Title: Loss of presenilin 2 function age-dependently increases susceptibility to kainate-induced acute seizures and blunts hippocampal kainate-type glutamate receptor expression

Authors: Larissa Robinson-Cooper[1,2*], Stephanie Davidson[1*], Rami Koutoubi[1], Kangni Zhang[1], Hannah Park[1], Melissa Barker-Haliski, PhD[1,2†]

*-co-first authors

†-corresponding author. Email: mhaliski@uw.edu

Affiliation:

1- Department of Pharmaceutics, School of Pharmacy, University of Washington
2- Graduate Program in Neuroscience, School of Medicine, University of Washington

Acknowledgements: This work was supported by NIA R01AG067788 (MBH).

Keywords:

Abstract.

Presenilin 2 (PSEN2) gene variants increase the risk of early-onset Alzheimer's disease (AD). AD patients with PSEN2 variants have increased risk of unprovoked seizures versus age-matched healthy controls, yet few studies have interrogated PSEN2 contributions to seizures, and fewer have done so with aging. PSEN2 variant mice also do not exhibit amyloid-β (Aβ) accumulation, allowing for the assessment of Aβ-independent contributions to seizure risk in AD. Critically, PSEN proteolytic capacity may regulate hippocampal kainate-type glutamate receptors (KARs), with PSEN deletion reducing KAR availability and synaptic transmission *in vitro* (Barthet et al 2022). Kainic acid (KA) is a naturally occurring KAR agonist that acutely evokes severe seizures in mice. We thus hypothesized that PSEN2 knockout (KO) mice would have reduced latency to acutely evoked seizures and status epilepticus (SE), increased convulsive SE burden, worsened 7-day survival, and altered hippocampal KAR expression vs age-matched wild-type (WT) mice. Using a repeated low-dose systemic KA administration paradigm, we quantified the latency to acute seizures and convulsive SE, then quantified neuropathology in 3–4-month-old and 12–15-month-old male and female PSEN2 KO versus WT mice. GluK2 and GluK5 KAR subunit expression was colocalized in astrocytes and neurons by immunohistochemistry 7 days after KA-SE or sham-SE to define the interaction between PSEN2 loss and acute seizures on hippocampal KARs. Regardless of sex, young PSEN2 KO mice were more susceptible to KA-induced acute seizures than WTs. Young PSEN2 KO mice of both sexes also entered SE sooner than age-matched WT mice. In aged mice, there was no significant difference in latency to first seizure or SE onset between genotypes in either sex. However, regardless of genotype, aged females entered SE sooner than young females and experienced greater mortality. This was not observed in males. Among young animals, there was no difference in KAR expression between genotypes and regardless of treatment group. In both genotypes, hippocampal CA3 astrocytes expressed GluK5 following KA-SE, however, astrocytic GluK2 expression only occurred in WT mice. GluK5 expression was significantly reduced in untreated aged PSEN2 KO mice versus untreated WT mice, while total GluK2 expression did not differ between genotypes or seizure groups. Following KA-SE, astrocytic GluK5 expression was only present in WT animals in CA3, while both genotypes presented with astrocytic GluK5 expression. This study highlights that KARs are an understudied contributor to seizures in aging and AD that warrant further investigation.

Highlights.

- Alzheimer’s disease (AD) and epilepsy share many pathophysiological links, but the contributions of distinct AD-risk factors are understudied.
- Aging also evokes long-term neurophysiological adaptations without hippocampal damage, which may be further worsened by acute seizures.
- Loss of normal presenilin 2 function is associated with AD; its contributions to seizure risk across the lifespan have been minimally evaluated.
- Loss of presenilin 2 increases susceptibility to kainic-acid induced acute seizures in young adult mice, mirroring susceptibility in late life.
- Kainate receptor expression in older mice is only changed with loss of presenilin 2 function.

1. Introduction

Older adults with Alzheimer's disease (AD) have a high risk of developing comorbid seizures. Seizure incidence is particularly high in those with early-onset AD (EOAD) and AD patients with seizures have worsened functional decline [1, 2]. People over age 65 represent the fastest growing demographic with an epilepsy diagnosis, underscoring an urgent need to better understand the conserved pathological mechanisms driving late life seizure risk. Several mechanistic contributors underlie the shared risk of seizures in older adults and in AD. Both epilepsy and AD are defined by glutamate excitotoxicity [3, 4]. Epilepsy patients have increased levels of hyperphosphorylated tau and amyloid-beta (Aβ) plaques[5]. Recurrent seizures reduce life expectancy and worsen cognition in dementia patients [6-8]. Furthermore, in some mouse AD models, seizures worsen the related hippocampal neuropathology [9]. Thus, AD and epilepsy share many pathological similarities such that studying seizures in AD may beneficially and bi-directionally improve disease outcomes.

AD has long been hypothesized to be associated with pathological Aβ accumulation[10]; accordingly, most investigations have focused on Aβ production. Several risk genes are implicated in autosomal dominant early-onset AD, but variants in Amyloid Precursor Protein (APP), Presenilin 1 (PSEN1), and Presenilin 2 (PSEN2) are the most well-known [11]. PSEN1 or PSEN2 form the catalytic core of the γ-secretase complex, and APP is their most well-studied substrate [12]. APP duplications and PSEN1 variants are the most investigated in seizure studies, while the impacts of PSEN2 variants on seizure susceptibility remain largely underexplored. This deficit is particularly concerning because these AD-associated genetic risk factors equivalently influence seizure risk [11]. Further, clinical PSEN1 variants are associated with AD onset as early as 39 years old, while PSEN2 variants tend to have later AD onset[13]. Some evidence even suggests that PSEN2 variants may be masked in the general late-onset AD population[14], potentially representing an understudied contributor to late-onset AD. As a result of this narrow focus on APP and PSEN1, preclinical seizure and hyperexcitability studies have been largely conducted in APP-overexpressing mouse AD models (e.g. 5xFAD and APP/PS1)[15-17]. These mice exhibit Aβ deposition at an early age, therefore, studies are primarily conducted in younger animals, limiting insight on how advanced age additively affects seizure risk in AD. Few studies have investigated the seizure-specific impact of loss of normal PSEN2 function in aged rodents. We thus aimed to fill this knowledge gap by defining the acute seizure risk in young and aged PSEN2 null mice to further uncover how Aβ-independent factors drive disease progression to better inform late-onset AD treatment.

PSEN2 is a particularly intriguing, understudied driver of AD pathology and seizure susceptibility. PSEN2 is the predominant γ-secretase in microglia and AD-associated variants in PSEN2 are associated with exaggerated microglial reactivity and a primed inflammatory phenotype in both microglia and astrocytes[18-20]. As inflammation is central to seizures in epilepsy [21], this phenotype may similarly influence seizure risk in AD. PSEN2 variant-derived astrocytes present with increased basal GFAP expression, which also occurs with normal

aging[20, 22]. Taken together, this suggests that loss of normal PSEN2 function may accelerate the onset of reactive gliosis. Human PSEN2 variants lead to a loss of normal γ-secretase function, making PSEN2 knockout (KO) mice useful to initially assess how loss of normal PSEN2 function influences seizure risk. PSEN2 KO mice have favorable breeding and longevity[23,24], providing an optimal preclinical platform on which to assess the influences of global PSEN2 deletion in an efficient, long-term manner. We have earlier reported age-related shift in sensitivity of PSEN2 KO mice to corneal kindling, a model of acquired chronic seizures and epileptogenesis[25]. In contrast, young PSEN2 KO mice have decreased electrically-evoked minimal clonic threshold, a model of acute limbic seizures, versus age-matched WT mice. Notably, PSEN2 KO mice also exhibit high-frequency oscillations (HFOs), a biomarker of epilepsy, that matches occurrence in two other amyloidogenic AD models[26]. Thus, loss of normal PSEN2 function may induce age-related changes in acute seizure risk, but disrupt integrity and connectivity of neuronal networks underlying chronic seizures. PSEN2 KO mice thus provide an excellent and untapped opportunity to assess seizures in the context of AD.

The long-term consequences of acute seizures in the aged, AD-associated brain are altogether understudied. Intriguingly, PSENs and APP may regulate expression of hippocampal kainate-type glutamate receptors (KARs) subunits[27]. We thus hypothesized that hippocampal KAR expression may differ in young and aged PSEN2 KO mice versus age-matched wild-type (WT) controls, which may underlie age-related changes in seizure susceptibility with loss of normal PSEN2 function. We thus sought to define the susceptibility of PSEN2 KO mice to the well-established kainic acid (KA)-induced acute seizure and status epilepticus (SE) model. KA is a potent chemoconvulsant that induces acute, generalized seizures through preferential activation of KARs that are expressed primarily in the hippocampus[28]. KA-induced SE evoked in rats is associated with significant effects of age; rats aged ~2 years or older are more sensitive to KA than younger adult rats[29, 30]. Sex differences also occur in rodent KA-SE. Aged female C57BL/6 mice are more vulnerable to KA-induced seizures than both age-matched male and younger female mice and exhibit worsened hippocampal neuronal damage and astrocytic reactivity [31]. Thus, our present study aimed to demonstrate how acute seizure susceptibility changes with age in male and female mice with and without loss of normal PSEN2 function, a known risk factor for EOAD.

2. Methods

2.1 Animals

Male and female PSEN2 KO mice were bred at the University of Washington from stock originally acquired from the Jackson Laboratory (stock #005617) as described[24, 25]. Wild-type (WT) C57BL/6J male and female control mice were purchased from Jackson Laboratory (Stock #000664) at 6-8 weeks-old and housed alongside PSEN2 KO mice until behavioral seizure testing. Mice were housed in a 14:10 light cycle (lights on at 06h00: off at 20h00) in ventilated cages with corncob bedding and food and water provided *ad libitum*, as previously

published[32], 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[33]. All behavioral seizure testing was performed during the hours of 09h00 and 17h00.

2.2 Kainic Acid-induced Acute Seizures and Status Epilepticus

Young (3-4 month old; male WT = 12; female WT = 10; male PSEN2 KO = 16; female PSEN2 KO = 15) mice and aged (12-15 month old; male WT = 10; male PSEN2 KO = 12; female WT = 10; female PSEN2 KO = 11) received KA (Tocris, Cat #0222; discontinued) prepared in saline solution at a concentration of 2 mg/mL (**Figure 1**). To reduce KA-induced mortality, we opted for systemic administration of KA through repeated low doses delivered via intraperitoneal injection [34, 35]. Mice received an initial injection of 10 mg/kg KA via the intraperitoneal (i.p.) route, with subsequent doses of 5 mg/kg every 20 minutes until SE onset (two generalized Racine stage 4+ seizures within 30 minutes), per our published protocol[36]. The dose was reduced to 2.5 mg/kg if a mouse only had a single Racine stage 4+ seizure within the 20-minute dosing window. Following SE onset, additional seizure activity was monitored and recorded for 1-hour, after which mice were given 1 mL of lactated Ringer's solution (subcutaneous; Hospira) and allowed to recover in their home cage for the remainder of the study. Post-SE survival and body weight change from baseline (pre-SE) was recorded 1-day, 3-days, and 7-days post KA-SE. Sham-SE mice (sterile PBS every 20-minutes for 1-hour) were included as control as follows: 3-4 month old (male WT = 10; female WT = 10; male PSEN2 KO = 13; female PSEN2 KO = 12) mice and 12-15 month old (male WT = 9; male PSEN2 KO = 13; female WT = 9; female PSEN2 KO = 12). A total of 68 young mice and 51 aged mice were sacrificed 7-days post KA-SE, with brains thus collected for histology.

2.3 Euthanasia

7-days after KA-SE or sham SE, all surviving mice were euthanized by live decapitation and brains collected into 4% PFA (CAT: PF101, FD NeuroTechnologies) for 24 hours. Brains were then transferred to 30% sucrose in PBS for 24-72 hours before being flash-frozen in 2-methylbutane on dry ice and stored at -80°C until sectioning on a Leica CM1850 or CM1860 cryostat into 30-µm thick coronal slices from Bregma -1.70 to -210 to capture the dorsal hippocampus for subsequent immunofluorescence. Two rostral and two caudal sections/mouse were mounted on a Superfrost slide (Fisher Scientific) and stored at -80°C until immunofluorescent processing.

2.4 Immunofluorescence

Cryosectioned slides were washed with 1X PBS 3 x 5 minutes before antigen retrieval. Briefly, 1X Antigen Retrieval Buffer (ab93678, Abcam) was heated to 95°C and slides were immersed in this heated buffer in the incubator for 20-minutes, then removed from the incubator and

allowed to come to room temperature in the buffer for 40 minutes (60 minutes in buffer in total). Slides were then washed in dH_2O 3 x 2 minutes before being incubated in 10% Goat Serum in 5% Triton-X PBS solution under coverwells in a humid chamber for 2 hours. Primary antibodies (NeuN (1:500; MAB377, MilliporeSigma), GFAP (1:500; MAB3402X, MilliporeSigma), GluK2 (1:300; ab66440, Abcam), GluK5 (1:500; ab67408, Abcam), and Iba-1 (1:500; 019-19741, Wako)) were applied under 200 µL coverwells in a 5% Goat Serum in 1X PBS solution overnight at 4°C, with an extra 2 hour room temperature incubation for GluK5 and GluK2. The following day, coverwells were removed and slides washed in 1X PBS 3 x 10 minutes. Secondary antibodies (1:500; Goat anti-Mouse IgG Alexa Fluor 488, ab150113, Abcam; 1:500; Goat anti-Rabbit IgG Alexa Fluor 555, ab150078, Abcam) were applied under 200 µL coverwells in 1X PBS for 2 hours at room temperature then washed in 1X PBS 3 x 10 minutes. Slides were then coverslipped with Prolong Gold with DAPI (P36935, ThermoFisher). Pictomicrographs were captured with a fluorescent microscope (Leica DM-6) with a 20x objective (80x final magnification). Acquisition settings were held constant throughout. AIVIA (Leica) cell classifier tool was used in combination with AVIA cell count tool to detect GFAP and Iba-1 expression. Signal thresholding was set using Fiji/ImageJ. A colocalization plugin was used to determine total colocalized area between GluK and GFAP expression (Credit: Pierre Bourdoncle, Institut Jacques Monod, Service Imagerie, Paris, France).

2.5 Statistical Analysis.

Behavioral seizures were assessed using a Log-rank (Mantel Cox) test for latency to first stage 4/5 seizure and SE onset, and using t-tests for behavioral seizure duration and amount of KA administered in young and aged mice. Acute survival post-SE insult was assessed using a Fisher's exact test. Body weight change after KA administration and SE onset was assessed using a two-way ANOVA, with Tukey's post-hoc tests. Immunofluorescence studies were assessed using two-way ANOVA followed by the Fisher's least square difference post-hoc test, using GraphPad Prism, v10.0 or later (GraphPad Software, San Diego, CA, USA). Statistical significance was defined as $p < 0.05$ for all tests. The sample size for histological analysis in the different experimental groups was always ≥6 (mixed sex), but for behavioral studies the minimum group size was always ≥10/sex because of known differences in seizure latency between males and females.

3. Results

Young PSEN2 KO mice are more susceptible to KA-induced acute seizures than age-matched wild-type mice

We first assessed the susceptibility of both WT and PSEN2 KO mice aged 3-4 months old to both KA-induced acute seizures and SE (**Figure 1A**). Young adult PSEN2 KO mice were significantly more susceptible to KA-induced seizures. Male WT mice took an average of 87.6

± 15.5 minutes to have their first acute stage 4/5 seizure, while male PSEN2 KO mice took an average of 61.8 ± 23.5 minutes (**Figure 1B**). Similarly, female WT mice took an average of 102 ± 16.9 minutes to have their first acute stage 4/5 seizure while female PSEN2 KO mice took an average of 61.3 ± 24.5 minutes (**Figure 1E**). While the latency to KA-induced SE did not achieve statistical difference as assessed by a Log-rank test (X^2 = 3.14, p = 0.08), the overall average time for male PSEN2 KO mice to enter SE was significantly sooner than male WT mice: 78.6 ± 25.3 minutes vs. 98.2 ± 16.0 minutes, respectively (p = 0.027; **Figure 1C**). Similarly, female PSEN2 KO mice had an average SE onset at 80.2 ±26.4 minutes, significantly sooner than their WT counterparts, which entered SE at an average of 112 ± 15.1 minutes following the first KA dose (p = 0.0001; **Figure 1F**). Further highlighting their increased sensitivity to KA, both male and female PSEN2 KO mice received significantly less KA than their matched WT counterparts (**Figure 1D and G**). Additionally, male PSEN2 KO had a significantly greater mortality than male WT mice (**Figure 2A**), whereas genotype did not influence female mouse mortality (**Figure 2C**). Notably, we found that 3-4 month-old WT females were significantly more susceptible to KA-induced mortality than matched WT males (**Supplemental figure 4A**). An acute reduction in body weight immediately following KA-SE, which we observed here, is typical of this model and genotype did not influence post-SE body weight change during the 7-day monitoring period (**Figure 2B and D**).

Loss of PSEN2 function does not alter aged mouse susceptibility to KA-induced acute seizures and SE

A cohort of mice aged 12-15 months were next tested to further explore age-related differences in acute seizure susceptibility with PSEN2 loss and to address how advanced age impacts seizure susceptibility. We found that aged PSEN2 KO and WT mice had a similar latency to both first stage 4/5 seizure and SE in both sexes (**Figure 3A-D**). Additionally, when comparing KA-induced acute seizure susceptibility between ages, we found that aged female mice of both genotypes had decreased latency to first stage 4/5 seizure versus their younger counterparts (**Figure 4**). Younger female WT mice took an average of 102 ± 16.9 minutes to have their first stage 4/5 seizure, while their aged counterparts took an average of 44.7 ± 14.8 minutes (**Figure 4C**) Similarly, aged female WT mice had faster latency to SE onset versus young WT females (112 ± 15.1 vs. 54.7 ± 14.1; **Figure 4G**). Aged PSEN2 KO female mice also took less time to have their first stage 4/5 seizure (61.3 ± 24.5 vs. 43.0 ± 15.9; **Figure 4D**) and less time to enter SE compared to young PSEN2 KO females (80.2 ±26.4 vs 53. 5 ± 13.7; **Figure 4H**). However, no differences were observed between age groups among male WT or PSEN2 KO mice (**Figure 4**). Thus, advanced age appears to significantly increase seizure susceptibility only in female mice regardless of genotype. Additionally, mortality in aged female mice of both genotypes was remarkably high (**Figure 3H**). While there were no genotype-related differences in mortality for either sex, both aged WT and PSEN2 KO females had increased mortality compared to their younger counterparts (**Figure 4K and L**). Additionally,

male WT, but not PSEN2 KO mice experienced increased age-related mortality (**Figure 4I and J**).

Hippocampal KAR expression does not differ between young adult WT and PSEN2 KO mice

PSENs regulate KAR expression[27], thus we hypothesized that a disruption in hippocampal KAR expression may underlie the increased susceptibility to KA we observed in 3-4 month-old PSEN2 KO mice. Brains of mice that survived to 7 days-post KA-SE and an additional cohort of sham animals that received saline instead of KA were examined by immunofluorescence. We quantified expression of two KAR subunits - GluK2 and GluK5 - due to their high hippocampal expression and known role in the initiation of KA-induced seizures in rodents[28, 37]. Surprisingly, we found no differences in total basal or post-SE expression of either subunit between WT and PSEN2 KO mice in the CA3 (**Figure 5B and C**). This was also the case in CA1 and DG regions (**See Supplemental Figure 5B&C and 6B&C**). As KA-SE is known to induce astrogliosis and microglial reactivity[38], and human variants in PSEN2 have been shown to alter microglial reactivity[39], we also examined potential differences in glial reactivity between genotypes. Both WT and PSEN2 KO mice showed an increase in Iba-1 expression, a marker of microglial reactivity, following KA-SE (**Figure 5H**). Additionally, GFAP expression, a marker of reactive astrogliosis, was increased for both genotypes following KA-SE (**Figure 5E**). This was consistent in both CA1 and DG (**See Supplemental Figures 5H&E and 6H&E**).

KA-SE in adult rats is also known to induce KAR expression on astrocytes[40]. While the function of astrocytic KARs remains unknown, it has been hypothesized that they may act as a glutamate sensor for astrocytes and play a role in their reaction to excessive glutamate at the synapse[40]. We were interested in determining if astrocytes also express KARs following KA-SE in mice and if loss of normal PSEN2 function had any effect on their expression. Therefore, we performed a colocalization analysis to assess astrocytic KAR expression (**See Supplemental Figure 5 for details**). KA-SE induced an increase in Gluk5 colocalized with GFAP in both WT and PSEN2 KO mice in the hippocampal CA3 region, however only WT animals had a significant increase in colocalized area of GluK2 with GFAP following KA-SE (**Figure 5F and G**). Conversely, only WT mice had increased astrocytic GluK2 in CA1 and DG after KA-SE, an effect not observed with PSEN2 KO mice. KA-SE only led to an increase in GluK5 colocalization with GFAP in the DG in PSEN2 KO mice, but not in WT mice (**See Supplemental Figure 6F&G and 7F&G**). While both WT and PSEN2 KO demonstrated astrocytic KAR expression in hippocampal structures following KA-SE, loss of normal PSEN2 function clearly influenced the post-SE changes in this response in young animals.

As KA-SE can lead to neuronal cell death[38], we also examined expression of NeuN, a marker of mature neuronal cell bodies[41]. Interestingly, we found that only WT mice had an increase in NeuN expression following KA-SE (**Figure 5D**), and that this increase was present in CA3, CA1, and DG regions of the hippocampus (**See Supplemental Figure 6D and 7D**). Thus, loss

of normal PSEN2 function disrupts the KA-induced increase in NeuN expression 7-days after an SE insult.

Loss of PSEN2 reduced hippocampal GluK5 expression in older animals

Despite no genotype-related difference in KA-induced acute seizure susceptibility in older mice, we observed reduced baseline GluK5 expression in aged PSEN2 KO mice versus matched WTs (**Figure 6C**). Baseline GluK5 expression was similarly reduced in the CA1 region of PSEN2 KO mice compared to their WT counterparts (**See Supplemental Figure 8C**). No differences in total GluK2 expression were observed between genotypes (**Figure 6B**). In the CA3 and CA1 regions, both WT and PSEN2 KO mice had increased astrocytic GluK2 expression 7-days after KA-SE (**Figure 7F and Supplemental Figure 8F**). However, astrocytic GluK2 expression was only increased in the DG region of PSEN2 KO mice (**Supplemental Figure 9F**). Astrocytic GluK5 expression increased following KA-SE in WT mice only in the CA3 region, while in CA1 only PSEN2 KO mice had increased astrocytic GluK5 expression following KA-SE (**Figure 7F and Supplemental Figure 8F**). Additionally, in the CA1 region, PSEN2 KO mice had reduced baseline astrocytic GluK5 expression relative to WT mice. While KA-SE increased GFAP and Iba-1 expression at 7 days post-insult for both genotypes in all three hippocampal regions, in the CA3 and DG, PSEN2 KO mice had increased baseline GFAP expression compared to WT mice (**Figure 6E, 6J and Supplemental Figure 8E and 9E**). Interestingly, KA-SE only induced NeuN expression in PSEN2 KO mice in the CA3 region (**Figure 6D**), an effect absent in aged WT mice.

4. Discussion

This current study significantly extends and expands our earlier work to assess the age-related seizure susceptibility associated with loss of normal PSEN2 function[24, 25]. It carries several important findings to better understand the intersection between seizures and AD in the context of PSEN2 deficiency. Most importantly, this study suggests that loss of normal PSEN2 function accelerates senescence, promoting greater seizure risk. First, we demonstrate that young adult mice lacking PSEN2 have increased KA-SE susceptibility. PSEN2 KO mice had both reduced latency to their first generalized tonic-clonic seizure and SE compared to age-matched WT mice. These data align well with our previous findings that 2–4-month-old PSEN2 KO mice have a reduced minimal clonic threshold, a model of acute forebrain focal seizures, compared to age-matched WT mice[25]. Secondly, this increased susceptibility to KA-induced acute seizures and SE disappears in aged mice, as mice aged 12-15 months had similar KA-SE susceptibility regardless of genotype. These data also align with our previous findings, as both WT and PSEN2 KO mice aged 8-months-old had a similar minimal clonic threshold. Taken together, these present data show that young adult PSEN2 KO mice are more susceptible to acute seizures versus age-matched WT mice. However, we have demonstrated previously that young adult PSEN2 KO mice seem to be resistant to formation of a hyperexcitable neuronal network via corneal kindling[24, 25]. This resistance to corneal kindling is

also age-dependent, as older PSEN2 KO and WT mice have a similar rate of kindling acquisition, suggesting that PSEN2 function influences neuronal hyperexcitability in an age-dependent manner. Additionally, here we reported that aged female mice are more susceptible to KA than young adult female mice, regardless of genotype. Another study looking into sex differences in the KA-SE model reported no differences in seizure susceptibility or mortality between males and females (2-3 months). However, this same study also found that gonadectomized mice were more susceptible to generalized seizures and SE compared to same-sex control mice. This effect as well as seizure-induced mortality was also significantly increased in gonadectomized males[42]. Our present study also found a dramatic increase in mortality among aged females of both genotypes, pointing to a potential synergistic effect of age and sex on susceptibility to KA-SE. These differences in KA-SE susceptibility across age groups in the WT female mice may be due to changes in the expression of PSENs during normal aging. Previous studies have shown that PSEN1 expression decreases in the brains of aged mice whereas PSEN2 expression increases[43]. Further studies will need to be done to determine how changes in PSEN1 expression due to loss of normal PSEN2 function impact seizure susceptibility.

Our study demonstrates that both age and AD genotype can influence KAR subunit expression differences. Administration of KA depolarizes hippocampal pyramidal cells via activation of KARs resulting in acute seizures[44, 45]. There are five distinct KAR subunits, GluK1-5, all of which are expressed in the mouse hippocampus[46]. When KA is delivered systemically, it enters the brain via passive diffusion through the blood-brain barrier resulting in low concentrations[47]. GluK5 has a high affinity for KA and thus is preferentially activated at lower concentrations[28]. Additionally, GluK5 requires dimerization with GluK2 to incorporate into the cell membrane meaning KARs containing dimerized GluK2/GluK5 subunits likely have the largest impact on the epileptogenic action of KA administered systemically. GluK2 KO mice are extremely resistant to KA-induced seizures and GluK2 KO cells exhibit a secondary loss of GluK5 [48, 49]. While KARs typically act in an ionotropic manner, GluK5 subunits may mediate inhibition of post-depolarization hyperpolarizing current through metabotropic mechanisms[50]. Mossy fiber KARs containing GluK2 and GluK3 act as autoreceptors that facilitate synaptic transmission and long and short-term plasticity[51, 52]. While KARs are known to play a role in epilepsy, how KARs regulate hippocampal neuronal activity with advanced age is still less clear. Importantly, PSEN and APP are known to regulate levels of KARs in the hippocampus [27]. Thus, our study had the secondary objective of determining how advanced age and loss of normal PSEN2 function influenced KAR expression prior to and after an acutely evoked seizure insult to define whether these receptors may influence subsequent epileptogenesis. We did not observe any morphological changes in young adult mice that could help explain the differences we see in KA-SE susceptibility across genotypes. Nonetheless, GluK5 expression was markedly lower in multiple regions of the hippocampus exclusively in aged PSEN2 KO mice. Barthet et al previously demonstrated that loss of both PSEN1 and PSEN2 in animals aged 5 to 8 weeks results in a decrease in hippocampal GluK2 expression, which is accompanied by decreased

KAR-EPSCs that may contribute to cognitive decline. Additionally, they found that GluK2 interacts with APP, suggesting that PSENs may regulate KAR expression through their cleavage of APP[27]. Taken together, these data show that PSEN2 may become more important for the regulation of KARs in aging and late life.

Astrocytes express KARs after both the seizure-free latent period and later during the onset of spontaneous seizures in the rat post-SE model[40], but the expression of KARs in mouse AD models has thus far not been robustly assessed. Here, we report that both WT C57BL6/J and PSEN2 KO mice also exhibit KAR colocalization on astrocytes 7 days after KA-induced SE. We observed astrocytic KAR expression in both WT and PSEN2 KO mice, however, the extent of expression was age-, genotype-, and hippocampal brain region-dependent. As this is only the second study to describe astrocytic KARs and these receptors do not appear to be expressed under basal conditions, very little is known about their function in health and disease. Vargas et al. hypothesized that these astrocytic KARs could either aid astrocytes in protecting neurons in excitotoxic conditions or further contribute to hyperexcitability through release of their own gliotransmitters due to increases in intracellular Ca^{2+} via activation of astrocytic glutamate receptors[40, 53-57]. Additionally, KA-SE is known to induce reactive astrogliosis that is highly linked with epilepsy [58]. While we did not observe a difference in astrogliosis between genotypes in the young adult animals, older PSEN2 KO mice presented with exaggerated reactive astrogliosis at baseline compared to WT mice. Previous studies have found that PSEN2 variant-derived astrocytes express more GFAP than WT controls and both PSEN2 variant-derived astrocytes and microglia show exaggerated secretion of pro-inflammatory cytokines[20]. Thus, loss of normal PSEN2 function may induce a ‘primed’, pro-inflammatory state. This primed state may act as a “first hit” that could further drive AD pathology when challenged by a secondary insult[59]. Future histological studies must thus further investigate how KA-induced acute seizures influence pathological hallmarks of AD, such as Aβ and p-tau.

While KA-SE is known to evoke neuronal death, this is highly dependent upon the model organism[38]. C57BL/6 mice, as presently utilized, has been shown to be resistant to KA-induced neurodegeneration[60]. In line with this, we did not observe any neurodegeneration following KA-SE in this study as assessed by Fluoro-Jade C staining (data not shown). In fact, we observed a consistent increase in NeuN expression across multiple regions of the hippocampus in young adult WT mice and aged PSEN2 KO mice. Neurogenesis following KA-SE has been reported previously in rat models and contributes to formation of an epileptogenic network[61, 62]. Interestingly, this increase in NeuN was not observed in young adult PSEN2 KO mice. Furthermore, among aged animals, PSEN2 KO mice showed an increase in NeuN expression following KA-SE, whereas WT mice did not. If this increase in NeuN expression is in fact due to the formation of aberrant connections that contribute to epileptogenesis, this may explain why we observed a resistance to corneal kindling among young but not aged PSEN2 KO mice in our group’s previous studies[24, 25].

Overall, we demonstrate that loss of normal PSEN2 function may influence increased seizure susceptibility by promoting an accelerated senescence phenotype in the adult brain. Loss of PSEN2 increases susceptibility to KA-induced seizures early in life, whereas both PSEN2 KO and WT animals present with similar KA susceptibility later in life. We also show that loss of PSEN2 later in life leads to exaggerated astrogliosis, which has previously been associated with aging, further supporting this hypothesis. While the differences we observed in seizure susceptibility could not be explained by differences in hippocampal KAR expression, loss of GluK5 expression in older PSEN2 KO mice may reflect an increased reliance on PSEN2 to regulate KAR expression with advanced age. We also observed an increase in NeuN expression following KA-SE only in WT animals, which could be indicative of differences in post-seizure neurogenesis. While this could help explain the resistance to epileptogenesis previously reported in PSEN2 KO mice, further studies will accordingly determine if this is truly due to neurogenesis.

5. **Figures and Tables**.

Figure 1.

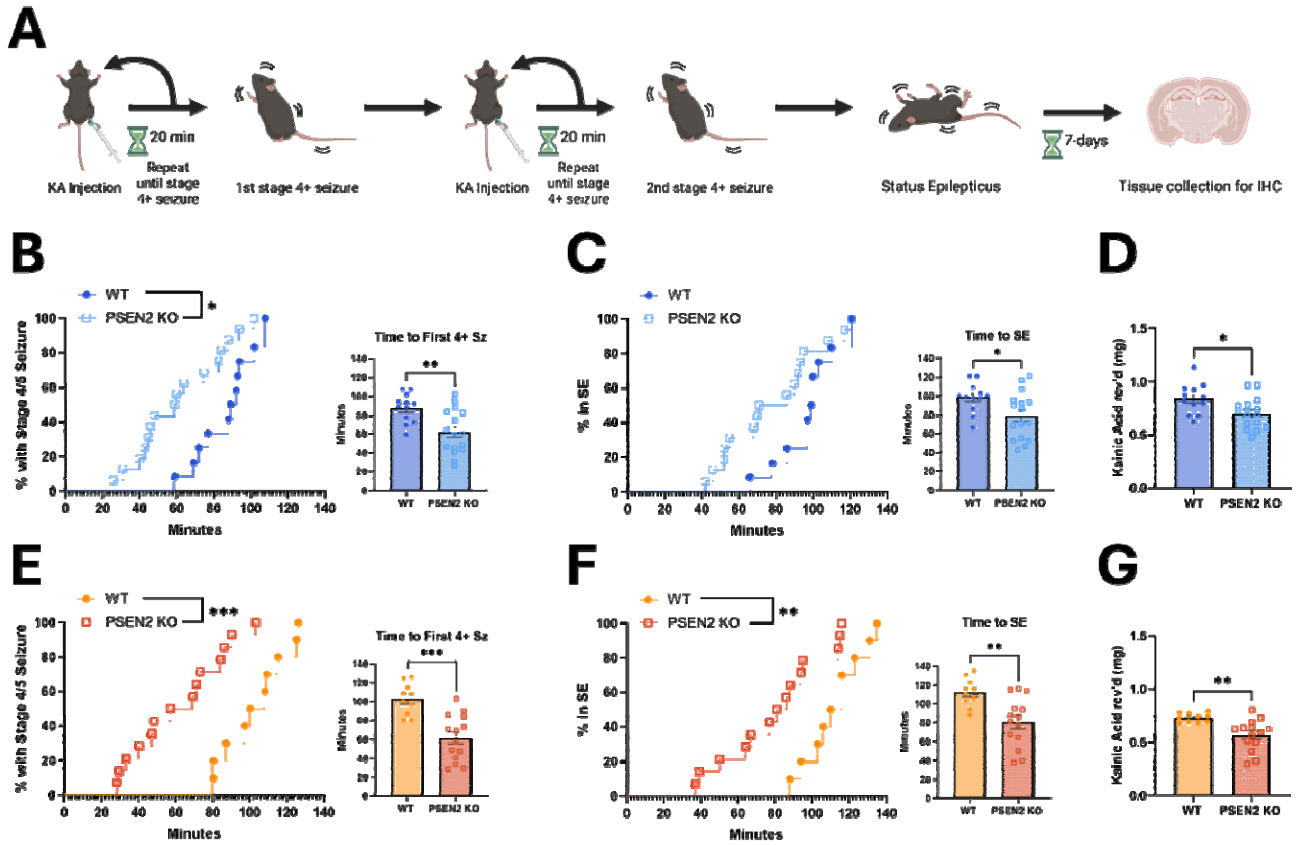

Figure 1. **Loss of normal PSEN2 function leads to significantly reduced latency to KA-induced acute seizures and status epilepticus in both male and female young mice relative to age-matched C57BL/6J mice.** A) Mice were given an initial dose of 10mg/kg KA followed by subsequent doses at 5mg/kg every 20 minutes until presentation of a stage 4 (bilateral forelimb clonus) or higher seizure on a modified Racine scale. After the first stage 4 or higher seizure, the dose was reduced to 2.5mg/kg. Once a mouse has had two stage 4 or higher seizures within a 30-minute window they were considered to be in status epilepticus. 7 days later the mice were sacrificed, and their brains were collected for histology. B) The latency to first Racine stage 4/5 seizure following repeated low-dose KA administration is significantly decreased in PSEN2 KO male mice aged 3-4 months old, as measured by a Log-rank (Mantel Cox) test $X^2 = 6.41$, p=0.011 and unpaired t-test p = 0.003. The WT mean latency to first acute Racine stage 4/5 seizure was 87.58 ± 15.52 minutes versus that of PSEN2 KO, which had a mean latency of 61.75 ± 23.46 minutes. C) The latency to KA-induced SE onset (as defined by 2 consecutive Racine stage 4/5 seizures within 30 min) was not significantly increased in young male PSEN2 KO mice relative to age-matched WT C57BL/6J mice as measured by a Log-rank (Mantel Cox) test $X^2 = 3.14$, p = 0.08. The WT male mean latency to

SE onset was 98.17 ± 15.97 minutes versus the PSEN2 KO male mean latency to SE onset of 78.62 ± 25.29 minutes. However, the average latency in each genotype of young male mice did significantly differ by unpaired t-test p = 0.027. D) Young male PSEN2 KO mice received significantly less KA to evoke an acute seizure and SE onset than age-matched WT mice, suggesting that increased seizure susceptibility was not a result of more KA administered during the study period, as measured by an unpaired t-test p value = 0.02. WT mean 0.84 ± 0.15 mg; PSEN2 KO mean 0.70 ± 0.14 mg E) The latency to first stage 4/5 seizure in young PSEN2 KO female mice was significantly reduced relative to age-matched WT female mice, as assessed by a Log-rank (Mantel Cox) test X^2 = 13.01, p = 0.0003, and an unpaired t-test p = 0.0001. The WT female mean latency to first Racine stage 4/5 seizure was 102 ± 16.92 minutes versus the latency of female PSEN2 KO mice, which was 61.29 ± 24.47 minutes. F) The latency to KA-induced SE onset in young female PSEN2 KO mice was significantly reduced versus that of WT age-matched female mice, as assessed by a Log-rank (Mantel Cox) test X^2 = 8.47, p = 0.0036 and unpaired t-test p = 0.0024. The female WT mean latency to SE onset was 112.2 ± 15.14 minutes versus the latency of PSEN2 KO of 80.21 ±26.40 minutes. G) Young female PSEN2 KO mice received significantly less KA to induce an acute stage 4/5 seizure and SE onset than age-matched WT females, as assessed by an unpaired t-test p value = 0.003, indicating that KA dose was not the cause of greater seizure susceptibility. WT mean 0.73 ± 0.043 mg; PSEN2 KO mean 0.57 ± 0.15 mg

Figure 2.

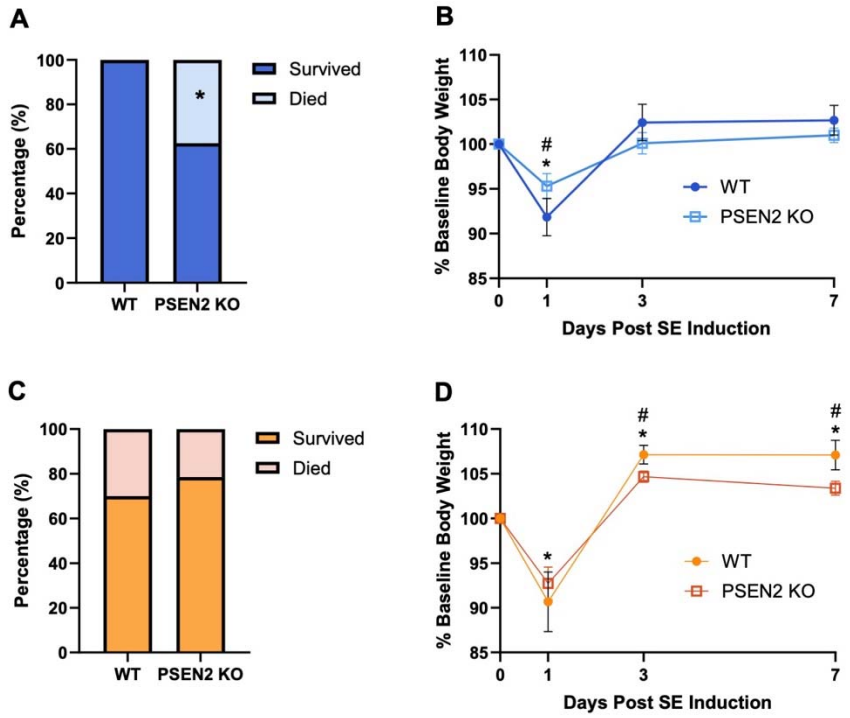

Figure 2. Following a KA-induced acute seizure and SE insult, young male and female PSEN2 KO mice showed significant sex-related differences in 24-hour survival and body weight change versus WT mice. A) Young PSEN2 KO male mice were significantly more likely to die following the KA SE insult than age-matched WT mice, as measured by a Log-rank (Mantel-Cox) test (X^2 = 5.52, p value = 0.019) and Fisher's exact test (p value = 0.0237). B) Immediately following the acute SE insult (1 day post-SE induction), young male WT and PSEN2 KO lost significantly more body weight relative to their pre-SE body weight (day 0 vs day 1 WT p value = 0.0112, PSEN2 KO p value = 0.0346), but there no significant changes in BW over the following 7-day monitoring period, as measured by a 2-way ANOVA. C) Young PSEN2 KO female mice were at no greater risk of KA SE-induced mortality than age-matched WT female mice, but all females were more sensitive to KA SE-induced mortality than young WT males, as measured by a Log-rank (Mantel-Cox) test X^2 = 0.22, p value = 0.64 and Fisher's exact test (p value = 0.67). D) Following an acute KA SE insult, young female mice were tracked for acute BW changes for 7 days. There was a significant change in BW over time, as assessed by 2- way ANOVA but no effect of genotype. Tukey's multiple comparison test – day 0 vs day 1 WT p value ns, PSEN2 KO p value = 0.0124

Figure 3.

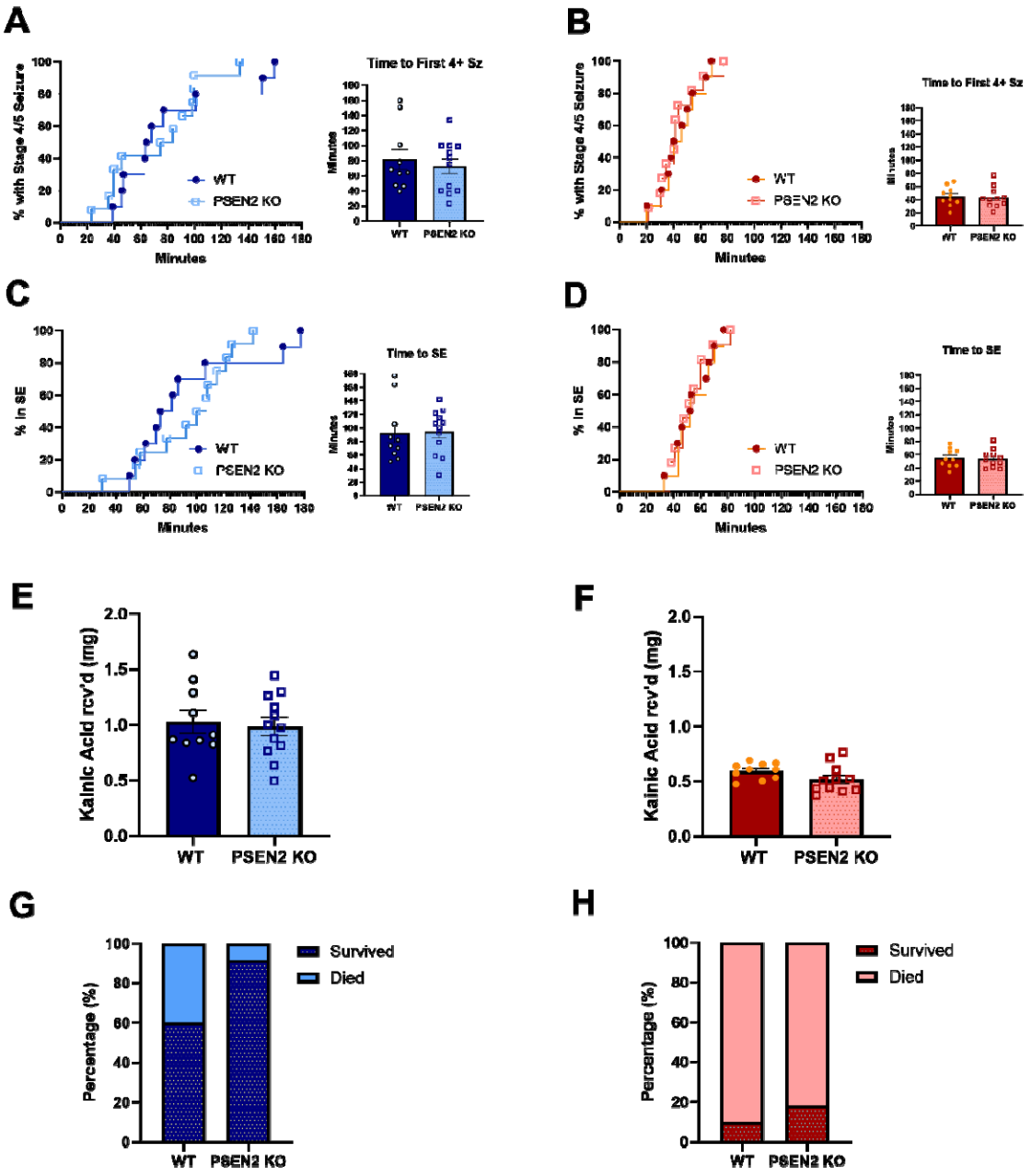

Figure 3. Loss of normal PSEN2 function does not adversely affect latency to first seizure or status epilepticus onset relative to age-matched WT mice, suggesting that early life decreases in seizure susceptibility in this strain may represent an accelerated aging phenotype. A) The latency to first stg 4/5 seizure in aged male PSEN2 KO does not differ from that of aged WT male mice, as measured by a Log-rank (Mantel Cox) test $X^2 = 0.66$, p value > 0.4, bar graph unpaired t-test p value > 0.5. WT mean latency to first stage 4/5 seizure onset was 81.6 ± 42.8 minutes versus that of PSEN2 KO, which had a mean latency of 72.3 ± 34.5 minutes. B) The

latency of aged female mice to first stage 4/5 seizure did not differ, as measured by a Log-rank (Mantel Cox) test $X^2 = 0.017$, p value > 0.9, and bar graph unpaired t-test p value > 0.8. The latency to first stage 4/5 seizure in WT female mice was 44.7 ± 14.8 minutes versus the latency of PSEN2 KO mean 43.0 ± 15.9 minutes. C) The latency to SE onset in aged male mice did not differ between genotypes, as measured by a Log-rank (Mantel Cox) test $X^2 < 0.01$, p value > 0.9, and bar graph unpaired t-test p value = 0.9. The WT mean latency to SE onset was 92.40 ± 44.34 minutes versus the latency of male PSEN2 KO of 94.4 ± 33.2 minutes. D) The latency to KA-induced SE onset in aged female mice did not differ between genotypes, as measured by a Log-rank (Mantel Cox) test $X^2 < 0.01$, p value > 0.9, and bar graph unpaired t-test p value > 0.8. The aged WT female mean latency to SE onset was 54.7 ± 14.0 Minutes versus the PSEN2 KO mean of 53.5 ± 13.7 minutes. E) The amount of KA received by both aged male WT and PSEN2 KO mice did not differ, as measured by unpaired t-test p value > 0.7. The aged WT mice received mean 1.02 ± 0.34 mg of KA whereas PSEN2 KO mice received a mean of 0.98 ± 0.29 mg of KA to induce an SE insult. F) The amount of KA received by both aged female WT and PSEN2 KO mice did not differ, as measured by an unpaired t-test p value > 0.1. The aged WT female mice received 0.60 ± 0.07 mg whereas the PSEN2 KO mice received 0.52 ± 0.13 mg. G) There was no significant difference between aged male mice genotypes in the 24-hour mortality following the acute KA SE insult, as measured by Log-rank (Mantel-Cox) test $X^2 = 3.15$, p = 0.065 and Fisher's exact test (p value = 0.1353). H) There was no significant difference between aged female mice genotypes in the 24-hour mortality following the acute KA SE insult, as measured by Log-rank (Mantel-Cox) test $X^2 = 1.38$, p value > 0.2401 and Fisher's exact test (p value>0.99).

Figure 4 -

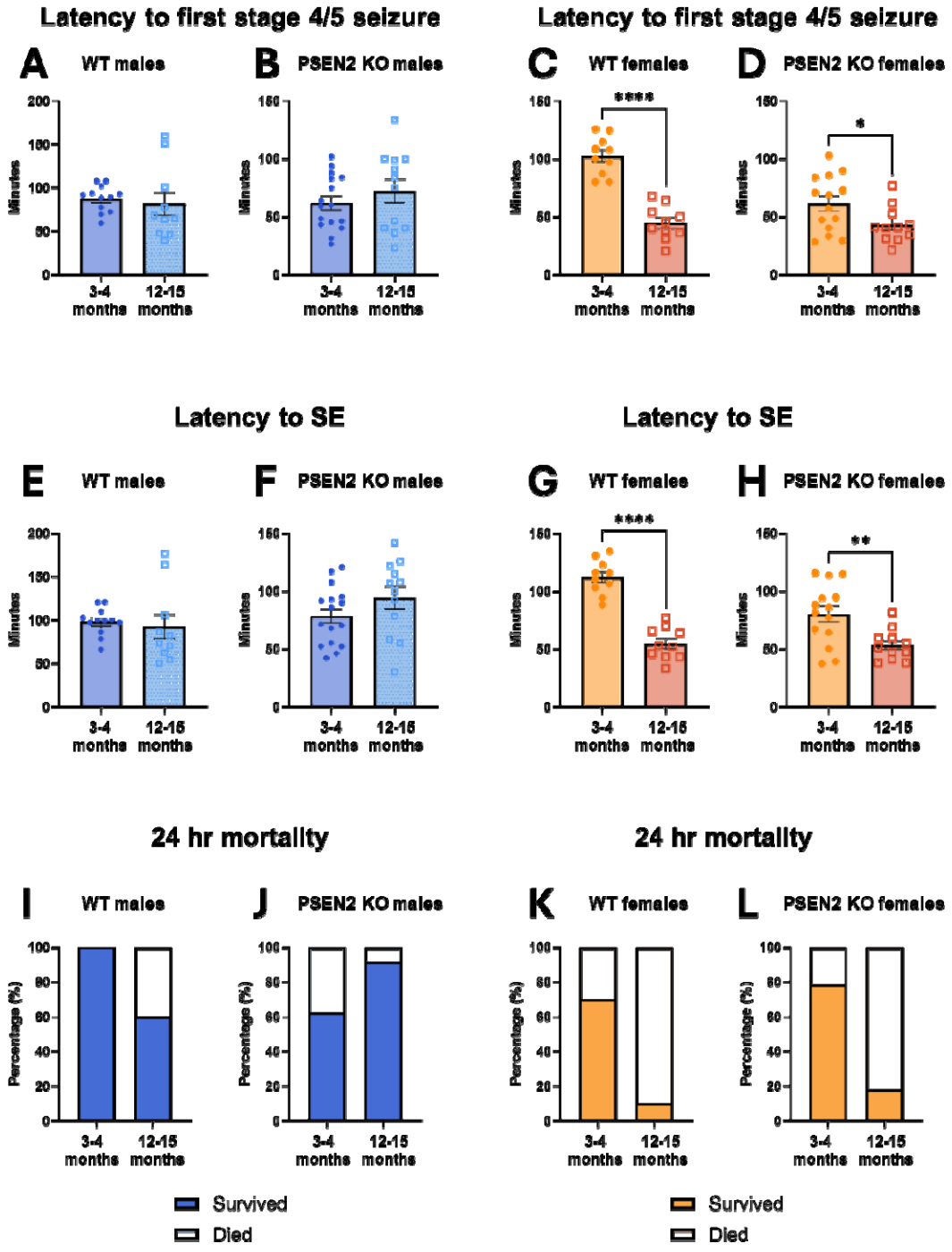

Figure 4. Only female mice showed worsened susceptibility to kainic acid with age. A) The latency to first stg 4/5 seizure in aged male WT does not differ from that of young WT male mice, as measured by an unpaired t test (p>0.05). B) The latency to first stg 4/5 seizure in aged male PSEN2 KO does not differ from that of young PSEN2 KO male mice, as measured by an unpaired t test (p>0.05). C) Latency to first Racine stage 4/5 seizure is significantly

decreased in 12-15 month old female WT mice compared to 3-4 month old female WT mice ($p<0.0001$). D) Latency to first Racine stage 4/5 seizure is significantly decreased in 12-15 month old female PSEN2 KO mice compared to 3-4 month old female PSEN2 KO mice ($p = 0.0428$). E) The latency to first SE onset in aged male WT does not differ from that of young WT male mice, as measured by an unpaired t test ($p>0.05$). F) The latency to SE onset in aged male PSEN2 KO does not differ from that of young PSEN2 KO male mice, as measured by an unpaired t test ($p>0.05$). G) Latency to SE onset is significantly decreased in 12-15 month old female WT mice compared to 3-4 month old female WT mice ($p<0.0001$). H) Latency to SE onset is significantly decreased in 12-15 month old female PSEN2 KO mice compared to 3-4 month old female PSEN2 KO mice ($p = 0.0057$). I) Aged male WT mice had increased 24-hour mortality compared to young male WT mice, as measured by Log-rank (Mantel-Cox) test $X^2 = 5.6$, $p = 0.02$ and Fisher's exact test (p value = 0.029). J) There was no significant difference between age groups among PSEN2 KO males in the 24-hour mortality following the acute KA SE insult, as measured by Log-rank (Mantel-Cox) test $X^2 = 3.1$, p value = 0.08 and Fisher's exact test (p value = 0.18). K) Aged female WT mice had increased 24-hour mortality compared to young female WT mice, as measured by Log-rank (Mantel-Cox) test $X^2 = 7.1$, $p = 0.008$ and Fisher's exact test (p value = 0.02). L) Aged female PSEN2 KO mice had increased 24-hour mortality compared to young female PSEN2 KO mice, as measured by Log-rank (Mantel-Cox) test $X^2 = 8.6$, $p = 0.003$ and Fisher's exact test (p value = 0.005).

Figure 5 - Gluk 2 + GFAP, Gluk5+ GFAP, Iba-1 + NeuN Young Mice

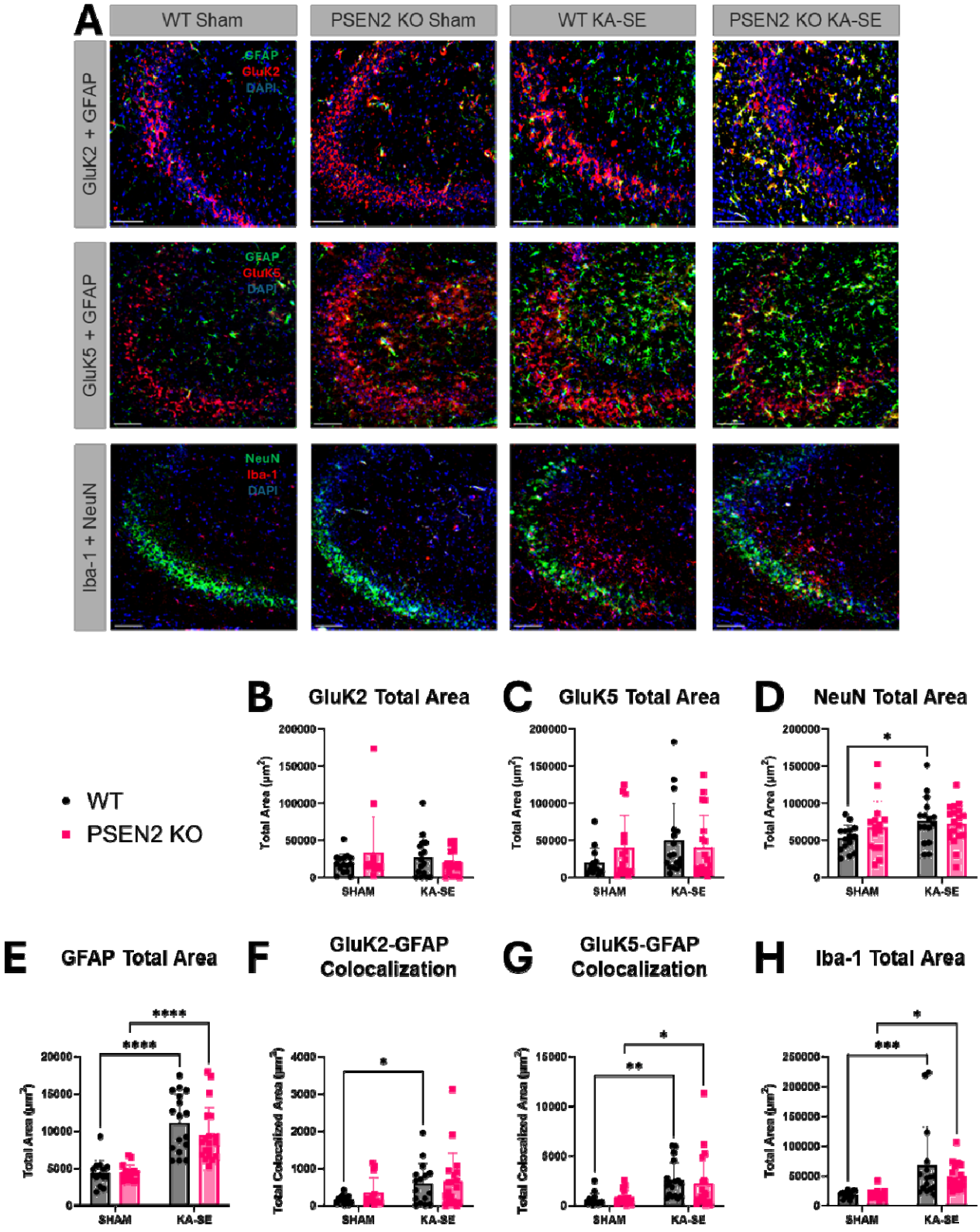

Figure 5. Loss of normal PSEN2 function did not impact total hippocampal expression of GluK2 and GluK5 subunits when compared to age-matched C57BL/6J mice aged 3-4 months. **A)** Representative images of GluK2 + GFAP, GluK5 + GFAP, and Iba-1 + NeuN staining at CA3 of WT and PSEN2 KO sham and KA-SE animals. **B,C)** There was no significant difference in expression of either GluK between genotypes or treatment groups. **E)** KA-SE resulted in increased GFAP expression regardless of genotype. **F,G)** Both genotypes showed a significant increase in astrocytic GluK5 expression following KA-SE, but only WT mice had increased astrocytic GluK2. **D)** NeuN expression increased following KA-SE in WT animals only. **H)** Iba-1 expression increased following KA-SE regardless of genotype. Bars represent the median and the SEM. Data were analyzed via two-way ANOVA followed by the Uncorrected Fisher's LSD Test (*$p<0.05$, **$p<0.01$, ***$p<0.005$ ****$p<0.0001$).

Figure 6 - Gluk 2 + GFAP, Gluk5+ GFAP, Iba-1 + NeuN Aged Mice

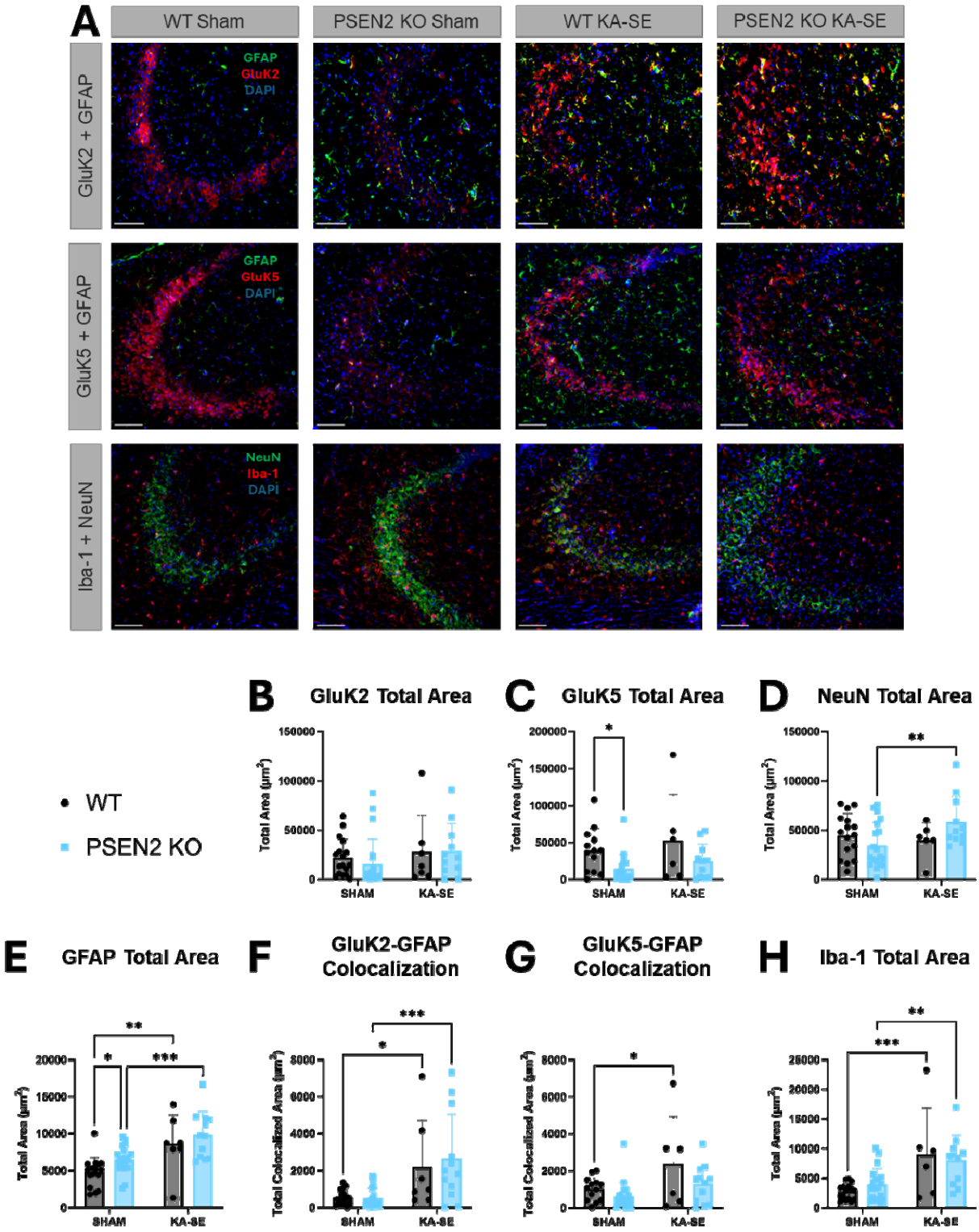

Figure 6. Loss of normal PSEN2 function resulted in decreased bassline expression of GluK5 compared to age-matched WT mice aged 12-15 months. **A)** Representative images of GluK2 + GFAP, GluK5 + GFAP, and Iba-1 + NeuN staining at CA3 of WT and PSEN2 KO sham and KA-

SE animals. **B,C)** Among sham animals, PSEN2 KO mice had significantly reduced hippocampal GluK5 expression compared to WT animals. No differences in GluK2 expression were noted between genotypes or treatment groups. **E)** KA-SE resulted in increased GFAP expression regardless of genotype. However, PSEN2 KO animals had significantly increased GFAP expression at baseline compared to WT animals. **F,G)** Both genotypes showed a significant increase in astrocytic GluK2 expression following KA-SE, but only WT mice had increased astrocytic GluK5. **D)** NeuN expression increased following KA-SE in PSEN2 KO animals only. **H)** Iba-1 expression increased following KA-SE regardless of genotype. Bars represent the median and the SEM. Data were analyzed via two-way ANOVA followed by the Uncorrected Fisher's LSD Test (*$p<0.05$, **$p<0.01$, ***$p<0.005$ ****$p<0.0001$).

1. Sen A, Jette N, Husain M, Sander JW. Epilepsy in older people. Lancet. 2020;395(10225):735-48. doi: 10.1016/s0140-6736(19)33064-8. PubMed PMID: 32113502.
2. Lehmann L, Lo A, Knox KM, Barker-Haliski M. Alzheimer's Disease and Epilepsy: A Perspective on the Opportunities for Overlapping Therapeutic Innovation. Neurochem Res.

2021;46(8):1895-912. Epub 20210430. doi: 10.1007/s11064-021-03332-y. PubMed PMID: 33929683; PMCID: PMC8254705.
3. Wang R, Reddy PH. Role of Glutamate and NMDA Receptors in Alzheimer's Disease. J Alzheimers Dis. 2017;57(4):1041-8. doi: 10.3233/jad-160763. PubMed PMID: 27662322; PMCID: PMC5791143.
4. Barker-Haliski M, White HS. Glutamatergic Mechanisms Associated with Seizures and Epilepsy. Cold Spring Harb Perspect Med. 2015;5(8):a022863. Epub 20150622. doi: 10.1101/cshperspect.a022863. PubMed PMID: 26101204; PMCID: PMC4526718.
5. Yan XX, Cai Y, Shelton J, Deng SH, Luo XG, Oddo S, Laferla FM, Cai H, Rose GM, Patrylo PR. Chronic temporal lobe epilepsy is associated with enhanced Alzheimer-like neuropathology in 3×Tg-AD mice. PLoS One. 2012;7(11):e48782. Epub 20121114. doi: 10.1371/journal.pone.0048782. PubMed PMID: 23155407; PMCID: PMC3498246.
6. Tai XY, Koepp M, Duncan JS, Fox N, Thompson P, Baxendale S, Liu JY, Reeves C, Michalak Z, Thom M. Hyperphosphorylated tau in patients with refractory epilepsy correlates with cognitive decline: a study of temporal lobe resections. Brain. 2016;139(Pt 9):2441-55. Epub 20160807. doi: 10.1093/brain/aww187. PubMed PMID: 27497924; PMCID: PMC5926008.
7. Joutsa J, Rinne JO, Hermann B, Karrasch M, Anttinen A, Shinnar S, Sillanpää M. Association Between Childhood-Onset Epilepsy and Amyloid Burden 5 Decades Later. JAMA Neurol. 2017;74(5):583-90. doi: 10.1001/jamaneurol.2016.6091. PubMed PMID: 28346588; PMCID: PMC5822199.
8. Zawar I, Quigg M, Ghosal S, Punia V, Calle-Lopez Y, Manning C, Kapur J. Association of Seizure Control with Mortality, Cognition, and Function in People With Dementia. Ann Neurol. 2025;97(2):358-68. Epub 20241030. doi: 10.1002/ana.27125. PubMed PMID: 39475168; PMCID: PMC11740283.
9. Del Pozo A, Knox KM, Lehmann LM, Davidson S, Rho SL, Jayadev S, Barker-Haliski M. Chronic evoked seizures in young pre-symptomatic APP/PS1 mice induce serotonin changes and accelerate onset of Alzheimer's disease-related neuropathology. Prog Neurobiol. 2024;235:102591. Epub 20240313. doi: 10.1016/j.pneurobio.2024.102591. PubMed PMID: 38484965.
10. Selkoe DJ, Hardy J. The amyloid hypothesis of Alzheimer's disease at 25 years. EMBO Mol Med. 2016;8(6):595-608. Epub 20160601. doi: 10.15252/emmm.201606210. PubMed PMID: 27025652; PMCID: PMC4888851.
11. Zarea A, Charbonnier C, Rovelet-Lecrux A, Nicolas G, Rousseau S, Borden A, Pariente J, Le Ber I, Pasquier F, Formaglio M, Martinaud O, Rollin-Sillaire A, Sarazin M, Croisile B, Boutoleau-Bretonnière C, Ceccaldi M, Gabelle A, Chamard L, Blanc F, Sellal F, Paquet C, Campion D, Hannequin D, Wallon D. Seizures in dominantly inherited Alzheimer disease. Neurology. 2016;87(9):912-9. Epub 20160727. doi: 10.1212/wnl.0000000000003048. PubMed PMID: 27466472.
12. Jiang H, Jayadev S, Lardelli M, Newman M. A Review of the Familial Alzheimer's Disease Locus PRESENILIN 2 and Its Relationship to PRESENILIN 1. J Alzheimers Dis. 2018;66(4):1323-39. doi: 10.3233/jad-180656. PubMed PMID: 30412492.
13. Jayadev S, Leverenz JB, Steinbart E, Stahl J, Klunk W, Yu CE, Bird TD. Alzheimer's disease phenotypes and genotypes associated with mutations in presenilin 2. Brain. 2010;133(Pt 4):1143-54. doi: 10.1093/brain/awq033. PubMed PMID: 20375137; PMCID: PMC2850581.

14. Course MM, Gudsnuk K, Keene CD, Bird TD, Jayadev S, Valdmanis PN. Aberrant splicing of PSEN2, but not PSEN1, in individuals with sporadic Alzheimer's disease. Brain. 2023;146(2):507-18. doi: 10.1093/brain/awac294. PubMed PMID: 35949106; PMCID: PMC10169283.
15. Sanchez PE, Zhu L, Verret L, Vossel KA, Orr AG, Cirrito JR, Devidze N, Ho K, Yu GQ, Palop JJ, Mucke L. Levetiracetam suppresses neuronal network dysfunction and reverses synaptic and cognitive deficits in an Alzheimer's disease model. Proc Natl Acad Sci U S A. 2012;109(42):E2895-903. Epub 20120806. doi: 10.1073/pnas.1121081109. PubMed PMID: 22869752; PMCID: PMC3479491.
16. Palop JJ, Chin J, Roberson ED, Wang J, Thwin MT, Bien-Ly N, Yoo J, Ho KO, Yu GQ, Kreitzer A, Finkbeiner S, Noebels JL, Mucke L. Aberrant excitatory neuronal activity and compensatory remodeling of inhibitory hippocampal circuits in mouse models of Alzheimer's disease. Neuron. 2007;55(5):697-711. doi: 10.1016/j.neuron.2007.07.025. PubMed PMID: 17785178; PMCID: PMC8055171.
17. Minkeviciene R, Rheims S, Dobszay MB, Zilberter M, Hartikainen J, Fülöp L, Penke B, Zilberter Y, Harkany T, Pitkänen A, Tanila H. Amyloid beta-induced neuronal hyperexcitability triggers progressive epilepsy. J Neurosci. 2009;29(11):3453-62. doi: 10.1523/jneurosci.5215-08.2009. PubMed PMID: 19295151; PMCID: PMC6665248.
18. Jayadev S, Case A, Eastman AJ, Nguyen H, Pollak J, Wiley JC, Möller T, Morrison RS, Garden GA. Presenilin 2 is the predominant γ-secretase in microglia and modulates cytokine release. PLoS One. 2010;5(12):e15743. Epub 20101229. doi: 10.1371/journal.pone.0015743. PubMed PMID: 21206757; PMCID: PMC3012089.
19. Jayadev S, Case A, Alajajian B, Eastman AJ, Möller T, Garden GA. Presenilin 2 influences miR146 level and activity in microglia. J Neurochem. 2013;127(5):592-9. Epub 20130930. doi: 10.1111/jnc.12400. PubMed PMID: 23952003; PMCID: PMC4346352.
20. Sullivan MA, Lane SD, McKenzie ADJ, Ball SR, Sunde M, Neely GG, Moreno CL, Maximova A, Werry EL, Kassiou M. iPSC-derived PSEN2 (N141I) astrocytes and microglia exhibit a primed inflammatory phenotype. J Neuroinflammation. 2024;21(1):7. Epub 20240104. doi: 10.1186/s12974-023-02951-2. PubMed PMID: 38178159; PMCID: PMC10765839.
21. Vezzani A, Aronica E, Mazarati A, Pittman QJ. Epilepsy and brain inflammation. Exp Neurol. 2013;244:11-21. Epub 20111001. doi: 10.1016/j.expneurol.2011.09.033. PubMed PMID: 21985866.
22. Rozovsky I, Finch CE, Morgan TE. Age-related activation of microglia and astrocytes: in vitro studies show persistent phenotypes of aging, increased proliferation, and resistance to down-regulation. Neurobiol Aging. 1998;19(1):97-103. doi: 10.1016/s0197-4580(97)00169-3. PubMed PMID: 9562510.
23. Herreman A, Hartmann D, Annaert W, Saftig P, Craessaerts K, Serneels L, Umans L, Schrijvers V, Checler F, Vanderstichele H, Baekelandt V, Dressel R, Cupers P, Huylebroeck D, Zwijsen A, Van Leuven F, De Strooper B. Presenilin 2 deficiency causes a mild pulmonary phenotype and no changes in amyloid precursor protein processing but enhances the embryonic lethal phenotype of presenilin 1 deficiency. Proc Natl Acad Sci U S A. 1999;96(21):11872-7. doi: 10.1073/pnas.96.21.11872. PubMed PMID: 10518543; PMCID: PMC18379.
24. Knox KM, Beckman M, Smith CL, Jayadev S, Barker-Haliski M. Chronic seizures induce sex-specific cognitive deficits with loss of presenilin 2 function. Exp Neurol. 2023;361:114321. Epub 20230109. doi: 10.1016/j.expneurol.2023.114321. PubMed PMID: 36634751.

25. Beckman M, Knox K, Koneval Z, Smith C, Jayadev S, Barker-Haliski M. Loss of presenilin 2 age-dependently alters susceptibility to acute seizures and kindling acquisition. Neurobiol Dis. 2020;136:104719. Epub 20191217. doi: 10.1016/j.nbd.2019.104719. PubMed PMID: 31862541; PMCID: PMC7462087.
26. Lisgaras CP, Scharfman HE. High-frequency oscillations (250-500 Hz) in animal models of Alzheimer's disease and two animal models of epilepsy. Epilepsia. 2023;64(1):231-46. Epub 20221129. doi: 10.1111/epi.17462. PubMed PMID: 36346209; PMCID: PMC10501735.
27. Barthet G, Moreira-de-Sá A, Zhang P, Deforges S, Castanheira J, Gorlewicz A, Mulle C. Presenilin and APP Regulate Synaptic Kainate Receptors. J Neurosci. 2022;42(49):9253-62. Epub 20221026. doi: 10.1523/jneurosci.0297-22.2022. PubMed PMID: 36288945; PMCID: PMC9761675.
28. Vincent P, Mulle C. Kainate receptors in epilepsy and excitotoxicity. Neuroscience. 2009;158(1):309-23. Epub 20080308. doi: 10.1016/j.neuroscience.2008.02.066. PubMed PMID: 18400404.
29. Dawson R, Jr., Wallace DR. Kainic acid-induced seizures in aged rats: neurochemical correlates. Brain Res Bull. 1992;29(3-4):459-68. doi: 10.1016/0361-9230(92)90083-a. PubMed PMID: 1393617.
30. Wozniak DF, Stewart GR, Miller JP, Olney JW. Age-related sensitivity to kainate neurotoxicity. Exp Neurol. 1991;114(2):250-3. doi: 10.1016/0014-4886(91)90042-b. PubMed PMID: 1748199.
31. Zhang XM, Zhu SW, Duan RS, Mohammed AH, Winblad B, Zhu J. Gender differences in susceptibility to kainic acid-induced neurodegeneration in aged C57BL/6 mice. Neurotoxicology. 2008;29(3):406-12. Epub 20080208. doi: 10.1016/j.neuro.2008.01.006. PubMed PMID: 18342945.
32. Meeker S, Beckman M, Knox KM, Treuting PM, Barker-Haliski M. Repeated Intraperitoneal Administration of Low-Concentration Methylcellulose Leads to Systemic Histologic Lesions Without Loss of Preclinical Phenotype. J Pharmacol Exp Ther. 2019;371(1):25-35. Epub 20190802. doi: 10.1124/jpet.119.257261. PubMed PMID: 31375638; PMCID: PMC11047094.
33. Percie du Sert N, Hurst V, Ahluwalia A, Alam S, Avey MT, Baker M, Browne WJ, Clark A, Cuthill IC, Dirnagl U, Emerson M, Garner P, Holgate ST, Howells DW, Karp NA, Lazic SE, Lidster K, MacCallum CJ, Macleod M, Pearl EJ, Petersen OH, Rawle F, Reynolds P, Rooney K, Sena ES, Silberberg SD, Steckler T, Würbel H. The ARRIVE guidelines 2.0: Updated guidelines for reporting animal research. PLoS Biol. 2020;18(7):e3000410. Epub 20200714. doi: 10.1371/journal.pbio.3000410. PubMed PMID: 32663219; PMCID: PMC7360023.
34. Umpierre AD, Bennett IV, Nebeker LD, Newell TG, Tian BB, Thomson KE, White HS, White JA, Wilcox KS. Repeated low-dose kainate administration in C57BL/6J mice produces temporal lobe epilepsy pathology but infrequent spontaneous seizures. Exp Neurol. 2016;279:116-26. Epub 20160216. doi: 10.1016/j.expneurol.2016.02.014. PubMed PMID: 26896834; PMCID: PMC5382800.
35. Tse K, Puttachary S, Beamer E, Sills GJ, Thippeswamy T. Advantages of repeated low dose against single high dose of kainate in C57BL/6J mouse model of status epilepticus: behavioral and electroencephalographic studies. PLoS One. 2014;9(5):e96622. Epub 20140506. doi: 10.1371/journal.pone.0096622. PubMed PMID: 24802808; PMCID: PMC4011859.

36. Knox KM, Zierath DK, White HS, Barker-Haliski M. Continuous seizure emergency evoked in mice with pharmacological, electrographic, and pathological features distinct from status epilepticus. Epilepsia. 2021;62(12):3076-90. Epub 20211008. doi: 10.1111/epi.17089. PubMed PMID: 34625953; PMCID: PMC8639616.
37. Wenthold RJ, Trumpy VA, Zhu WS, Petralia RS. Biochemical and assembly properties of GluR6 and KA2, two members of the kainate receptor family, determined with subunit-specific antibodies. J Biol Chem. 1994;269(2):1332-9. PubMed PMID: 8288598.
38. Rusina E, Bernard C, Williamson A. The Kainic Acid Models of Temporal Lobe Epilepsy. eNeuro. 2021;8(2). Epub 20210409. doi: 10.1523/eneuro.0337-20.2021. PubMed PMID: 33658312; PMCID: PMC8174050.
39. Fung S, Smith CL, Prater KE, Case A, Green K, Osnis L, Winston C, Kinoshita Y, Sopher B, Morrison RS, Garden GA, Jayadev S. Early-Onset Familial Alzheimer Disease Variant PSEN2 N141I Heterozygosity is Associated with Altered Microglia Phenotype. J Alzheimers Dis. 2020;77(2):675-88. doi: 10.3233/jad-200492. PubMed PMID: 32741831; PMCID: PMC7592656.
40. Vargas JR, Takahashi DK, Thomson KE, Wilcox KS. The expression of kainate receptor subunits in hippocampal astrocytes after experimentally induced status epilepticus. J Neuropathol Exp Neurol. 2013;72(10):919-32. doi: 10.1097/NEN.0b013e3182a4b266. PubMed PMID: 24042195; PMCID: PMC3880830.
41. Moon J, Ahn JH, Won MH. NeuN expression in health and disease: A histological perspective on neuronal heterogeneity. Histol Histopathol. 2025:18965. Epub 20250711. doi: 10.14670/hh-18-965. PubMed PMID: 40697072.
42. Lawande NV, Conklin EA, Christian-Hinman CA. Sex and gonadectomy modify behavioral seizure susceptibility and mortality in a repeated low-dose kainic acid systemic injection paradigm in mice. Epilepsia Open. 2023;8(4):1512-22. Epub 20230923. doi: 10.1002/epi4.12828. PubMed PMID: 37715318; PMCID: PMC10690657.
43. Kaja S, Sumien N, Shah VV, Puthawala I, Maynard AN, Khullar N, Payne AJ, Forster MJ, Koulen P. Loss of Spatial Memory, Learning, and Motor Function During Normal Aging Is Accompanied by Changes in Brain Presenilin 1 and 2 Expression Levels. Mol Neurobiol. 2015;52(1):545-54. Epub 20140910. doi: 10.1007/s12035-014-8877-4. PubMed PMID: 25204494; PMCID: PMC4362879.
44. Ben-Ari Y. Limbic seizure and brain damage produced by kainic acid: mechanisms and relevance to human temporal lobe epilepsy. Neuroscience. 1985;14(2):375-403. doi: 10.1016/0306-4522(85)90299-4. PubMed PMID: 2859548.
45. Nadler JV. Minireview. Kainic acid as a tool for the study of temporal lobe epilepsy. Life Sci. 1981;29(20):2031-42. doi: 10.1016/0024-3205(81)90659-7. PubMed PMID: 7031398.
46. Bureau I, Bischoff S, Heinemann SF, Mulle C. Kainate receptor-mediated responses in the CA1 field of wild-type and GluR6-deficient mice. J Neurosci. 1999;19(2):653-63. doi: 10.1523/jneurosci.19-02-00653.1999. PubMed PMID: 9880586; PMCID: PMC6782223.
47. Gynther M, Petsalo A, Hansen SH, Bunch L, Pickering DS. Blood-brain barrier permeability and brain uptake mechanism of kainic acid and dihydrokainic acid. Neurochem Res. 2015;40(3):542-9. Epub 20141209. doi: 10.1007/s11064-014-1499-4. PubMed PMID: 25488153.
48. Christensen JK, Paternain AV, Selak S, Ahring PK, Lerma J. A mosaic of functional kainate receptors in hippocampal interneurons. J Neurosci. 2004;24(41):8986-93. doi: 10.1523/jneurosci.2156-04.2004. PubMed PMID: 15483117; PMCID: PMC6730072.

49. Ruiz A, Sachidhanandam S, Utvik JK, Coussen F, Mulle C. Distinct subunits in heteromeric kainate receptors mediate ionotropic and metabotropic function at hippocampal mossy fiber synapses. J Neurosci. 2005;25(50):11710-8. doi: 10.1523/jneurosci.4041-05.2005. PubMed PMID: 16354929; PMCID: PMC6726035.
50. Rodríguez-Moreno A, Sihra TS. Kainate receptors with a metabotropic modus operandi. Trends Neurosci. 2007;30(12):630-7. Epub 20071105. doi: 10.1016/j.tins.2007.10.001. PubMed PMID: 17981346.
51. Pinheiro PS, Perrais D, Coussen F, Barhanin J, Bettler B, Mann JR, Malva JO, Heinemann SF, Mulle C. GluR7 is an essential subunit of presynaptic kainate autoreceptors at hippocampal mossy fiber synapses. Proc Natl Acad Sci U S A. 2007;104(29):12181-6. Epub 20070709. doi: 10.1073/pnas.0608891104. PubMed PMID: 17620617; PMCID: PMC1924597.
52. Contractor A, Swanson G, Heinemann SF. Kainate receptors are involved in short- and long-term plasticity at mossy fiber synapses in the hippocampus. Neuron. 2001;29(1):209-16. doi: 10.1016/s0896-6273(01)00191-x. PubMed PMID: 11182092.
53. Bezzi P, Carmignoto G, Pasti L, Vesce S, Rossi D, Rizzini BL, Pozzan T, Volterra A. Prostaglandins stimulate calcium-dependent glutamate release in astrocytes. Nature. 1998;391(6664):281-5. doi: 10.1038/34651. PubMed PMID: 9440691.
54. Fellin T, Pascual O, Gobbo S, Pozzan T, Haydon PG, Carmignoto G. Neuronal synchrony mediated by astrocytic glutamate through activation of extrasynaptic NMDA receptors. Neuron. 2004;43(5):729-43. doi: 10.1016/j.neuron.2004.08.011. PubMed PMID: 15339653.
55. Parpura V, Basarsky TA, Liu F, Jeftinija K, Jeftinija S, Haydon PG. Glutamate-mediated astrocyte-neuron signalling. Nature. 1994;369(6483):744-7. doi: 10.1038/369744a0. PubMed PMID: 7911978.
56. Tian GF, Azmi H, Takano T, Xu Q, Peng W, Lin J, Oberheim N, Lou N, Wang X, Zielke HR, Kang J, Nedergaard M. An astrocytic basis of epilepsy. Nat Med. 2005;11(9):973-81. Epub 20050814. doi: 10.1038/nm1277. PubMed PMID: 16116433; PMCID: PMC1850946.
57. Cornell-Bell AH, Finkbeiner SM, Cooper MS, Smith SJ. Glutamate induces calcium waves in cultured astrocytes: long-range glial signaling. Science. 1990;247(4941):470-3. doi: 10.1126/science.1967852. PubMed PMID: 1967852.
58. Purnell BS, Alves M, Boison D. Astrocyte-neuron circuits in epilepsy. Neurobiol Dis. 2023;179:106058. Epub 20230301. doi: 10.1016/j.nbd.2023.106058. PubMed PMID: 36868484; PMCID: PMC10334651.
59. Zhu X, Lee HG, Perry G, Smith MA. Alzheimer disease, the two-hit hypothesis: an update. Biochim Biophys Acta. 2007;1772(4):494-502. Epub 20061027. doi: 10.1016/j.bbadis.2006.10.014. PubMed PMID: 17142016.
60. Kasugai M, Akaike K, Imamura S, Matsukubo H, Tojo H, Nakamura M, Tanaka S, Sano A. Differences in two mice strains on kainic acid-induced amygdalar seizures. Biochem Biophys Res Commun. 2007;357(4):1078-83. Epub 20070419. doi: 10.1016/j.bbrc.2007.04.067. PubMed PMID: 17467670.
61. Parent JM, Yu TW, Leibowitz RT, Geschwind DH, Sloviter RS, Lowenstein DH. Dentate granule cell neurogenesis is increased by seizures and contributes to aberrant network reorganization in the adult rat hippocampus. J Neurosci. 1997;17(10):3727-38. doi: 10.1523/jneurosci.17-10-03727.1997. PubMed PMID: 9133393; PMCID: PMC6573703.

62. Buckmaster PS, Zhang GF, Yamawaki R. Axon sprouting in a model of temporal lobe epilepsy creates a predominantly excitatory feedback circuit. J Neurosci. 2002;22(15):6650-8. doi: 10.1523/jneurosci.22-15-06650.2002. PubMed PMID: 12151544; PMCID: PMC6758164.