female mice (Sárvári et al., 2017), while estradiol reduces microglia reactivity in the hippocampus of aged ovariectomized animals (Lei et al., 2003). Additionally, ovariectomy elevates macrophage antigen complex-1, another marker of reactive microglia, in the hippocampus of aged mice (Benedusi et al., 2012). In AD mouse models, chronic estrogen deficiency is linked to heightened microglial activation and neurodegeneration (Prat et al., 2011). Given these findings, ovarian failure may alter hippocampal Iba1 expression during amyloidosis, however, direct evidence remains limited.

The density of Iba1 was examined in CA1, CA3, DG and subiculum subregions within the rostral and caudal hippocampus (Figures 5A,B). Consistent with our prior studies (Milner et al., 2022; Platholi et al., 2023), Iba1-labeled cells were scattered throughout all lamina in the CA1, CA3 and DG; however, the pyramidal and granule cell layers contained less Iba1 labeling (Figures 5,6,). Representative micrographs show the distribution of Iba1 labeling in the rostral CA1 (Figures 5C–H), caudal CA1/subiculum (Figures 5J–O), rostral DG (Figures 6A–F), and caudal DG (Figures 6H–M).

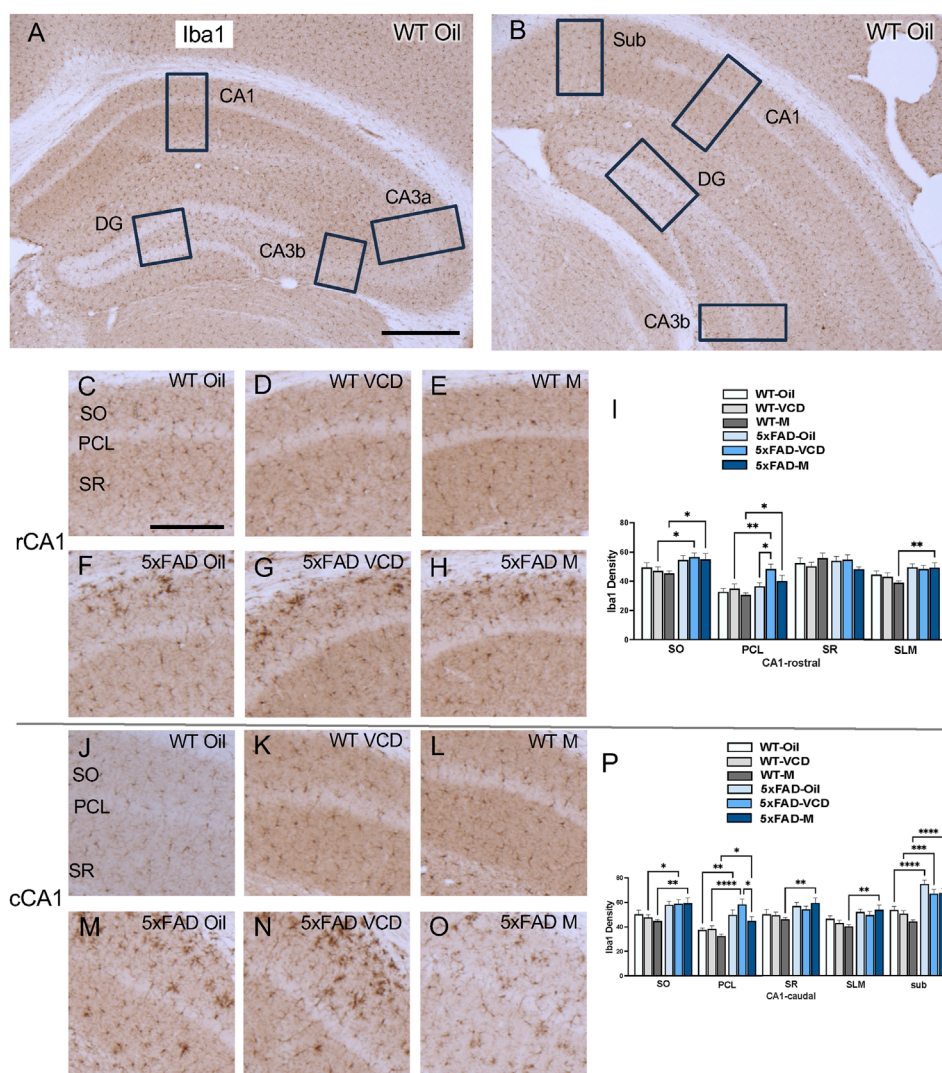


FIGURE 5

Increased Iba1 labeling is associated with peri-AOF and/or 5xFAD genotype in select regions of the hippocampus. (A,B) Low-magnification photomicrographs of Iba1 labeling in the rostral (A) and caudal (B) hippocampus. Boxes indicate regions of the CA1, CA3a, CA3b, dentate gyrus (DG), and subiculum (Sub) that were sampled. (C–H) Representative photomicrographs showing Iba1 labeling in the rostral CA1 of WT-oil (C), WT-VCD (D), WT-male (E), 5xFAD-oil (F), 5xFAD-VCD (G), and 5xFAD-male mice (H). (I) In the rostral CA1 SO and PCL, the density of Iba1 was increased in 5xFAD-VCD female and 5xFAD-male mice compared to WT-VCD female and WT-male mice, respectively. In the PCL, Iba1 density was greater in 5xFAD-VCD female mice than 5xFAD-oil female mice. In the SLM, 5xFAD-male mice had more Iba1 labeling than WT-male mice. (J–O) Representative photomicrographs showing Iba1 labeling in the caudal CA1 of WT-oil (J), WT-VCD (K), WT-male (L), 5xFAD-oil (M), 5xFAD-VCD (N), and 5xFAD-male mice (O). (P) In the caudal CA1 SO, 5xFAD-VCD female and 5xFAD-male mice had increased Iba1 density compared to WT-VCD and WT-male mice, respectively. In the PCL, the density of Iba1 was increased in 5xFAD mice compared to WT mice irrespective of sex/AOF treatment. 5xFAD-VCD female mice also showed greater Iba1 labeling than 5xFAD-male mice. In the SR and SLM, 5xFAD-male mice had more Iba1 labeling than WT-male mice. In the Sub, 5xFAD mice had increased Iba1 density compared to WT mice irrespective of sex/AOF treatment. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$ by two-way ANOVA with Tukey's *post hoc* multiple comparison analysis. Data are expressed as mean \pm SEM, $n = 11$ animals per experimental group. Scale bars (A,B) = 500 μm , (C,D,E,F,G,H,I,K,L,M,N,O) = 200 μm .

CA1 and subiculum: In the rostral CA1 SO region, there was a significant main effect of genotype ($F_{\text{genotype}} = 10.16$, $p = 0.0023$) on Iba1 labeling. Post-hoc analysis showed that Iba1 density was greater ($p < 0.05$) in 5xFAD-VCD and 5xFAD-male than their WT counterparts (Figure 5I). In the PCL region, there was a main effect of genotype ($F_{\text{genotype}} = 14.12$, $p = 0.0004$) and treatment ($F_{\text{treatment}} = 3.449$, $p = 0.038$) on Iba1 labeling. As in the SO region, post-hoc analysis showed increased labeling in 5xFAD-VCD and 5xFAD-male mice ($p < 0.05$) compared to WT mice (Figure 5I).

Additionally, 5xFAD-VCD mice exhibited significantly more Iba1 labeling compared to 5xFAD-oil mice ($p = 0.016$) (Figure 5I). In the SLM region of the rostral CA1, a main effect of genotype was observed ($F_{\text{genotype}} = 10.89$, $p = 0.002$), with post-hoc analysis showing significantly more Iba1 labeling in 5xFAD-male mice than WT-male mice ($p = 0.006$) (Figure 5I).

In the caudal CA1 SO region, there was a significant main effect of genotype ($F_{\text{genotype}} = 19.61$, $p < 0.0001$). Post-hoc analysis showed that Iba1 density was greater ($p < 0.05$) in 5xFAD-VCD and 5xFAD-male

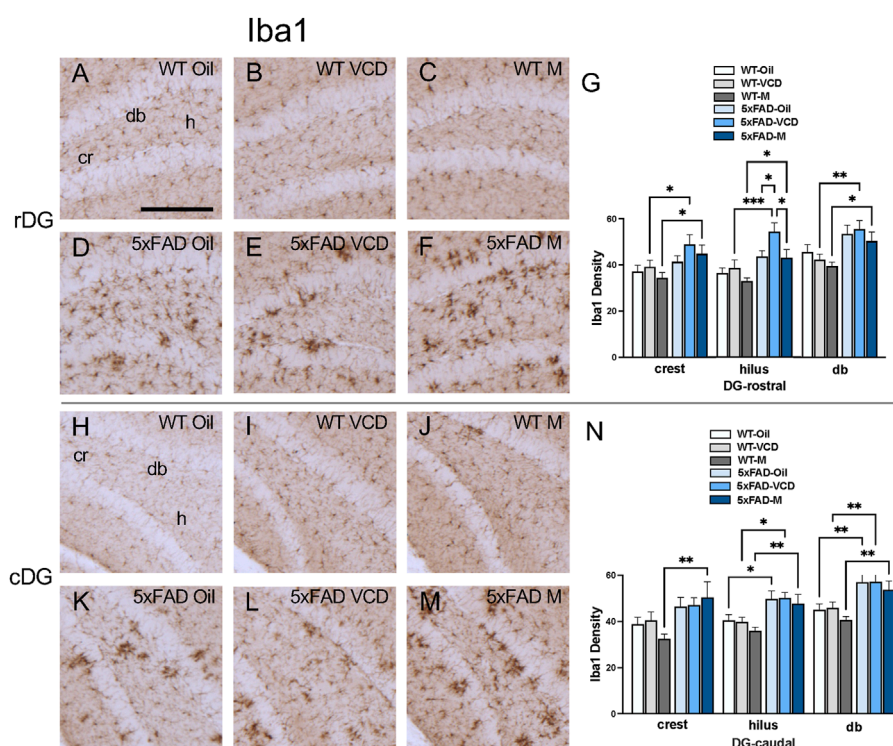


FIGURE 6

Increased Iba1 labeling is associated with peri-AOF and/or 5xFAD genotype in select regions of the hippocampus. (A–F) Representative photomicrographs showing Iba1 labeling in the rostral DG of WT-oil (A), WT-VCD (B), WT-male (C), 5xFAD-oil (D), 5xFAD-VCD (E), and 5xFAD-male mice (F). (G) In the rostral DG crest and db, 5xFAD-VCD female and 5xFAD-male mice showed greater Iba1 labeling than WT-VCD and WT-male mice, respectively. In the hilus, the density of Iba1 labeling was increased in 5xFAD mice compared to WT mice, irrespective of sex/AOF treatment. 5xFAD-VCD female mice also showed greater Iba1 labeling than 5xFAD-male mice. (H–M) Representative photomicrographs showing Iba1 labeling in the caudal DG of WT-oil (H), WT-VCD (I), WT-male (J), 5xFAD-oil (K), 5xFAD-VCD (L), and 5xFAD-male mice (M). (N) In the caudal DG crest, 5xFAD-male mice had greater Iba1 labeling than WT-male mice. In the hilus and db, the density of Iba1 was increased in 5xFAD mice compared to WT mice, irrespective of sex/AOF treatment. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$ by two-way ANOVA with Tukey's *post hoc* multiple comparison analysis. Data are expressed as mean \pm SEM, $n = 11$ animals per experimental group. Scale bar = 200 μ m.

mice than their WT counterparts (Figure 5P). In the PCL region, a main effect of genotype ($F_{\text{genotype}} = 30.99$, $p < 0.0001$) and treatment ($F_{\text{treatment}} = 4.272$, $p = 0.014$) was observed. Multiple comparisons revealed significantly more Iba1 labeling in 5xFAD mice than in WT mice ($p < 0.05$) (Figure 5P). Additionally, 5xFAD-VCD mice exhibited greater Iba1 labeling in the PLC than 5xFAD-male mice, ($p = 0.014$) (Figure 5P). In the SR region of the caudal CA1, a main effect of genotype ($F_{\text{genotype}} = 9.983$, $p = 0.003$) was found, with post-hoc analysis showing significantly more Iba1 in 5xFAD-male mice than in WT-male mice (Figure 5P). In the SLM, there was also a main effect of genotype ($F_{\text{genotype}} = 14.19$, $p = 0.0004$), with 5xFAD-male mice exhibiting greater Iba1 labeling than WT-male mice (Figure 5P). Similarly, in the subiculum, a significant main effect of genotype ($F_{\text{genotype}} = 67.47$, $p < 0.0001$) and treatment ($F_{\text{treatment}} = 3.996$, $p = 0.024$) was found, with post-hoc analysis revealing significantly more Iba1 labeling in 5xFAD mice than WT mice ($p < 0.05$) (Figure 5P).

CA3: There was no effect of treatment or genotype on the density of Iba1 labeling in any of the sublayers of CA3a or CA3b (Supplementary Figures S2O–AB).

DG: In the rostral DG, there was a significant main effect of genotype ($F_{\text{genotype}} = 9.923$, $p = 0.0025$) on Iba1 labeling in the crest. Post-hoc analysis showed greater Iba1 density ($p < 0.05$) in

5xFAD-VCD and 5xFAD-male mice than in WT mice (Figure 6G). In the hilus, there was a main effect of genotype ($F_{\text{genotype}} = 20.20$, $p < 0.0001$) and treatment ($F_{\text{treatment}} = 4.349$, $p = 0.017$). Post-hoc analysis showed significantly more Iba1 labeling ($p < 0.05$) in 5xFAD-VCD and 5xFAD-male mice compared to their WT counterparts (Figure 6G). Additionally, 5xFAD-VCD mice exhibited increased Iba1 density compared to 5xFAD-oil mice ($p = 0.036$) and 5xFAD-male mice ($p = 0.027$) (Figure 6G). In the db of the rostral DG, a main effect of genotype ($F_{\text{genotype}} = 16.39$, $p = 0.0002$) was observed, with post-hoc analysis showing greater density of Iba1 ($p < 0.05$) in 5xFAD-VCD and 5xFAD-male mice compared to WT mice (Figure 6G).

In the caudal DG, a main effect of genotype ($F_{\text{genotype}} = 10.04$, $p = 0.002$) was found in the crest, with 5xFAD males exhibiting greater Iba1 density than WT male mice ($p = 0.003$) (Figure 6N). In the caudal hilus, a significant main effect of genotype ($F_{\text{genotype}} = 20.42$, $p < 0.0001$) was observed, with post-hoc analysis showing greater Iba1 labeling ($p < 0.05$) in 5xFAD mice than in their WT counterparts (Figure 6N). Similarly, a significant main effect of genotype ($F_{\text{genotype}} = 25.07$, $p < 0.0001$) was determined in the db of the DG, with multiple comparisons revealing significantly more Iba1-labeling ($p < 0.01$) in 5xFAD mice than in WT mice (Figure 6N).

These results suggest that the 5xFAD genotype at peri-AOF influences microglia activation in multiple subregions of rostral and caudal CA1 and DG compared to peri-AOF WT mice. Additionally, peri-AOF further exacerbates microglia activation in 5xFAD mice in rostral CA1 PCL and DG hilus, suggesting sex-dependent vulnerability. These findings, in concert with increased GFAP labeling in select hippocampal regions, suggest enhanced susceptibility to neuroinflammation at the intersection of AD and perimenopause.

Cognitive impairment varies with genotype, sex and AOF

Neuroinflammation is intrinsically linked to the progression of cognitive impairment and dementia (Heneka et al., 2015). However, the impact of early ovarian failure on cognitive function in VCD-treated 5xFAD mice, and how this compares to males, remains unknown. To address this, we assessed the behavioral consequences of early ovarian decline in 5xFAD mice compared to non-VCD treated females and males using different cognitive tests sensitive to A β pathology, including the Y-maze alternation test, novel object recognition, and spatial navigation in the Barnes maze.

Y-maze

No significant differences were observed in arm alternation behavior (Figure 7A) or in the total number of arm entries in all 3 arms during the testing period (Figure 7B) between 5xFAD and WT mice. No differences were observed in the % novel arm entries respect to total arm entries and in the time spent in the novel arm (data not shown). This may suggest preservation of short-term working memory or that other cognitive domains might be more affected in 5xFAD mice and in VCD-treated females at a young age.

Novel object

An effect of AOF was seen in locomotor activity as assessed by total distance travelled ($F(5, 60) = 2.42$, $p = 0.05$). 5xFAD-male compared to 5xFAD-VCD female mice had a lower total distance ($p = 0.06$), although not significant (Figure 7C). No significant differences across groups were observed in the total object exploration time or in Novel Object Recognition Index (Figures 7D,E). Interestingly, significant increase in time spent exploring the novel object vs. the familiar object was observed between WT-male ($p < 0.0001$) and 5xFAD-male mice ($p = 0.001$) (Figure 7F). For females, significant differences in time spent exploring familiar and novel objects were found between treatment in WT mice (WT-female ($p = 0.018$); WT-VCD female ($p = 0.014$)), but did not extend to 5xFAD females (Figure 7F).

Barnes maze

There were no significant differences in motor activity, as assessed by distance traveled and mean speed between 5xFAD

and WT mice, regardless of genotype or treatment (Figures 8A,B). Although, a significance difference was observed in mean speed between WT females and VCD-treated WT females at day 1 only ($p = 0.0086$). Also, no differences were observed in learning measures as shown for latency to find the escape and path efficiency (Figures 8C,D).

When cognitive performance was tested 24 h after the final acquisition training session, VCD-treated WT-females spent significantly less time in the target quadrant where the escape hole was located, and more time in non-target quadrants compared to both WT-oil or 5xFAD-VCD females (Figures 8E,F).

Given that the Barnes maze is a measure of spatial learning and memory, the altered performance in VCD-treated WT females suggests a potential adverse effect of early ovarian failure on spatial memory. This effect appears to be mitigated in 5xFAD-VCD females.

Overall, these behavioral data demonstrate that VCD treatment impairs performance in select cognitive tasks. Combined with our anatomical findings on A β deposition and glia activation in the hippocampus, these results suggest that early ovarian failure contributes to early-stage AD-like neurobehavioral pathology.

Discussion

The relationship between early ovarian failure and neuroinflammation in the hippocampus was investigated using a model of perimenopausal AD that combined chemically-induced AOF with transgenic 5xFAD mice. Age-matched males were also tested in tandem. Mice at approximately 4-months of age showed complex effects on production of A β as well as signs of astrocyte and microglia activity that varied by genotype, VCD treatment (AOF) and sex. We found that A β expression was elevated in female 5xFAD mice, but only further increased in peri-AOF mice in select hippocampal subregions. Further, increases in glial activation also paralleled A β pathology but only in discrete areas of the hippocampus. Assessment of cognitive function showed no effect of peri-AOF in the Y-maze, in the Novel Object Recognition tests, or across Barnes Maze training days (days 1–4). Interestingly, peri-AOF reduced the time spent in the Barnes Maze target quadrant during the testing day (day 5) in WT but not in 5xFAD females, suggesting a treatment-genotype interaction effect. These data provide preclinical experimental evidence supporting the contention that peri-menopause is a sensitive period for neuroinflammation and cognitive function in women.

AD, the most prevalent of the dementias (Alzheimer's, 2016), is classically characterized by A β deposition and cognitive impairment. However, AD also involves a complex set of neurodegenerative processes, including neuroinflammation (Lista et al., 2024). Importantly, the incidence, progression, and severity of the disease are greater in women (Pike, 2017; Laws et al., 2016). Further, perimenopause may be a critical period for the emergence of AD, suggesting that altered gonadal hormone levels contribute to the increased AD risk (Gong et al., 2022). Although perimenopause represents a potential turning point in AD pathology, the impact of associated hormonal changes on hippocampal pathology remains unclear. To investigate the role of early ovarian decline in AD pathology, we exposed female 5xFAD mice to the ovotoxin VCD to induce AOF and tested them at an

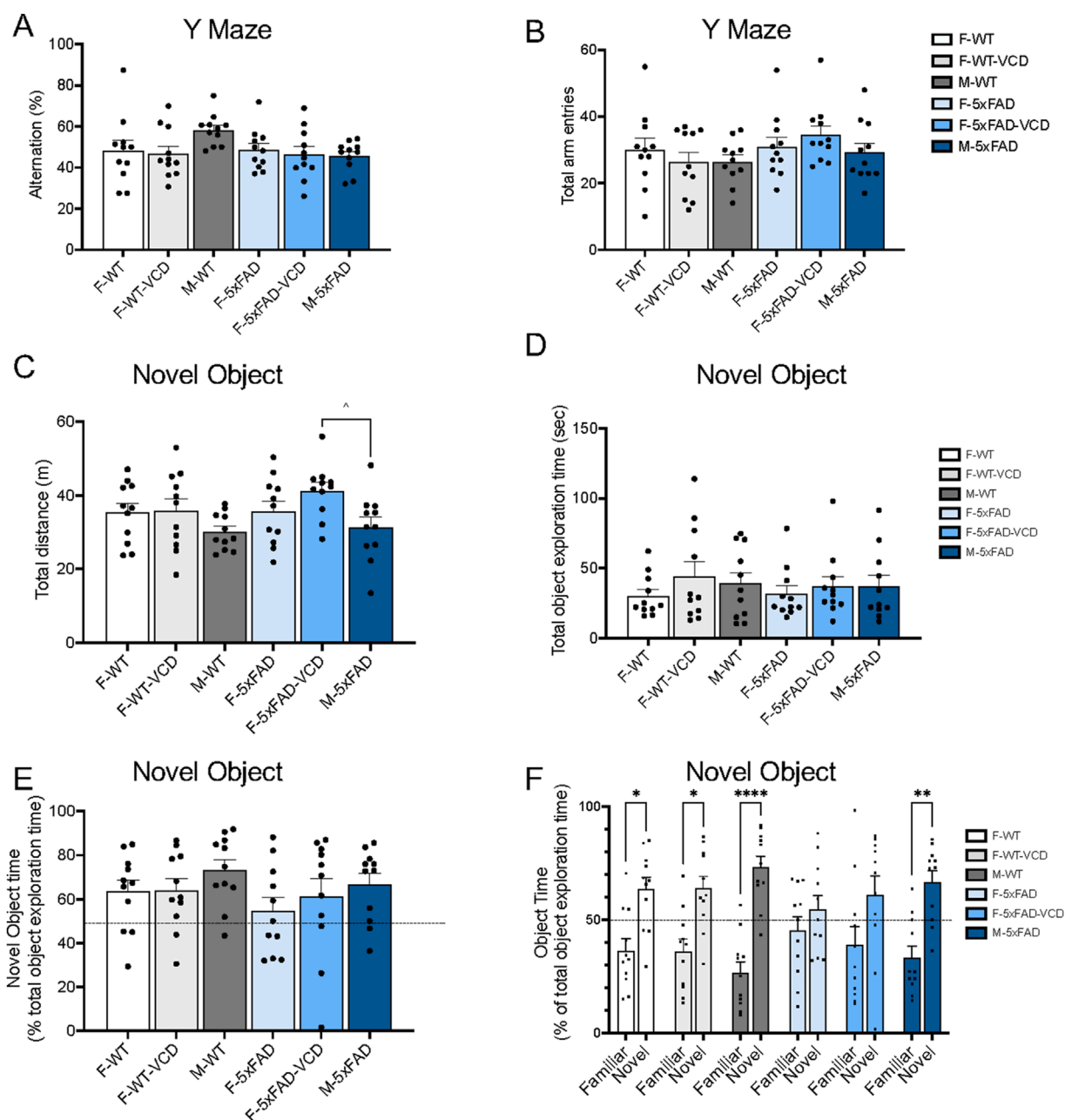


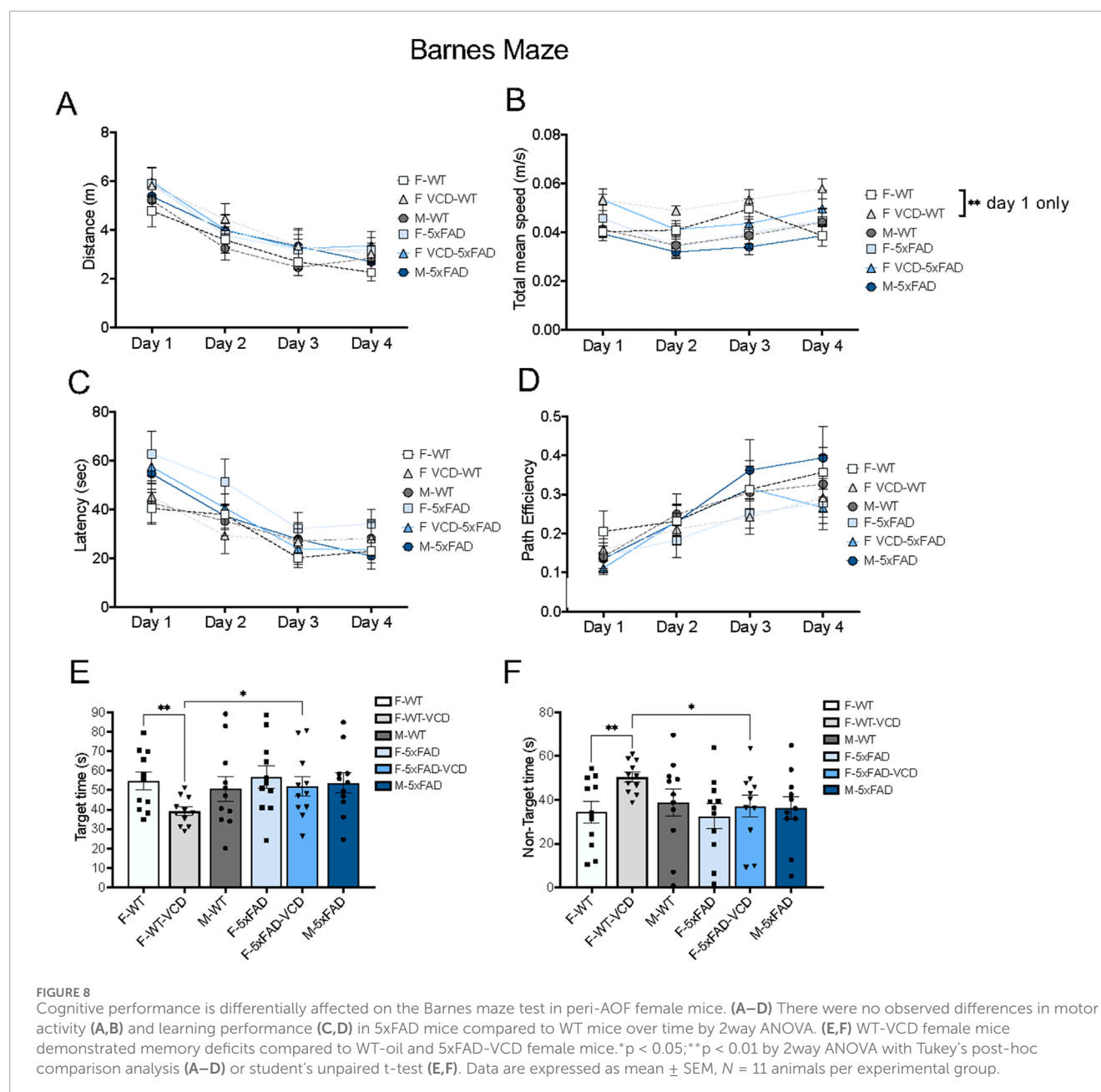
FIGURE 7

Cognition is not impaired in the Y-maze but is in the novel object recognition test in 5xFAD compared to WT mice. (A,B) In the Y-maze test, there were no significant differences in arm alternation behavior (A) or in the number of arm entries (B) between 5xFAD and WT mice. (C) Spontaneous motor activity tended to be decreased in 5xFAD-male mice compared to 5xFAD-VCD female mice. (D) total object exploratory time was not changed across experimental groups. (E) No significant differences were observed between groups in the percentage time spent exploring the novel object. (F) Significant increase in time spent with the novel object vs familiar object was observed for WT male and female (oil and VCD), as well as in 5xFAD male mice. This difference is lost in 5xFAD females. $^{\wedge}p = 0.06$; * $p < 0.05$; ** $p < 0.01$; **** $p < 0.0001$ by one-way ANOVA with Sidak's *post hoc* multiple comparison analysis. Data are expressed as mean \pm SEM, $n = 11$ animals per experimental group.

age when brain amyloid deposition and ovarian failure are both at early stages. Age-matched WT mice and 5xFAD male mice were also studied to isolate the impact of AOF independently of amyloidosis and to characterize sex differences, respectively.

We found that A β expression was elevated in female 5xFAD peri-AOF mice (injected with VCD) compared to intact females and males injected with sesame oil, in select hippocampal subregions

such as the CA1 and CA3b, but not CA3a. The mechanism underlying increased A β levels in CA3b compared to CA3a in VCD-treated 5xFAD mice is unclear at present. Presumably, these regional differences are mediated by neurophysiological (Hemond et al., 2009; Wittner and Miles, 2007), transcriptional (Cembrowski et al., 2016; Thompson et al., 2008), connectonal (Wu et al., 1998), morphological (Wittner and Miles, 2007) and



functional (Sun et al., 2017) divergence across these CA3 subfields, which in turn may be modulated by sex and estrogens. For example, CA3b and CA3a each show unique changes in expression patterns of both stress and plasticity-related molecules following stress, a modulator of dementia (Yakemow et al., 2024), and do so in an estrogen-dependent manner (Van Kempen et al., 2014; Pierce et al., 2014; Van Kempen et al., 2013). These results suggest that the pathways linking A β pathology with estrogen signaling within the CA3 are subregion-specific, and further research is needed to clarify their specific contributions to learning and memory during ovarian failure.

In VCD-injected WT mice, GFAP density was increased compared to oil-treated WT mice in the caudal CA1 PCL and compared to males in the rostral CA1 PCL. No differences

were detected with regard to Iba-1 labeling in any hippocampal region between the WT mice. The increased astrogliosis observed during peri-AOF may result from the loss of astrocyte estrogen signaling. Both resting and activated astrocytes are prominent among the non-neuronal hippocampal cell types that express estrogen receptors alpha and beta (Mitterling et al., 2010; Azcoitia et al., 1999; Sakuma et al., 2009) as well as G-protein estrogen receptors (Waters et al., 2015). Further, the expression and cellular location of estrogen receptors in the hippocampus are influenced by estrogen levels (Rune et al., 2002; Adams et al., 2002). Estrogen modulates GFAP expression (Stone et al., 1998) and regulates the expression of genes involved in astrocyte proliferation (Ma et al., 2014). Importantly, the CA1 PCL is an estrogen receptor-expressing region of the hippocampus (Mitterling et al.,

2010; Azcoitia et al., 1999; Sakuma et al., 2009) and is a major hippocampal output that plays a critical role in both spatial and non-spatial memory processes (Cinalli et al., 2023). The functional impact of elevated astrocyte activation in the context of reduced estrogen signaling remains unclear, although it may involve the loss of estrogen-mediated neuroprotection and/or the activation of compensatory mechanisms. This is supported by findings that estrogen reduces glucose- and oxygen-deprivation-induced apoptosis in astrocyte-neuron co-cultures, and that estrogen replacement attenuates the increased apoptosis in the CA1 produced by cerebral ischemia (Ma et al., 2016). Given the protective role of estrogen against astrocytosis-associated neural dysfunction, its loss in the CA1 PCL region suggests an increased vulnerability to stress or insult and a heightened propensity for neural pathology.

In addition to their critical metabolic role, astrocytes exert important effects on neuronal signaling and plasticity, particularly via modulation of pyramidal cells (Bosson et al., 2017; Dong et al., 2020). CA1 pyramidal cell hyperexcitability is a hallmark of neurodegeneration and, in particular, is observed in AD models (Šišková et al., 2014; Phelan et al., 2024; Lei et al., 2012). Significantly, astrocytes contribute to CA1 pyramidal cell activity (Courtney et al., 2023; Bohmbach et al., 2022; Wang et al., 2021), and reactive astrocytes are associated with decreased inhibitory synaptic currents and CA1 hyperexcitability (Ortinski et al., 2010). Thus, increased astrocyte activity in the CA1 PCL during early AOF, in the context of disrupted estrogen signaling, may contribute to conditions favoring pyramidal cell dysfunction.

We examined astrogliosis and A β expression in peri-AOF 5xFAD mice. Compared to WT VCD-treated mice, 5xFAD VCD mice did not show a further increase in GFAP in the CA1 PCL. However, GFAP density was increased in the CA1 SO and subiculum in VCD-treated 5xFAD mice compared to similarly treated WT mice. Additionally, 5xFAD peri-AOF mice showed higher GFAP density in the crest and hilus of the rostral DG compared to WT mice.

Microglia activation was also observed. Iba-1 levels were higher in peri-AOF 5xFAD mice compared to peri-AOF WT mice in the CA1 PCL, SO and subiculum as well as the different subregions of the DG. These results indicate that microglia are recruited in non-pyramidal cell regions of the CA1. Significantly, microglia activation was greater in CA1 PCL and DG hilus cells in 5xFAD peri-AOF mice compared to 5xFAD females and males.

The increased 4G8 density observed in 5xFAD mice was expected to be coupled with elevated GFAP and Iba-1 levels. We found that in VCD-treated mice only the CA3b PCL showed increased A β compared to both oil-treated female and male controls. However, there were no differences in either GFAP or Iba-1 across any treatment in any CA3 field. Additionally, in both oil and VCD 5xFAD mice, 4G8 in the CA1 PCL was higher compared to males. Yet, this was associated with increased Iba-1 only in peri-AOF mice, suggesting that this region may be particularly sensitive in response to AOF and A β aggregation. The lack of coupling between increased neuroinflammatory markers and A β levels in several hippocampal subregions suggests that peri-AOF affects glial activity independently of amyloid aggregation in these areas. Alterations in brain function have been described prior to

A β plaque or fibril formation in other APP overexpressing mouse models (Park et al., 2004). For example, functional hyperemia is impaired in young mice prior to A β aggregation (Park et al., 2004) and deficits in autoregulation occur (Niwa et al., 2002). These effects may be attributed to the actions of soluble oligomeric A β (Tolar et al., 2021), which are detectable in 5xFAD mice at the age range studied here (Habashi et al., 2022). Notably, oligomeric A β can impact astrocytes (Matafora et al., 2023) and microglia (Tong et al., 2024).

Our findings in 5xFAD mice contrast with similar experiments in SwDI mice, a model of cerebral amyloid angiopathy (Xu et al., 2014a; Xu et al., 2007). In SwDI VCD mice, we found increased 4G8 in the CA1 SO and DG. Further, astrocyte and microglia activation was observed in the CA1 SO, and astrocytes in the DG crest. Pyramidal cell layers of the CA1 and CA3 were not affected. These results indicate that dementia models with A β pathology in either parenchyma or cerebral vasculature exhibit distinct patterns of hippocampal amyloid deposition and glial cell activation with notable differences in pyramidal and non-pyramidal regions.

The present results demonstrate that distinct hippocampus regions exhibit differential susceptibility to AOF and A β in 5xFAD mice. The CA1 of 5xFAD mice, particularly the pyramidal cell layer, was vulnerable to the neuroinflammatory effects of peri-AOF. This aligns with evidence that the CA1 is the earliest hippocampal subregion to exhibit neuronal loss in AD (Padurariu et al., 2012; Kerchner et al., 2012) and that astrocyte-pyramidal cell interactions in the CA1 are disrupted in AD models (Pirttimäki et al., 2013).

Interestingly, the pyramidal cell layer of the CA3b region uniquely demonstrated elevated A β in 5xFAD peri-AOF mice. The CA3 is a region that expresses estrogen receptors (Mitterling et al., 2010; Mitra et al., 2003; Weiland et al., 1997; Shughrue and Merchant, 2000; Tsurugizawa et al., 2005), contains estrogen-sensitive neurons (Van Kempen et al., 2014), and may be protected against A β -mediated degeneration by estrogen (Pike, 1999). Together with our findings in WT mice, these results suggest that pyramidal cell layers of the CA1 and CA3 fields are particularly sensitive to decreased estrogen and increased A β during early AOF in female mice. In sum, these findings highlight the complex interplay between ovarian hormone loss, neuroinflammation, and amyloid pathology, underscoring the need for further research into the mechanisms by which perimenopause contributes to heightened AD susceptibility in women.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding authors.

Ethics statement

The animal study was approved by Weill Cornell Medicine Institutional Animal Care and Use Committees. The study was

conducted in accordance with the local legislation and institutional requirements.

Author contributions

RM: Formal Analysis, Investigation, Methodology, Visualization, Writing – original draft, Writing – review and editing. JP: Formal Analysis, Investigation, Methodology, Visualization, Writing – original draft, Writing – review and editing. LP: Formal Analysis, Methodology, Supervision, Writing – review and editing, Writing – original draft. FY: Data curation, Investigation, Methodology, Writing – original draft, Writing – review and editing. GS: Data curation, Investigation, Visualization, Writing – original draft, Writing – review and editing, Formal Analysis. CW: Writing – original draft, Writing – review and editing. TM: Methodology, Supervision, Project administration, Conceptualization, Writing – original draft, Funding acquisition, Validation, Visualization, Resources, Writing – review and editing, Investigation. MG: Supervision, Conceptualization, Funding acquisition, Software, Investigation, Writing – review and editing, Visualization, Project administration, Writing – original draft, Validation, Resources, Data curation.

Funding

The author(s) declare that financial support was received for the research and/or publication of this article. Work was supported by NIH grants R01 HL136520S1 (TM and MG) and HL135498 (MG), R01 HL135428 (MG), R01 GM130722 (JP), R21 AG064455 (RM), R01 NS097805 (LP).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Generative AI statement

The author(s) declare that no Gen AI was used in the creation of this manuscript.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fmolb.2025.1597130/full#supplementary-material>

SUPPLEMENTARY FIGURE S1

4G8 labeling is not altered in select regions of the hippocampus of across WT and 5xFAD experimental groups. **(A–C)** Representative photomicrographs showing 4G8 labeling in the caudal CA1 of 5xFAD-oil **(A)**, 5xFAD-VCD **(B)**, and 5xFAD-male mice **(C)**. **(D)** In the caudal CA1, there were no significant differences in 4G8 labeling between 5xFAD-oil, 5xFAD-VCD female, and 5xFAD-male mice. **(E–G)** Representative photomicrographs showing 4G8 labeling in the rostral CA3a of 5xFAD-oil **(E)**, 5xFAD-VCD **(F)**, and 5xFAD-male mice **(G)**. **(H)** In the CA3a, there were no significant differences in 4G8 labeling observed between 5xFAD-oil, 5xFAD-VCD female, and 5xFAD-male mice. **(I–K)** Representative photomicrographs showing 4G8 labeling in the rostral DG of 5xFAD-oil **(I)**, 5xFAD-VCD **(J)**, and 5xFAD-male mice **(K)**. **(L)** In the rostral DG, there were no significant differences in 4G8 labeling observed between 5xFAD-oil, 5xFAD-VCD female, 5xFAD-male mice. **(M–O)** Representative photomicrographs showing 4G8 labeling in the caudal DG of 5xFAD-oil **(M)**, 5xFAD-VCD **(N)**, and 5xFAD-male mice **(O)**. **(P)** In the caudal DG, there were no significant differences in 4G8 labeling between 5xFAD-oil, 5xFAD-VCD female, and 5xFAD-male mice. Statistics was calculated by One-way ANOVA with Tukey's post-hoc multi comparison analysis. Data are expressed as mean \pm SEM, $n = 11$ mice/group. Scale bar = 200 μ m.

SUPPLEMENTARY FIGURE S2

GFAP and Iba1 labeling is unchanged in the CA3a and CA3b regions of the hippocampus of WT and 5xFAD female and male mice. **(A–F)** Representative photomicrographs showing GFAP labeling in the rostral CA3a of WT-oil **(A)**, WT-VCD **(B)**, WT-male **(C)**, 5xFAD-oil **(D)**, 5xFAD-VCD **(E)**, and 5xFAD-male mice **(F)**. **(G)** In the CA3a, there were no significant differences in GFAP labeling across groups. **(H–M)** Representative photomicrographs showing GFAP labeling in the rostral DG of WT-oil **(H)**, WT-VCD **(I)**, WT-male **(J)**, 5xFAD-oil **(K)**, 5xFAD-VCD **(L)**, and 5xFAD-male mice **(M)**. **(N)** In the CA3b, there were no significant differences in GFAP labeling across groups. **(O–T)** Representative photomicrographs showing Iba1 labeling in the rostral DG of WT-oil **(O)**, WT-VCD **(P)**, WT-male **(Q)**, 5xFAD-oil **(R)**, 5xFAD-VCD **(S)**, and 5xFAD-male mice **(T)**. **(U)** In the CA3a, there were no significant differences in Iba1 labeling across groups. **(V–Z)**, **(AA)** Representative photomicrographs showing Iba1 labeling in the rostral DG of WT-oil **(V)**, WT-VCD **(W)**, WT-male **(X)**, 5xFAD-oil **(Y)**, 5xFAD-VCD **(Z)**, and 5xFAD-male mice **(AA)**. **(AB)** In the CA3a, there were no significant differences in Iba1 labeling across groups. Statistics was calculated by two-way ANOVA with Tukey's post hoc multiple comparison analysis. Data are expressed as mean \pm SEM, $n = 11$ animals per experimental group. Scale bar = 200 μ m.

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