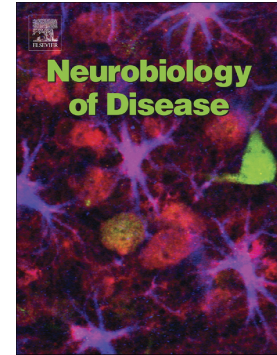


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Loss of Presenilin 2 Age-Dependently Alters Susceptibility to Acute Seizures and Kindling Acquisition

Running Title: Effect of PSEN2 Loss on Seizure Models

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Abstract

Patients with Alzheimer's disease (AD) experience seizures at higher rates than the general population of that age, suggesting an underexplored role of hyperexcitability in AD. Genetic variants in presenilin (PSEN) 1 and 2 genes lead to autosomal dominant early-onset AD (ADAD); patients with PSEN gene variants also report seizures. Pharmacological control of seizures in AD may be disease-modifying. Preclinical efficacy of FDA-approved antiseizure drugs (ASDs) is well defined in young adult rodents; however, the efficacy of ASDs in aged rodents with chronic seizures is less clear. The mechanism by which ADAD genes lead to AD remains unclear, and even less studied is the pathogenesis of epilepsy in AD. PSEN variants generally all result in a biochemical loss of function (De Strooper, 2007). We herein determined whether well-established models of acute and chronic seizure could be used to explore the relationship between AD genes and seizures through investigating whether loss of normal PSEN2 function age-dependently influenced susceptibility to seizures and/or corneal kindling acquisition. PSEN2 knockout (KO) and age-matched wild-type (WT) mice were screened from 2- to 10-months-old to establish age-dependent focal seizure threshold. Additionally, PSEN2 KO and WT mice aged 2- and 8-months-old underwent corneal kindling such that mice were aged 3- and 9-months old at the beginning of ASD efficacy testing. We then defined the dose-dependent efficacy of mechanistically distinct ASDs on kindled seizures of young versus aged mice to better understand the applicability of corneal kindling to real-world use for geriatric patients. PSEN2 KO mice demonstrated early-life reductions in seizure threshold. However, kindling acquisition was delayed in 2-month-old PSEN2 KO versus WT mice. Young male WT mice took 24.3 ± 1.3 (S.E.M.) stimulations to achieve kindling criterion, whereas age-matched PSEN2 KO male mice took 41.2 ± 1.1 stimulations ($p < 0.0001$). The rate of kindling acquisition of 8-month-old mice was no longer different from WT. This study demonstrates that loss of normal PSEN2 function is associated with age-dependent changes in the *in vivo* susceptibility to acute seizures and kindling. Loss of normal PSEN2 function may be an underexplored molecular contributor to seizures. The use of validated models of chronic seizures in aged rodents may uncover age-related changes in susceptibility to epileptogenesis and/or ASD efficacy in mice with AD-associated genotypes, which may benefit the management of seizures in AD.

Keywords: Seizures; lamotrigine; carbamazepine; valproic acid; levetiracetam; diazepam; epilepsy; Alzheimer's; presenilin; corneal kindling; aging

Introduction

The relative risk of unprovoked seizures markedly increases in autosomal dominant early-onset Alzheimer's disease (ADAD) relative to individuals without dementia (Amatniek et al. 2006; Vossel et al. 2013). Seizures have been documented in clinical ADAD cases (Lam et al. 2017b; Vossel et al. 2017; Zarea et al. 2016), and preclinical EEG monitoring of mice that overexpress amyloid-precursor protein (APP; (Palop et al. 2007; Sanchez et al. 2012; Ziyatdinova et al. 2011)) and tau (Hall et al. 2015; Roberson et al. 2011). However, *presenilin 2* (*PSEN2*) gene variants also result in ADAD, albeit with a later onset than as occurs in individuals with *APP* or *presenilin 1* (*PSEN1*) variants. Patients with *PSEN2* variants also commonly experience seizures; one study reported nearly 29% of *PSEN2* patients have at least one seizure within 5 years of diagnosis (Zarea et al. 2015). Despite the high incidence of seizures in patients with *PSEN2*-associated ADAD (Jayadev et al. 2010a), studies to define whether loss of normal *PSEN2* function influences age-related seizure susceptibility *in vivo* are as yet absent.

AD and epilepsy share many pathological similarities: neuroinflammation, gliosis, and neurodegeneration (Ackermann et al. 1986; Cook et al. 1992; Fazekas et al. 1989; Heppner et al. 2015; Struble et al. 2010). *PSEN* ADAD variants have been generally shown to confer a biochemical loss of function (De Strooper 2007) that in some cases leads to elevated amyloid β ($A\beta_{42}$) formation or increased $A\beta_{42:40}$ ratios (Oyama et al. 1992; Walker et al. 2005). However, *PSEN*s do not directly increase APP expression and amyloid plaque deposition (Elder et al. 2010). *PS2* protein normally mediates cellular processes distinct from *PS1* protein (Kaja et al. 2015; Lee et al. 1996; Meckler and Checler 2016; Payne et al. 2015). *PSEN2* itself is the predominate γ -secretase within microglia; loss of normal *PSEN2* function in primary microglia derived from perinatal *PSEN2* KO mice can promote exaggerated expression of proinflammatory cytokines (Jayadev et al. 2010b) and increase the expression of inflammatory mediators (Jayadev et al. 2013). Loss of normal *PSEN2* function may thus disrupt neuroinflammatory mechanisms that underlie both AD pathophysiology and seizure susceptibility. Unlike *PSEN1* knockout (KO) mice, *PSEN2* KO mice exhibit normal long-term viability, (Herreman et al. 1999). *PSEN2* KO mice are thus useful to *a priori* define how the loss of normal *PSEN2* function age-dependently impacts susceptibility to seizures and epilepsy in an $A\beta$ -independent manner within an intact organism across its lifespan.

Few studies have extensively examined the link between AD and epilepsy in preclinical AD models (Palop et al. 2007; Sanchez et al. 2012; Verret et al. 2012; Ziyatdinova et al. 2011; Ziyatdinova et al. 2016). Loss of normal *PSEN2* function and the resulting chronic proinflammatory neurological

milieu could itself change the susceptibility to seizures and epilepsy (Broekaart et al. 2018). PSEN2 is an intriguing molecular target to interrogate this pathological overlap for two key reasons: 1) loss of normal PSEN2 function *in vitro* increases expression of TNF- α , IL-1 β , and IRAK-1 (Jayadev et al. 2010b; Jayadev et al. 2013); and 2) resected tissues from patients and animal models with epilepsy consistently exhibit increased expression of these same proinflammatory cytokines (M. L. Barker-Haliski et al. 2017a; Kirkman et al. 2010). Further, LPS administration can accelerate kindling acquisition in rats (Auvin et al. 2010b; Auvin et al. 2010a), demonstrating that inflammation can promote epileptogenesis (Vezzani et al. 2013). Given that loss of normal PSEN2 expression is proinflammatory in microglia *in vitro* (Jayadev et al. 2010b), we hypothesized that loss of normal PSEN2 expression would alter the age-dependent susceptibility to acute seizures and formation of an epileptic network *in vivo*. A primary goal of the present work was to thus define the extent to which loss of normal PSEN2 function affects seizure susceptibility and/or risk for corneal kindling acquisition in an intact neuronal network in an age-dependent manner.

One proposed therapeutic approach in AD has been to administer ASDs to patients (Vossel et al. 2013). Untreated focal seizures may worsen the cognitive deficits of AD (Tai et al. 2016), as occurs in patients with epilepsy (Brooks-Kayal et al. 2003). However, there are conflicting reports around the anticonvulsant efficacy of ASDs on spontaneous seizures in rodent AD models; some studies suggest that sodium channel-blocking ASDs, i.e. carbamazepine (CBZ), are effective against seizures (Ziyatdinova et al. 2011), whereas others suggest an opposite, and even exacerbating, effect, i.e. phenytoin (Sanchez et al. 2012). Further, there is little information to understand whether ASD efficacy and tolerability in rodent models of AD is impacted by age. The majority of preclinical ASD development is conducted in young adult, neurologically-intact rodents (White and Barker-Haliski 2016). In this regard, the second major objective of this study was to establish the age-dependent acute anticonvulsant efficacy of mechanistically distinct ASDs in PSEN2 KO and age-matched wild-type (WT) mice using a preclinical model of epilepsy that has contributed to the identification of numerous ASDs (Matagne and Klitgaard 1998): the corneal kindled mouse (CKM). This study thus also provides a comprehensive assessment of the age-dependent seizure susceptibility of PSEN2 KO mice and provides new information concerning ASD effects in aged rodents, which may better guide ASD trials for the management of seizures in AD patients.

Materials and 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). This study was not testing the effects of sex-hormones on seizure susceptibility or ASD efficacy, thus we did not track estrous cycles of female mice or dominant/subordinant status of male mice. PSEN2 KO mice are viable long-term and breed normally (Herreman et al. 1999); therefore, all breeding at UW was between PCR-confirmed PSEN2 KO pairs. Age-matched male and female WT (stock # 000664) were acquired from a similarly established breeding colony at UW, which was matched in age and housing conditions for PSEN2 KO mice. Animals were housed on a 14:10 light cycle (lights on at 06h00; off at 20h00) in ventilated cages with corncob bedding in a manner consistent with the *Guide for the Care and Use of Laboratory Animals* and all animal work was approved by the UW Institutional Animal Care and Use Committee and conformed to ARRIVE guidelines (Drummond et al. 2019). Animals were provided free access to irradiated chow (PicoLab Rodent Diet 20 – 5053), water, and enrichment, except during the periods of behavioral manipulation. All behavioral testing was performed during the hours of 10h00 and 17h00.

Seizure threshold tests

Focal seizures have been documented in clinical AD cases (Lam et al. 2017b), thus our study used two distinct rodent models of focal seizure (Barton et al. 2001). Discrete electrical stimulation protocols can differentiate limbic (6 Hz) and forebrain (minimal clonic) seizure thresholds. Minimal clonic seizures are characterized by rhythmic face and forelimb clonus, ventral neck flexion, and may include rearing and falling but not maximal tonic hindlimb extension (Otto et al. 2004; Otto et al. 2009), characteristic of a maximal electroshock seizure (MES; (Goodman et al. 1953)). A 6 Hz seizure is characterized by forelimb clonus, twitching of the vibrissae, behavioral arrest, and Straub tail (Barton et al. 2001; Toman 1951). The minimal clonic and 6 Hz seizure thresholds were determined in male and female PSEN2 KO and WT mice aged up to 10-months-old according to the staircase procedure (Finney 1952). Briefly, an electrical current was delivered bilaterally to anesthetized (0.5% tetracaine) corneas for the specific seizure model (minimal clonic or 6 Hz). A mouse was considered to have had a seizure if it presented with the model-specific behavioral seizure. The seizure result of each mouse dictated the stimulation current delivered to the next mouse in each group until at least 5 current groups were established between the limits of 0% and 100% of mice with seizure to quantify median convulsant current (CC50) and 95% confidence intervals (M. Barker-Haliski et al. 2018).

Group sizes for threshold determinations: male WT = 44; female WT = 30; male PSEN2 KO = 24; female PSEN2 KO = 32. For the minimal clonic threshold test, a 0.2 sec, 60 Hz sinusoidal electrical pulse of varying current was delivered bilaterally. In the 6 Hz seizure threshold test, a 3 sec, 6 Hz square-wave electrical pulse was delivered bilaterally. Age- and genotype-dependent changes in threshold was determined at 2-, 4-, 6-, 8- and 10-months old, with 1-week between tests. This range was selected to define the age-related changes in seizure threshold throughout early to mid-life; ages that correspond to ASD screening in young mice (M. L. Barker-Haliski et al. 2017b)) and aged rodent models of AD. Animals were randomized to stimulation current group during each session. For each seizure model, mice were tested every 3-4 days until the 95% confidence intervals were calculated to fall between the limits of the minimal and maximal stimulation currents tested (Finney 1952).

Minimal clonic threshold shift test

The minimal clonic threshold shift test defines the acute effect of an ASD on the seizure threshold of a population of animals using the staircase procedure (M. Barker-Haliski et al. 2018). The minimal clonic threshold of mice aged, untreated 10- to 12-months-old was first defined. After a one-week recovery, the same animals were administered 150 mg/kg (i.p.) valproic acid (VPA) and then again tested for minimal clonic seizure threshold 15 min after drug administration. This dose of VPA was selected because it is known to exceed that which is necessary to block both a 6 Hz and corneal kindled seizure in mice, but low enough to not completely inhibit seizures in the MES model of generalized-tonic clonic seizures (M. L. Barker-Haliski et al. 2017b). The CC50 and total shift (delta) was calculated for both untreated and VPA-treated mice.

Corneal kindling

Two groups of either young adult (2-months-old at initiation) or mature (7- to 8-months-old at initiation) PSEN2 KO and WT mice were used for corneal kindling studies. This was a separate cohort of mice from the seizure threshold cohort. This range was selected to define the age-related changes in kindling acquisition in early to mid-life; ages that correspond to ASD screening (young mice; (M. L. Barker-Haliski et al. 2017b)) and aged rodent models of AD. Mice were aged at least 3- or 9-months-old at the beginning of ASD dose-response studies, thus falling within the defined seizure threshold ranges. Group sizes for young adult mice: male WT = 23; female WT = 21; male PSEN2 KO = 30; female PSEN2 KO = 19. Group sizes for mature adult mice: male WT = 19; female WT = 22; male PSEN2 KO

= 14; female PSEN2 KO = 22. Tetracaine (0.5%) was bilaterally applied to the corneas immediately prior to each twice-daily electrical stimulation with a 3 sec, 60 Hz sinusoidal pulse (M. L. Barker-Haliski et al. 2016). Stimulation intensity was based on the subconvulsive minimal clonic seizure thresholds for age-, sex- and genotype-matched mice. Young females were stimulated throughout at 2.0 mA. Young males were initially stimulated at 1.6 mA. After 9 stimulations sessions, when young male WT mice were clearly experiencing focal seizures and KO males were not, the stimulation current for young PSEN2 KO males was increased to 3.0 mA. Mature female mice were kindled throughout with 2.6 mA and mature male mice were kindled throughout with 3.0 mA. Kindled seizure severity was scored according to the Racine scale (Racine 1972).

The duration of the kindled seizure was recorded from all fully kindled mice following a 5-day stimulation-free period at the conclusion of the kindling acquisition period. The total number of mice for each young age group experiencing a Racine stage 5 seizure for which behavioral arrest duration was recorded was: male WT = 8; male KO = 14; female WT = 13; female KO = 13. The total number of mice for each aged group experiencing a Racine stage 5 seizure for which behavioral arrest duration was recorded was: male WT = 9; male KO = 8; female WT = 10; female KO = 7. A stopwatch was started at the conclusion of the electrical stimulation and the time until a mouse resumed normal exploratory behavior was recorded by an investigator blinded to genotype. Only mice that presented with a Racine stage 5 seizure during this testing were included in the analysis of seizure duration. Behavioral seizure duration included the time to initiation of a stage 5 seizure, actual stage 5 seizure, and post-ictal immobility until the mouse returned to normal exploratory behavior (e.g. forepaws on the tabletop, grooming, ambulation, responsive to external stimuli.). The timer was stopped if a mouse did not resume normal behaviors after 120 sec.

Acute antiseizure drug efficacy

ASD efficacy studies in the same cohort of CKM commenced at least 5-7 days after achieving the fully kindled state. Seizure scores ≤ 2 were considered protected. Mice were allowed a minimum of 3 days' washout between a dose of each ASD. ASDs were administered in a cross-over drug administration protocol to account for drug and seizure history (Koneval et al. 2018a). Where possible, the ED50 for each ASD was calculated by the Probit method (M. Barker-Haliski et al. 2018; Finney 1952).

Antiseizure Drugs

ASDs were administered by the intraperitoneal (i.p.) route. ASDs were tested at a single dose (minimal clonic test) or dose ranges (CKM) within the previously established time of peak anticonvulsant activity for each compound and based on previously defined median effective doses in mice (M. L. Barker-Haliski et al. 2017b; Koneval et al. 2018b; Rowley and White 2010) and as available from the NINDS Epilepsy Therapy Screening Program PANACHE database (<https://panache.ninds.nih.gov>). ASDs were selected to represent distinct pharmacological classes: sodium channel blockers (CBZ and LTG), broad spectrum (VPA), benzodiazepines (DZP), and SV2A modulators (LEV). All ASDs were formulated in 0.5% methylcellulose (Sigma Aldrich #M0430, St. Louis, MO USA; Table 2), except for VPA, which was formulated in 0.9% saline (Koneval et al. 2018a). Tetracaine HCl (0.5%; Sigma Aldrich #T7508, St. Louis, MO USA) was formulated in 0.9% saline.

Statistical Analysis

The CC50 and 95% confidence intervals required to produce the desired seizure endpoint at each age range were calculated by Probit based on binary outcomes in the specific seizure model (seizure/no seizure; (Finney 1952)). Corneal kindling acquisition was compared by repeat measures ANOVA. ED50s were estimated by Probit based on binary outcome (protection/no protection). All statistical analysis was conducted by Graphpad Prism 6.0 software, with $p < 0.05$ significant.

Results

Focal seizure threshold is reduced in young PSEN2 KO mice

To first evaluate whether loss of normal PSEN2 function impacts susceptibility to acute seizure, we determined the minimal clonic and 6 Hz seizure thresholds of WT and PSEN2 KO mice up to 10-months-old (Figure 1). As early as 4-months-old, there were notable reductions in minimal clonic threshold in PSEN2 KO mice. The CC50 of WT versus PSEN2 KO male mice were found to significantly differ at 4-month-old: 7.54 mA [95% CI: 6.91-8.36] vs 6.51 mA [95% CI: 6.25-6.78], respectively (Figure 1A). The CC50s of female mice aged 4-months were also significantly different: 6.51 mA [95% CI: 6.25-6.78] vs 5.09 mA [95% CI: 4.17-5.55], respectively (Figure 1C). These differences in threshold were no longer apparent by 8 months-old (Figure 1C).

In the 6 Hz model of limbic seizures, there was a similar trend for reductions in early-life thresholds in male, but not female, PSEN2 KO mice versus WT mice (Figure 1B and 1D). There was also a reduction in later life threshold in this assay in both male and female PSEN2 KO mice. Because the seizure threshold of PSEN2 KO mice aged ≥ 10 -months-old mice exceeded the current stimulation capacity of our 6 Hz stimulator (not shown), we were only able to determine the 6 Hz seizure threshold in PSEN2 KO and WT mice aged up to 8-months-old. By 8-months of age, the 6 Hz CC50s differed between genotypes of both sexes. The 6 Hz CC50 of male WT mice was 55.3 mA [52.0-59.4] versus that of PSEN2 KO, which was 44.5 mA [42.2 – 49.4]. The 6 Hz CC50 in WT females was 49.9 mA [47.8-51.9] versus 41.6 mA [40.2-42.7] in PSEN2 KO (Figure 1B and 1D).

Acute administration of valproic acid does not change seizure threshold of aged mice

ASDs can exert anticonvulsant actions through the attenuation of seizure spread or the modification of seizure threshold (Piredda et al. 1985). Thus, at the conclusion of longitudinal seizure threshold testing, we defined whether single acute administration of the broad-spectrum ASD, valproic acid (VPA), differentially impacted minimal clonic threshold of mice aged 10- to 12-months-old (Table 1). There were no significant differences in minimal clonic threshold between PSEN2 KO and WT mice when tested at peak efficacy time, 15 minutes after administration of 150 mg/kg VPA (i.p.; Table 1), a dose known to block 6 Hz and CKM seizures in young male mice (M. L. Barker-Haliski et al. 2017b). Thus, loss of normal PSEN2 function did not impact sensitivity to a single anticonvulsant dose of VPA in aged mice.

Loss of PSEN2 delays kindling acquisition in young, but not mature, adult mice

Kindling generally models the chronic network hyperactivity associated with epilepsy and engages different neuronal processes than those involved in a single, acute minimal clonic or 6 Hz seizure (Albertini et al. 2017; Barton et al. 2001). We found significant age-dependent differences in kindling acquisition between WT and PSEN2 KO mice at 2- and 8-months-old of age. Young adult PSEN2 KO and WT mice of both sexes acquired kindling criterion of five consecutive stage 5 seizures (Figure 2). However, the number of stimulations necessary to achieve criterion was significantly greater in 2-month-old PSEN2 KO mice relative to WT mice. Male WT mice required 24.3 ± 1.3 (S.E.M.) stimulations to achieve kindling criterion, whereas age matched PSEN2 KO male mice took 41.2 ± 1.1 stimulations ($p < 0.0001$). Similarly, WT female mice took 26.5 ± 0.6 stimulations to achieve criterion, but

PSEN2 KO females required 34.9 ± 1.1 stimulations ($p < 0.0001$). This difference in the rate of kindling acquisition was no longer apparent in 8-month-old mice. Aged male WT mice required 22.4 ± 0.8 stimulations versus PSEN2 KO males required 22.9 ± 1.0 stimulations ($p > 0.6$). Aged WT females required 19.4 ± 0.7 stimulations and PSEN2 KO females required 19.7 ± 0.8 stimulations ($p > 0.8$). Further, PSEN2 KO mice showed a greater slope in the change in kindling acquisition rate with age (Figure 2E and 2F).

To further characterize the phenotype of the kindled seizure in young versus aged mice, the duration of a single kindled behavioral seizure was recorded following corneal stimulation (Figure 3). In male mice, loss of PSEN2 was associated with significant increases in seizure duration ($F_{(1, 35)} = 8.149$, $p = 0.007$). Young male PSEN2 KO mice experienced the longest duration: 59.8 ± 7.6 versus 30.0 ± 2.4 seconds for young male WT mice. In female mice, there was a significant genotype x age interaction on seizure duration ($F_{(1, 42)} = 11.88$, $p < 0.001$). Young PSEN2 KO female mice also experienced prolonged seizure duration: 50.7 ± 3.5 versus 37.8 ± 2.6 seconds in young WT females. Tukey's *post-hoc* analysis also demonstrated that aged female PSEN2 KO mice experienced shorter seizures than young female PSEN2 KO mice (34.9 ± 3.1 seconds; $p = 0.005$). Thus, loss of normal PSEN2 function leads to an age-related difference in kindled seizure duration.

Acute administration of antiseizure drugs dose-dependently reduces seizure severity in kindled PSEN2 KO mice

ASD administration may be a disease-modifying strategy in AD (Vossel et al. 2013), yet few preclinical evaluations are conducted in aged rodents to inform ASD efficacy and tolerability studies in aged patients (M. Barker-Janski 2019; Kelly 2010). We thus sought to establish the pharmacological profile of several mechanistically distinct ASDs to determine whether there is differential anticonvulsant efficacy in young versus aged mice with and without normal PSEN2 function. Despite notable age-dependent differences in kindling acquisition and kindled seizure duration between young PSEN2 KO and WT mice, there was no difference in pharmacosensitivity to the ASDs lamotrigine (LTG) and VPA in young versus aged mice (Figure 4, Figure 5, and Table 2). CBZ demonstrated a marked, age-related increase in potency in young versus aged male PSEN2 KO mice. Nonetheless, these findings are consistent with the efficacy of VPA in the minimal clonic shift assay (Table 1). Specifically, these ASDs were able to dose-dependently attenuate the acute seizure severity of PSEN2 KO CKM to a similar degree as in aged-matched WT mice.

In contrast to the dose-dependent efficacy of VPA, CBZ, and LTG in PSEN2 KO and WT mice at both ages, diazepam (DZP) and levetiracetam (LEV) demonstrated a genotype-dependent reduction in potency within the sexes (Figure 4, males, and Figure 5, females). While an ED50 for DZP was calculated for some of the genotype and age groups, DZP did not consistently or dose-dependently attenuate acute seizure severity. In fact, the ED50 of DZP was determined to exceed the highest dose tested for aged male WT mice (Table 2). Further, LEV demonstrated marked age-related variability in efficacy (Table 2). For example, young male WT mice attained significant seizure control following administration of 30 mg/kg LEV (6/8 protected), whereas the maximum protection in aged WT male mice was 2/8 mice following a 60 mg/kg dose. The potency of LEV in young male PSEN2 KO was low; the maximum observed protection was following a 120 mg/kg dose of LEV (1/8 mice). LEV was also ineffective in aged male PSEN2 KO mice. LEV demonstrated differential efficacy in young versus aged female WT and PSEN2 KO mice. There were no other notable differences in ASD efficacy in CKM with regards to sex. Thus, the acute efficacy of various ASDs against the secondarily generalized kindled seizure was highly divergent within age, sex, and genotype.

It is important to note that doses of the ASDs administered were selected to be below the known motor impairing doses in WT, young-adult CF-1 mice so as to minimize the potential for adverse effects (M. L. Barker-Haliski et al. 2017b; Rowley and White 2010). Nonetheless, we observed significant and uncontrollable tremors following administration of ≥ 17 mg/kg LTG to aged male and female mice, regardless of genotype; an effect that was not apparent in any young adult mice treated with ≥ 17 mg/kg LTG. Thus, aged CKM were more sensitive to LTG than young adult CKM. No other ASDs tested exerted any overt age- or genotype-related dose-dependent adverse effects in this study.

Discussion

Herein, we report that loss of normal PSEN2 function is associated with age-related differential susceptibility to both acute seizures and formation of a hyperexcitable neuronal network *in vivo*. Our findings suggest a role for normal PSEN2 activity on seizure susceptibility that is independent of A β deposition. An important novel finding of this work is that young PSEN2 KO mice demonstrated significant delays in kindling acquisition versus WT age-matched mice, whereas aged PSEN2 KOs that were no different from WT. Minimal clonic seizure threshold was lower in young adult PSEN2 KO mice compared to WT, in contrast to aged mice that showed no effect of genotype. Both young and aged PSEN2 KO mice conversely demonstrated reduced 6 Hz seizure threshold, attesting to the brain region-

specific difference in the mechanisms underlying these focal seizure models (Barton et al. 2001). Minimal clonic seizures engage structures beyond the limbic system (e.g. thalamus, basal ganglia) suggesting that loss of PSEN2 expression may more meaningfully disrupt limbic excitability in an age-related manner. Our findings demonstrate a differential impact of loss of normal PSEN2 function on susceptibility to a single acute seizure versus formation of a chronically hyperexcitable neuronal network. We herein illustrate that PSEN2 is an unexplored age-related molecular contributor to seizure risk, which may underlie the increased prevalence of seizures in patients with PSEN2 variant-associated AD (Jayadev et al. 2010a; Zarea et al. 2016).

Loss of normal PSEN2 function may contribute to AD and neurodegeneration in the absence of direct effects on APP protein expression (Beglopoulos et al. 2004). PSEN2 variants do increase the A β 42:40 ratio, but do not directly induce the expression of APP protein. APP-overexpressing mice experience spontaneous seizures and these seizures are sensitive to ASDs (Sanchez et al. 2012; Ziyatdinova et al. 2011). However, the present studies are the first to demonstrate an age-dependent change in acute seizure susceptibility in the context of loss of normal PSEN2 function. Further, this is the first study to demonstrate any age-related change in susceptibility to kindling acquisition in animals with loss of an AD-associated gene. While other studies have indeed demonstrated a more hyperexcitable phenotype in PSEN2-N141I homozygous mice (Fontana et al. 2017), no study has yet established whether loss of normal PSEN2 expression itself may affect susceptibility to acute seizures and/or formation of a chronically hyperexcitable neuronal network. In this regard, we herein establish a novel moderate-throughput approach to interrogate the increasingly recognized pathological overlap between AD and epilepsy using well-defined evoked seizure models that have been instrumental to ASD development (seizure threshold and CKM; (M. Barker-Haliski 2019)).

The 60 Hz CKM is a well-established model of epileptogenesis and epilepsy (Matagne and Klitgaard 1998) with clear neuroinflammation (Loewen et al. 2016) and cognitive deficits (M. L. Barker-Haliski et al. 2016; Koneval et al. 2018a; Remigio et al. 2017) that is frequently used for ASD discovery (M. L. Barker-Haliski et al. 2017b; Matagne and Klitgaard 1998) and to define how a genetic variant impacts susceptibility to chronic network hyperexcitability (Otto et al. 2009; Singh et al. 2008). Importantly, WT CKM do not exhibit neurodegeneration and microgliosis (Loewen et al. 2016), such that any effects of neuronal death or microglial activation on cell type-specific gene or microRNA expression are likely minimal, unlike other acquired epilepsy models (Henshall 2014). While seizure threshold is known to change with advanced age (Engstrom et al. 1986), to our knowledge, no study has

yet demonstrated an age-related effect on kindling acquisition in genetically modified mice. The present study indicates that loss of normal PSEN2 expression impedes kindling acquisition in young, but not aged, PSEN2 KO mice; the mechanism by which remains to be defined.

Dose-dependent efficacy of ASDs in PSEN2 KO mice

Patients with AD have undiagnosed seizures (Lam et al. 2017b), which may increase the burden of AD (Vossel et al. 2017). ASD administration has been hypothesized to be a disease-modifying strategy in early AD (Vossel et al. 2017) that could extend the period of independent functioning. Yet previous pharmacological studies in AD-associated rodent models were insufficient. First, the ASDs administered to J20 mice to evaluate effects on spontaneous electrographic seizures were not pharmacologically equivalent (Sanchez et al. 2012); the dose of phenytoin was over 17-fold higher than the MES ED₅₀ for this drug, whereas the dose of VPA was just over 1.1-fold greater (Bialer et al. 2004). Such a high dose of phenytoin is known to reduce seizure threshold in mice (Piredda et al. 1985), which could have also confounded interpretation of potential efficacy in a seizure-prone animal. Second, EEG monitoring of single-housed rodents (Sanchez et al. 2012; Ziyatdinova et al. 2011) can produce chronic social isolation stress, which can itself reduce seizure threshold (Swinyard et al. 1963) and provoke spontaneous seizures. While our present study was not designed to address whether spontaneous behavioral or electrographic seizures arise in PSEN2 KO mice in any regard, a primary goal of this work was to demonstrate the feasibility of using moderate-throughput seizures models to evaluate ASD efficacy in an AD-associated neurological substrate at an etiologically-relevant age; a practice that is currently absent in standard ASD discovery (M. Barker-Haliski 2019).

Ours is the first study to demonstrate dose-dependent seizure control with pharmacologically-equivalent doses of ASD standards-of-care in the context of loss of normal PSEN2 function. Loss of normal PSEN2 function does not impact the efficacy of ASDs with sodium channel blocking-mechanisms (i.e. CBZ, LTG, and VPA). Aged WT rodents also did not lose sensitivity to these ASDs (Figure 4 and Figure 5). Further, we demonstrate that VPA, which is a frontline treatment for generalized tonic-clonic and focal seizures in humans, increases the minimal clonic seizure threshold of aged PSEN2 KO mice to the same extent as WT mice (Table 1). Further, CBZ is also frontline therapy for focal and generalized tonic-clonic seizures and LTG is recommended for aged adults with epilepsy (Glauser et al. 2006; Shih et al. 2017). Generalized tonic-clonic seizures are commonly reported in ADAD patients at least once within the 5 years post-diagnosis (Zarea et al. 2016), whereas focal seizures

may be more frequent but their subtle nature makes them difficult to identify. Hyperexcitability and seizures may be a manageable feature of AD (Cretin et al. 2016; Lam et al. 2017a; Vossel et al. 2013): seizures in the elderly are generally not drug-resistant (Hernandez-Ronquillo et al. 2018). Thus, our present study demonstrates that kindled PSEN2 KO mice remain sensitive to CBZ, LTG, and VPA, even with advanced age. These findings for sustained efficacy of sodium channel-blocking ASDs are consistent with prior pharmacological studies (Ziyatdinova et al. 2011; Ziyatdinova et al. 2015) and sodium channel knockdown studies (Hu et al. 2017) in APP-overexpressing mice, suggesting that modulation of sodium channels with ASDs is effective against acute seizures in multiple AD-associated rodent models, regardless of age.

DZP was ineffective in young, and to a lesser degree, aged PSEN2 KO CKM. DZP suppresses seizures through allosteric modulation of GABA_A-receptor channel opening (Twyman et al. 1989), thus loss of normal PSEN2 function may more meaningfully impact inhibitory signaling through a GABAergic mechanism such that DZP is no longer effective in PSEN2 KO, compared to WT, CKM. Neither LEV nor DZP ever attained significant dose-dependent efficacy in PSEN2 KO mice, further attesting to the unique physiological changes associated with loss of normal PSEN2 function on neuronal excitability. The kindled seizures of young, but not aged PSEN2 KO mice were also of significantly longer duration, likely further contributing to the reduced potency of LEV and DZP observed in young mice. One likely reason for the inefficacy of LEV is due to the discrete neuronal compartmentalization of PSEN1 versus PSEN2 proteins in hippocampal neurons (Meckler and Checler 2016). The specific mechanism of LEV remains heavily debated, but it has been widely attributed to the regulation of vesicular release of neurotransmitters through the SV2A protein (M. Barker-Haliski et al. 2014; Lynch et al. 2004; Yang et al. 2007). PSEN2 itself is concentrated within intracellular vesicular structures in hippocampal neurons *in vitro*, and thus loss of normal PSEN2 expression may directly impact the LEV binding site within presynaptic terminals and contribute to the inefficacy of this ASD in aged PSEN2 KO mice.

Potential role for PSEN2 on acute and chronic seizures

Loss of normal PSEN2 function may affect neuronal processes underlying our presently reported age-dependent changes in rate of kindling acquisition. PSEN1 and PSEN2 exert differential (Kaja et al. 2015; Payne et al. 2013; Payne et al. 2015), age-dependent roles on Ca²⁺ signaling (Wu et al. 2013) and vesicular trafficking underlying normal neuronal function (Meckler and Checler 2016). Loss of PSENs

reduce ryanodine receptor (RyR) expression and Ca^{2+} release *in vitro* (Wu et al. 2013). Aging itself also normally modulates RyR expression and generally increases intracellular Ca^{2+} concentrations (Xu et al. 1998). The PSEN2 N-terminal fragment has been shown to increase the dynamic range of RyR-mediated Ca^{2+} release into the toxic range by blocking Ca^{2+} autoinhibition of the RyR. In the context of loss of normal PSEN2 function, PSEN1 may still regulate normal RyR-induced Ca^{2+} release, but only until a certain age, at which point PSEN2 would normally predominate. PSEN2 itself normally regulates the auto-inhibition of RyR-mediated increases in Ca^{2+} concentration. Thus, PSEN2 may be more meaningful on RyR function in late life, whereas PSEN1 is likely more relevant to RyR function in early life, which is further supported by our present findings with the kindling rates of young versus aged PSEN2 KO CKM. Loss of normal PSEN2 function in early age can increase PSEN1 expression in primary microglia (Jayadev et al. 2010b), but whether this also leads to global changes in neuronal PSEN1 expression in an intact organism should be addressed. Corneal kindling itself is not known to influence microglial activation in WT, young-adult mice (Jewen et al. 2016), thus it is unlikely that microglial activation contributed in any regard to the presently observed effects. PSEN2 levels do normally decrease with age, thus our findings that aged PSEN2 KO CKM are no different from WT in their kindling acquisition rate at 8-months-old further emphasize the differential role of PSEN1 and PSEN2 in early versus late age. Nonetheless, these present studies illustrate that loss of normal PSEN2 function may be useful to identify novel age-related mechanisms underlying chronic network hyperexcitability, as well as to further interrogate the relationship between AD and seizures.

Conclusions

Despite the increased risk of comorbid seizures in patients with AD, few studies have been conducted to understand how seizures and susceptibility to epilepsy arise in rodent models deficient in an AD-associated gene and in an age-dependent manner (Palop et al. 2007; Palop and Mucke 2009). This novel study demonstrates how the loss of normal PSEN2 function may contribute to the susceptibility to seizures seen in ADAD patients. The PSEN2 activity in modulating pro-inflammatory cytokines and the age-dependent effect on RyR are compelling factors underlying the connection between seizures and ADAD. We herein contribute significant drug efficacy data of prototype ASDs relevant to aged and ADAD patients, which has been previously underrepresented. We also presently demonstrate a novel, age-dependent role that loss of normal PSEN2 function can play on susceptibility to formation of an epileptic network and risk for acute seizures across the lifespan. The intersection

between AD and epilepsy affords new and, as-yet, untapped opportunities to identify novel molecular targets that may influence ictogenesis and epileptogenesis, as well as define how age and AD-associated risk factors ultimately contribute to the burden of AD.

Funding

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Tables.

Table 1. Shift in minimal clonic seizure threshold of aged (10- to 12-months-old) PSEN2 KO and WT mice 15 minutes after administration of 150 mg/kg valproic acid (VPA; i.p.). Group sizes for seizure threshold determinations were: male WT = 44; female WT = 30; male PSEN2 KO = 24; female PSEN2 KO = 32. CC50 = median convulsant current; CI = confidence interval.

| Sex and Genotype | CC50 and 95% CI (untreated); mA | CC50 and 95% CI (150 mg/kg VPA); mA | Δ CC50 (aged-young); mA |
|------------------|---------------------------------|-------------------------------------|--------------------------------|
| Male WT | 8.22 [7.57 – 8.55] | 10.8 [9.95-11.2] | 2.58 |
| Male KO | 8.05 [7.72-8.33] | 10.8 [9.84-11.3] | 2.75 |
| Female WT | 7.11 [6.55-7.92] | 9.40 [9.10 - 9.63] | 2.29 |
| Female KO | 7.04 [6.41-7.50] | 10.4 [9.13-10.9] | 3.36 |

Table 2. Calculated median effective dose (ED50) of prototype ASDs in young and aged PSEN2 KO mice.

| Sex and Genotype | Young - ED50 and 95% CI; mA | Aged - ED50 and 95% CI; mA | Δ ED50 (aged-young); mA |
|--|------------------------------------|-------------------------------------|--------------------------------|
| <u>Valproic acid (mg/kg [95% CI]) – Sigma Aldrich, catalogue #P4543</u> | | | |
| Male WT | 87.2 [33.3-125] | 186 [120-353] | 98.8 |
| Male PSEN2 KO | 181 [102-381] | 188 [129-365] | 87.0 |
| Female WT | 161 [120-208] | 170 [NC] | 9.00 |
| Female PSEN2 KO | 130 [82.7-176] | 165 [114-337] | 35.0 |
| <u>Carbamazepine (mg/kg [95% CI]) – Sigma Aldrich, catalogue #C4024</u> | | | |
| Male WT | 8.26 [4.35-12.5] | 8.35 [5.15-12.3] | 0.09 |
| Male PSEN2 KO | > 30 (4/8 protected at 30 mg/kg) | 7.56 [4.47-11.2] | < -22.4 |
| Female WT | 18.7 [15.0-27.2] | 10.5 [3.65-68.1] | - 8.2 |
| Female PSEN2 KO | 11.3 [7.50-19.4] | 9.17 [1.0-37.5] | - 2.13 |
| <u>Lamotrigine (mg/kg [95% CI]) – AK Scientific, catalogue #K499</u> | | | |
| Male WT | 8.14 [3.89-23.5] | 16.5 [8.58-29.9] | 8.36 |
| Male PSEN2 KO | 25.5 [13.0-563] | 9.77 [0.28-15.4] | 15.7 |
| Female WT | ~ 30 (4/8 protected at 30 mg/kg) | ~ 30 (4/8 protected at 30 mg/kg) | Not Defined |
| Female PSEN2 KO | 21.3 [14.6-36.5] | 14.1 [8.36-20.2] | - 7.20 |
| <u>Diazepam (mg/kg [95% CI]) – Hospira, NDC-0409-3213-12</u> | | | |
| Male WT | 0.41 [0.67-0.87] | > 2.0 (2/9 protected at 2.0 mg/kg) | > 1.59 |
| Male PSEN2 KO | > 4.0 (3/8 protected at 4.0 mg/kg) | > 2.0 (5/10 protected at 2.0 mg/kg) | < -2.00 |
| Female WT | 0.73 [0.15-1.33] | 1.04 [NC] | 0.31 |

| | | | | |
|--|-------|--|------------------------------------|---------|
| Female KO | PSEN2 | 1.87 [NC] | 1.64 [1.07-6.87] | - 0.23 |
| Levetiracetam (mg/kg [95% CI]) - Tokyo Chemical Industry, L0234 | | | | |
| Male WT | | < 30 mg/kg (6/8 protected at 30 mg/kg) | > 60 (2/8 protected at 60 mg/kg) | > 30 |
| Male PSEN2 KO | | > 120 (1/8 protected at 120 mg/kg) | > 60 (2/8 protected at 60 mg/kg) | > -60.0 |
| Female WT | | > 120 (3/8 protected at 120 mg/kg) | > 60 (3/7 protected at 60 mg/kg) | > -60.0 |
| Female KO | PSEN2 | Max protection at 60 mg/kg (5/8 protected) | > 120 (2/8 protected at 120 mg/kg) | < 60.0 |

Figures

Figure 1. Male and female wild-type (WT) and PSEN2 knockout (PSEN2 KO) mice generally demonstrate an age-dependent increase in minimal clonic (A, C) and 6 Hz seizure threshold (B, D), but susceptibility to these specific focal seizure induction parameters differs with age and genotype. Data are expressed as the median convulsant current (CC50) with 95% confidence intervals (CI) for each age.

A) The threshold for a minimal clonic seizure, a model of forebrain seizures, of male PSEN2 KO mice is significantly less than the threshold of male WT mice at 4-months-old (PSEN2 KO CC50: 5.66 mA [CI: 5.13-6.24]; WT CC50: 7.54 mA [6.91-8.36]), but this difference is lost with advanced age. B) The threshold for a 6 Hz seizure, a model of limbic seizures, of male PSEN2 KO mice is significantly less than WT mice in both early (2-months-old) and late life (8-months-old). The threshold in 2-month-old PSEN2 KO male mice was 32.8 mA [30.3-35.8] versus 2-month-old WT male mice, which was 42.5 mA [38.6-45.7]. By 8-months-old, the 6 Hz seizure threshold was again different between genotypes; PSEN2 KO: 44.5 mA [42.2-49.5] versus WT: 55.3 mA [52.0-59.4]. C) The threshold for a minimal clonic seizure of female PSEN2 KO mice is significantly less than the threshold of male WT mice at 4-months-old (PSEN2 KO CC50: 7.06 [6.70-7.41], WT CC50: 7.51 mA [7.06-7.91]), but this difference is lost with advanced age. D) The threshold for a 6 Hz seizure of female PSEN2 KO mice is only significantly less than WT in late life (8-month-old): PSEN2 KO: 41.6 mA [40.2-42.7] versus WT: 49.9 mA [47.8-51.9].

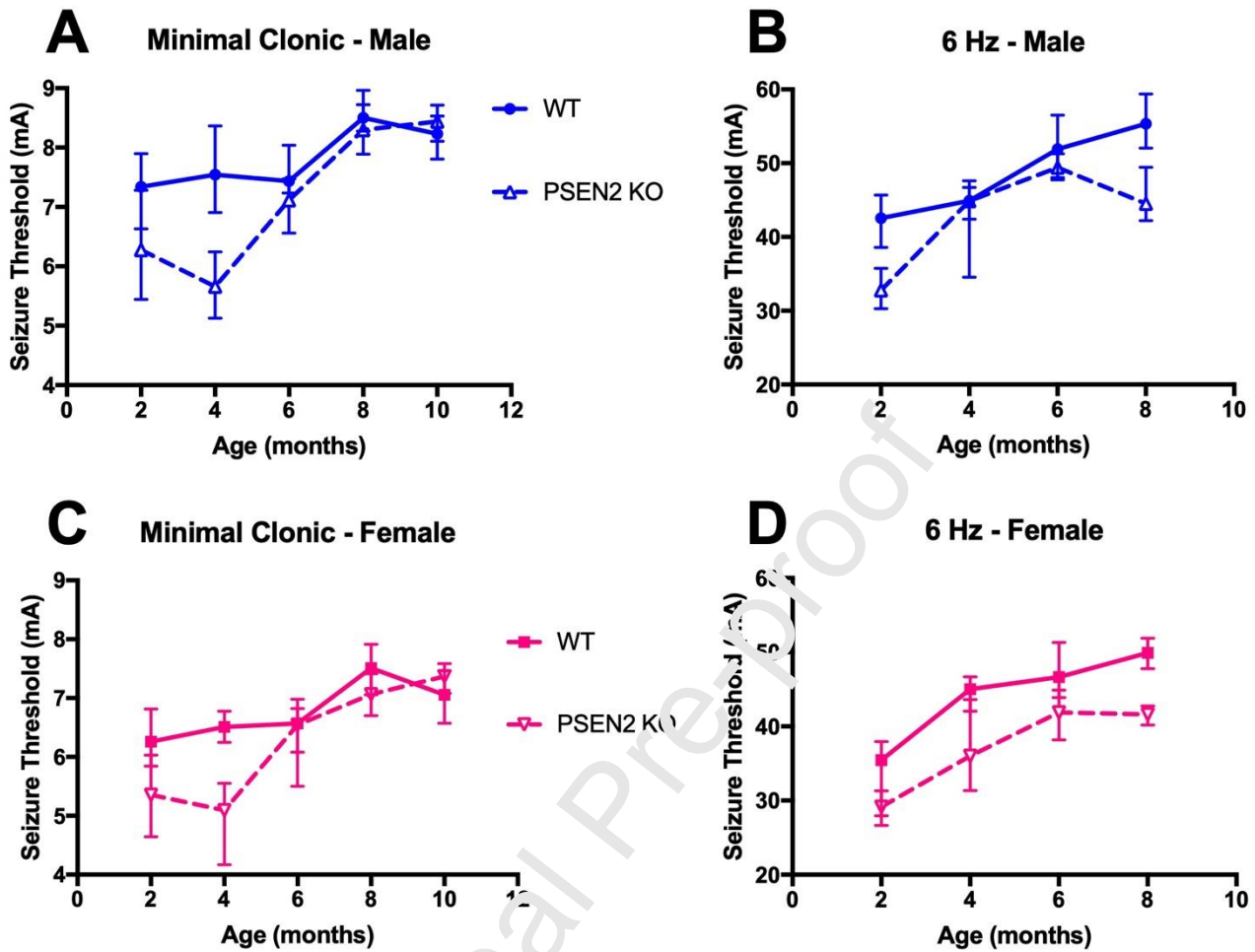


Figure 2. Corneal kindling acquisition rates of young A) male and B) female wild-type (WT) and PSEN2 KO mice. Corneal kindling is a well-established preclinical model of epilepsy that is induced by an initially benign electrical stimulus that elicits secondarily generalized Racine stage 5 seizures after 2-4 weeks of 2x/day corneal stimulation. Kindling criterion is defined a presentation of five consecutive Racine stage 5 seizures. A and B) Young (2-months-old) PSEN2 KO mice demonstrate significant delays in acquisition of the fully kindled state relative to WT mice. * indicates first stimulation session when significant differences in kindled seizure score are apparent, $p < 0.05$. Corneal kindling acquisition in aged C) male and B) female WT and PSEN2 KO mice is not impacted by genotype. The total number of stimulation sessions needed to achieve kindling criterion is significantly increased in young PSEN2 KO E) male and F) female mice relative to age-matched WT mice, * indicates significant difference from age-matched WT mice, $p < 0.0001$. There is no significant difference in the number of stimulations sessions necessary to achieve criterion in aged E) male and F) female mice. However, there is a significant age-dependent decrease in the number of kindling sessions to achieve criterion in female WT and PSEN2 KO mice, but this age-related decrease is only apparent in PSEN2 KO male mice. # indicates significantly different from genotype-matched young mice, $p < 0.0001$.

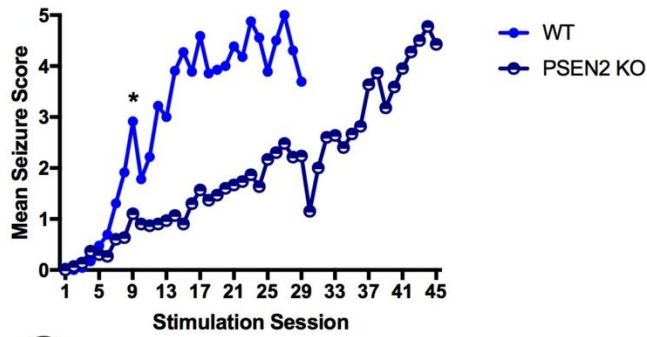
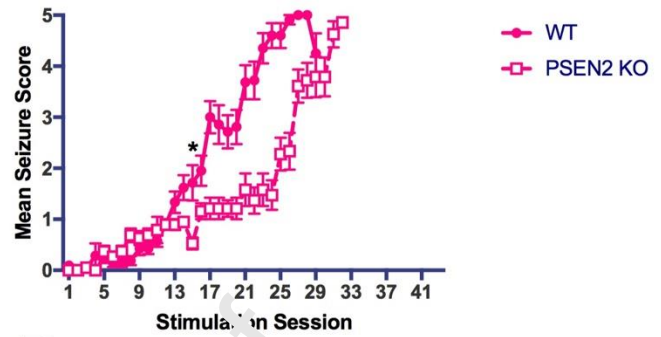
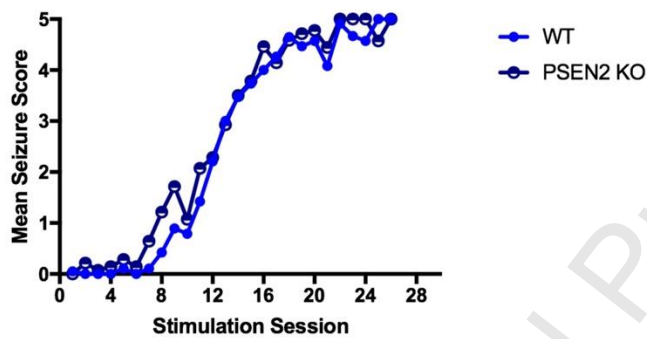
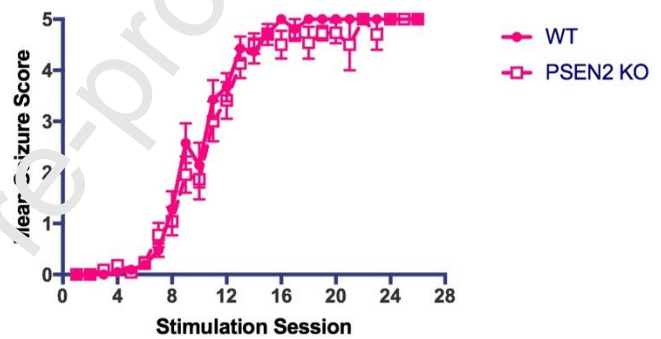
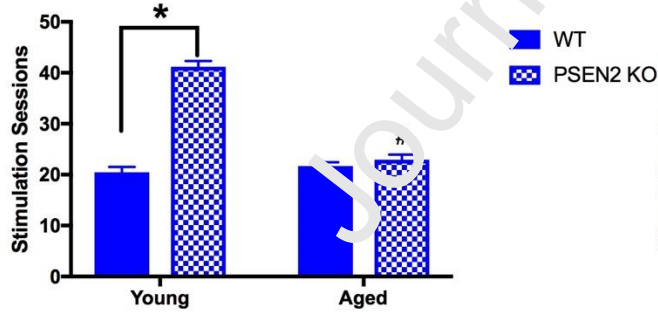
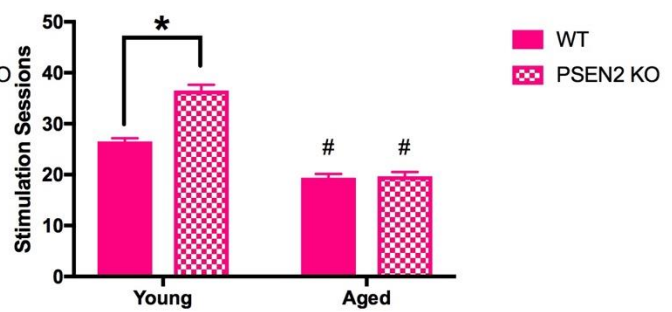
A**Male Kindling Acquisition - 2 months****B****Female Kindling Acquisition - 2 months****C****Male Kindling Acquisition - 8 months****D****Female Kindling Acquisition - 8 months****E****Sessions to Criterion - Male****F****Sessions to Criterion - Female**

Figure 3. The duration of the kindled behavioral seizure and post-ictal behavioral arrest was recorded from all fully-kindled mice following a 5-day stimulation-free period after the conclusion of the kindling acquisition period. Behavioral seizure duration included the time to initiation of a Racine stage 5 seizure, actual stage 5 seizure, and post-ictal immobility until the mouse returned to normal exploratory behavior on the tabletop (e.g. forepaws on the tabletop, grooming, ambulation, responsive to external stimulation, etc.). The timer was stopped if a mouse did not resume normal behaviors after 120 sec. There was a significant increase in the duration of time from corneal stimulation to resumption of normal exploratory behavior in young, but not aged, A) male and B) female PSEN2 KO mice versus age-matched WT mice (* indicates significantly different from WT, $p < 0.05$). There was no such significant difference in the duration of behavioral arrest in aged mice.

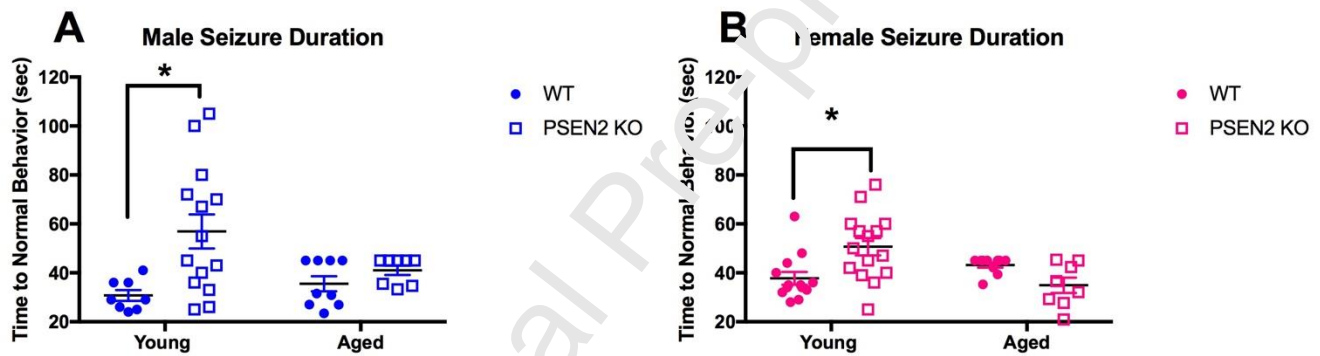
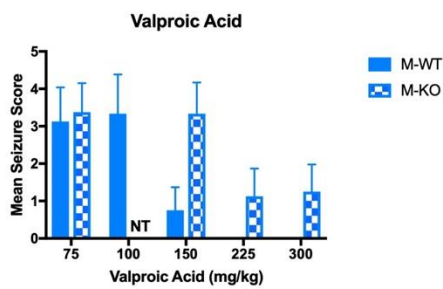
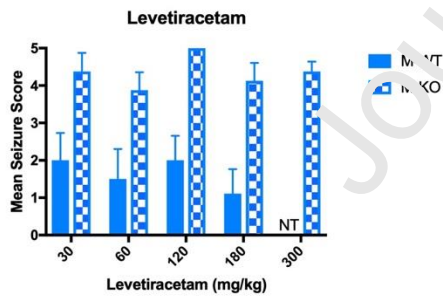
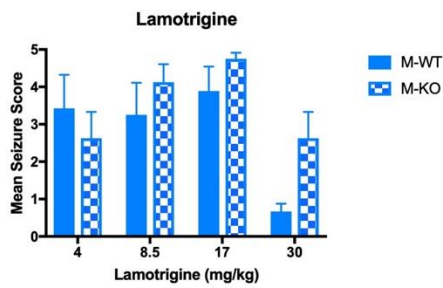
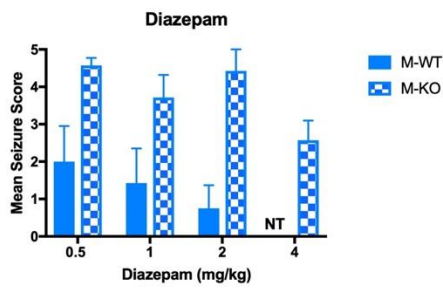
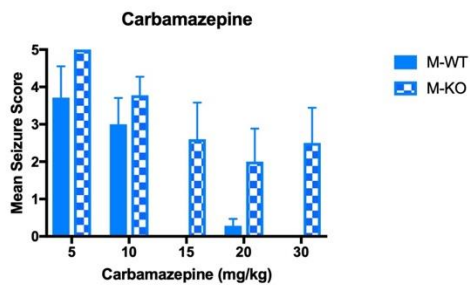


Figure 4. Several mechanistically-distinct prototype antiseizure drugs demonstrated dose-dependent efficacy against secondarily generalized focal seizure in young and old PSEN2 KO versus WT male mice. Carbamazepine, lamotrigine, and valproic acid demonstrated consistent and dose-dependent efficacy against corneal kindled seizures in both young and aged PSEN2 KO male mice that generally matched the activity for each compound in WT male mice. Diazepam and levetiracetam were ineffective at the doses tested in young male PSEN2 KO mice, whereas both ASDs demonstrated dose-dependent attenuation of kindled seizure severity in young male WT mice. At the doses tested, neither diazepam nor levetiracetam exerted dose-dependent reductions in mean seizure score in aged male PSEN2 KO or WT mice. Aged male mice treated with ≥ 17 mg/kg (i.p.) lamotrigine displayed prominent tremors regardless of genotype. NT = dose not tested.

2-months-old



8-months-old

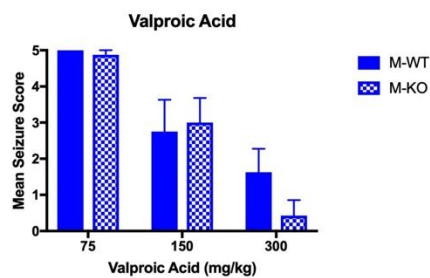
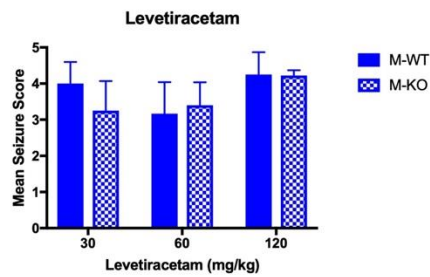
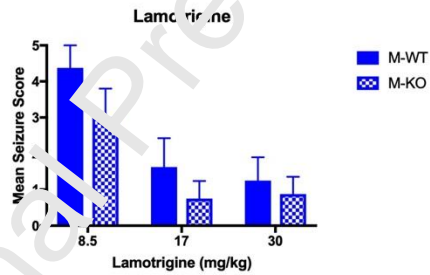
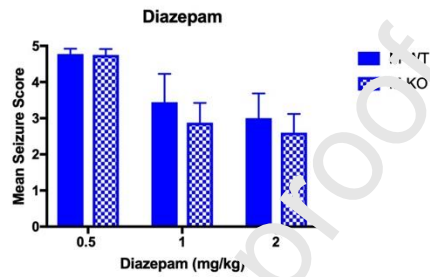
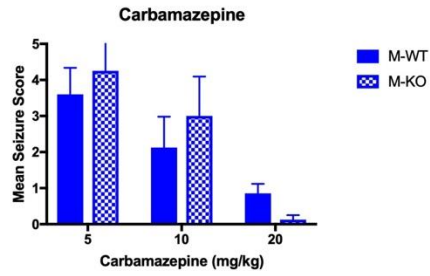
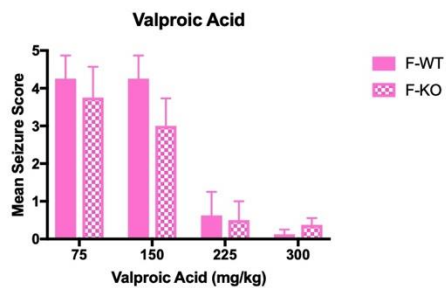
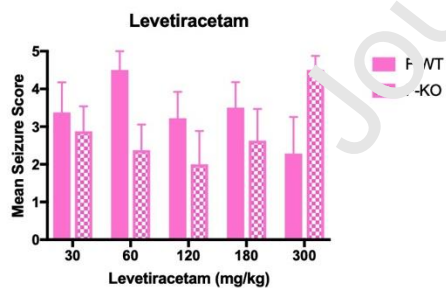
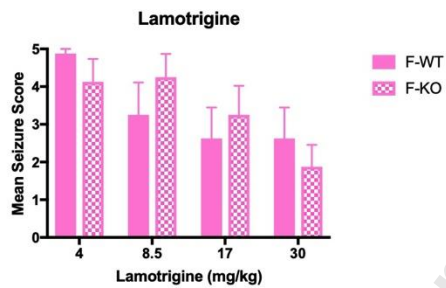
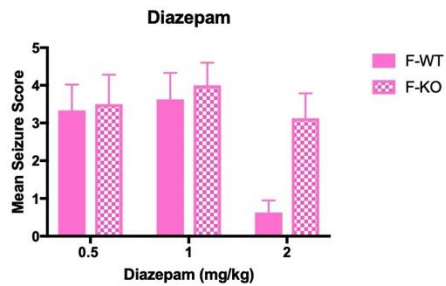
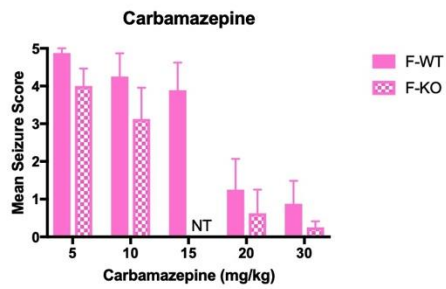
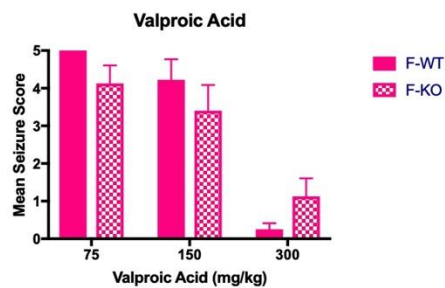
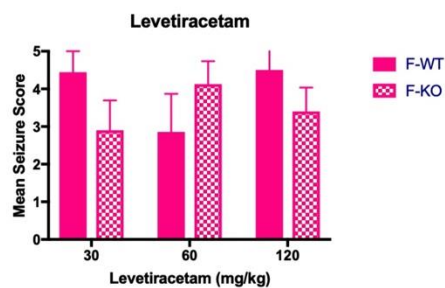
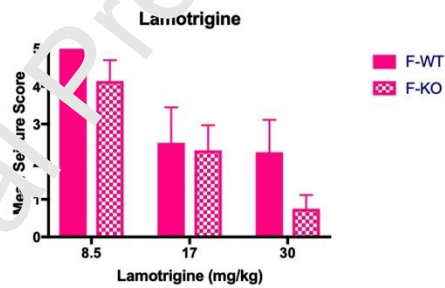
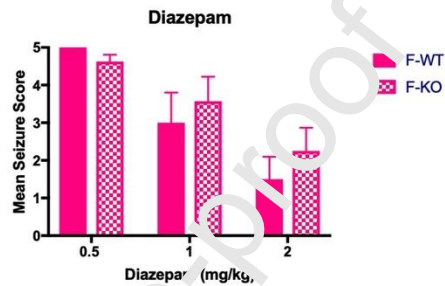
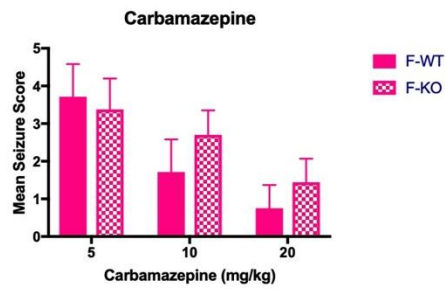


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2-months-old



8-months-old



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Highlights

- Alzheimer's disease and epilepsy share pathological similarities that need study.
- Seizures in PSEN2 KO mice were used to explore the AD and epilepsy relationship.
- Young adult PSEN2 KO mice were resistant to corneal kindling acquisition.
- PSEN2 KO mice had age-related changes in sensitivity to anticonvulsant drugs.
- PSEN2 is an underexplored molecular contributor to seizures and hyperexcitability.

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