Title Page

Title: Chronic Seizures Worsen Cognitive Function with Loss of Presenilin 2 Function in a Sex-Related Manner

Abbreviated title: Impact of Chronic Seizures with PSEN2 Knockout

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Number of pages: 25

Number of figures: 6

Number of tables: 1

Number of words for abstract: 250

Number of words for introduction: 645

Number of words for discussion: 1325

Conflict of interest statement: The authors declare no competing financial interests.

Acknowledgements: This work was supported by the University of Washington Royalty Research Fund (MBH).

Abstract

Patients with early-onset Alzheimer's disease (EOAD) are at elevated risk for seizures, including patients with variants in presenilin 2 (PSEN2). Like patients with epilepsy, untreated seizures may also worsen cognitive function in AD. We therefore sought to define the impact of loss of normal *PSEN2* function and chronic seizures on cognitive function in the aged brain. Male and female PSEN2 KO and age- and sex-matched wild-type (WT) mice were sham or corneal kindled beginning at 6-months-old. Kindled and sham-kindled mice were then challenged up to 6 weeks later in a battery of cognitive tests: non-habituated open field (OF), T-maze spontaneous alternation (TM), and Barnes maze (BM), followed by immunohistochemistry for markers of neuroinflammation and neuroplasticity. PSEN2 KO mice required significantly more stimulations to kindle (males: $p<0.02$; females: $p<0.02$). Chronic seizures more significantly worsened anxiety-like behaviors of female PSEN2 KO mice as measured by percentage of total time exploring the center of an OF. TM performance was significantly worsened in kindled female ($p=0.024$), but not male, PSEN2 KO mice. Male BM performance was generally worsened by seizures ($p=0.038$), but not by genotype. Conversely, kindled PSEN2 KO females made the most BM errors ($p=0.007$). Chronic seizures also significantly altered expression of hippocampal neuroinflammation and neuroplasticity markers in a sex-specific manner. This study demonstrates that hippocampus-dependent cognitive function may be only worsened by uncontrolled chronic seizures in female, but not male, PSEN2 KO mice. Our work aligns with clinical evidence of sex-specific worsening of AD burden and supports sex-specific anticonvulsant interventions in AD.

Significance Statement

It is critical to understand the interaction between Alzheimer's disease (AD)-associated genetic risk factors (i.e., PSEN2) and chronic seizures to implement disease modifying strategies for AD. Patients with EOAD are at up to 87-fold elevated risk of having focal impaired awareness seizures, but whether the seizures are a cause, or a consequence of functional decline is less clear. This study demonstrates that investigator-initiated chronic seizures specifically worsen cognitive function in female mice challenged in a battery of tasks, as well as shines renewed focus on the disproportionate burden of AD in the female brain. This work provides clear evidence that chronic seizures worsen cognitive performance in mice with an AD-associated genotype and suggests that seizure-induced functional deficits may be sex-specific.

Introduction

Alzheimer's disease (AD) is a devastating neurological condition that is irreversible, unstoppable, and ultimately fatal. Most cases first become symptomatic in old age (65+), but early-onset AD (EOAD; ≤ 50 years old) has an annual incidence rate of 6.3/100,000 (Bickel et al., 2006). People with EOAD typically experience worse severity and more rapid disease progression. These patients experience 87-times higher risk of seizures versus same-aged people without AD (Amatniek et al., 2006; Vossel et al., 2013). Although not widely recognized as a comorbidity of AD, seizures may accelerate functional decline in patients and dramatically increase caregiver burden (Vossel et al., 2017). Seizures in AD are often unprovoked focal impaired awareness seizures, meaning they have no obvious trigger or clinical signs, and therefore can go unrecognized (Lam et al., 2017). Failure to recognize and treat seizures may compound neuropathology in AD patients. This is especially tragic because antiseizure medications (ASMs) may improve cognitive function in older people with mild cognitive impairment (Bakker et al., 2012), and seizures in elderly patients with epilepsy are generally well-controlled (Hernandez-Ronquillo et al., 2018), suggesting that rational selection of ASMs could slow seizure-related functional decline in EOAD. However, few studies have interrogated the direct functional impacts of chronic seizures within AD-related genotypes to spur intervention trials. As a result, clinical studies for ASMs in AD patients have been largely unsuccessful (Lehmann et al., 2021).

EOAD is associated with variants in the homologous presenilin 1 (*PSEN1*) and presenilin 2 (*PSEN2*) genes (Herreman et al., 1999). Presenilin (PS) proteins are intramembrane proteases of the catalytic component of γ-secretase, mutations of which promote the cleavage of amyloid-beta (Aβ) oligomers from the amyloid precursor protein (Wiley et al., 2005). PS2 mediates cell signaling pathways distinct from PS1 (Lee et al., 1996). Unlike PSEN1 knockout (KO) models, PSEN2 KO mice demonstrate normal development and are viable over 12-months-old (Herreman et al., 1999). The mechanisms by which *PSEN* variants cause AD remains unclear, however, it is well established that they lead to loss of normal γ-secretase complex function (Sannerud et al., 2016). AD-associated PSEN variants may thus lead to impaired synaptic plasticity, memory, and age-dependent neurodegeneration (Saura et al., 2004; Xia et al., 2015; Qin et al., 2017). Individuals with AD harboring the most common PSEN2 variant also exhibit a high incidence of seizures (Jayadev et al., 2010a). We have previously applied the corneal kindled mouse (CKM) model of chronic focal seizures to define the dose-related anticonvulsant efficacy of ASMs in young versus aged PSEN2 KO mice (Beckman et al., 2020). Importantly, this investigator-controlled model of chronic focal seizures provides an unique platform on which to directly assess functional impacts of chronic seizures with AD-associated genotypes (Lehmann et al., 2021).

Neuroinflammation and the sustained expression of inflammatory cytokines may worsen cognitive decline in both epilepsy and AD. Elevated levels of the cytokine, IL-1b, may underlie cognitive worsening, especially on hippocampus-mediated tasks (Rachal Pugh et al., 2001). IL-1b overexpression can disrupt long-term contextual and spatial memory but does not affect short-term and non-hippocampal memory (Hein et al., 2010), potentially through suppression of brain-derived neurotrophic factor (BDNF). BDNF itself plays an indirect role in regulating AMPA-type glutamate receptor localization to activated neuronal synapses required for learning and memory (Bramham et al., 2008; Barker-Haliski et al., 2012; Pevzner et al., 2012). Expression of BDNF correlates to behavioral performance (Bramham et al., 2009). However, limited preclinical studies are available in AD models to define the additive impact of chronic seizures on cognitive performance and neuropathology (Palop et al., 2007). No study has yet demonstrated how loss of normal PSEN2 function, which primes an inflammatory response (Jayadev et al., 2010b; Jayadev et al., 2013), may impact cognitive function with chronic seizures. Therefore, this study sought to define how hippocampally-mediated cognitive performance and neuropathology in the brains of aged PSEN2 KO mice (>6 months old) were directly impacted by chronic, untreated focal seizures.

Methods

Animals – Male and female PSEN2 KO mice were bred at the University of Washington (UW) from stock originally acquired from the Jackson Laboratory (stock #005617). All breeding at UW was between PCR-confirmed PSEN2 KO pairs. Age-matched male and female C57Bl/6J WT (stock #000664) were acquired from a similarly established breeding colony at UW, which was matched in housing conditions with the PSEN2 KO mice. This study was not testing the effects of sex hormones on behavior or protein expression; thus, we did not track estrous cycles of females or the dominant/subordinate status of males. Mice were housed on a 14:10 light cycle (on at 6 h00; off at 20 h00) in ventilated cages in corncob bedding in a manner consistent with the *Guide for the Care and Use of Laboratory Animals*. All animal work was approved by the UW Institutional Animal Care and Use Committee (protocol 4387-01) and conformed to ARRIVE guidelines (Drummond et al., 2010). Animals were provided free access to irradiated chow (PicoLab Rodent Diet 20-5053), filtered water, and enrichment, except during periods of behavioral manipulation. All behavioral testing was performed between 7h00 and 19h00.

Corneal Kindling – Two groups of 6-month-old male and female mice from each genotype (PSEN2 KO and WT) were used for corneal kindling studies. Group sizes for the kindling procedure were as follows: male WT = 55 (32 kindled, 23 sham); male KO = 51 (32 kindled, 19 sham); female WT = 41 (23 kindled, 18 sham); female KO = 53 (32 kindled, 21 sham). Tetracaine-HCl (0.5%) was applied bilaterally to the corneas immediately preceding electrical stimulation with a 3 s, 60 Hz sinusoidal pulse. Behavioral manipulation was identical for the kindled and sham groups, except no electrical stimulation was delivered to the sham mice. Kindling stimulation intensity was based on the subconvulsive minimal clonic seizure thresholds for age-, sex- and genotype-matched mice (Beckman et al., 2020). Males were stimulated at 3.0 mA; females were stimulated at 2.6 mA. Kindled seizure severity was scored according to the modified Racine scale (Racine, 1972). Mice were kindled until they achieved criterion, defined as five consecutive Racine stage 5 seizures (Beckman et al., 2020). The total number of mice that attained the kindled state for each genotype group were: male WT = 25; male KO = 24; female WT = 21; female KO = 22. Mice that did not achieve the fully kindled state were excluded from further behavioral testing. Following corneal or sham kindling, mice were divided into two testing cohorts. Cohort 1 completed the open field (OF) test and T-maze continuous alternation task (TM). Male group sizes for the OF/TM cohort were: kindled WT = 12; kindled KO = 12; sham WT = 10; sham KO = 8. Female group sizes for the OF/TM were: kindled WT = 12; kindled KO = 9; sham WT = 9; sham KO = 9. Cohort 2 completed the Barnes maze (BM). Male group sizes for the BM cohort were: kindled WT = 13; kindled KO = 12; sham WT = 13; sham KO = 11. Female group sizes for the BM were: kindled WT = 9; kindled KO = 13; sham WT = 9; sham KO = 12.

Seizure Duration – The kindled seizure duration was recorded from fully kindled mice from Cohort 1 following the 17-day stimulation-free period in which OF and TM tasks were completed to confirm the stability of the fully kindled state. The duration of seizure-induced behavioral arrest for mice that presented with a secondarily generalized (Racine stage 4 or 5) seizure at this time point post-kindling was recorded by an investigator blinded to genotype and kindling status, consistent with published methods (Beckman et al., 2020).

Open Field (OF) – In this assay, each mouse was placed into an OF Plexiglass chamber (40L × 40W × 30H cm) equipped with infrared sensors to detect animal movement for 10 min. During the 10-min period, the total distance travelled (cm), vertical activity counts, and horizontal activity counts in both the center and perimeter of the OF were automatically recorded by the computer system, consistent with our reported methods with kindled mice (Barker-Haliski et al., 2016b; Barker-Haliski et al., 2016a; Koneval et al., 2018; Meeker et al., 2019; Koneval et al., 2020).

T-maze (TM) – The TM continuous alternation task is a land-based task well-suited to assess hippocampally-mediated spatial working memory of mice (Deacon and Rawlins, 2006). Each TM test consisted of 15 trials. Mice were allowed to alternate spontaneously 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) throughout a 15-trial session. On the testing day, each mouse was contained in a starting box (10 x 8 cm) at the entry to the start arm. At the start of each trial, a partition door was opened to allow a mouse to move down the starting arm and choose between 2 goal arms, left or right. Once a mouse entered a particular goal arm, a door was lowered quietly to block entry into the opposite arm. Arm choices were recorded manually by a non-interfering observer. After 30 seconds, the mouse was returned to the starting box and the start alley and goal arms were cleaned with 30% isopropyl alcohol and dried to remove any olfactory cues. The spontaneous alternation rate was calculated as the ratio between the alternating choices and total number of choices during all trials, with 60% considered successful TM performance (Wenk, 2001).

Barnes Maze (BM) – The BM is a test of spatial learning and memory for rodents (Barnes et al., 1980; Barnes et al., 1996), and is particularly well suited for rodents with epilepsy. The platform consisted of a 68.6 cm diameter, white Plexiglass circular platform with twenty equally spaced escape holes and a hidden, black escape chamber attached below one of the twenty holes. Extra-maze spatial cues remained in a constant location, including the investigator. Bright lights (~1200 lux) were placed overhead of the maze as an aversive stimulus. The mildly aversive stimulus formed the basis to motivate each mouse to escape and thus learn the location of the escape chamber. The location of the escape hole remained the exact same for each individual mouse during the four acquisition days, but was randomly assigned to one of 8 coordinates (N, NW, S, SW, E, NE, W, SE) for each mouse in each experimental group. Before each trial, the BM platform, the escape chamber, and the start tube were cleaned with 30% isopropyl alcohol and allowed to air dry to remove any olfactory cues.

Testing on the BM proceeded in three phases (Patil et al., 2009): the habituation phase (day 0), an acquisition phase (days 1-4), and a probe trial of long-term memory (day 7). The habituation day was designed to familiarize each mouse with the escape chamber to reinforce escape from the bright lights illuminating the maze. The acquisition phase was designed to teach each mouse the location of the escape chamber over a series of twelve trials. During the probe trial, the escape chamber was removed, and one trial performed to determine if the animal remembered where the target escape hole was located. The following information was recorded manually by a non-interfering observer during each acquisition and probe trial: the latency to first encounter with escape chamber hole, number of holes explored before first encounter with escape chamber hole, and total latency to enter the escape chamber (in sec).

Euthanasia – One hour after the conclusion of TM and BM testing, mice were euthanized by live decapitation in accordance with IACUC guidelines and brains were collected, flash frozen on dry ice, and stored at –80°C. Brains were later sectioned on a Leica CM1860 cryostat into 20-μm thick coronal slices from Bregma –1.70 to –2.10 to capture the dorsal hippocampus for subsequent immunohistochemistry. Two rostral and two caudal sections/mouse were mounted on a Superfrost slide.

Immunohistochemistry – After cryosectioning, slides were fixed in 4% PFA for 10 minutes (22°C) then dehydrated in 50% ethanol for 30 min. After dehydration, the slides were washed (3 x 10 min) in 0.1 M PBS before being incubated in a 10% Goat Serum in 5% Triton-X PBS solution under coverwells in a humid chamber for 2 hours. Briefly, NeuN (1:300; MAB377X, Millipore) and GFAP (1:1000; C9205 Sigma-Aldrich) antibodies

were applied under 200 mL coverwells in a 5% Goat Serum/1xPBS solution overnight at 4°C (Barker-Haliski et al., 2016b; Loewen et al., 2016a). The following day, coverwells were removed and slides washed in PBS (3 x 10 min) before being coverslipped with Prolong Gold with DAPI.

Slides were similarly processed for BDNF with the exception that slides were blocked with 10% sheep serum in 5% Triton-X PBS under coverwells in a humid chamber for 2 hours. Next, the slides were incubated with primary antibodies for BDNF (1:500; ab226843, Abcam) and NeuN (1:300; MAB377X, Millipore) for 2 hours. Slides were then washed in 1x PBS (3 x 10 min). The secondary antibody (1:1000; C2306 Sigma Aldrich) was applied in 5% sheep serum/1x PBS under coverwells overnight at 4°C in a humid chamber. The next day, coverwells were removed and slides washed in 1x PBS (3 x 10 min) before being coverslipped with Prolong Gold with DAPI.

Slides were imaged after a minimum 24 hours with a fluorescent microscope (Leica DM-4) with a 20x objective (80x final magnification). Acquisition settings were held constant throughout. NeuN, GFAP, and BDNF expression levels, given as average area percentage, were automatically measured using Leica Thunder software.

Statistics – Percent of mice that attained kindling criterion was compared by repeat measures ANOVA. Total numbers of stimulations to attain kindling criterion and kindled seizure duration was compared between genotypes within sexes by t-test. Performance on the OF, TM, and BM were compared by two-factor ANOVA (kindling x genotype) within sexes, with *post-hoc* Tukey's tests when applicable. Immunohistochemical assessments were also compared by two-factor ANOVA (kindling x genotype) within sexes, with *post-hoc* Tukey's tests when applicable. All analysis was conducted with GraphPad Prism version 8.0 or later, with $p<0.05$ considered significant.

Results

Kindling acquisition is significantly delayed in 6-month-old PSEN2 KO mice.

Corneal kindling of 6-month-old male and female PSEN2 KO mice was well-tolerated, consistent with the kindling of age-matched WT mice (Figure 1). There was a significant time x genotype interaction on kindling acquisition in both male and female PSEN2 KO mice (males: $F_{(11,682)}=2.216$, $p=0.0123$; females: $F_{(6,306)}=3.384$, $p=0.003$), demonstrating that loss of normal PSEN2 function substantially slows kindling rate (Figure 1A and Figure 1B). The percentage of male and female PSEN2 KO mice that attained kindling criterion was also significantly different (Figure 1C and 1D; males: $F_{(23, 23)} = 32.50$, $p<0.0001$; females: $F_{(23, 23)} = 14.10$, $p<0.0001$). The differences in percentage of fully kindled mice between genotypes were apparent by the 13th (males) and 18th stimulation (females). Both male and female PSEN2 KO mice aged 6-months required significantly more stimulations to achieve the kindled state (Figure 1E and 1F - males: KO 17.1±0.808 vs WT 14.6±0.689, $t=2.32$, $p=0.0243$; female KO 11.3± 0.9 vs female WT 8.85±0.5, $t=2.59$, $p<0.02$). Thus, loss of normal PSEN2 function leads to resistance to corneal kindling, like our previous findings in PSEN2 KO mice aged 2 months-old (Beckman et al., 2020).

Open field exploration is worsened in female PSEN2 KO mice with chronic kindled seizures

The OF task is useful to assess cognitive function in mice. There was a significant main effect of both kindling and genotype, but no interaction, on the total distance traveled by male PSEN2 KO versus WT mice (Figure 2A; kindling - $F_{(1, 38)}=39.52$, $p<0.0001$, genotype - $F_{(1, 38)}=4.682$, $p=0.0368$). *Post-hoc* analysis did not reveal any significant differences between sham-kindled PSEN2 KO and WT male mice ($p>0.05$), nor was there

any difference between kindled PSEN2 KO and WT male mice ($p>0.05$). There was also a significant main effect of both kindling and genotype, but no interaction, on the total distance traveled by female PSEN2 KO relative to WT mice within an OF (Figure 2B; kindling - $F_{(1, 35)}=7.758$, $p=0.0086$, genotype - $F_{(1, 35)}=18.07$, $p=0.0002$). However, *post-hoc* tests did reveal significant differences between sham-kindled PSEN2 KO and WT female mice ($p=0.035$), and between kindled PSEN2 KO and WT female mice ($p=0.0155$). Therefore, genotype-related differences in total OF exploration were only significantly affected by seizure history in female mice.

The OF is also useful to assess anxiety-like behavioral comorbidities, including in fully kindled mice (Barker-Haliski et al., 2016a), through the evaluation of thigmotaxis (Prut and Belzung, 2003). Assessment post-kindling demonstrated a significant kindling x genotype interaction on percentage of total time exploring the center of the OF (Figure 2C and 2D), suggestive of worsened anxiety-like behaviors in PSEN2 KO mice with kindled seizures versus sham-kindled mice (males: $F_{(1, 37)}=11.24$, $p<0.002$; females: $F_{(1, 34)}=11.75$, $p<0.002$). Kindled females generally spent less of their total percentage of time in the OF center versus sham-kindled mice (Figure 2D). Interestingly, *post-hoc* tests revealed that sham-kindled female PSEN2 KO mice spent more time in the center of the OF versus WT sham-kindled mice ($p=0.0005$), yet kindling caused female PSEN2 KO mice to spend the same amount of their total time in the center of the OF versus WT mice ($p>0.9$). Thus, there was a substantially greater degree of change in percentage of total time in the OF center in kindled PSEN2 KO female mice, suggesting that kindling more significantly worsens anxiety-like behavior of female PSEN2 KO mice.

T-maze continuous alternation task performance was worsened by chronic kindled seizures only in PSEN2 KO female mice

Performance of mice on the land-based TM continuous alternation task was compared within sexes (Figure 3A and 3B). The number of response errors on the TM task was not significantly impacted in kindled male ($F_{(1, 40)} = 0.054$, $p>0.8$, Figure 3A). Conversely, there was a significant genotype x kindling interaction in female mice ($F_{(1, 36)}=5.52$, $p=0.024$, Figure 3B). *Post-hoc* tests demonstrated that fully kindled PSEN2 KO mice performed worse than sham kindled PSEN2 KO female mice ($p=0.0221$) and there was a non-significant trend for worsening of performance relative to WT kindled female mice ($p=0.086$). Thus, performance on the TM was only impaired in female PSEN2 KO mice with a history of chronic kindled seizures. Performance of male PSEN2 KO mice on the TM was not negatively affected by chronic kindled seizures.

Behavioral seizure duration was not different between fully kindled PSEN2 KO versus WT mice

Mice in Cohort 1 had a 17-day stimulation-free period during the period of OF and TM testing. The duration and stability of fully kindled behavioral seizures was thus recorded by an investigator blinded to genotype and kindling status after behavioral studies concluded (Beckman et al., 2020). The fully kindled seizure duration was not significantly different between genotypes in either sex (not shown): male PSEN2 KO (mean±SEM – 37.2±2.43 sec) vs age-matched WT mice (38.1±3.13 sec); female PSEN2 KO (45.6±3.89 sec) versus age-matched WT mice (38.6±2.48 sec). Thus, evoked seizures were both stably induced and no different in duration between genotypes in either sex up to 17 days post-kindling.

Performance of PSEN2 KO female mice on the Barnes maze task was worsened by chronic kindled seizures

Cohort 2 of fully kindled (or sham kindled) male and female PSEN2 KO and WT mice were used to assess performance on the BM task at 4-6 weeks after corneal kindling. Performance on the BM for each sex was assessed by the number of holes explored to locate the escape chamber and latency to first encounter with the escape hole over the four trial days and final probe trial on Day 7 (Figure 4). There was no significant effect of kindling or genotype on the latency to first encounter with the escape platform of male WT and PSEN2 KO mice

(Figure 4A), as there was only a significant improvement with time on the BM ($F_{(4,176)}$=21.59, p<0.0001). However, the number of erroneously explored escape holes was generally worsened in males by kindling (Figure 4C; $F_{(3, 44)}$=7.15, p=0.0005) but there was no interaction between the kindling groups and number of days on the BM ($F_{(12,176)}$=0.56, p>0.8). Conversely, in the female PSEN2 KO and WT mice, there were marked differences in BM performance due to chronic seizures and genotype (Figure 4B and 4D). First, there was a significant kindling group x trial day interaction on the latency to first encounter with the correct escape hole (Figure 4B; $F_{(12,148)}$=2.142, p=0.0175). *Post-hoc* test demonstrated that sham-kindled WT female mice were significantly slower to initially locate the hidden platform relative to kindled WT female mice (p=0.0001) and kindled PSEN2 KO mice were slower than kindled WT female mice (p=0.0138), but sham KOs were not different from kindled KOs. Second, there was a significant main effect of kindling in female mice on the number of erroneously explored escape holes (Figure 4D; $F_{(3,37)}$=12.92, p<0.0001). *Post-hoc* tests demonstrated significant differences in the number of erroneously explored escape holes between kindled WT and kindled PSEN2 KO female mice on the first trial day (p=0.001) and between sham-kindled WT and kindled WT female mice on the probe trial day (Day 7, p=0.0107). Thus, only the BM performance of female PSEN2 KO mice was significantly impacted by chronic kindled seizures; kindled male PSEN2 KO mice were unaffected.

Further scrutiny of the total number of errors made on the first trial day demonstrated sex-related differences in the impacts of kindled seizures on short-term memory in mice aged over 6-months old (Figure 4E and 4F). Specifically, kindling was generally associated with worsened performance of male mice on the first testing day, regardless of genotype ($F_{(1,44)}$=4.676, p=0.0361) but there was no effect of genotype $F_{(1, 44)}$ = 0.554, p>0.4). Conversely in female mice, there was a significant kindling x genotype interaction (Figure 4F; $F_{(1, 36)}$ = 11.91, p=0.0014), with kindled PSEN2 KO females committing more errors on the first trial day relative to kindled WT females (p=0.0002). Thus, a history of chronic kindled seizures most substantially worsened the Day 1 performance of PSEN2 KO female mice aged over 6-months old, again revealing sex-specific differences in the functional impact of chronic kindled seizures in PSEN2 KO mice.

BDNF expression is reduced with chronic kindled seizures in PSEN2 KO mice

Kindled female, but not male, PSEN2 KO mice were most significantly impaired on the BM and TM tasks, suggesting that hippocampally-mediated memory deficits are specifically worsened by chronic seizures and loss of normal PSEN2 function only in female mice. We thus sought to define the extent to which the molecular marker of synaptic plasticity (BDNF) was impacted by chronic kindled seizures in WT and PSEN2 KO mice (Figure 5). There was a significant kindling x genotype effect on BDNF expression in male mice in areas CA1 (Figure 5A; $F_{(1,72)}$=4.087, p=0.0469) and CA3 of dorsal hippocampus (Figure 5B; ($F_{(1,72)}$=4.504, p=0.037). *Post-hoc* tests revealed significant differences in BDNF expression in area CA1 only between sham and kindled WT male mice (p=0.0348) and between kindled WT and kindled PSEN2 KO mice (p=0.0272), suggesting that chronic kindled seizures do not upregulate BDNF expression in PSEN2 KO mice. There were no significant differences in BDNF expression in the DG of kindled and sham kindled male mice of either genotype.

In female mice, PSEN2 genotype significantly influenced BDNF expression in area CA1 (Figure 5D, $F_{(1,46)}$=4.607, p=0.038), whereas kindling significantly influenced this expression in CA3 (Figure 5E, $F_{(1,46)}$=5.716, p=0.021). Lastly, there were both effects of kindling and PSEN2 KO genotype on BDNF expression in DG (Figure 5F, genotype: $F_{(1,46)}$=6.068, p=0.0177; kindling: $F_{(1,46)}$=4.734, p=0.0349), but no significant interaction between factors. Further, *post-hoc* tests only revealed significant differences in DG between sham kindled and kindled WT female mice (p=0.0358), as well as sham kindled WT mice versus kindled PSEN2 KO female mice. While WT male mice demonstrated seizure-induced increases in BDNF expression in area CA1, this was not similarly observed in female WT mice with kindled seizures. Notably, female PSEN2 KO mice did not show

kindling-induced reductions in DG BDNF expression as was observed in WT female mice, further demonstrating that chronic kindled seizures differentially impact synaptic plasticity-associated protein expression with loss of normal PSEN2 expression.

Fully kindled PSEN2 KO female mice demonstrate blunted seizure-induced astrogliosis

Chronic kindled seizures are associated with reactive astrogliosis (Loewen et al., 2016a). Thus, we sought to quantify the degree of reactive astrogliosis within dorsal hippocampus of male and female WT and PSEN2 KO kindled, and sham-kindled, mice following Day 7 BM testing (Figure 6A-K), consistent with previous studies in much younger kindled WT male mice (Loewen et al., 2016a). Kindling-induced astrogliosis in aged male mice was detected within both genotypes in CA1 and DG (Figure 6A and 6B), with a significant effect of kindling in CA1 ($F_{(1, 67)} = 20.65$, $p<0.0001$) and DG ($F_{(1, 68)} = 32.23$, $p<0.0001$). There were no significant differences in CA3 for any group assessed. *Post-hoc* evaluation revealed significant differences between sham and kindled male mice within each genotype ($p<0.05$), indicating that kindling induced significant astrogliosis in both PSEN2 KO and WT male mice over 6-months-old. There were no significant changes in NeuN immunoreactivity in male PSEN2 KO or WT kindled mice versus sham in either analyzed brain region (Figure 6C and 6D).

In female mice, there was a significant main effect of kindling ($F_{(1, 44)} = 7.576$, $p=0.0086$) and genotype ($F_{(1, 44)} = 9.868$, $p=0.003$; Figure 6E) on GFAP immunoreactivity in area CA1. *Post-hoc* analysis demonstrated stimulation-induced increases in GFAP immunoreactivity in WT female mice only ($p=0.030$). There was only a main effect of genotype on DG astrogliosis ($F_{(1, 45)} = 7.057$, $p=0.0109$; Figure 6F), with only WT female mice exhibiting kindling induced increased GFAP immunoreactivity ($p=0.0157$). As observed in male mice, there were no significant changes in NeuN immunoreactivity in either analyzed brain region.

Discussion

Consistent with our prior findings in young adult (2-3 months-old) PSEN2 KO mice (Beckman et al., 2020), we now report that loss of normal PSEN2 function, as may occur in patients with EOAD, may disrupt the formation of an epileptic network in mice aged 6- to 7-months-old. Despite our presently detected resistance to kindling at this older age, seizures were not differentially severe in PSEN2 KO mice, and the fully kindled seizure was stable until at least 17 days post-acquisition. However, we herein demonstrate that hippocampus-dependent cognitive function was only significantly worsened by evoked chronic seizures in aged female, but not male, PSEN2 KO mice. These data align with clinical findings suggesting a greater susceptibility to AD in women compared to men (Udeh-Momoh et al., 2021). Further, this present study provides clear demonstration that investigator-controlled induction of chronic focal seizures in an AD-associated preclinical model is suitable to interrogate functional and neuropathological impacts on disease trajectory, which may substantially benefit future disease modification strategies in patients with AD.

Our present study provides the first conceptional demonstration of how the corneal kindling model of epileptogenesis can be applied to better define the functional impacts of seizures in the AD-associated brain. CKM is a moderate-throughput, minimally invasive model that is commonly used in epilepsy research (Matagne and Klitgaard, 1998; Rowley and White, 2010; Barker-Haliski et al., 2016a; Barker-Haliski et al., 2017b; Loscher, 2017). Unlike the resource-intensive recordings of spontaneous electrographic seizures by video-EEG with single-housed mice with AD-associated amyloid precursor protein (APP) overexpression (Palop et al., 2007; Palop and Mucke, 2009; Ziyatdinova et al., 2011; Verret et al., 2012; Ziyatdinova et al., 2015; Ziyatdinova et al., 2016; Brown et al., 2018), CKM reproduces chronic network hyperactivity in an investigator-controlled setting wherein seizures can be clearly identified; i.e. epileptogenesis can be slowed to time-dependently and directly resolve how seizure history affect behavioral and neuropathological outcomes at discrete time points (Loewen et al., 2016a).

Spontaneous seizures in animal models of epilepsy are infrequent and unpredictable (Hellier and Dudek, 1999; Stewart et al., 2010), therefore directly attributing behavioral changes to uniform seizure history is difficult, highly individualized, and requires large groups sizes, and long-duration, resource-intensive monitoring to have sufficient power to resolve an effect of seizures on behavior or disease modification (Barker-Haliski et al., 2021; Mizuno et al., 2021). CKM can generate large cohorts of AD-associated mice with uniform seizure history that are suitable to evaluate behavioral outcomes and pharmacological interventions. Additionally, unlike long-term recordings of EEG-implanted rodents, CKM are group housed throughout their lifespan to minimize social isolation stress, which may itself induce behavioral deficits (Ieraci et al., 2016) and reduce seizure threshold of rodents (Swinyard et al., 1963).

Inflammation represents a significant risk factor for seizure induction and maintenance (Vezzani et al., 2013), as well as AD pathogenesis (Heppner et al., 2015). There is significant upregulation of inflammatory cytokines in human patients with epilepsy and preclinical seizure and epilepsy models (Maroso et al., 2010; Vezzani et al., 2015). PSEN2 is a novel and presently underexplored molecular contributor to neuroinflammation due to the conserved regulation between PSEN2 and NFκB and molecules of the Toll-like receptor signaling pathway (Yagi et al., 2008). Both pathways are heavily implicated in the inflammatory response (Vezzani et al., 2013). Microglia derived from PSEN2 KO mice are primed to exhibit enhanced inflammatory cytokine release in response to insult *in vitro* (Jayadev et al., 2010b), possibly enhancing susceptibility to neurological damage with chronic seizures. Indeed, the most common PSEN2 variant (N141I) worsens inflammatory response of primary microglia (Fung et al., 2020). While our study presently did not address the changes in seizure-induced microglial response, future work is needed to evaluate how chronic seizures influence neuroinflammation in an AD-associated brain. Neuroinflammation is critical to seizure induction and maintenance (Vezzani et al., 2013; Barker-Haliski et al., 2017a) and underlies the onset of reactive gliosis in preclinical models of chronic network hyperexcitability and seizures (Loewen et al., 2016b). Neuroinflammation is also a key contributor to AD (Heppner et al., 2015). In this regard, models that are characterized by an inflammatory phenotype are particularly interesting to study additive impacts of seizures and AD-associated genetic risk factors to better understand how seizures affect disease course. Our study indicates that PSEN2 KO mice aged over 6-months-old fill an important gap to define the impact of chronic seizure history and AD-associated genotypes on neuroinflammation.

In this study, we demonstrated significant sex-related differences in the hippocampal region-specific BDNF expression with loss of normal PSEN2 function. BDNF is a critical component of neuroplasticity that has been shown to enhance α secretase and inhibit β secretase activities to effectively reduce Aβ production (Nigam et al., 2017). While we did not assess Aβ levels in this study, it is known that PSEN2 KO mice do not demonstrate accumulated Aβ plaques (Elder et al., 2010). BDNF also has critical roles as a neuroprotectant in excitotoxic and ischemic stress conditions (Arancibia et al., 2008; Tapia-Arancibia et al., 2008; Lau et al., 2015; Kowianski et al., 2018), as well as being disease-modifying in a rodent model of acquired epilepsy (Liu et al., 2013). BDNF may thus reduce AD burden and delay disease pathology (Choi et al., 2018). Given this neuroprotective and cognitive-sparing role of BDNF in the AD-associated brain, our present results demonstrate that BDNF expression in CA1 of the male hippocampus is not markedly upregulated in PSEN2 KO mice with chronic seizures, in stark contrast to the seizure-induced increased expression in age-matched WT mice (Figure 5A). Further, female mice did not show BDNF changes in CA1 in either genotype with kindling, suggesting dimorphic sex-specific impacts on the expression of this neurotrophic factor with chronic seizures. There were also only significant reductions in BDNF expression in the DG of WT female mice, but there were no concomitant seizure-induced changes in PSEN2 KO mice. These data suggest that aged PSEN2 KO mice generally demonstrate disruptions in seizure-related changes in BDNF expression, with WT female mice generally exhibiting reduced hippocampal BDNF expression with kindling. This dysregulated BDNF expression

in kindled female PSEN2 KO mice may contribute to the presently observed cognitive deficits.

PSEN2 KO mice offer a unique platform on which to model the sequelae of chronic seizures in an AD-associated genetic background (Lehmann et al., 2021). The use of PSEN2, rather than other models of AD that rely on pathological APP overexpression (Palop et al., 2007; Palop and Mucke, 2009), allows for specific interrogation of the role that inflammation may play on comorbidities of AD, including focal seizures. Importantly, because PSEN2 KO mice are not defined by Aβ accumulation, this model provides an opportunity to better define the Aβ-independent mechanisms associated with cognitive decline in AD. Further, PSEN2 variants are associated with later onset of disease and may even be masked as sporadic AD cases (Blauwendraat et al., 2016), suggesting that PSEN2 variants may be particularly relevant to late-onset AD. There are no issues with the long-term viability of PSEN2 KO mice, which can reach over 12 months of age without attrition ((Herreman et al., 1999) and our own experiences indicate even older), unlike APP overexpressing models. This robust viability allows for ample numbers of PSEN2 KO mice to reach late adulthood and even geriatric time points; time points that will better model the additive effects of aging, AD-associated mutations, and chronic seizures. Aged mice are not routinely used for epilepsy research (Kelly, 2010) or ASD development (Barker-Haliski, 2019; Lehmann et al., 2021). Without well-characterized models of seizures in models with aging-related neurological pathology, safe and effective therapies for patients with AD and comorbid seizures will never reach clinical use. Indeed, a significant percentage of patients with the most common AD-associated variant in PSEN2 also report seizures (32% of cases with the N141I variant; (Jayadev et al., 2010a). In this regard, corneal kindled PSEN2 KO mice are an intriguing platform on which to directly define the functional impacts of chronic seizures in the context of aging and AD-associated genetic risk factors.

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Figures and Tables

Figure 1. Loss of normal PSEN2 function delays corneal kindling acquisition in both male and female 6-month-old mice. There was a significant delay in the corneal kindling acquisition rate for both A) male ($F_{(11,682)}$=2.216, p=0.0123); and B) female ($F_{(6,306)}$=3.384, p=0.003) PSEN2 KO mice compared to WT mice. The percentage of C) male ($F_{(23, 23)}$ = 32.50, p<0.0001) and D) female ($F_{(23, 23)}$ = 14.10, p<0.0001) PSEN2 KO mice showed a delay in reaching their fully kindled state when compared to WT mice. The differences in percentage of fully kindled mice between genotypes were apparent by the 13th (males) and 18th stimulation (females). (E-F) Both male (E) and female (F) PSEN2 KO mice required significantly more stimulation sessions to reach kindling criterion (XXX). * Indicates significantly different, p<0.05.

Figure 2. Male and female mice with loss of normal PSEN2 function (PSEN2 KO) aged over 6-months-old underwent corneal kindling or sham kindling to induce a history of chronic seizures before being challenged in the non-habituated open field (OF) test of spatial memory and anxiety-like behaviors. A) Fully kindled male PSEN2 KO and WT mice generally exhibited more total distance travelled when compared to genotype-matched sham controls ($F_{(1, 38)}$=39.52, p<0.0001). There were no post-hoc differences between kindled PSEN2 KO and WT mice. B) There was a significant effect of kindling on total distance travelled for both female PSEN2 KO and WT mice when compared to genotype-matched sham controls ($F_{(1, 35)}$=7.758, p=0.0086). There was also a main effect of genotype ($F_{(1, 35)}$=18.07, p=0.0002) and *post-hoc* tests demonstrated that both sham and kindled female PSEN2 KO mice travelled less total distance when compared to kindling-matched WT controls. C) Analysis of center versus perimeter OF exploration (i.e., thigmotaxis) demonstrated a kindling x genotype interaction, with kindled male mice spending significantly less time in the center of the open field compared to sex-matched sham-kindled mice ($F_{(1, 37)}$=11.24, p<0.002;). D) Analysis of center versus perimeter OF exploration demonstrated a kindling x genotype interaction, with kindled female mice spending significantly less time in the center of the open field compared to sex-matched sham-kindled mice ($F_{(1, 34)}$=11.75, p<0.002). However, this change in OF exploration preference was most measured in female PSEN2 KO kindled mice. * Indicates significantly different from WT, p<0.05; # indicates significantly different from genotype-matched sham kindled group, p<0.05.

Figure 3. Male and female mice with loss of normal PSEN2 function (PSEN2 KO) aged over 6-months-old underwent corneal kindling or sham kindling to induce a history of chronic seizures before being challenged in the T-maze continuous alternation test of spatial working memory. A) Chronic seizures do not worsen the performance of PSEN2 KO male mice in the T-maze continuous alternation task. There was no significant genotype x kindling interaction in the male mice ($F_{(1, 40)} = 0.054$, $p>0.8$). B) Conversely, there was a significant genotype x kindling interaction in female mice ($F_{(1, 36)}=5.52$, $p=0.024$). Female kindled PSEN2 KO mice made significantly more errors than the age-matched kindled female WT mice. # Indicates significantly different from genotype-matched sham kindled group, $p<0.05$.

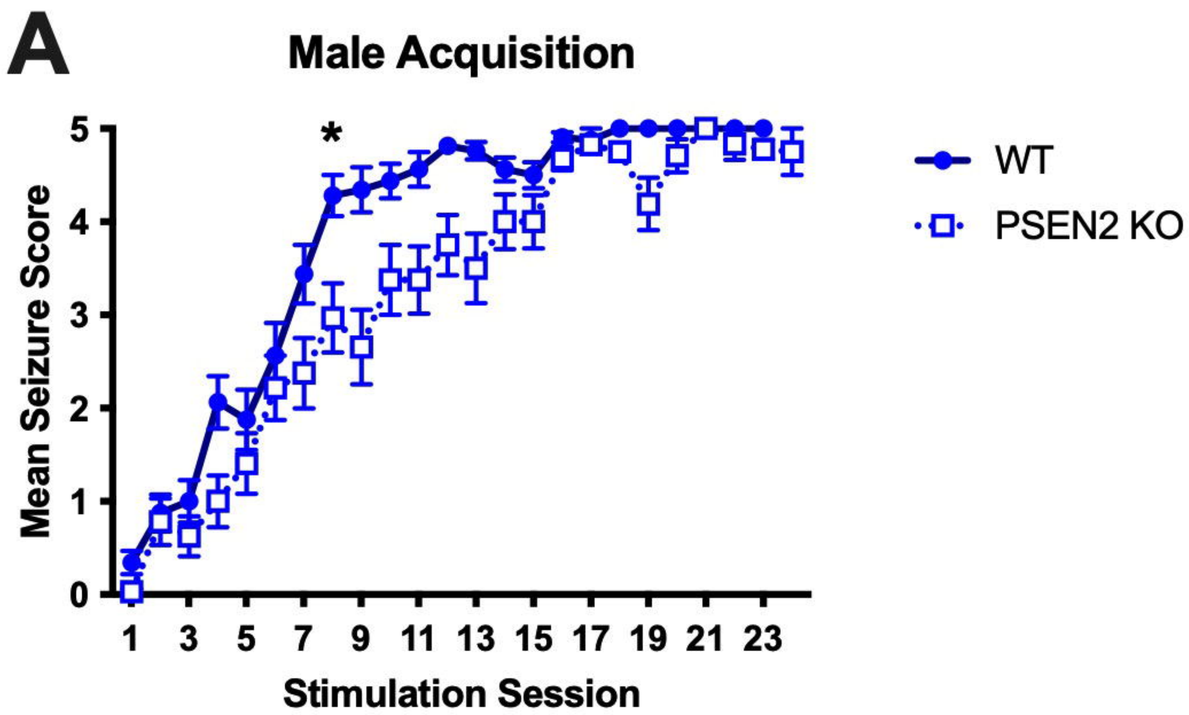
Figure 4. Male and female mice with loss of normal PSEN2 function (PSEN2 KO) aged over 6-months-old underwent corneal kindling or sham kindling to induce a history of chronic seizures before being challenged in the land-based, hippocampally-mediated Barnes maze (BM) test of learning and memory. BM performance was only worsened by chronic kindled seizures in PSEN2 KO female mice. A) Male mice generally demonstrated a time-related reduction in latency to first encounter with the escape chamber ($F_{(4,176)}=21.59$, $p<0.0001$). However, the number of erroneously explored escape holes was generally worsened in males by kindling (Figure 4C; $F_{(3, 44)}=7.15$, $p=0.0005$) but there was no interaction between the kindling groups and number of days on the BM ($F_{(12,176)}=0.56$, $p>0.8$). B) Female mice generally demonstrated a time-related reduction in latency to first encounter with the escape chamber ($F_{(12,148)}=2.142$, $p=0.0175$). *Post-hoc* test demonstrated that sham-kindled WT female mice were significantly slower to initially locate the hidden platform relative to kindled WT female mice ($p=0.0001$) and kindled PSEN2 KO mice were slower than kindled WT female mice ($p=0.0138$), but sham KOs were not different from kindled KOs. D) There was also a significant main effect of kindling in female mice on the number of erroneously explored escape holes (Figure 4D; $F_{(3,37)}=12.92$, $p<0.0001$). E) Kindling was generally associated with worsened performance of male mice on the first testing day, regardless of genotype ($F_{(1,44)}=4.676$, $p=0.0361$) but there was no effect of genotype $F_{(1, 44)} = 0.554$, $p>0.4$). F) On the converse, there was a significant kindling x genotype interaction in female mice ($F_{(1, 36)} = 11.91$, $p=0.0014$), with kindled PSEN2 KO females committing more errors on the first trial day relative to kindled WT females ($p=0.0002$). * indicates significantly different from WT mice, $p<0.05$; # indicates significantly different from genotype-matched mice, $p<0.05$.

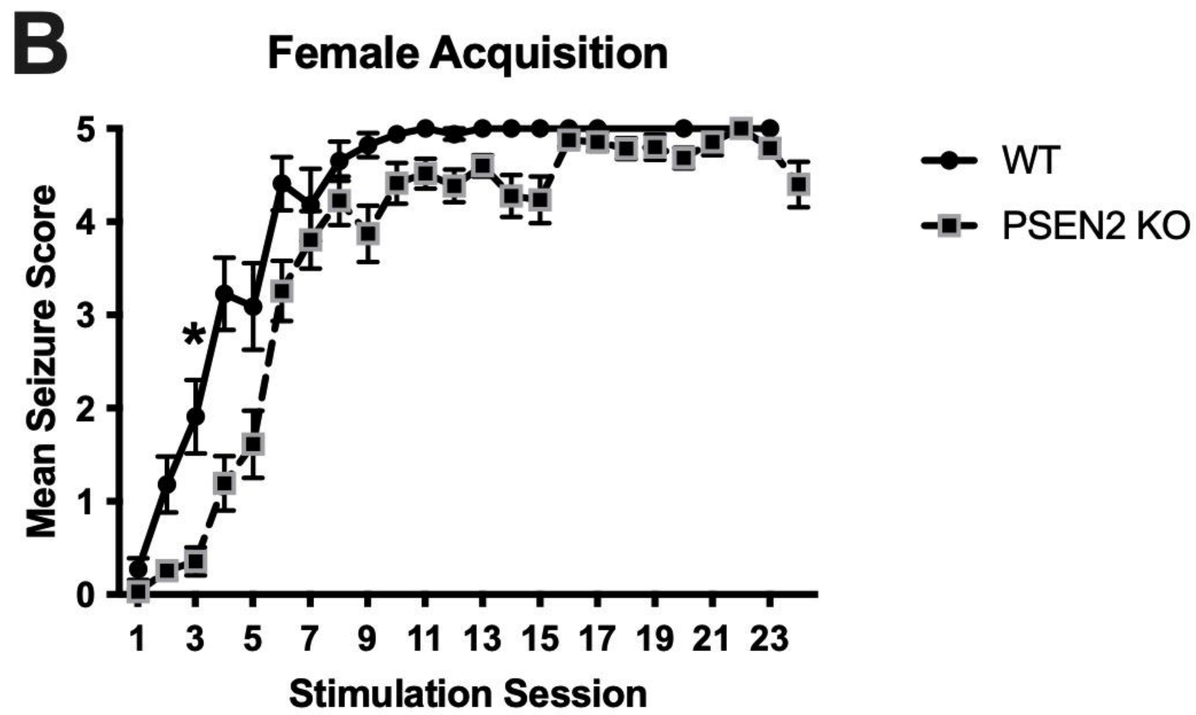
Figure 5. Male and female mice with loss of normal PSEN2 function (PSEN2 KO) aged over 6-months-old underwent corneal kindling or sham kindling to induce a history of chronic seizures before being challenged in a BM learning and memory test and euthanized 1 hour after the final testing day. The expression of brain derived neurotrophic factor (BDNF) was then assessed by immunohistochemistry in dorsal hippocampal structures (CA1, CA3, and Dentate Gyrus (DG)). A) There was a significant kindling x genotype effect on BDNF expression in male mice in area CA1 ($F_{(1,72)}$=4.087, p=0.0469). *Post-hoc* tests revealed significant differences in BDNF expression between sham and kindled WT male mice (p=0.0348) and between kindled WT and kindled PSEN2 KO mice (p=0.0272). B) There was a significant kindling x genotype effect on BDNF expression in male mice in area CA3 of dorsal hippocampus ($F_{(1,72)}$=4.504, p=0.037). C) There were no significant differences in BDNF expression in the DG of kindled and sham kindled male mice of either genotype. D) There was a significant kindling x genotype effect on BDNF expression in female mice in area CA1 ($F_{(1,46)}$=4.607, p=0.038). E) There was a significant main effect of kindling on BDNF expression in female mice in area CA3 ($F_{(1,46)}$=5.716, p=0.021). F) Both kindling and PSEN2 KO genotype influenced BDNF expression in DG (genotype: $F_{(1,46)}$=6.068, p=0.0177; kindling: $F_{(1,46)}$=4.734, p=0.0349), but there was no significant interaction between factors. Further, *post-hoc* tests only revealed significant differences in DG between sham kindled and kindled WT female mice (p=0.0358). G) Sample photomicrographs of BDNF (pink), NeuN (green), and DAPI (blue) from sham kindled WT, corneal kindled WT, sham kindled PSEN2 KO, and corneal kindled PSEN2 KO female mice from areas CA1, CA3, and Dentate Gyrus. Scale bar indicates 100 µm. * Indicates significantly different, p<0.05.

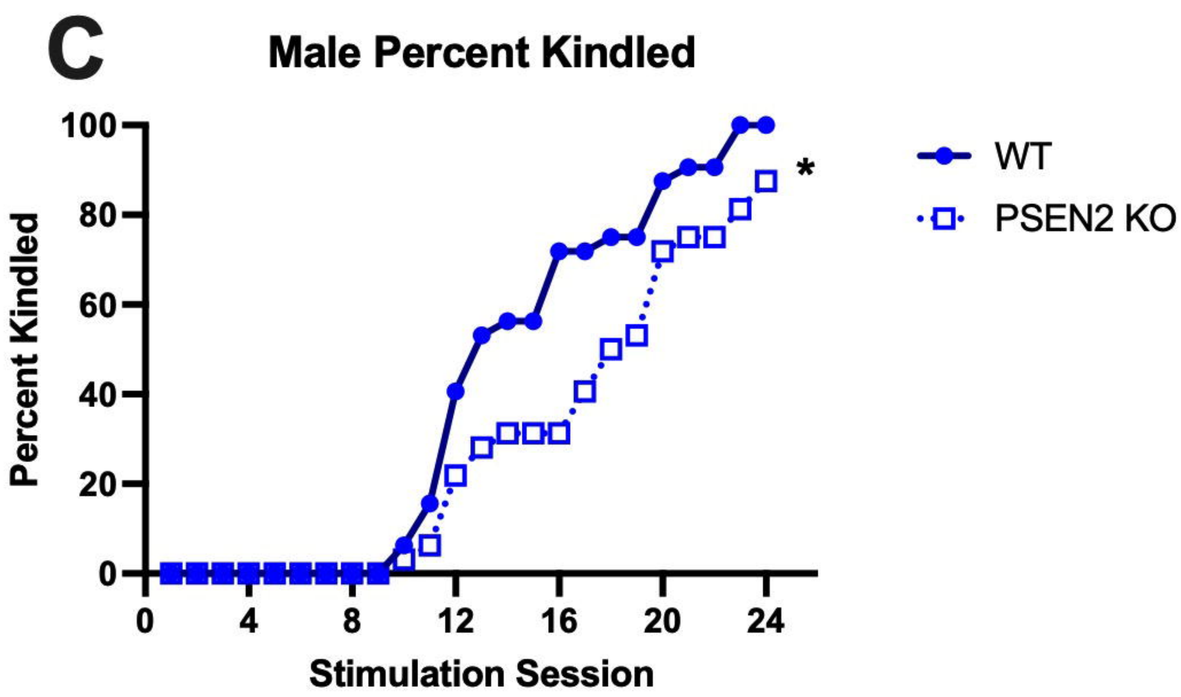
Figure 6. Male and female mice with loss of normal PSEN2 function (PSEN2 KO) aged over 6-months-old underwent corneal kindling or sham kindling to induce a history of chronic seizures before being challenged in a BM learning and memory test and euthanized 1 hour after the final testing day. The extent of reactive gliosis (as measured by GFAP, A-B; E-F) and neuronal density (as measured by NeuN; C-D: G-H) were evaluated in dorsal hippocampal structures (CA1 and Dentate Gyrus (DG)). There were no significant differences in area CA3, thus this region is not presented. A) There were significant kindling-induced increases in GFAP immunoreactivity in CA1 in both WT and PSEN2-KO mice ($F_{(1, 67)}$ = 20.65, p<0.0001). B) There were significant kindling-induced increases in GFAP immunoreactivity in DG in both WT and PSEN2-KO mice ($F_{(1, 68)}$ = 32.23, p<0.0001). C and D) There were no significant differences in NeuN immunoreactivity in CA1 and DG in male mice. E) There were significant kindling-induced increases in GFAP immunoreactivity in CA1 in both WT and PSEN2-KO female mice ($F_{(1, 44)}$ = 7.576, p=0.0086), as well as a main effect of genotype ($F_{(1, 44)}$ = 9.868, p=0.003) on GFAP immunoreactivity in area CA1. *Post-hoc* analysis demonstrated stimulation-induced increases in GFAP immunoreactivity in WT female mice only (p=0.030). F) There was only a main effect of genotype on DG astrogliosis ($F_{(1, 45)}$ = 7.057, p=0.0109; Figure 6F), with only WT female mice exhibiting kindling induced increased GFAP immunoreactivity (p=0.0157). G and H) There were no significant differences in NeuN immunoreactivity in CA1 and DG in female mice. * Indicates significantly different, p<0.05. I) Sample photomicrographs of GFAP (pink), NeuN (green), and DAPI (blue) from sham kindled WT, corneal kindled WT, sham kindled PSEN2 KO, and corneal kindled PSEN2 KO male and female mice from areas CA1 and Dentate Gyrus (DG). Scale bar indicates 100 µm.

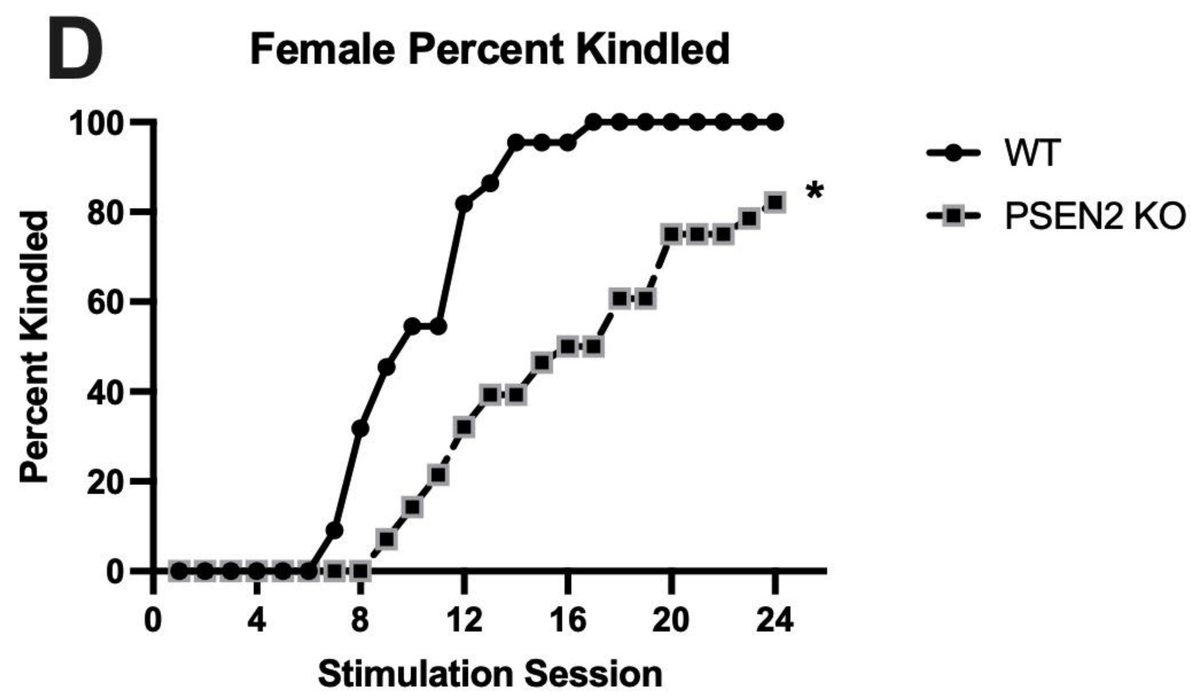
Table 1. Male and female mice with loss of normal PSEN2 function (PSEN2 KO) aged over 6-months-old underwent corneal kindling or sham kindling to induce a history of chronic seizures before being challenged in several behavioral tasks to assess behavioral and functional impacts of chronic kindled seizures. Outcomes on each test are summarized below.

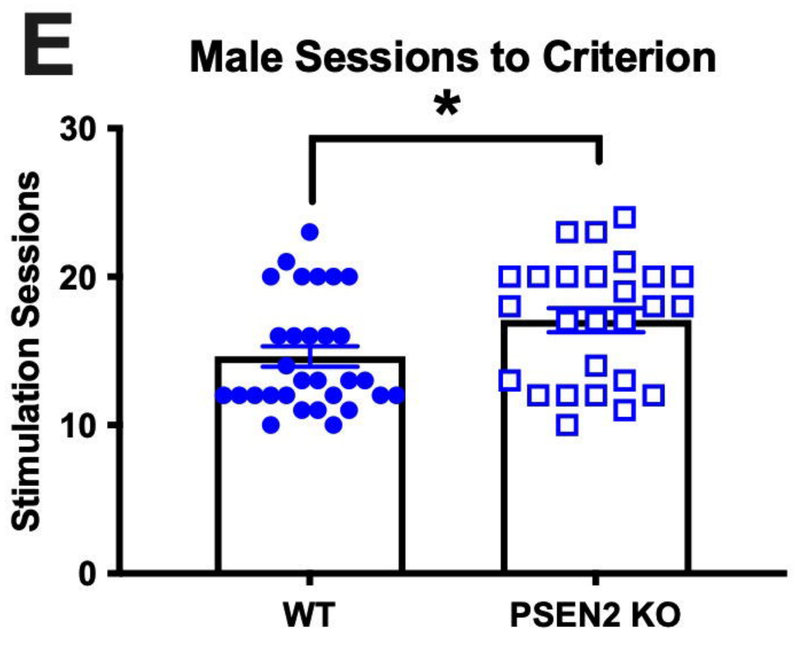
Behavioral Test	**PSEN2 KO Kindled Male vs Sham**	**PSEN2 KO Kindled Female vs Sham**
T-maze continuous alternation (working memory)	No difference	**Increased Response Errors**
Barnes maze (learning and memory)	No difference	**Increased Day 1 Errors**
Open field exploration (working memory)	No difference	**Increased Exploration**
Anxiety-like behavior	**Increased Perimeter-Seeking**	**Increased Perimeter-Seeking**

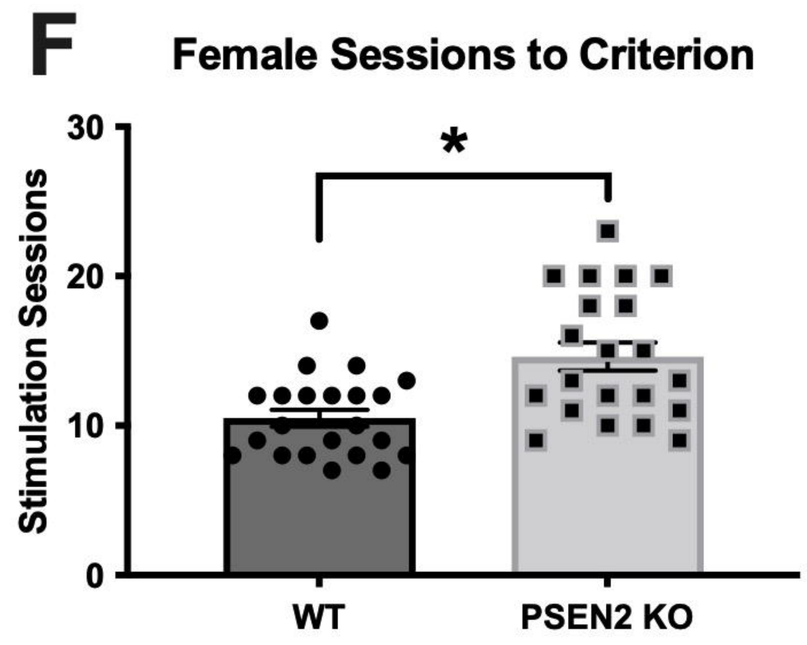

A
Male Acquisition
Mean Seizure Score
Stimulation Session
WT
PSEN2 KO
*

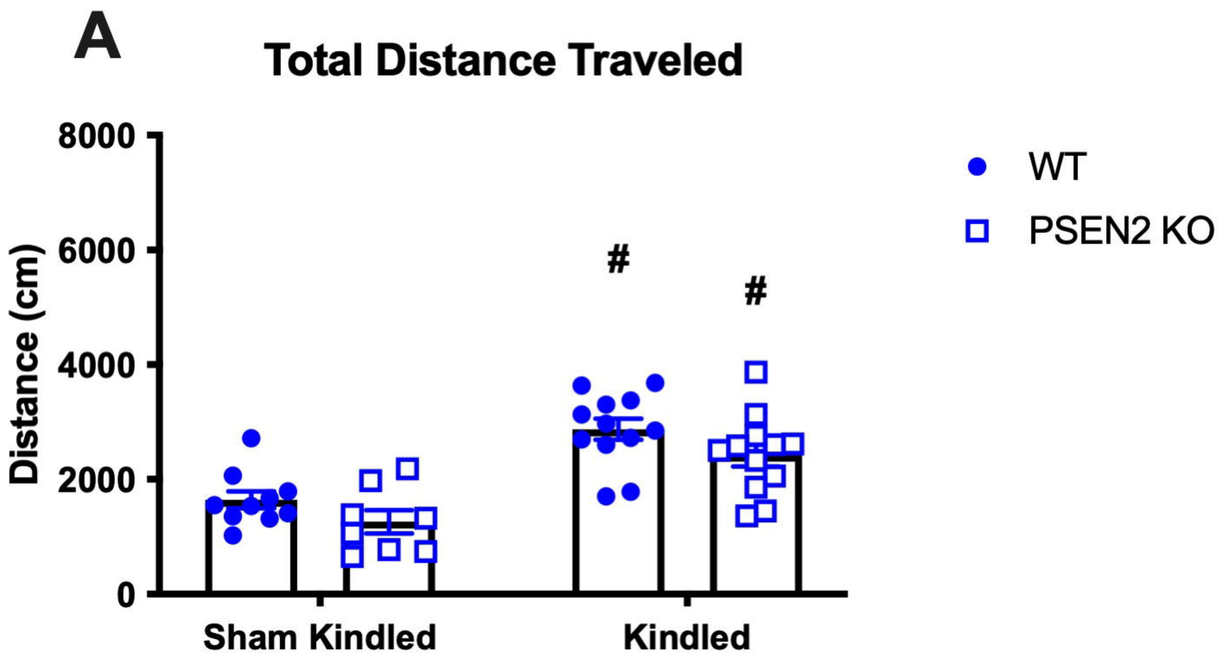

B
Female Acquisition
Mean Seizure Score
Stimulation Session
WT
PSEN2 KO
*

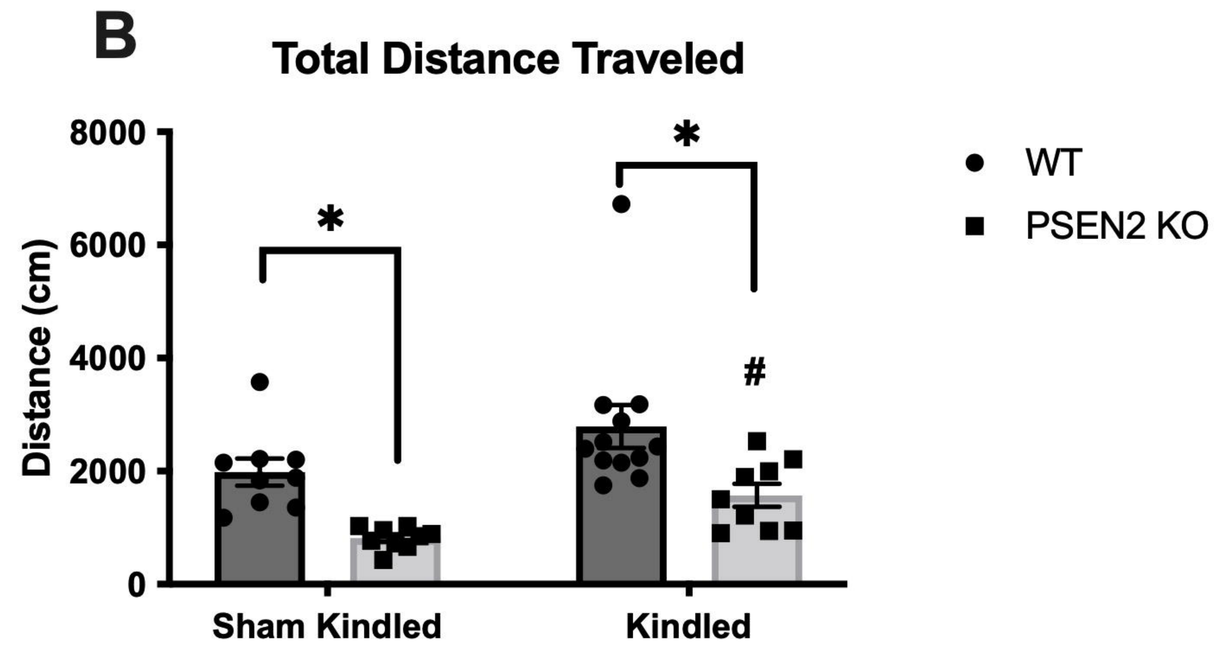

C
Male Percent Kindled
Percent Kindled
Stimulation Session
WT
PSEN2 KO
*

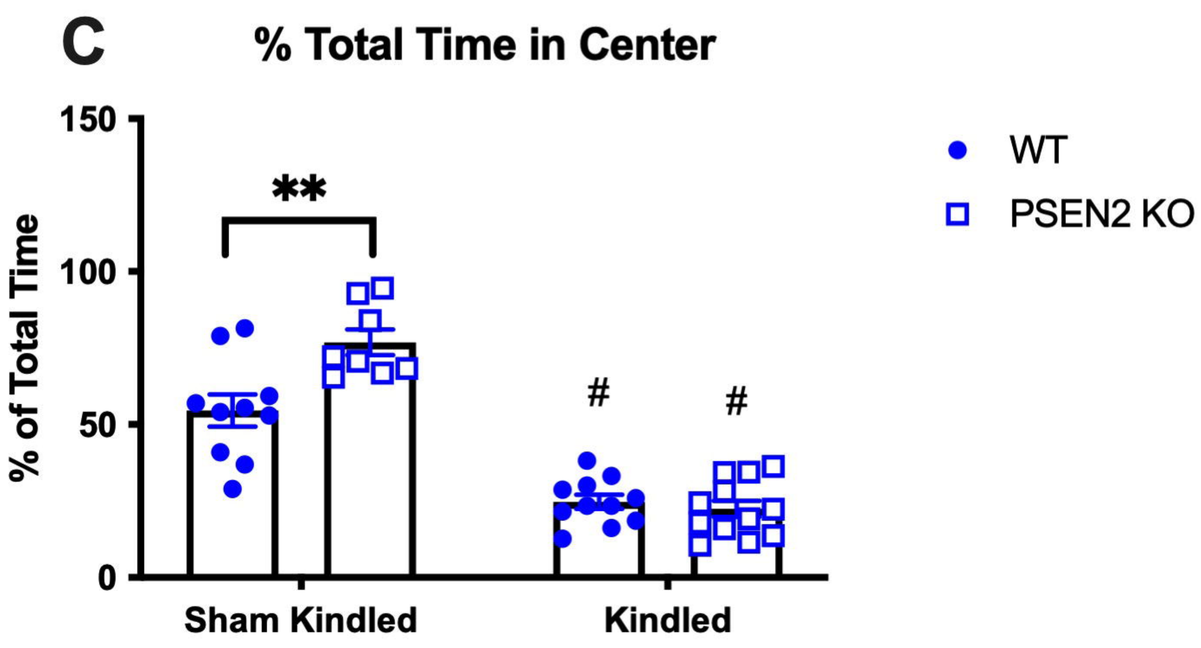

D
Female Percent Kindled
Percent Kindled
Stimulation Session
WT
PSEN2 KO
*

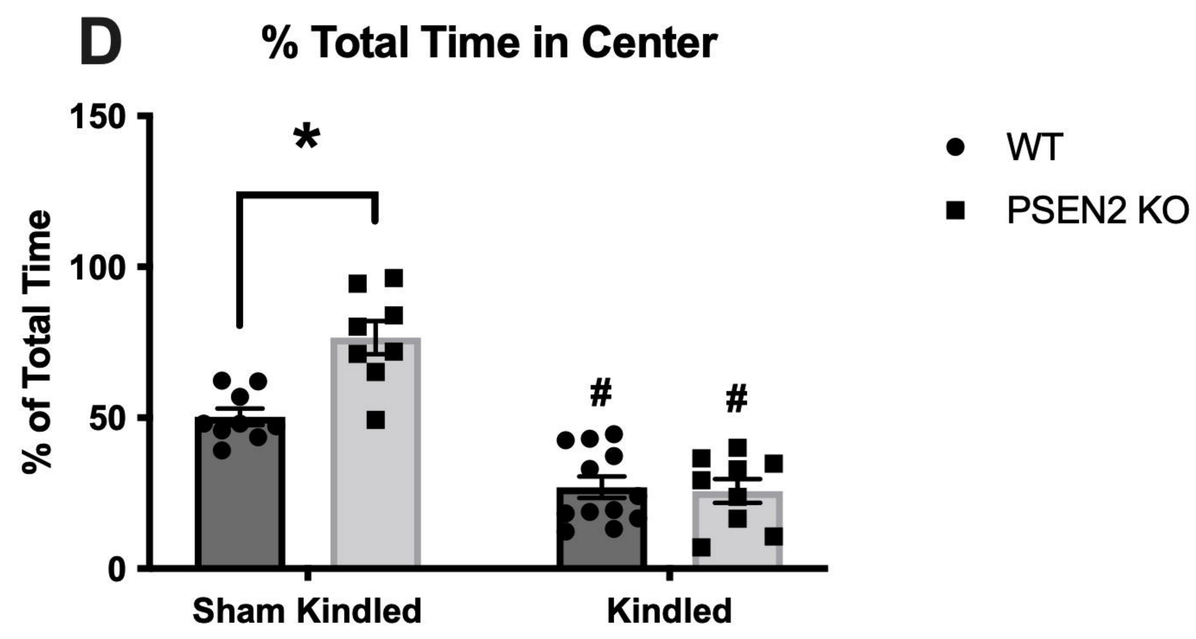

E
Male Sessions to Criterion
Stimulation Sessions
*
WT
PSEN2 KO

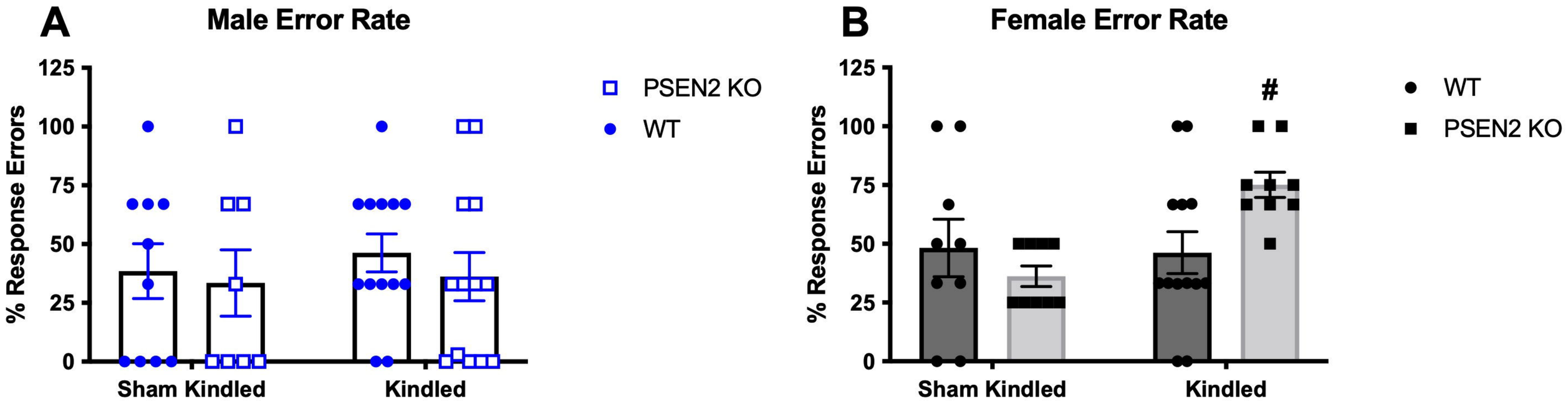

F
Female Sessions to Criterion
Stimulation Sessions
*
WT
PSEN2 KO

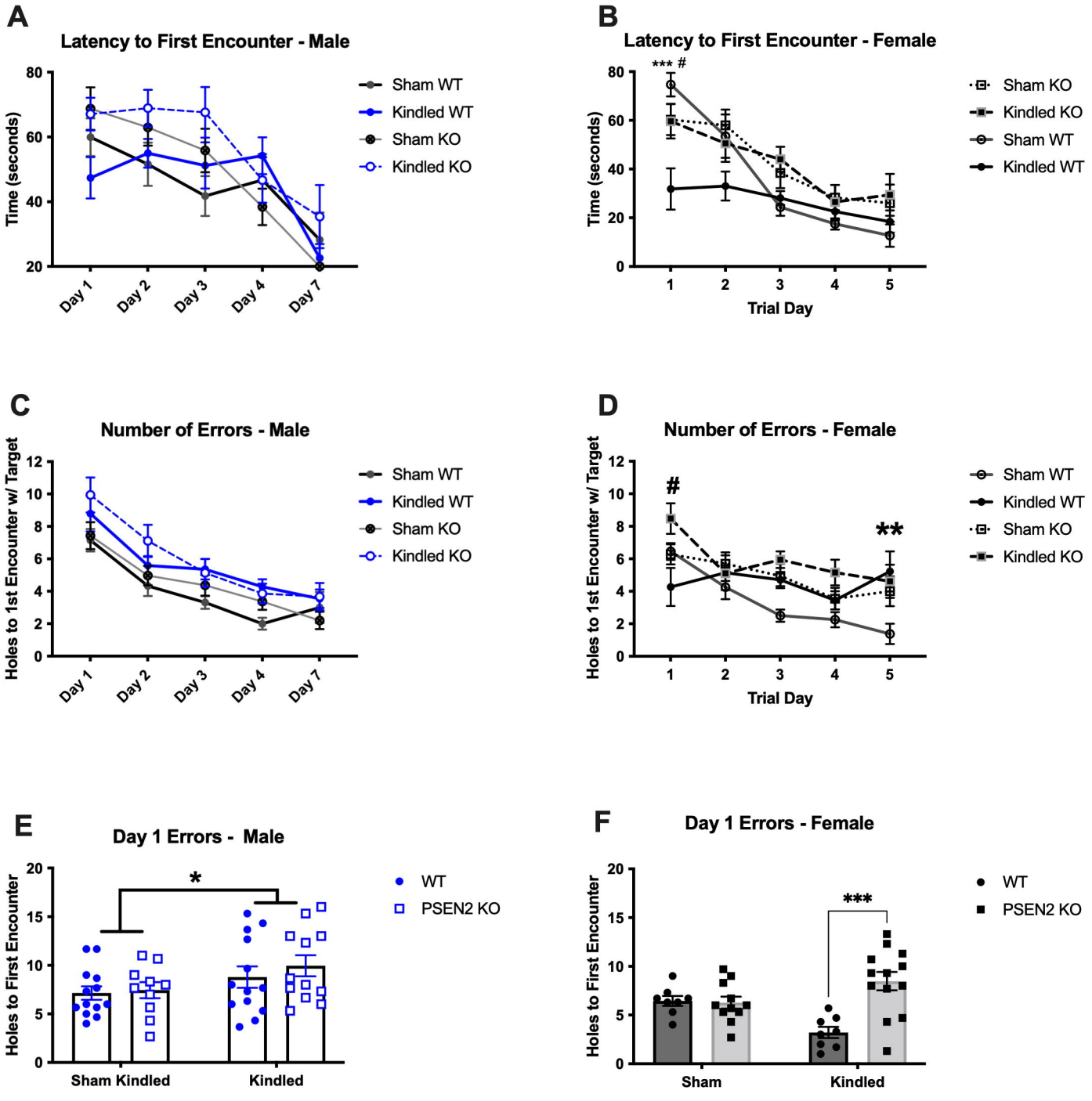

A
Total Distance Traveled
Distance (cm)
8000
6000
4000
2000
0
#
#
Sham Kindled
Kindled
WT
PSEN2 KO

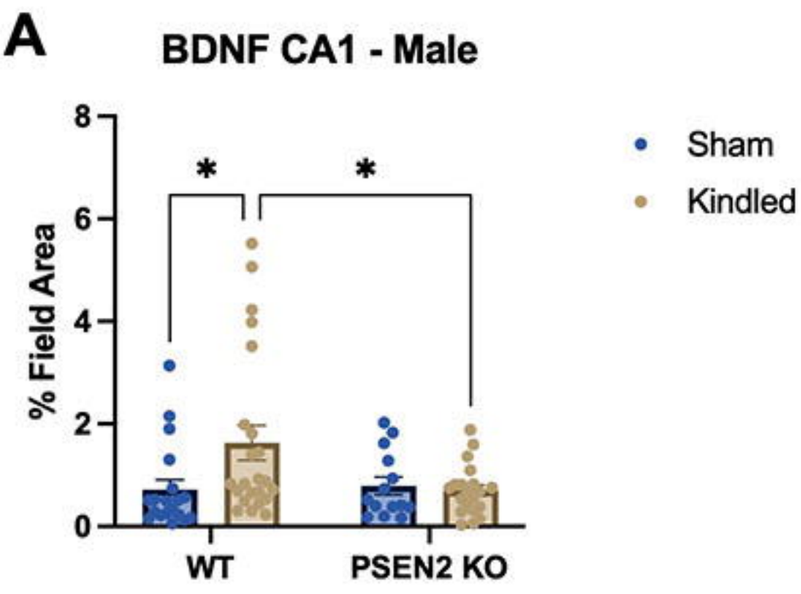

B
Total Distance Traveled
Distance (cm)
8000
6000
4000
2000
0
*
*
#
Sham Kindled
Kindled
WT
PSEN2 KO

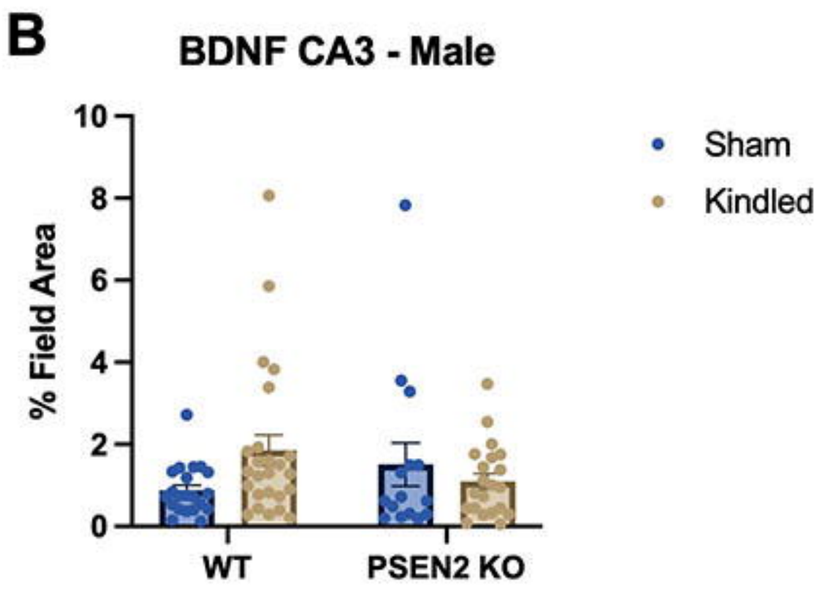

C
% Total Time in Center
% of Total Time
150
100
50
0
**
#
#
Sham Kindled
Kindled
WT
PSEN2 KO

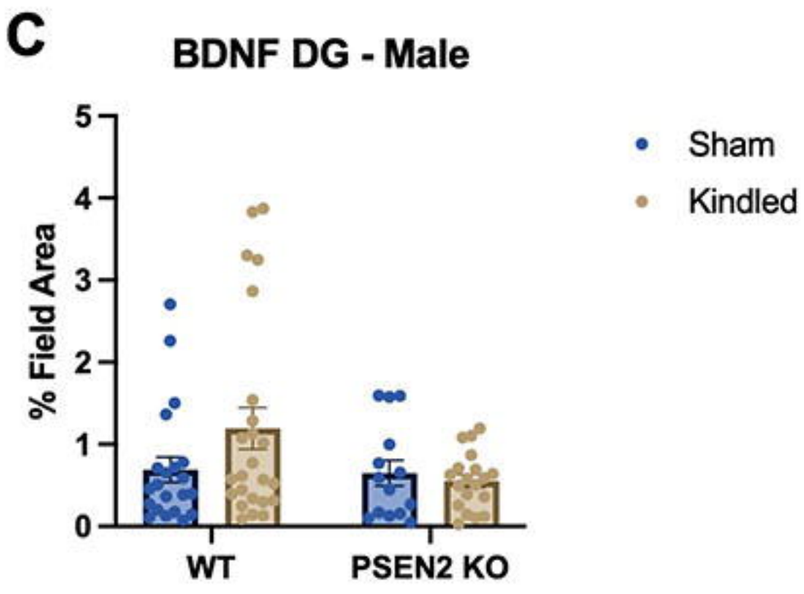

D
% Total Time in Center
% of Total Time
150
100
50
0
*
#
#
Sham Kindled
Kindled
WT
PSEN2 KO

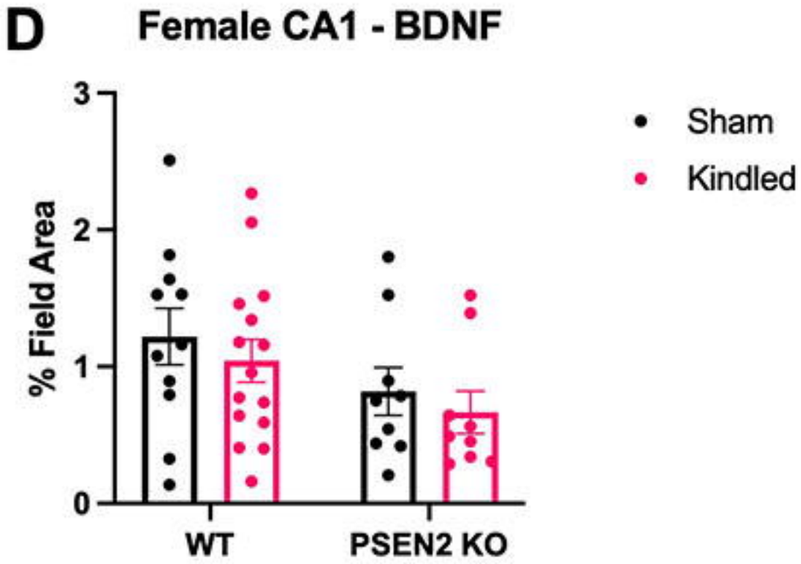

A
Male Error Rate
% Response Errors
125
100
75
50
25
0
Sham Kindled
Kindled
PSEN2 KO
WT
B
Female Error Rate
% Response Errors
125
100
75
50
25
0
#
Sham Kindled
Kindled
WT
PSEN2 KO

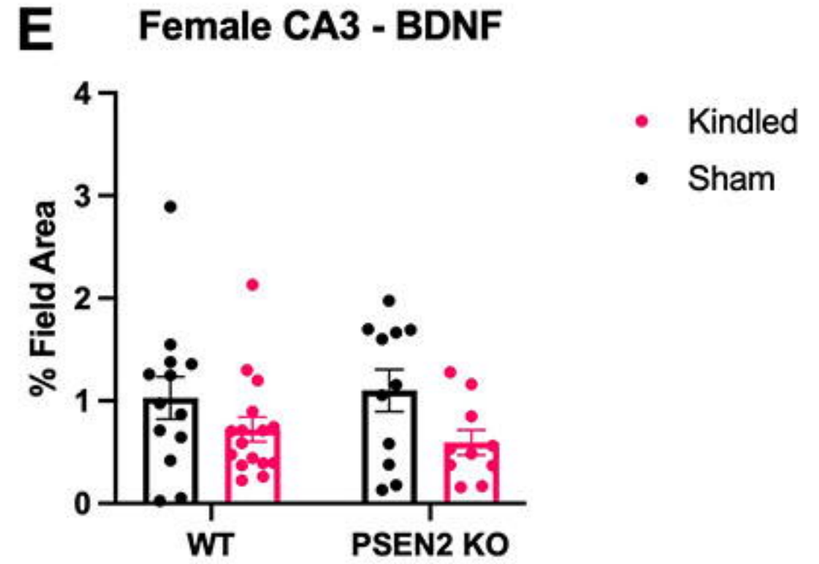

A
Latency to First Encounter - Male
Time (seconds)
80
60
40
20
Day 1
Day 2
Day 3
Day 4
Day 7
Sham WT
Kindled WT
Sham KO
Kindled KO
B
Latency to First Encounter - Female
*** #
Time (seconds)
80
60
40
20
0
1
2
3
4
5
Trial Day
Sham KO
Kindled KO
Sham WT
Kindled WT
C
Number of Errors - Male
Holes to 1st Encounter w/ Target
12
10
8
6
4
2
0
Day 1
Day 2
Day 3
Day 4
Day 7
Sham WT
Kindled WT
Sham KO
Kindled KO
D
Number of Errors - Female
#
**
Holes to 1st Encounter w/ Target
12
10
8
6
4
2
0
1
2
3
4
5
Trial Day
Sham WT
Kindled WT
Sham KO
Kindled KO
E
Day 1 Errors - Male
*
Holes to First Encounter
20
15
10
5
0
Sham Kindled
Kindled
WT
PSEN2 KO
F
Day 1 Errors - Female
***
Holes to First Encounter
20
15
10
5
0
Sham
Kindled
WT
PSEN2 KO

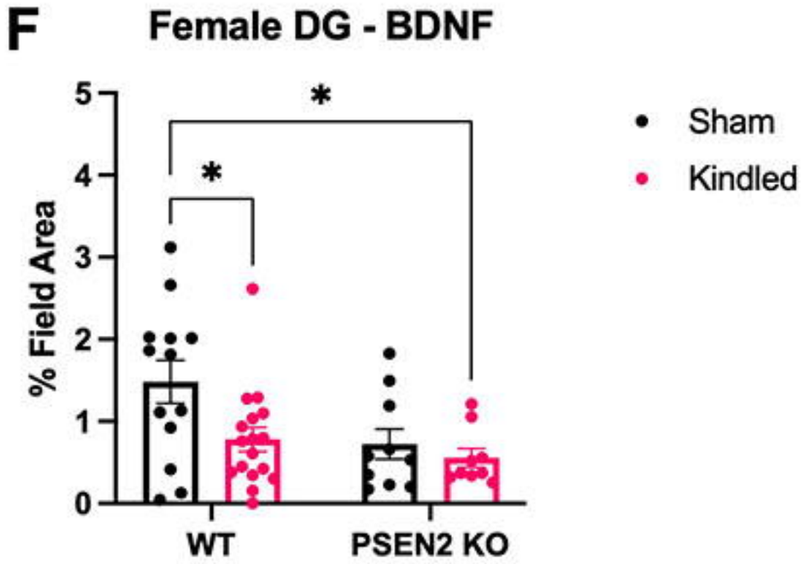

A
BDNF CA1 - Male
% Field Area
0
2
4
6
8
*
*
WT
PSEN2 KO
Sham
Kindled

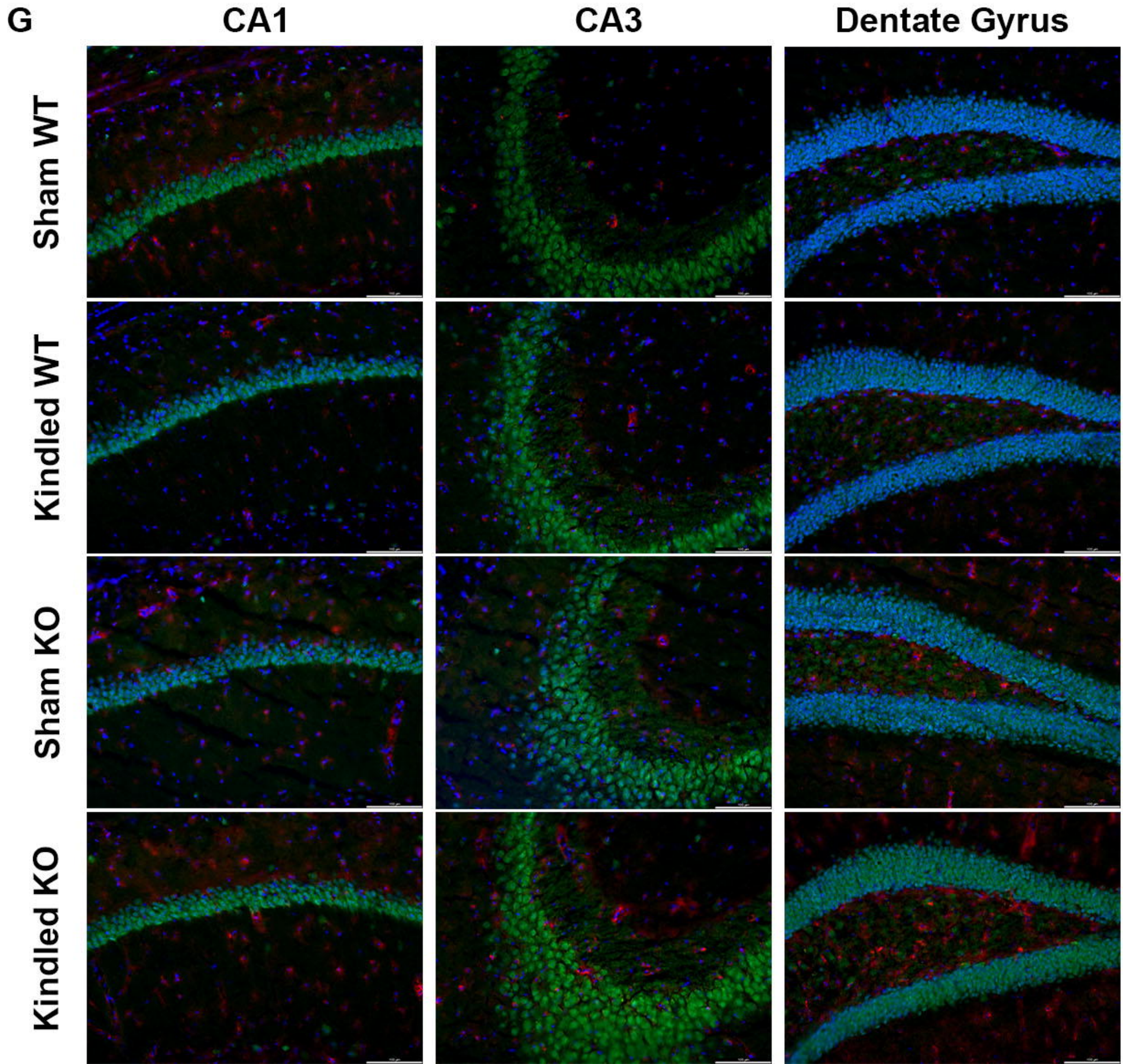

B
BDNF CA3 - Male
% Field Area
0
2
4
6
8
10
WT
PSEN2 KO
Sham
Kindled

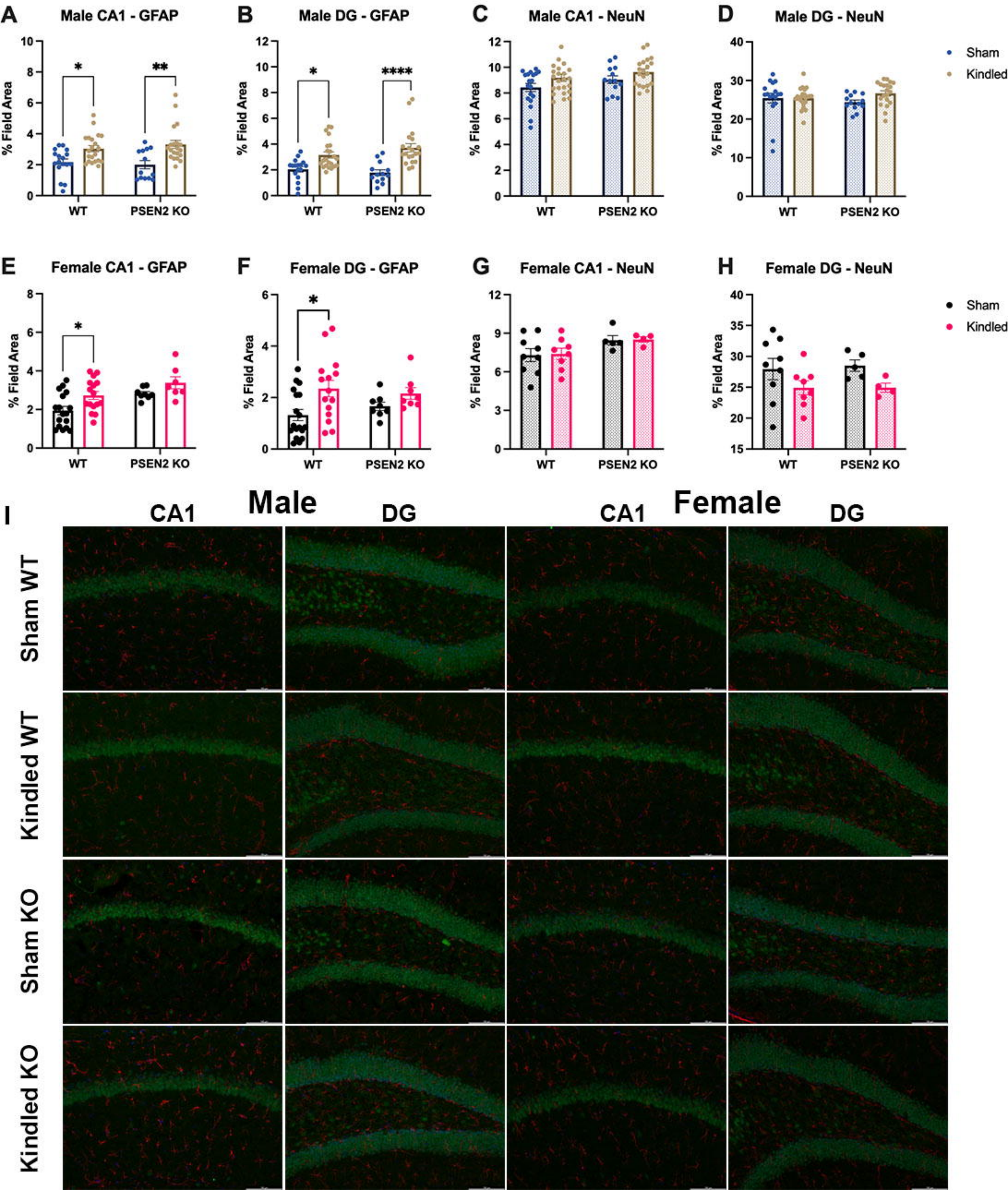

C
BDNF DG - Male
% Field Area
0
1
2
3
4
5
WT
PSEN2 KO
Sham
Kindled


D
Female CA1 - BDNF
% Field Area
0
1
2
3
WT
PSEN2 KO
Sham
Kindled


E
Female CA3 - BDNF
% Field Area
0
1
2
3
4
WT
PSEN2 KO
Kindled
Sham


F
Female DG - BDNF
% Field Area
0
1
2
3
4
5
*
*
WT
PSEN2 KO
Sham
Kindled


G
CA1
CA3
Dentate Gyrus
Sham WT
Kindled WT
Sham KO
Kindled KO


A
Male CA1 - GFAP
B
Male DG - GFAP
C
Male CA1 - NeuN
D
Male DG - NeuN
E
Female CA1 - GFAP
F
Female DG - GFAP
G
Female CA1 - NeuN
H
Female DG - NeuN
% Field Area
WT
PSEN2 KO
Sham
Kindled
I
Male
Female
CA1
DG
Sham WT
Kindled WT
Sham KO
Kindled KO