

Pregnancy History Impacts Cognitive Function and Neurobiology in Aged Presenilin 2 Null
Female Mice

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Abstract

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Rationale

Alzheimer's disease (AD) is an age-related condition that exhibits well-documented sex differences, with females experiencing greater lifetime AD burden, worsened pathology, and more severe cognitive decline. Accordingly, it is necessary to study how female-specific experiences interact with AD-related risk factors to impact disease trajectory. For example, pregnancy exerts dramatic influences on the maternal immune system, behavior, and neuroplasticity, specifically in the hippocampus and limbic system. However, clinical evidence reports conflicting findings on the role of pregnancy history (parity) on late-life AD outcomes. Thus, to investigate the biological impacts of parity on disease outcomes, we hypothesized that parity would differentially impact cognitive function and neurobiology with loss of normal function of the AD-related risk factor presenilin 2 (PSEN2) in advanced age.

Methods

Female PSEN2 knockout (KO) and wild-type (WT) mice at 10-15 months-old with (multiparous) and without (nulliparous) pregnancy history were challenged in the T-maze (TM) and Open Field Test (OFT). This was followed by immunofluorescence for markers of neuronal circuitry and neuroinflammation.

Results

Neither pregnancy history nor loss of normal PSEN2 function impacted spatial working memory in the TM of aged female mice. However, both PSEN2 KO and multiparous mice exhibited decreased OFT locomotor behavior. Multiparous mice also showed reduced exploration in the OFT center ($p=0.0045$) suggesting an anxious phenotype. Pregnancy history was generally associated with increased inhibitory marker expression in the dorsal hippocampus ($p=0.017$), prefrontal cortex ($p=0.021$), and amygdala ($p=0.035$).

Conclusion

This work suggests that although pregnancy history and loss of PSEN2 function exert individual differing age-related effects on cognition and brain pathology, they do not act synergistically.

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INTRODUCTION

Aging elicits sex-specific differences in cognitive decline and associated neurobiological changes. Consequently, advanced age and female sex are two major non-modifiable risk factors for Alzheimer's disease (AD). AD is not only the most well-known age-related neurodegenerative disorder but also exhibits a well-documented sex burden disparity ("2025 Alzheimer's Disease Facts and Figures," 2025). Females demonstrate greater prevalence, more rapid cognitive decline following the mild cognitive impairment stage, worsened neuropathological progression, and more sex-specific AD-related risk factors (Emrani & Sundermann, 2025; Pike, 2017). Furthermore, despite the rapidly aging worldwide population driving the increased AD prevalence, the age-related changes that underlie sex-specific difference in cognitive burden are poorly understood. To better understand the cognitive burden difference present in AD between males and females, it is crucial to examine how female-specific life experiences, such as pregnancy history, interact with AD-associated risk factors to impact cognitive function in late life.

AD is the most common cause of dementia, accounting for 60-80% of cases, and is characterized by progressive memory loss, executive function decline, and behavioral changes ("2025 Alzheimer's Disease Facts and Figures," 2025; Sheppard & Coleman, 2020). Synapse loss and neuronal atrophy occur in the hippocampus and cerebral cortex of the brain, and the hallmark amyloid-beta and hyperphosphorylated tau protein aggregates accumulate broadly throughout various brain regions. While most AD cases are late-onset, about 5-10% manifest before the age of 65 and are thus classified as early onset AD (EOAD). There are 3 autosomal dominant risk

genes associated with EOAD, including amyloid precursor protein (APP) duplication events and gene variants encoding the homologous proteins, Presenilin 1 and 2 (PSENs 1 and 2). PSEN2 variants are the least common genetic cause of EOAD, however, this could potentially be due the fact that these variants typically result in a later age of onset and may thus be masked in the late-onset AD population (Course et al., 2022; Jayadev, et al., 2010). Furthermore, when compared with other AD-associated risk genes, PSEN2 remains relatively understudied and insufficiently characterized, necessitating increased research on how PSEN2 contributes to AD pathophysiology. Like late-onset AD, EOAD also appears to exhibit sex differences. While the prevalence of EOAD does not seem to differ between males and females, there is greater cognitive impairment and broader atrophy burden in female EOAD patients versus males, when compared to same-sex healthy controls (Contador et al., 2022; Peeters et al., 2022). This suggests that despite the largely genetic causes of EOAD, it may share links to the more common late-onset AD when the influence of biological sex is considered on the clinical and pathological disease progression.

PSEN2 functions as a γ -secretase subunit that cleaves APP into the β -amyloid peptide form that can typically aggregate into the characteristic plaques of AD (Cai et al., 2015). The most common PSEN2 gene variants lead to a biochemical loss of normal γ -secretase function such that deletion of PSEN2 gene expression (i.e., PSEN2 knockout – KO) results in a similar biochemical outcome *in vivo* (Jayadev et al, 2010; 2013). Thus, PSEN2 KO mouse models provide a suitable platform on which to *a priori* investigate how loss of normal PSEN2 function may impact long-term physiological processes in health and disease. Despite this, PSEN2 KO mice do not significantly alter APP metabolism or β -amyloid plaque deposition in the aged

rodent brain (Herreman et al., 1999; Jayadev et al., 2010; Robinson-Cooper et al., 2026), suggesting that loss of normal PSEN2 function may also contribute to AD development in an amyloid-independent manner (Herreman et al., 1999; Jayadev et al., 2010). Instead, AD-associated PSEN2 variants have been associated with increased microglia reactivity and heightened inflammatory responses in glial cells (Jayadev et al., 2010; Jayadev et al., 2013). Other non-amyloidal functions of PSEN2 involve modulation of the β -catenin and the Wnt signaling pathway, regulation of cellular Ca^{2+} homeostasis, gene transcription via Notch signaling pathways, and cleavage of voltage-gated sodium channels (Jurisch-Yaksi et al., 2013; Pizzo et al., 2020; Kim et al., 2005). We have also previously reported PSEN2 KO mice exhibit differential sex-based cognitive burden when combined with experimentally induced neuronal hyperexcitability (Knox et al., 2023), further demonstrating the value of this model to investigate what other factors illicit sex differences. One critical adaptation the body must undergo during pregnancy is modulation of the maternal immune system to tolerate the half-foreign fetus while still retaining capacity to combat pathogens, a feat that heavily involves microglia (Gumusoglu & Stelzer, 2025). Microglia number, morphology, and dynamics were found to be vastly altered in the postpartum brain (Rim et al., 2026). Thus, the PSEN2 KO model provides an interesting platform to study how short-term changes in immunological processes during a pregnancy interact with AD-associated risk factors to mediate long-term changes in neuroinflammation, which may lead to greater cognitive burden in late life.

Pregnancy history (also known as parity) confers conflicting effects on the development and risk for clinical AD diagnosis and disease burden. Some evidence in women indicates that the number of pregnancies is associated with severity of AD neuropathological lesions (Beerli et al.,

2009), decreased age of onset for AD (Sobow & Kloszewska, 2003), and increased AD risk (Colucci et al., 2006; Jang et al., 2018), suggesting that pregnancy may worsen AD outcomes. However, others have more recently reported no interaction between parity and AD development in females. Some report an inverted U-shaped association of parity and dementia risk between women and men (Basit et al., 2023; Gong et al., 2022), indicating that sociocultural influences, rather than biological factors, in females mediates any AD-pregnancy interaction. On the other end, research supporting neuroprotective effects of parity on AD also exists; women with more cumulative months pregnant have lower AD risk (Fox et al., 2018). A potential explanation for these contrasting findings is that societal and behavioral factors, such as variation in child rearing and family life, may confound clinical study findings (Beeri et al., 2009). Yet, rodent studies investigating this interaction are limited. Cui et al. (2014) demonstrated that although wild-type non-breeder females outperformed wild-type breeders in various memory parameters, AD-associated breeders failed to improve cognition compared to genotype-matched non-breeders. Lee et al. (2024) found that in a rat model of AD, performance of parous rodents in a spatial memory task involved search strategies that suggested decreased hippocampal activation. Consolidating this data, the interaction between parity and AD-associated risk factors on long-term functional and cognitive outcomes is still relatively unknown in humans and in animal models.

During pregnancy, not only does the maternal immune system adapt, but the maternal brain also undergoes significant changes, notably altering cognition (Duarte-Guterman et al., 2023; Eid et al., 2019; Trainer et al., 2006). At the cellular neuroplasticity level, neurogenesis, synaptic remodeling, and changes in dendrite morphology, spine density, and astrocytic density occur to

support maternal behavior (Barba-Müller et al., 2019). Additionally, estrogen and progesterone flux dramatically modify the GABA- (γ -aminobutyric acid) and glutamate-mediated inhibition and excitation systems (Gilfarb & Leuner, 2022; Zhao & Gammie, 2014). Central GABA concentrations change dramatically depending on pregnancy term and during the postpartum period (Gilfarb & Leuner, 2022). However, the mechanisms that underlie parity-related changes in memory and behavior with AD-related risk factors across the lifespan remain largely understudied. The hippocampus, largely associated with the process of episodic memory, not only exhibits neuroplasticity with pregnancy (Pawluski & Galea, 2006), but also experiences significant neurodegeneration in AD. Moreover, the broader limbic system and prefrontal cortex play a critical role in higher brain function, mediating cognitive control, emotion, and memory. There appears to be an effect of pregnancy on electrophysiology and synaptic activity in the hippocampal CA1 region, the basolateral amygdala (BLA), and the medial prefrontal cortex (MPFC) (Anastasiades & Carter, 2021; Bin & Luo, 2025). We therefore hypothesized that pregnancy history (parity) and loss of normal PSEN2 function, as occurs in a subset of EOAD patients, would alter neuroinflammation, neuronal circuitry, and neurotransmitter homeostasis in brain regions belonging to the limbic system and prefrontal cortex. Our overall study objective was to better understand how pregnancy history affects brain aging in conjunction with an AD-associated genotype.

METHODS

Animals

Female 10-15-month-old PSEN2 KO mice were bred at the University of Washington (UW) from stock originally acquired from the Jackson Laboratory (stock #005617), as previously reported (Beckman et al., 2020). PSEN2 KO mice are viable long-term and breed normally (Herreman et al., 1999; Knox et al., 2023; Robinson-Cooper et al., 2026). All breeding at UW was between PCR-confirmed PSEN2 KO pairs. Age-matched female WT (stock #000664) were acquired from Jackson Labs at 12 weeks-old. Multiparous females were retired breeders such that experimental testing was conducted upwards of 7 weeks after last litter, with the number of pregnancies unknown. We did not track estrous cycles of female mice. Animals were housed on a 14:10 light cycle (lights on at 06 h00; off at 20 h00) in ventilated cages with corncob bedding in a manner consistent with the *Guide for the Care and Use of Laboratory Animals* (protocol 4387-01) and all animal work was approved by the UW Institutional Animal Care and Use Committee and conformed to ARRIVE guidelines (Sert et al., 2020). Animals were provided free access to irradiated chow (PicoLab Rodent Diet 20–5053), water, and enrichment, except during the periods of behavioral manipulation. Multiparous females received a high-fat breeder chow (PicoLab Rodent Diet 20–5058) from breeder status initiation (7-10 weeks) until retirement (~10 months). While both formulas contain roughly 20% protein, 5058 is a high-energy diet formulated with double the fat content (9.0%) compared to 5053 (4.5%) to support demanding mouse breeding cycles and transgenic strains. All behavioral testing was performed between 10h00 and 17h00.

Open Field (OFT) Behavioral Testing of Anxiety-Like Behavior

Ambulation, locomotion, and exploratory behavior were assessed using the Open Field Test (OFT), as previously published (Knox et al., 2023). An infrared-sensitive motion detection system (SuperFlex Open Field, Omnitech Electronics) was used to assess exploratory behavior and locomotion. Each mouse was placed into an OFT Plexiglas chamber (40 L x 40 W x 30H cm) for 10 mins, while their movements were monitored with the Fusion v6.5 (Omnitech) software. Locomotor chambers were cleaned with water after each animal test period.

OFT parameters recorded include total distance travelled (cm), vertical activity counts, and horizontal activity counts in both the center and the perimeter regions. Preference for the OFT chamber center versus perimeter portion is useful to assess thigmotaxis as a measure of anxiety-like behaviors (Seibenhener & Wooten, 2015).

T Maze (TM) Testing of Working Memory

The TM spontaneous alternation test is a land-based task used to test hippocampal-mediated spatial working memory in mice (Deacon & Rawlins, 2006), as previously shown (Knox et al., 2023). TM was conducted 2 days following the OFT. Each TM test consisted of 9 total sessions per mouse, with 3 sessions per day for 3 days. Mice were challenged to spontaneously alternate between the left and right goal arms (30 x 10 cm) of a T-shaped epoxy-coated wooden maze (start alley 30 x 10 cm). On each behavioral testing day, each mouse was placed into a starting box (10 x 8 cm) at the entry of the start arm for 30 s. Then, a partition door was opened to allow the mouse to enter the start arm and choose between the left and right goal arms. Once the mouse entered a goal arm, a door was quietly lowered to prevent entry into the opposite arm, with the

goal arm selection and latency to choose (in seconds) recorded. After 30 seconds, the mouse was returned to the starting box, and the start alley and goal arms were cleaned with water. The mouse was again challenged to explore the maze, and the outcome recorded; an error defined as whether the mouse failed to alternate arm choices on this second trial. The latency to choose a goal arm was recorded for both trials. The spontaneous alternation rate was calculated as the ratio between alternating choices and total number of choices during all trials, with 60% considered successful TM performance.

Brain Tissue Collection and Processing

Mice were euthanized approximately 1 h after the last TM session was completed, and brains were collected and hemi-sectioned longitudinally. The left brain hemisphere was flash frozen in 2-methylbutane overlaying dry ice and stored in -80°C; the right brain hemisphere was fixed for 24 h in 4% paraformaldehyde at 4°C, then stored in 30% sucrose with 10-20 uL sodium azide for cryoprotection and preservation at 4°C. The sucrose-submerged right hemisphere was then flash frozen for sectioning on a cryostat (Leica CM1850) into 30-um thick slices from the prefrontal cortex (AP from Bregma: 1.98 to 1.70 mm) and the dorsal hippocampus (AP from Bregma: -1.58 to -2.10 mm). A total of 20 prefrontal cortex sections and 20 rostral and 20 caudal hippocampal sections were collected sequentially from each mouse, with 2 sequential sections per brain region mounted on SuperFrost slides (Fisher) per mouse. The slides were stored at -80°C until immunofluorescent processing.

Immunofluorescence

Slides were washed with 1X PBS (3 x 5 min) before undergoing antigen retrieval with citrate buffer (#ab93678; Abcam) heated at 95 °C for 10 min, then resting at room temperature for 20 min. GAD65+67 and PV underwent antigen retrieval for 20 min at 95 °C, then 40 min at room temperature. The slides were then washed with dH₂O (3 x 2 min). Antibodies for all molecular targets are detailed in Table 1. Slides immunolabeling for GAD65+67 and PV was permeabilized with 0.3% Triton X-100 in 1X PBS for 30 min before undergoing blocking incubation with 10% goat serum with 0.03% Triton-X 100 in 1X PBS with 200 uL coverwells for 2 hr at RT. For other markers, a different blocking reagent (10% goat serum with 5% Triton-X 100 in 1X PBS) was applied for 2 hr at RT. Next, the GAD65+67 and PV antibodies were applied to the slides in a 1% goat serum, and 0.03% Triton-X in 1X PBS solution with coverwells. Other antibodies were applied with 5% Goat Serum in 1X PBS. Slides for GAD65+67, PV, and Plp underwent primary antibody incubation at 4 °C overnight, whereas slides for NeuN, EAAT2, and Iba1 were incubated for 2 hr at RT. The next day, the slides were washed with 1X PBS for 3 x 5 min and incubated with either anti-rabbit secondary antibody or anti-mouse secondary antibody, with addition of conjugated GFAP g . For GAD65+67 and PV, the secondary antibody was applied in 1% goat serum, 0.03% Triton-X in 1X PBS. For remaining markers, secondary incubation was conducted in 5% Goat Serum in 1X PBS. Slides staining for GAD65+67, PV, Plp, GFAP underwent secondary antibody incubation for 2 hr at RT, whereas slides for NeuN, EAAT2, and Iba1 were incubated at 4 °C overnight. A final wash was done with 1X PBS (3 x 5 min) before the slides were coverslipped with Prolong Gold Antifade mounting reagent with DAPI (#P36934; Thermo Fisher).

Photomicrographs were captured with a fluorescent microscope (Leica DM-6) with a 20x objective (80x final magnification) a minimum of 24 hr after coverslip mounting. Acquisition settings were held constant throughout. Plp, NeuN, PV, GAD65+67, EAAT2, Iba1, and GFAP expression levels, given as total field area coverage with fluorescent signal for the molecular marker of interest, were calculated with ImageJ (Fiji). Brain regions assessed included prefrontal cortex, subregions of the dorsal hippocampus, the somatosensory cortex overlaying CA1 of the dorsal hippocampus, and the basolateral amygdala.

Statistical Analyses

Performance on the OFT and TM were compared in a two-way ANOVA (parity x genotype), with post-hoc Dunnett's test when applicable. Immunohistochemical assessments were conducted similarly with two-way ANOVA with post-hoc Dunnett's test, comparing total area coverage with positive signal. All analyses were conducted with GraphPad Prism v10.0 with $p < 0.05$ denoting statistical significance.

RESULTS

Experimental design was conducted as visualized in Fig 1A . Body weight was found to be significantly higher in the multiparous females compared to the nulliparous females (Fig 1B; $F(1, 64) = 60.8, p < 0.0001$), likely due to the high fat content of the diet. Both the WT and PSEN2 KO MP groups weighed significantly more than their genotype-matched counterparts (WT; $p < 0.0001$, PSEN2 KO; $p < 0.0001$).

Pregnancy history does not impact short-term spatial working memory, but does significantly increase performance task recall latency of aged female mice

Performance of 10-15-month-old WT versus PSEN2 KO mice was assessed on the land-based T Maze (TM) continuous alternation task (Fig 2). The number of TM response errors did not significantly differ between genotypes nor parity history (Fig 2A), and all groups exhibited a response rate similar to typical alternation rates of healthy WT mice, which is reported as an error rate equal to or less than 40% (Deacon & Rawlins, 2006). Similarly, there was no significant difference between the number of responders, defined as the mice that met this healthy WT criteria, between the various groups (Fig 2B).

Genotype significantly influenced the latency to initiate the task (i.e., to “start” the trial; $F(1, 64) = 6.63, p = 0.012$), where PSEN2 mice took a significantly longer amount of time to complete the initial goal arm choice, compared to the WT mice (Fig 2C). Parity significantly increased the recall latency ($F(1, 61) = 4.44, p = 0.04$), meaning that multiparous females took significantly longer to initiate the secondary arm decisions and/or recall previous arm choice (Fig 2D). Since

response accuracy was not affected in the TM, these increased latencies may indicate elevated stress or decreased motivation and/or interest in the task. Furthermore, parity also influenced the delay between trial start and decision (i.e. the start-recall latency, Fig 2E; $F(1, 61) = 11.61$, $p=0.001$). Post-hoc analysis revealed that PSEN2 KO multiparous females had a significantly greater start-recall latency than PSEN2 KO nulliparous females ($p=0.034$), meaning the nulliparous mice took longer to choose a goal arm in the second recall trial, relative to the initial start trial, compared to the genotype-matched multiparous mice. Overall, neither genotype nor parity affected hippocampal-mediated spatial working memory capacity as demonstrated by no difference in error rates between groups. However, prior pregnancy may be associated with memory and recall delays regardless of genotype.

Pregnancy history and presenilin 2-associated genotype exert differing long-term effects on open field locomotion and anxiety-like behaviors.

Both exploratory and ambulatory behaviors were assessed in the OFT, revealing that both genotype and parity significantly influenced locomotor activity (Fig 3A). Specifically, AD-related genotype was associated with significantly decreased total distance traveled (Fig 3B; genotype: $F(1, 61) = 41.6$, $p<0.0001$) and total horizontal activity (Fig 3C; genotype: $F(1, 61) = 27.2$, $p<0.0001$) in the OFT. Further, pregnancy history was also generally associated with reduced OFT total distance traveled (Fig 3B; $F(1, 61) = 6.68$, $p=0.012$) and reduced horizontal activity (Fig 3C; $F(1, 61) = 13.0$, $p=0.0006$). Post-hoc analyses revealed that for both total distance traveled and total horizontal activity, WT mice were significantly different from PSEN2 KO in both nulliparous (total distance; $p<0.0001$, horizontal activity; $p=0.005$) and multiparous mice (total distance; $p=0.0009$, horizontal activity; $p=0.044$). Furthermore, WT nulliparous mice

showed significantly more total distance traveled and horizontal exploratory activity versus WT multiparous mice (total distance; $p=0.018$, horizontal activity; $p=0.0009$), but this trend was not observed in the PSEN2 KO mice. While genotype and parity exert effects on locomotion, such that multiparous status and loss of normal PSEN2 function each reduced ambulation independently, there was no genotype x parity interaction in either total distance traveled or horizontal activity (Figs 3B and 3C). Additionally, loss of normal PSEN2 function was associated with generally reduced total vertical activity relative to parity history-matched WT mice (Fig 3D; $F(1, 61) = 22.7$, $p<0.0001$). Vertical activity indicates exploratory behaviors such as rearing, where the mouse stands on its hind paws, suggesting that the PSEN2 KO mice exhibit reduced motivation to explore novel environments. Post-hoc analyses revealed that the WT and PSEN2 KO females were significantly different in both the nulliparous ($p=0.0019$) and the multiparous groups ($p=0.015$; Fig 3D) reflecting a general effect in the PSEN2 null aged females that is consistent with our prior studies in this model (Knox et al., 2023).

The percentage of total time or total distance in the OFT center can be used to quantify thigmotaxis and indicate anxiety-like behaviors. There was a main effect of parity, where multiparous mice spent more time (Fig 3E; $F(1, 61) = 10.1$, $p=0.002$) and distance (Fig 3F; $F(1, 61) = 7.13$, $p=0.001$) in the maze periphery, indicating elevated anxiety-like behaviors. Post-hoc analysis revealed that PSEN2 KO multiparous mice spent a significantly lower percent of their total time in the OFT center compared to PSEN2 KO nulliparous mice (Fig 3E; $p=0.018$; Fig 3E). There was also a main effect of genotype on percentage of time in the center (Fig 3E; $F(1, 61) = 7.37$, $p=0.009$), with PSEN2 KO mice spending a greater amount of time in the OFT center; however, there was no genotype x parity interaction. Overall, PSEN2 genotype and parity

did not synergistically worsen OFT performance and instead seemed to moderate cognitive performance in differing ways; genotype more significantly influenced motor activity and exploratory behaviors (Fig 3B-3D), whereas parity had a larger influence on anxiety-like behavior (Figs 3E-3F).

Neuronal structure and integrity are differentially impacted by loss of normal PSEN2 function and pregnancy history.

Proteolipid protein (Plp) is the primary protein component of the central nervous system (CNS) myelin sheath, facilitating rapid neural transmission and signalling, and is thus expressed by oligodendrocytes (*Gene - NCBI*, n.d.). Importantly, Plp expression was found to be elevated in aged female mice hippocampus and up-regulation of Plp in oligodendrocytes improved cognition in old female mice, compared to male counterparts (Gadek et al., 2025). Given that myelin damage and impairment has been clinically reported in AD (Papuć & Rejdak, 2018), we thus hypothesized that PSEN2 deficiency and pregnancy history would influence this protein expression in aged female mice (Fig 4). There was a main effect of genotype in the hippocampal dentate gyrus (DG) region (Fig 5B; $F(1, 40)$), with PSEN2 KO mice exhibiting elevated Plp expression relative to WT. Post-hoc analyses reported DG Plp levels of multiparous PSEN2 KO mice were significantly elevated compared to multiparous WT mice ($p=0.04$). The CA3 region demonstrated a similar main effect (Fig 5C; $F(1, 38) = 10.64$, $p=0.002$), with the PSEN2 KO groups being associated with higher Plp levels. As for pregnancy history, Plp expression was significantly impacted by parity in the hippocampal CA1 region (Fig 4D; $F(1, 40) = 5.29$ $p=0.027$) and the medial prefrontal cortex (Fig 4E; MPFC; $F(1, 36) = 4.43$, $p=0.042$), with multiparous females exhibiting decreased Plp. Specifically in the WT groups, MPFC Plp

expression was significantly greater in the nulliparous mice compared to multiparous mice ($p=0.007$), but this was not observed in the PSEN2 KO groups. This suggests that parity status does not downregulate Plp expression in PSEN2 KO mice in the MPFC, a region heavily associated with cognitive control and learning. In the somatosensory cortex, there was a significant genotype x parity interaction (Fig 4F; $F(1, 39) = 5.89, p=0.02$), but no specific differences between the assessed groups. Lastly, the basolateral amygdala demonstrated no main effects or significant changes in Plp expression (Fig 5G), indicating that neither genotype nor parity history modulates Plp expression in the BLA in these aged females.

In contrast, neuronal nuclei (NeuN), which identifies post-mitotic, mature neurons in the CNS (Moon et al., 2026), only exhibited differential expression in the dorsal hippocampus (Fig 4H-4J). There was a genotype x parity interaction reported in both the CA3 (Fig 4I; $F(1, 38) = 6.48; p=0.015$) and the CA1 regions (Fig 4J; $F(1, 39) = 6.05, p=0.018$) such that the PSEN2 genotype-related upregulation of NeuN expression in the nulliparous mice was not seen in the multiparous mice, suggesting pregnancy history may dampen PSEN2 KO associated changes in neuron density. Within the CA3 region, in addition to the interaction, genotype also exerted an influence on NeuN expression ($F(1, 38) = 4.90, p=0.033$). CA3 NeuN expression in nulliparous PSEN2 KO mice was significantly increased from nulliparous WT mice ($p=0.024$), but this was not observed in the multiparous groups, further demonstrating this dampening effect. Post-hoc analyses also demonstrated that NeuN immunoreactivity levels in the CA1 region in the nulliparous PSEN2 KO mice were significantly greater than the multiparous PSEN2 KO mice ($p=0.029$), highlighting the long-lasting impacts of pregnancy on individual brain regions well

into late life. All other regions (hippocampal DG, MPFC, cortex, and BLA; Fig 4H, 4K-M) demonstrated no differences in NeuN expression due to genotype or parity.

Pregnancy history is associated with generally increased inhibitory signaling throughout analyzed brain regions in aged female mice, irrespective of PSEN2 KO genotype.

EAAT2, or the excitatory amino acid transporter 2, plays a critical role in glutamate clearance from the synaptic cleft and is primarily expressed in astrocytes (Lin et al., 2012). EAAT2 expression was moderately affected by PSEN2 KO genotype and pregnancy history, mainly in the dorsal hippocampus. Genotype significantly impacted EAAT2 density in CA3 (Fig 5C; $F(1, 38) = 5.55, p=0.024$), with nulliparous PSEN2 KO mice having significantly greater EAAT2 immunoreactivity than nulliparous WT mice ($p= 0.044$), indicating reduced excitatory transmission or potentially increased number of astrocytes. The same trend was not seen in the multiparous group, suggesting that pregnancy history could blunt EAAT2 overexpression in PSEN2 KO. In the CA1 region, multiparous mice showed an increase of EAAT2 expression compared to nulliparous mice (Fig 5D; $F(1, 38) = 7.32, p=0.01$); however, there were no significant differences between genotype-matched parity groups measured with post-hoc analyses. Remaining brain regions analyzed displayed no effects of genotype nor parity on EAAT2 immunoreactivity.

GABA, the major inhibitory neurotransmitter, is synthesized from glutamate in an enzymatic reaction catalyzed by glutamic acid decarboxylase (GAD), which exists in two isoforms with molecular weights of 65 and 67 kDa (aptly named GAD65+67) (Martin & Rimvall, 1993)6/13/26 9:33:00 PM. Thus, GAD65+67 expression provides insight into inhibitory balance

and GABA production in the brain. Parity history consistently influenced GAD65+67 levels in the CA1 (Fig 6D; $F(1, 40) = 6.15, p=0.017$), the MPFC (Fig 7E; $F(1, 38) = 5.84, p=0.021$), the CTX (Fig 6F; $F(1, 40) = 7.61, p=0.009$), and the BLA (Fig 5G; $F(1, 38) = 4.77, p=0.035$), where multiparous females had increased GAD65+67 signal. In those same brain regions, post-hoc analyses revealed that the multiparous WT females had greater GAD65+67 immunoreactivity compared to nulliparous WT females (CA1; $p=0.03$, MPFC; $p=0.035$, CTX; $p=0.01$, BLA; $p=0.046$) but the same difference was not reported for the PSEN2 KO groups. The remaining hippocampal DG and CA3 regions (Fig 6B, C) did not demonstrate any significant differences in GAD65+67 expression across the groups. Overall, pregnancy history appears to exert a broad and consistent influence on inhibitory neurotransmitter synthesis in WT and PSEN2 KO female mice.

Parvalbumin (PV) is a calcium binding protein expressed in the largest subpopulation of GABAergic inhibitory interneurons (Bjerke et al., 2020). PV provides an additional marker of the inhibitory signaling in discrete brain regions involved in cognitive function and anxiety-like behaviors. Parity history in aged female mice significantly affected PV expression in the hippocampal DG region (Fig 6H; $F(1, 40) = 9.43, p=0.004$); multiparous mice generally had more PV-positive signal. However, there were no individual group differences found with post-hoc analysis. Similarly, there was a main effect of both parity and genotype in the CA3 (Fig 6I; parity: $F(1, 40) = 5.43, p=0.025$, genotype; $F(1, 40) = 11.74, p=0.001$) and BLA (Fig 6M; parity: $F(1, 38) = 6.09, p=0.018$, genotype: $F(1, 38) = 8.42, p=0.006$), with both pregnancy history and the PSEN2 KO genotype being associated with increased PV immunoreactivity. There was no parity x genotype interaction in these regions. Specifically in the multiparous groups, PSEN2 KO

mice had significantly more PV expression compared to parous-matched WT mice (CA3; $p=0.004$, BLA; $p=0.012$). These findings further suggest that pregnancy history can upregulate inhibitory signaling well into late life.

Neuroinflammatory changes in the brain in response to presence of pregnancy history and/or loss of PSEN2 function.

The expression of ionized calcium-binding adapter molecule 1 (Iba1) is upregulated in activated microglia and its function assists with membrane ruffling and phagocytosis by microglia (Ohsawa et al., 2004). Thus, Iba1 serves as a marker for reactive microgliosis and neuroinflammation, albeit it cannot differentiate resident microglia from infiltrating macrophages. Nonetheless, we sought to examine whether prior pregnancy or AD-related loss of normal PSEN2 function would exert any lasting changes on Iba1 immunoreactivity in late life. Iba1 expression was upregulated in the hippocampal CA3 region (Fig 7C) with pregnancy history ($F(1, 40) = 7.32$, $p=0.01$) and PSEN2 KO genotype ($F(1, 40) = 11.20$, $p = 0.002$). Specifically, post-hoc analyses revealed that the PSEN2 KO multiparous females had significantly higher Iba1 signal compared to both the WT multiparous females ($p=0.0001$) and the PSEN2 KO nulliparous females ($p= 0.02$). Similarly, there was also a main effect of genotype in the somatosensory cortical region overlaying dorsal hippocampus (Fig 7F; $F(1, 40) = 4.53$, $p=0.04$), with PSEN2 KO females generally displaying increased microgliosis, however there were no pregnancy history-related differences. The remaining brain regions did not display any significant differences in Iba1 microglial activation. This association between greater microgliosis and loss of normal PSEN2 function in late life aligns with previous literature

demonstrating that loss of PSEN2 function may promote exaggerated inflammatory responses (Jayadev et al., 2010; Jayadev et al., 2013).

Glial Fibrillary Acidic Protein (GFAP) is the hallmark intermediate filament protein in astrocytes, a cell type critical for metabolic balance, energy homeostasis and neuronal signaling in the CNS (Hol & Pekny, 2015). Reactive astrogliosis, a characteristic change in the function and morphology of astrocytes seen in neurological diseases such as AD, is associated with upregulation of GFAP. We thus investigated whether pregnancy history or loss of PSEN2 function would alter astrogliosis in the brain. In all three regions of the dorsal hippocampus, pregnancy history exerted a significant main effect on GFAP expression. Multiparous females exhibited increased GFAP-mediated astrogliosis in the DG (Fig 8B; $F(1, 40) = 15.61$, $p=0.0003$), the CA3 (Fig 8C; $F(1, 40) = 20.0$, $p<0.0001$), and the CA1 (Fig 8D; $F(1, 41) = 6.903$, $p=0.012$) regions, compared to the nulliparous groups. In the DG, the WT multiparous group had significantly elevated GFAP compared to the WT nulliparous group ($p= 0.006$), a trend that was preserved in the CA3 ($p=0.018$) and CA1 ($p= 0.042$) regions. Furthermore, GFAP expression in the PSEN2 KO multiparous females was also significantly greater than that of the PSEN2 KO nulliparous females in the CA3 ($p= 0.008$). Thus, increased reactive astrogliosis was broadly observed throughout the hippocampus due to pregnancy history. Other cortical and amygdala brain regions did not display visible GFAP immunoreactivity and were thus not analyzed.

DISCUSSION

This present study is the first, to our knowledge, to evaluate the interaction between parity and loss of PSEN2 function on cognitive performance and neuroplasticity protein expression in the aged brain. Spatial working memory and spontaneous alternation was assessed with the T Maze and while neither pregnancy history nor the PSEN2 null genotype was sufficient to alter task performance, these variables may be associated with impaired motivation or affect. We report that parity and AD-associated genotype exerted similar influences on OFT-mediated locomotor parameters, both reducing ambulation activity and movement, but exert opposing trends in center and peripheral zone preference. PSEN2 null mice spent a greater proportion of their exploratory time and distance in the OFT center, whereas parity status increased activity in the periphery, inferring more anxiety-like behaviors. Pregnancy history and the PSEN2 KO genotype may impact cognitive performance and neurobiological markers independently; however, they do not act synergistically to worsen disease outcomes. Cui and colleagues found that in APP23 mice, an amyloidogenic model, 12-month multiparous females displayed reduced Y-Maze alternation rate compared to age and genotype-matched controls whereas in the WT groups, multiparous females experienced improved alternation compared to nulliparous (Cui et al., 2014). Another study found that primiparous (one birth) 12–13-month rodents with the hAPOEε4 genotype, a late onset AD-associated risk factor, were more likely to utilize a non-spatial strategy during the radial arm maze task, indicating decreased hippocampal recruitment to complete this task (Lee et al., 2024). This suggests that parity-mediated impairment to hippocampal-mediated spatial working memory performance in AD is mechanism-dependent and may involve amyloidogenic (APP23) or lipid homeostasis (APOEε4) processes. Our study contributes to an important, yet

understudied field of research and demonstrates that loss of PSEN2 function and pregnancy history each seem to exert their own individual impacts on locomotor activities and anxiety-like behaviors but do not synergistically impair cognition.

Within the brain, across various regions, pregnancy history is associated with broad inhibitory signaling indicated by increased expression of EAAT2-mediated glutamate re-uptake, GAD65+67-catalyzed GABA synthesis, and inhibitory PV interneuron density. We herein demonstrate that pregnancy history is associated with more GAD65+67 immunoreactivity in CA1, BLA, and cortical (somatosensory cortex and MPFC) regions of WT, but not PSEN2 KO female mice, suggesting that these earlier reproductive events lead to genotype-specific lasting and sustained remodeling of neuronal inhibition. However, there was only a measurable shift in PV neuron expression in the CA3 and BLA of multiparous PSEN2 KO mice, indicating that this inhibitory interneuron population was not responsible for the observed shift in GAD65+67 levels. PV only identifies a subset of interneurons and with the numerous classes of non-PV expressing interneurons present in the brain, it is possible that upregulation of another GABAergic neuron subtype or increased inhibitory output from existing interneurons may be responsible for this effect. Additionally, previous work has reported elevated GAD expression in the lateral septum, accessory olfactory tract bed nucleus, and MPFC, brain regions heavily associated with anxiety and maternal behaviors, in postpartum mice compared to virgin females. (Ragan et al., 2022; Rodríguez et al., 2004; Zhao et al., 2012). This further establishes the interaction between pregnancy history and inhibitory modulation. Furthermore, Page et. al., found that chronic stress increased prefrontal PV cell activity and chronic activation of these prefrontal PV cells induced an anxiety-like phenotype in female mice, demonstrating the link

between upregulation of inhibitory cells with anxiety-related behaviors (Page et al., 2019). This aligns closely with our findings, indicating that GABA modifications long after pregnancy are complex and that pregnancy history may act similarly to chronic stress, inducing inhibitory modulation and potentially mediating increased anxiety-like behaviours. Inhibitory signaling also displays vast regional differences in circuitry involvement and regulation. PV neurons are implicated in parahippocampal pathways facilitating spatial information and temporal encoding for episodic memory, the prefrontal cortex depends heavily on the excitatory and inhibitory balance for normal executive functioning, and GAD expression displays region-specific regulation in response to stress (Bjerke et al., 2020; Bowers et al., 1998; Ferguson & Gao, 2018). Thus, the regional differences in PV and GAD65+67 expression reported in this study may reflect the vast, diverse functions of the inhibitory system and its contributions to various behavioural and cognitive processes. To supplement these findings, future studies should include a more diverse range of inhibitory neurons and investigate glutamatergic pathways to study how pregnancy history affects excitatory-inhibitory signaling.

Pregnancy is a highly complex immunological event, with the maternal immune system performing specialized and precisely timed alterations. To investigate whether those changes are long-lasting and continue into late life, microglial and astrogliosis responses were investigated. Iba1 levels were elevated in the CA3 and somatosensory cortex regions of PSEN2 KO mice, supporting findings that loss of normal PSEN2 signaling in the brain is associated with inflammation and that PSEN2 is the primary γ -secretase in microglia (Jayadev et al., 2010). This may translate to altered synaptic pruning in CA3 that can underlie the observed deficits in the ability to rapidly recall spatial memories in the TM (Fig 2E). Microglia demonstrate vast

heterogeneity in density and morphology across various brain regions with the hippocampus being well-documented as a site of high microglia density (Tan et al., 2020). Thus, it is possible that variability in microglial distribution, spatial arrangement, and physiology across different brain regions could facilitate the regional effects in Iba1 immunoreactivity. These findings are also consistent with our earlier studies suggesting that female PSEN2 KO mice are more detrimentally affected by repeated seizure history than males, suggesting some sex-specific vulnerability to neuroinflammatory-associated stressors in this model (Knox et al 2023). Yet, parity history was associated with increased reactive astrogliosis across all three hippocampal brain regions. In the hippocampus, astrocytes regulate synaptic transmission and plasticity by modulating neurotransmitter release, ATP secretion, and cytokine response and these processes facilitate memory formation and anxiety-related behaviors in the brain (Cho et al., 2022; Wang et al., 2022). Thus, increased hippocampal astrogliosis could contribute to the behavioural outcomes and cognitive impairment observed with pregnancy history. Furthermore, astrocytes may receive input from sex steroids from reproductive hormone regulator kisspeptin, suggesting a potential interaction between pregnancy-mediated fluctuations in estrogen-related hormones and astrocyte activation ([Abbara & Dhillon, 2024](#)).

Female cognitive advantage and resilience is increasingly documented in normal brain aging conditions, with women generally experiencing reduced age-related declines in cognitive function and brain atrophy relative to age-matched men (Ravndal et al., 2025; Xu et al., 2000). However, studies of the impact of pregnancy history between men and women in late life is less well established and subject to greater variability in societal influence. Thus, well-controlled animal studies are informative to better define how pregnancy and biological sex interact on

cognitive function long-term, including in the setting of AD-related risk factors. Indeed, studies of past reproductive experience in rodents suggest that pregnancy is associated with dendrite and neuron proliferation and remodeling in the hippocampus (Pawluski & Galea, 2006; Trainer et al., 2006), increased synaptic plasticity (Duarte-Guterman et al., 2023) and enhanced performance in learning and memory tasks (Kinsley et al., 1999; Zimmerknopf et al., 2011; Macbeth et al., 2008). In this current study, we found that pregnancy history did not impact spatial working memory capacity and was instead associated with changes in anxiety-like behaviors and locomotion. To our knowledge, our present study had the advantage over other prior studies that used rodents that were much younger, the oldest being 7 months, and tested animals shortly after weaning (3 weeks). As a result, our present data suggest that advanced age and increased time post-parturition, which was upwards of 7 weeks in our study, may modulate or reverse the initially neuroprotective effects of pregnancy.

Female cognitive resilience may involve the X chromosome; male cells contain the maternal X and paternal Y chromosomes, whereas female cells contain one randomly active X chromosome from either the mother or father (Bourzac, 2025). An X chromosome genetic meta-analysis identified a X chromosome gene locus involved with secretory and endosomal function, and potentially downstream amyloid accumulation (Belloy et al., 2024). This may also explain the minimal impact of the PSEN2 KO genotype on memory and cognitive outcomes, as the model lacks amyloid accumulation or pathology. Evidence reports that aging induces the activation of the silent female X chromosome, upregulating genes associated with neural health, facilitating improved cognition (Gadek et al., 2025). Similar to how Abdulai-Saiku et al. concluded that epigenetic gene silencing in the maternal X chromosome was associated with cognitive aging

(Abdulai-Saiku et al., 2025), it is possible that the epigenetic modifications that occur during pregnancy, partly in response to hormonal signaling, may act to silence genes associated with brain health (Andrawus et al., 2022). Gadek and colleagues also found increased Plp1 expression in the hippocampus of aged females and correlations between Plp1 levels and improved cognition (Gadek et al., 2025). Our finding that parity history was associated with both decreased Plp in the hippocampus and medial prefrontal cortex in conjunction with measurable shifts in behavioral performance on two tasks (i.e. increased latency to initiate goal choice in the TM (Fig 2D-E) and elevated anxiety-like behaviors in the OFT (Fig 3)) may align with this previous study and further points towards Plp and X-chromosome associated markers as contributors towards resilience in aging in the female brain.

This study has several limitations that must be considered when interpreting the research conclusions and informing future studies. The majority of the human studies have found differential risk for AD and dementia outcomes based on the number of children, with more pregnancies (some reporting 5 children or more) being associated with larger risk for AD (Colucci et al., 2006; Jang et al., 2018). Thus, further experiments would benefit from the inclusion of primiparous mice (1 pregnancy) versus multiparous mice (multiple pregnancies) to evaluate whether reproductive history acts in an additive manner with regards to cognitive outcomes in AD. One limitation of this current study is that the multiparous mice utilized were retired breeders, meaning that the exact number of pregnancies each female experienced was variable across the individual mice. This could contribute to the variation in behavioral task performance and biomarker expression, especially if the phenomenon of number of pregnancies being positively correlated with magnitude of AD risk from humans holds true in animal models.

The retired breeders are also placed on a high-fat chow to compensate for the metabolic requirements of breeding, whereas the nulliparous mice were provided with a normal diet. This diet likely resulted in the higher body weight observed in the multiparous females and, with evidence suggesting the relevance of diet-mediated cognitive impairments, could contribute to the behavioural outcomes reported here. Additionally, the hormone fluctuations necessary during pregnancy are unparalleled by any other neuroendocrine events in a healthy woman's life, with estrogen rising to 300–1000-fold increases before rapidly dropping to basal levels shortly after delivery (Deems & Leuner, 2020). Future studies should monitor estrogen and progesterone levels to investigate whether pregnancy history can impart lasting changes to sex hormone levels and whether this hormone flux correlates to cognitive performance. Inclusion of sex hormones may also provide a more comprehensive understanding of the differences that biological sex may induce on later disease progression, particularly in the context of other endocrine transitions, such as menopause, which are known to influence brain aging and neurodegenerative disease risk.

If this current study were to be altered or changed, one variable that would benefit from standardization would be the amount of time between the multiparous female's last pregnancy and initiation of behavioural testing, alongside the number of pregnancies. Due to the variability of when the breeding females would retire, it was difficult to match the retirement age and experimental timing across all animals. However, intentionally testing at a preset duration of time post-partum would ensure all cognitive and pathological effects observed were due to pregnancy history and not acute effects during the post-partum period. The diet would also be standardized across all animals, ensuring the high-fat chow was provided for all experimental

groups to eliminate any possible confounding effects. Furthermore, I would include more complex behavioural tests, such as the Barnes Maze or the Radial Arm Maze. Given that T-Maze spontaneous alternation rate was neither altered by pregnancy history nor PSEN2 KO genotype, it's possible that tasks with more sophisticated parameters and involved training may be necessary to elicit a response. Investigating search strategies and patterns may better elucidate how parity or AD-associated genotype impact hippocampal mediated memory and learning.

In summary, this study demonstrates that pregnancy history can alter late life cognitive and behavioral changes, both with and without the loss of normal PSEN2 function. Specifically, we observe that pregnancy history leads generally to reduced anxiety-like behaviors (reduced OFT center zone time) and increased recall latency, suggesting reduced motivation. Further, we report that pregnancy history with an AD-related genotype (PSEN2 null) reduced locomotor activity and increases transition between behavioral tasks (start-recall latency). This study demonstrates that sex-specific experiences can lead to long-lasting changes in brain circuitry and cognitive processing. Furthermore, although there was no impact to spatial working memory performance on the TM task, the PSEN2 KO genotype modulated other behavioral task performance metrics, suggesting that PSEN2 may more largely contribute to other behavioral symptoms of AD, separate from memory formation. Importantly, pregnancy history and PSEN2 KO do not synergistically affect disease outcomes, but this observation may be specific to this PSEN2 model. Pregnancy history generally was associated with decreased neuronal structural integrity and health, as well as upregulation of inhibitory activity in the brain and increased astrogliosis. This impaired neuronal health and signaling may contribute to the poor cognitive performance observed in the multiparous animals and provide insight into potential mechanisms. This study

demonstrates that while pregnancy history does impart effects on cognition and brain aging, it does not appear to interact with PSEN2 in contributing to the significant sex differences observed in AD. Future studies should investigate how pregnancy history interacts with other mechanistic contributors, such as amyloidogenic, tauopathic, or autophagic pathways, to AD progression. The effect of parity in the brain is both complex and diverse, further emphasizing the importance of studying female-specific experiences in disease.

FIGURES AND TABLES

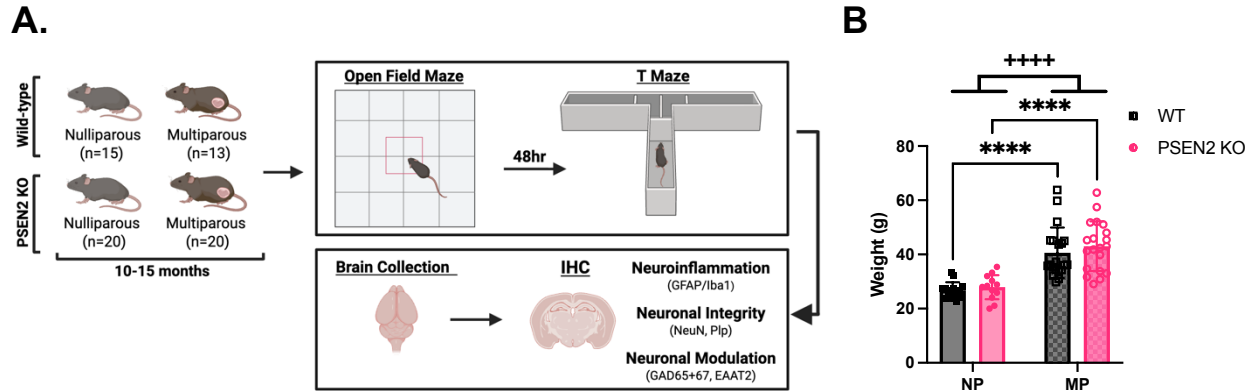


Figure 1. Experimental design to assess the impact of prior pregnancy history and AD-related genotype (PSEN2 knockout – KO) on late life cognitive function and neuropathology in aged female mice (10-15 months-old). A) Behavioral task performance was assessed in WT and PSEN2 KO mice, both nulliparous (NP) and multiparous (MP), before immunofluorescence was conducted for various neuroinflammatory and neuroplasticity markers. We hypothesized that AD-related risk factors, specifically PSEN2, and parity history would differentially impact cognitive function and neuropathology with advanced age. B) Body weight was significantly higher in the multiparous mice compared to the nulliparous mice, irrespective of genotype. All comparisons were analyzed with two-way ANOVA. + indicates main effect of parity, * indicates individual group differences, (****/++++ p<0.0001).

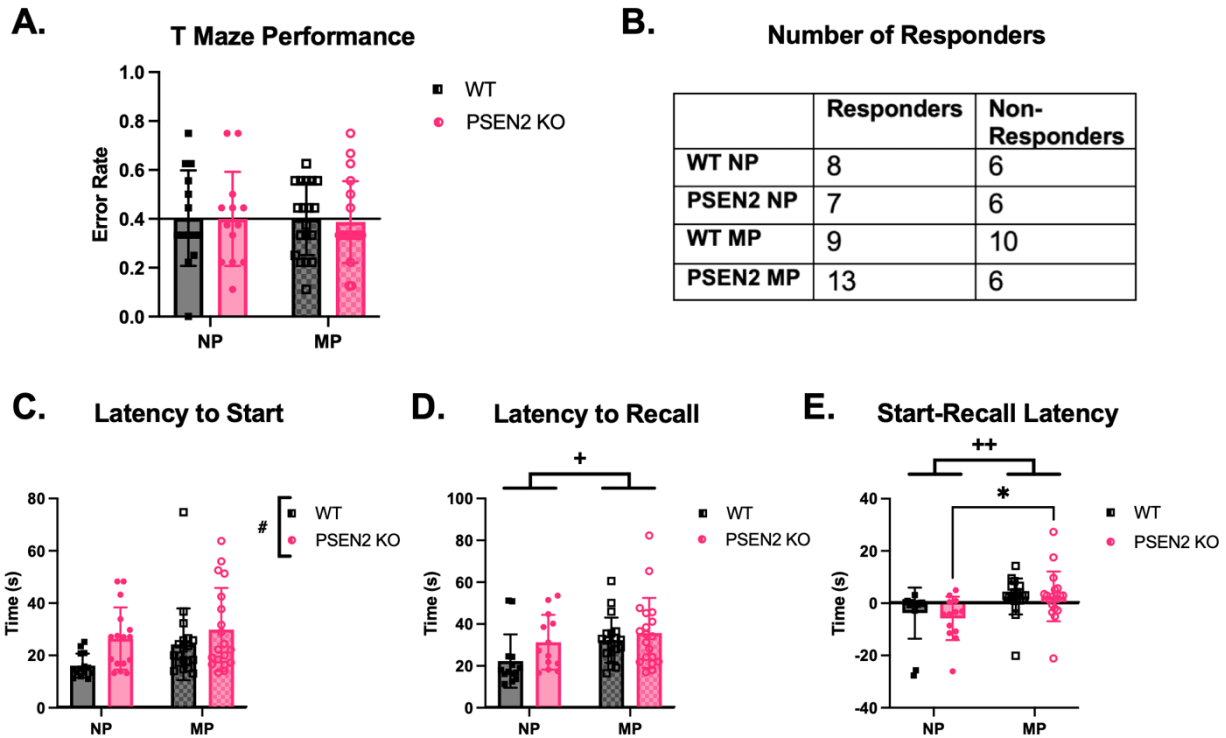


Figure 2. Nulliparous (NP) and multiparous (MP) female mice with loss of normal PSEN2 function (PSEN2 KO) were challenged in the spatial working memory TM task to assess cognitive function at age 10-15 months-old. A) T maze spontaneous alternation error rate was determined in nulliparous and multiparous mice and was found to have no statistical difference because of genotype or parity status. All groups performed around that of a healthy WT mouse (defined as error rate <40%), indicated by the horizontal line. B) The numbers of mice in each experimental group that met responder criteria of >60% correct responses were assessed and neither genotype nor pregnancy history influenced responder rate. Genotype exerted a main effect on the C) latency to start, where PSEN2 KO mice exhibited a longer latency than WT mice. There was a main effect of parity on the D) latency to recall and the E) start-recall latency, where multiparous females took a significantly longer time to complete the task. The latency to start and latency to recall are defined as the time it took for each mouse to choose a goal arm, either on the initial or secondary trial respectively. The start-recall latency is the difference between these values. PSEN2 KO multiparous mice had a significantly higher start-recall latency compared to PSEN2 KO nulliparous mice. This means the PSEN2 KO multiparous mice are taking longer to complete the second arm choice, relative to the initial trial, whereas the PSEN2 KO nulliparous mice exhibit the opposite trend. All comparisons were analyzed with two-way ANOVA. # indicates main effect of genotype, + indicates main effect of parity, * indicates individual group differences, (*/+/# $p < 0.05$, ++ $p < 0.01$).

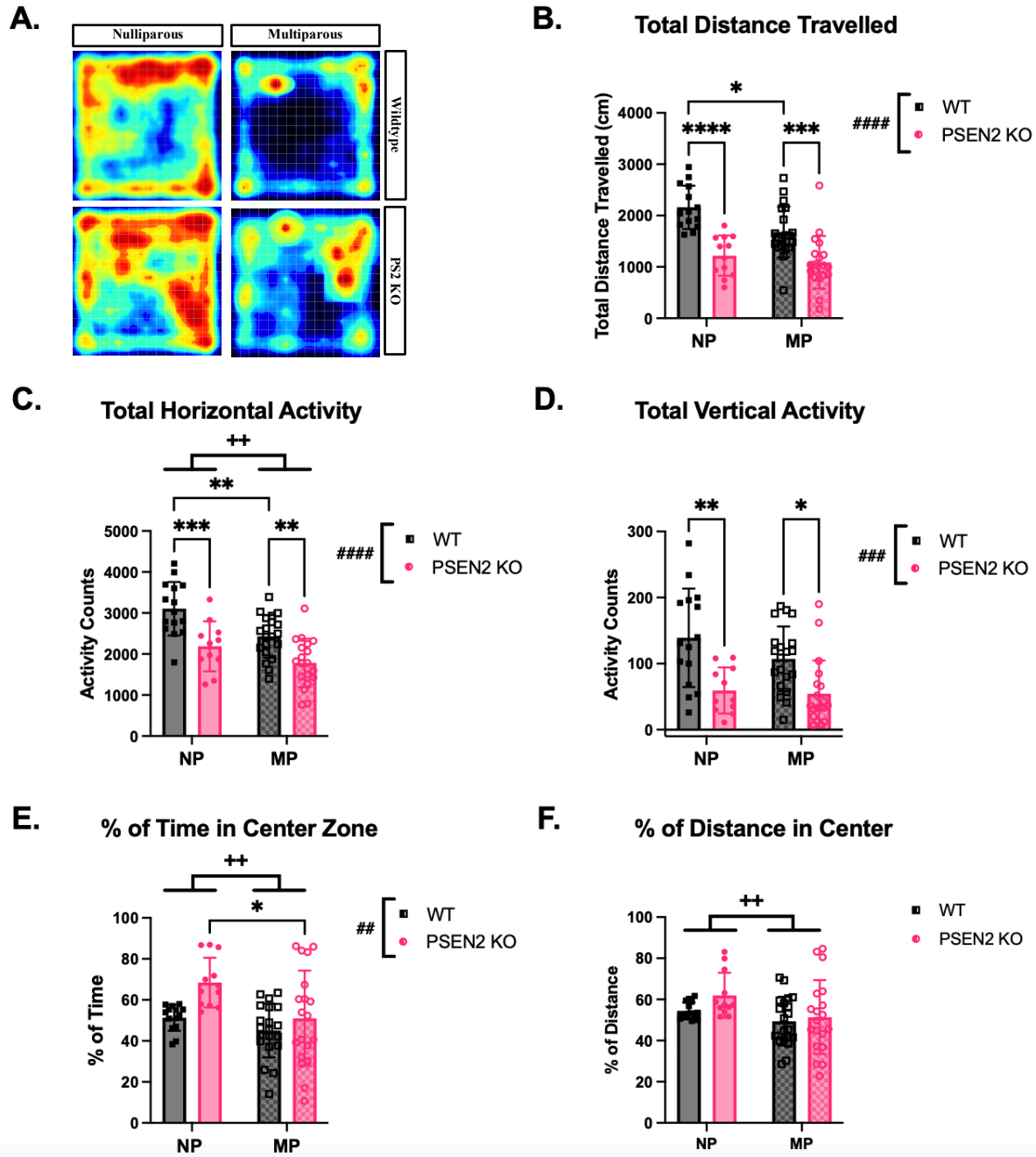
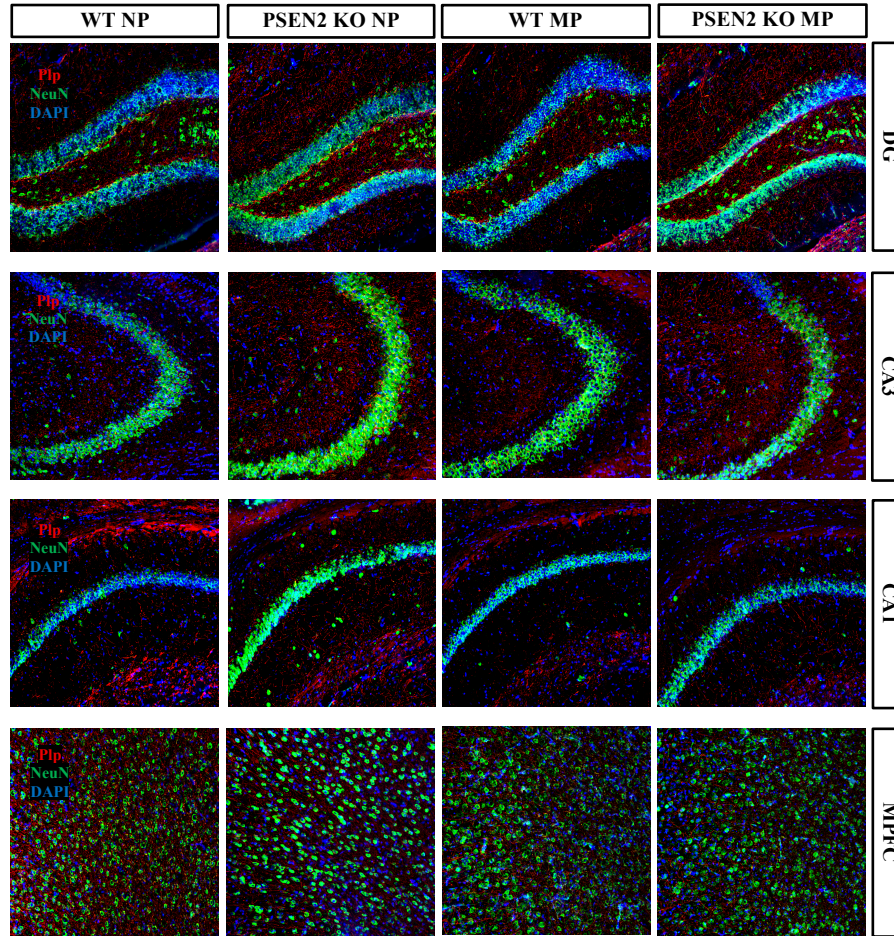


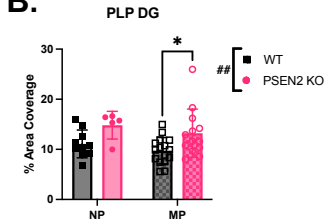
Figure 3. Genotype and parity status of 10-15-month-old WT and PSEN2 KO female mice exerted differing effects on Open Field Task mediated parameters for ambulation, locomotion, and anxiety like behaviors. PSEN2 KO and WT mice with (multiparous – MP) and without (nulliparous – NP) pregnancy history were challenged in a non-habituated open field test. A) Representative heat map images of open field maze exploration over 10 minutes depicts behavioral differences in activity and preference zones. B) There was a significant effect of genotype and parity in total distance travelled, with PSEN2 KO mice exhibiting decreased distance travelled compared to WT, in both nulliparous and multiparous groups. Furthermore, for the WT groups, multiparous mice had significantly decreased distance travelled compared to the

nulliparous mice. Similar trends for total distance travelled was observed for C) total horizontal activity. D) For total vertical activity, PSEN2 KO genotype significantly influenced activity counts. For both nulliparous and multiparous groups, PSEN2 KO mice exhibited decreased vertical activity compared to parous-matched WT mice. E), F) Analysis of center versus perimeter open field maze exploration, which serves as an indicator for thigmotaxis, was determined with percentage of time and distance in the center zone. E) With percentage of time in the center, both parity and genotype exerted a significant main effect with both multiparity and PSEN2 KO status reducing center preference. This effect was most evident in the PSEN2 KO nulliparous versus the genotype-matched multiparous mice. F) For percentage of distance in the center, multiparous females traveled less distance in the center compared to nulliparous, however there were no individual group differences. All comparisons were analyzed with two-way ANOVA. # indicates main effect of genotype, + indicates main effect of parity, * indicates individual group differences, (*/+ $p < 0.05$, **/++/## $p < 0.01$, ***/#### $p < 0.001$, ****/##### $p < 0.0001$).

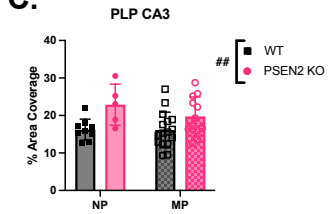
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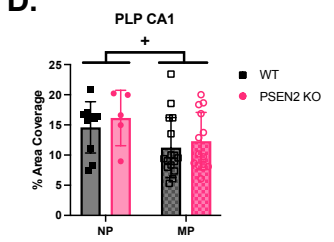
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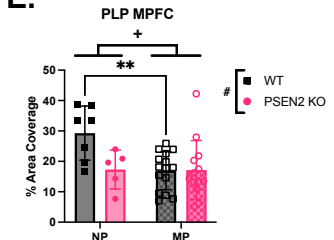
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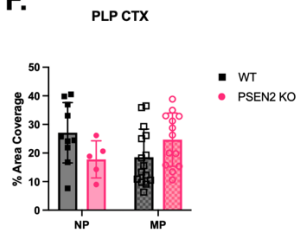
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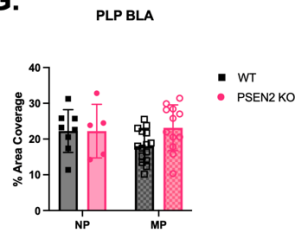
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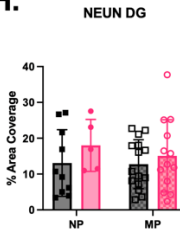
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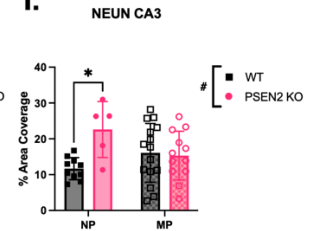
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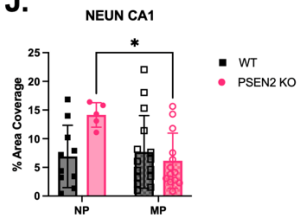
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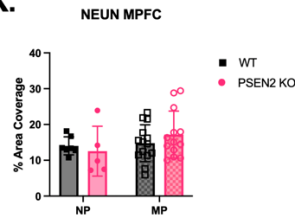
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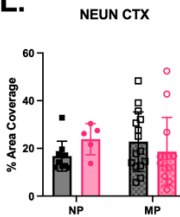
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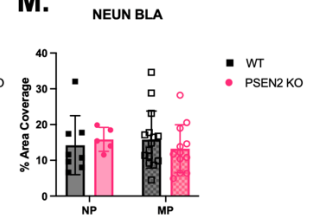


Figure 4. Proteolipid Protein (Plp) and Neuronal Nuclei (NeuN) expression in 10-15-month-old WT and PSEN2 KO mice with (multiparous - MP) and without (nulliparous - NP) pregnancy history after learning and memory behavioral testing. Myelin sheath integrity (as measured by Plp; red; B to G) and mature neuron density (as measured by NeuN; green; H) to

M)) was analyzed in dorsal hippocampus (Dentate Gyrus (DG), CA3, CA1; B to D, H to J), medial prefrontal cortex (MPFC; E, K), somatosensory cortex (CTX; F, L), and basolateral amygdala (BLA; G, M). DAPI nuclear counterstain was also applied (blue). A) Representative photomicrographs of immunofluorescent labeling of Plp and NeuN in mouse brain in WT and PSEN2 KO nulliparous and multiparous mice. PSEN2 KO genotype significantly influenced myelin sheath structure in the B) DG and C) CA3 region, with PSEN 2 KO females displaying increased Plp expression in the DG when compared to parous-matched WT. D) There were significant parity-induced decreases in Plp expression in the CA1 region, with multiparous females exhibiting lower Plp area coverage. E) In the MPFC, there was a main effect of both pregnancy history and PSEN2 genotype on Plp expression, but no parity x genotype interaction. WT nulliparous females were found to have significantly greater Plp coverage in the MPFC versus WT multiparous females. There were no significant differences in Plp levels in the F) CTX and the G) BLA. For NeuN signaling in the I) hippocampal CA3 region, PSEN2 KO mice had increased NeuN expression compared to the WT mice, specifically in the nulliparous group. J) there was a significant parity x genotype interaction in CA1 region, with the PSEN2 nulliparous mice having greater neuronal density compared to the WT multiparous mice, but this trend was not observed in genotype or parity-matched pairs. The H) DG, K) MPFC, L) CTX, and M) BLA regions did not exhibit any significant differences in NeuN expression. All comparisons were analyzed with two-way ANOVA. # indicates main effect of genotype, + indicates main effect of parity, * indicates individual group differences, (*+/# p<0.05, **/## p<0.01).

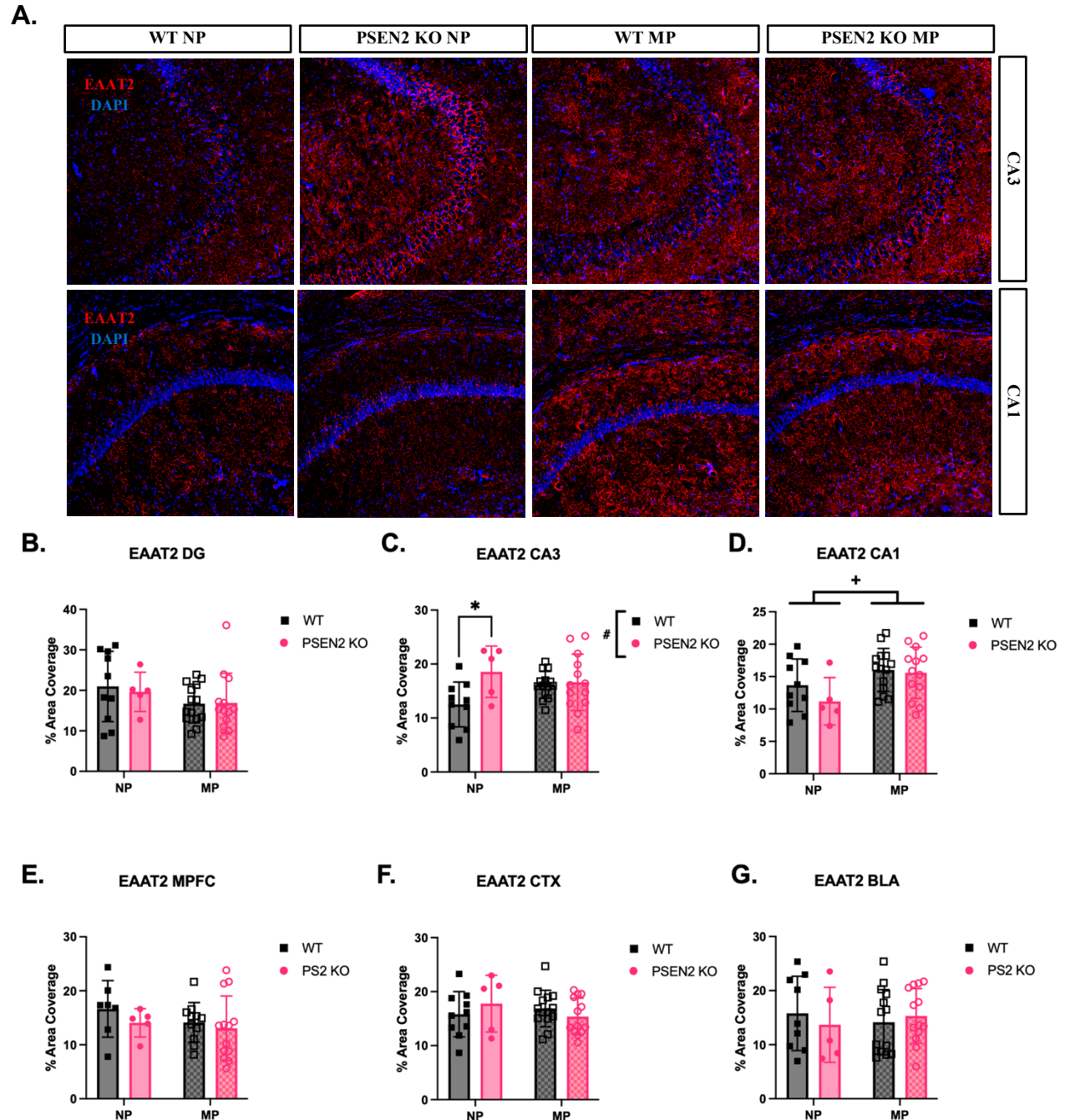


Figure 5. Excitatory amino acid transporter 2 (EAAT2) expression in 10-15-month-old WT and PSEN2 KO female mice with (multiparous - MP) and without (nulliparous - NP) pregnancy history after the conclusion of learning and memory behavioral testing. EAAT2 is responsible for glutamate reuptake from the synaptic cleft and serves to prevent excitotoxicity. EAAT2 expression (red) was analyzed in dorsal hippocampus (Dentate Gyrus (DG), CA3, CA1; B to D), medial prefrontal cortex (MPFC; E), somatosensory cortex (CTX; F), and basolateral amygdala (BLA; G). DAPI nuclear counterstain was applied (blue). A) Representative photomicrographs of immunofluorescent staining of EAAT2 in mouse brain in WT and PSEN2

KO nulliparous and multiparous mice. C) EAAT2 was significantly influenced by genotype in the CA3 region. Post-hoc analysis revealed that PSEN2 KO mice had higher EAAT2 expression versus WT in the nulliparous groups specifically. D) In the CA1 region, multiparous females were found to have significantly increased EAAT2 levels compared to nulliparous, regardless of genotype, indicating greater inhibitory tone with pregnancy history. The B) DG, E) MPFC, F) CTX, and G) BLA did not report any significant differences in EAAT2 signal because of parity of genotype status. All comparisons were analyzed with two-way ANOVA. # indicates main effect of genotype, + indicates main effect of parity, * indicates individual group differences, (*/+/# $p < 0.05$).

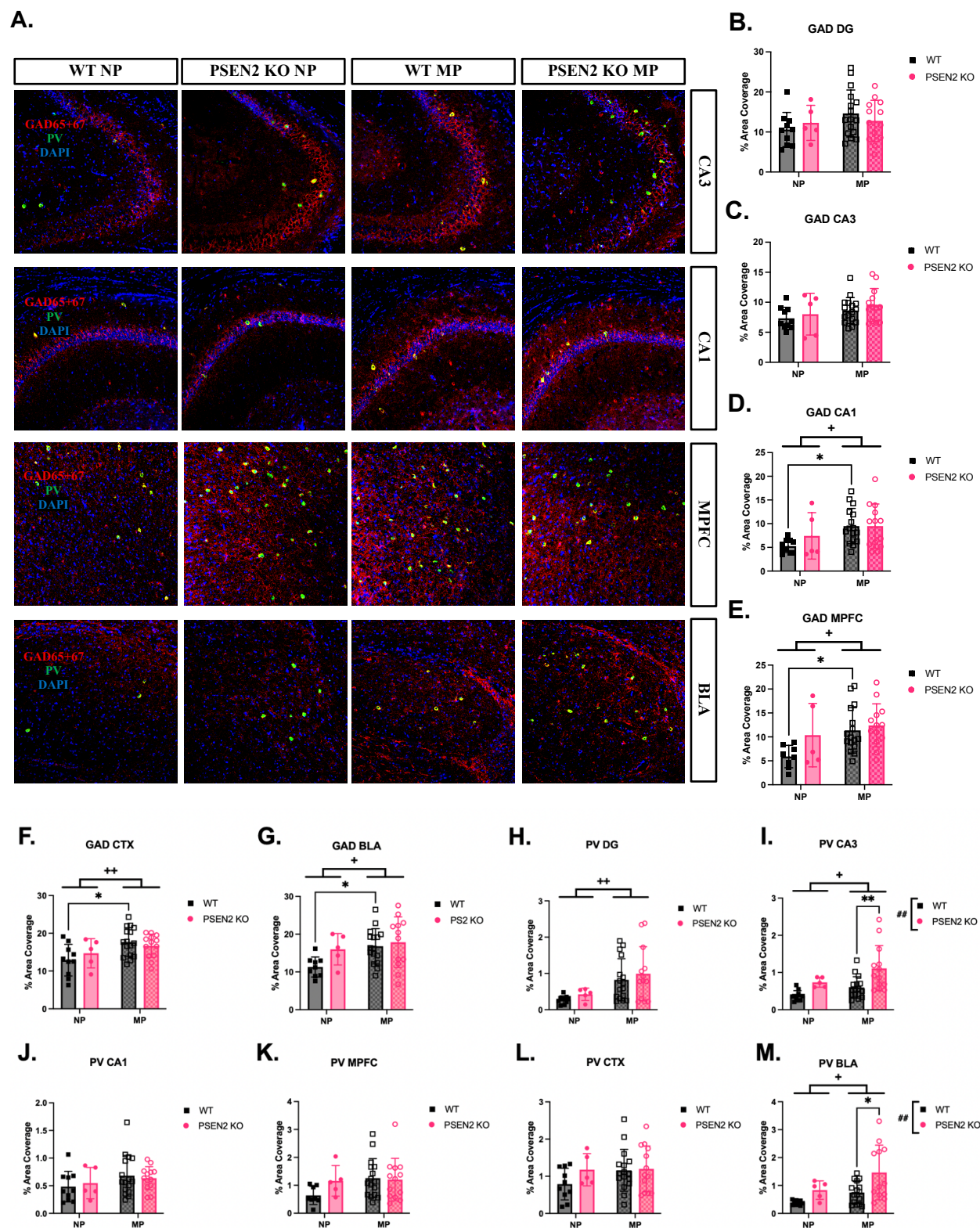
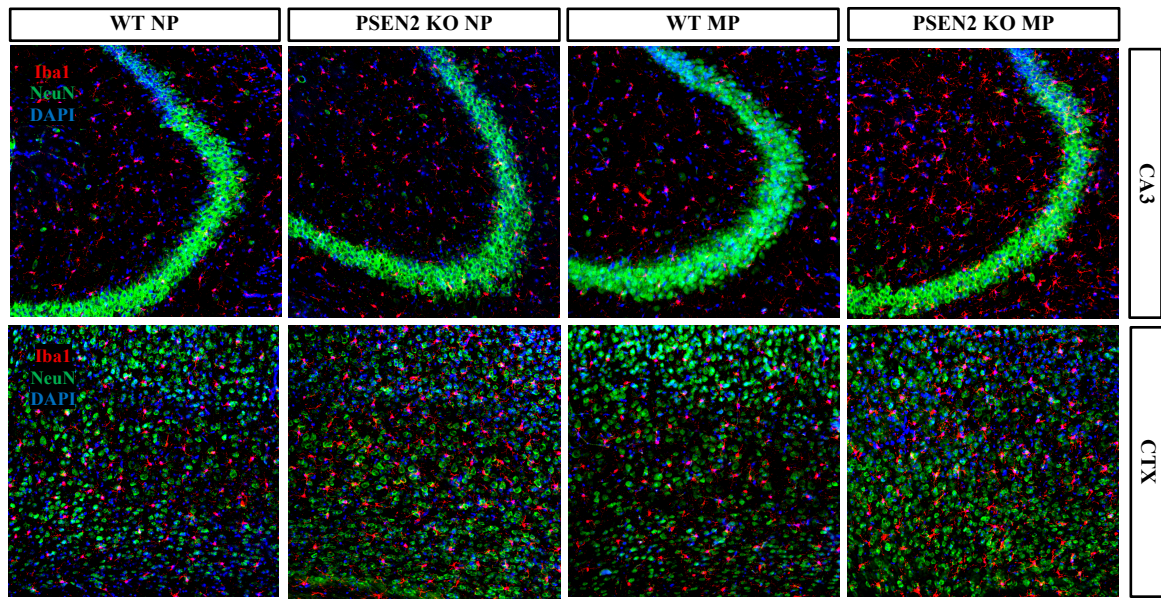


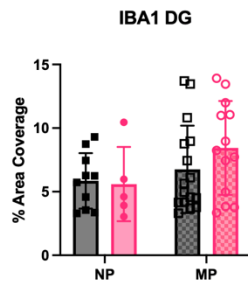
Figure 6. Inhibitory signaling measured via glutamic acid decarboxylase 65+67 (GAD65+67) and parvalbumin (PV) expression in 10-15-month-old WT and PSEN2 KO female mice with (multiparous - MP) and without (nulliparous - NP) pregnancy history

after the conclusion of learning and memory behavioral testing. GAD65+67-mediated GABA synthesis (red; B to G) and inhibitory PV interneuron density (green; H) to M)) was analyzed in dorsal hippocampus (Dentate Gyrus (DG), CA3, CA1; B to D, H to J), medial prefrontal cortex (MPFC; E, K), somatosensory cortex (CTX; F, L), and basolateral amygdala (BLA; G, M). DAPI nuclear counterstain was also applied (blue). A) Representative photomicrographs of immunofluorescent labeling of GAD65+67 and PV-positive cells in the brains of WT and PSEN2 KO nulliparous and multiparous mice. Parity influence on GAD65+67 expression throughout various brain regions in multiparous females in the D) CA1, E) MPFC, F) CTX, and G) BLA, compared to nulliparous. Within these brain regions, specifically the WT multiparous females were found to have consistently higher GAD65+67 levels compared to genotype-matched nulliparous mice. B) DG and C) CA3 did not experience any parity or genotype-mediated differences in GAD65+67 expression. Looking at PV interneuron coverage, the H) DG exhibited a main effect of pregnancy, with multiparous females displaying greater PV signal compared to nulliparous, although there were no individual group differences. I) CA3 and M) BLA exhibited similar trends; both parity and genotype exerted a significant main effect, with PSEN2 KO females showing increased PV expression compared to WT, specifically in the multiparous groups. Remaining brain regions (J - L) reported no difference in PV levels across groups. All comparisons were analyzed with two-way ANOVA. # indicates main effect of genotype, + indicates main effect of parity, * indicates individual group differences, (*/+ $p < 0.05$, **/++/### $p < 0.01$).

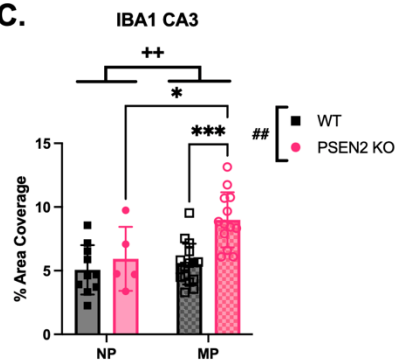
A.



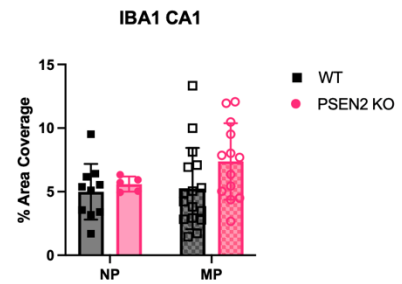
B.



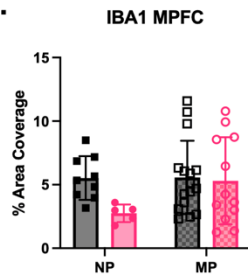
C.



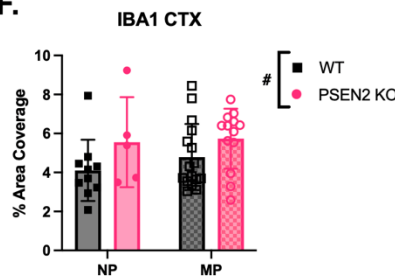
D.



E.



F.



G.

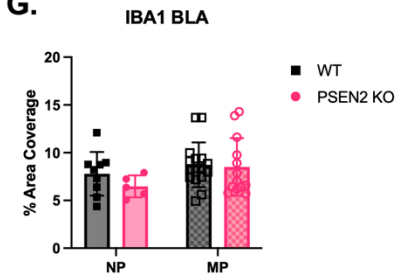


Figure 7. Aged (10-15-month-old) PSEN2 KO multiparous female mice exhibited greater reactive microgliosis in area CA3 of dorsal hippocampus relative to both PSEN2 nulliparous (NP) and WT multiparous (MP) animals. The expression of Ionized Calcium-Binding Adaptor Protein (Iba1; red) was analyzed by immunofluorescence in the dorsal hippocampus (Dentate Gyrus (DG), CA3, CA1; B to D), medial prefrontal cortex (MPFC; E), somatosensory cortex (CTX; F) and basolateral amygdala (BLA; G) to mark microgliosis. NeuN (green) co-labelling and DAPI nuclear counterstain was also applied (blue). A) Representative photomicrographs of immunofluorescent labeling of Iba1 and NeuN in WT and PSEN2 KO

nulliparous and multiparous mice brains. In the C) hippocampal CA3 region, both parity history and PSEN2 genotype increased Iba1 signal, indicating elevated microglia activation. The microgliosis in the PSEN2 KO multiparous mice was significantly greater than both the WT multiparous mice and the PSEN2 KO nulliparous mice. There was also a main effect of genotype in the F) cortex, with PSEN2 KO mice exhibiting elevated Iba1 expression compared to wildtype in both multiparous and nulliparous groups. The remaining brain regions (B, D, E, G) displayed no differences in Iba1-associated microglia activation. All comparisons were analyzed with two-way ANOVA. # indicates main effect of genotype, + indicates main effect of parity, * indicates individual group differences, (*/# $p < 0.05$, ++/## $p < 0.01$, *** $p < 0.001$).

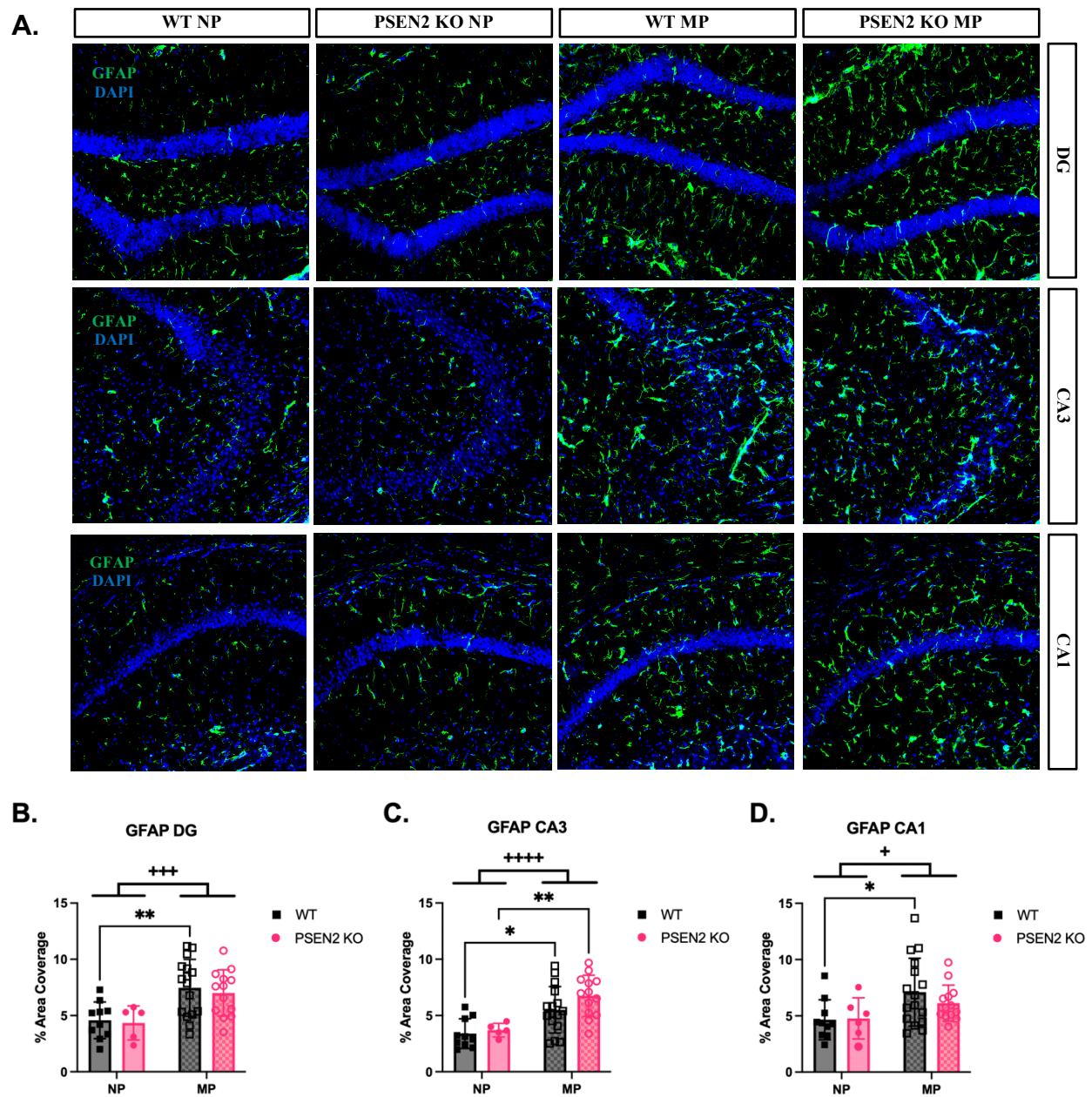


Figure 8. Aged (10-15-month-old) multiparous (MP) females demonstrated increased astrogliosis throughout various regions of the dorsal hippocampus compared to nulliparous (NP) females, irrespective of genotype. Glial Fibrillary Acidic Protein (GFAP; green) was utilized to assess reactive astrocyte activation with immunofluorescence in the dorsal hippocampus (Dentate Gyrus (DG), CA3, CA1; B to D). DAPI nuclear counterstain was also applied (blue). A) Representative photomicrographs of immunofluorescent labeling of GFAP in WT and PSEN2 KO nulliparous and multiparous mice hippocampal tissue. B) In the hippocampal DG region, pregnancy history exerted a significant effect on GFAP expression, with multiparous WT females exhibited increased astrogliosis compared to nulliparous WT

females. A similar effect was seen in the C) CA3, with elevated GFAP levels observed in the multiparous females. In the CA3, both the WT and PSEN2 KO multiparous groups displayed significantly greater GFAP immunofluorescence compared to genotype-matched nulliparous animals. D) The same trend persisted in the CA1 region. For the WT animals, there was increased GFAP in the multiparous CA1 compared to nulliparous. Remaining brain regions (CTX, MPFC, BLA) were not assessed for GFAP due to lack of signal across all animals. All comparisons were analyzed with two-way ANOVA. # indicates main effect of genotype, + indicates main effect of parity, * indicates individual group differences, (*/+ $p < 0.05$, ** $p < 0.01$, +++ $p < 0.001$, ++++ $p < 0.0001$).

Antibody	Company	ID	Lot #	Dilute	Function
EAAT2	Abcam	Ab271970	1087731-1	1:1000	Synaptic glutamate clearance
GFAP	Sigma-Aldrich	MAB3402X	4162265	1:500	Astrocyte activation
GAD65+67	Abcam	Ab183999	1071027-52	1:1000	Gamma-aminobutyric acid (GABA) inhibitory signaling
PV	Sigma-Aldrich	SAB4200545	0000453498	1:1000	Inhibitory interneuron
Plp	Abcam	Ab254363	1051615-31	1:500	Myelin integrity
NeuN	Sigma-Aldrich	MAB377	4158921	1:500	Identifies mature neurons
Iba1	Wako	019-19741	SEP6459	1:500	Microglial activation
Goat anti-rabbit 555	Abcam	Ab150078	1135748-6	1:500, 1:1000	Binds rabbit & fluoresces at 555 nm
Goat anti-mouse 488	Sigma-Aldrich	SAB4600042	22C0706	1:500, 1:1000	Binds mouse & fluoresces at 488 nm

Table 1. Antibodies and their target functions used for immunofluorescence analyses.

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