Title: Chronic seizure-induced serotonin pathway dysregulation coincides with mortality in Alzheimer's disease mouse models

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Abstract

Objective: People with early-onset Alzheimer's disease (AD) with amyloid precursor protein (APP) duplications or presenilin (PSEN) variants are at elevated seizure risk within 5 years of diagnosis. Further, seizures in AD may increase neuropsychiatric comorbidity burden. We thus hypothesized that disruptions in serotonin pathway-related protein expression was impacted by 60 Hz corneal kindled seizures in an AD-genotype-related manner.

Methods: Male and female 2-3-month-old APP/PS1, PSEN2-N141I, and respective transgenic (Tg) control mice underwent corneal or sham kindling for 2 weeks until reaching kindling criterion defined as five consecutive Racine stage 5 seizures. Chronic seizure-induced changes in serotonin pathway protein expression in hippocampus were then quantified by western blot.

Results: Young female APP/PS1 mice kindled significantly faster than Tg- controls, whereas PSEN2-N141I mice kindled no differently versus their Tg controls. APP/PS1 mice subjected to corneal kindling were at extremely elevated mortality risk relative to kindled Tg- controls, as well as sham-kindled APP/PS1 and Tg- control mice, whereas PSEN2-N141I mice were not adversely affected by kindling. Kindled APP/PS1 mice demonstrated a marked downregulation of hippocampal tryptophan hydroxylase 2 and monoamine oxidase A protein expression versus kindled Tg- and sham-kindled APP/PS1 groups. Serotonin pathway protein expression in PSEN2-N141I mice was unchanged from Tg. Importantly, all changes in serotonin pathway protein expression in kindled APP/PS1 mice occurred in the absence of amyloid β (Aβ) deposition.

Significance: The co-occurrence of seizures in the APP/PS1 mouse is sufficient to evoke unexpected mortality well ahead of pathological Aβ deposition synonymous with a symptomatic AD model. The presence of another AD-associated variant (PSEN2-N141) does not lead to seizure-induced mortality, suggesting that AD-associated risk genes differentially influence vulnerability to chronic seizure-associated mortality. Further, disruptions in serotonin pathway synthesis coincide with heightened mortality risk exclusively in adult APP/PS1 mice, suggesting a possible non-canonical sudden unexpected death in epilepsy (SUDEP)-related phenotype.

Key Words: SUDEP; tryptophan hydroxylase 2; monoamine oxidase A; amyloid; corneal kindling; epilepsy

Key points (3-5 total):

- Sudden unexpected death is a devasting consequence of uncontrolled epilepsy
- Serotonin pathway dysfunction may increase risk of premature mortality in epilepsy
- APP/PS1 mice are at higher risk of seizure-induced mortality versus other AD models
- Seizures downregulate hippocampal serotonin pathway proteins solely in APP/PS1 mice
- Seizure-induced mortality in APP/PS1 mice does not require amyloid β accumulation

Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disease [1] characterized by the accumulation of a pathological protein hallmark: amyloid β (Aβ). Undiagnosed and unprovoked seizures occur 8 to 10 times more frequently in people with AD compared to unaffected age-matched adults [2-5]. The relative risk of unprovoked seizures is particularly high in individuals with autosomal dominant early-onset Alzheimer's disease (EOAD; [1,6-8]). EOAD can arise due to duplications or genetic variance in three key deterministic risk genes – amyloid precursor protein (*APP*), and the homologous presenilin 1 (*PSEN1*) and 2 (*PSEN2*) genes. Untreated seizures may accelerate AD-associated cognitive decline and neuropathological hallmarks [9]. Concerningly, mortality in individuals with epilepsy and coincident AD has increased 200% in recent years even though epilepsy-related mortality alone has decreased in this same time [2]. Thus, excessive neuronal hyperexcitability and comorbid seizures in individuals with AD may promote conserved pathological mechanisms that increase the neuropsychiatric and behavioral burden of disease, as well as exacerbate mortality risk.

Sudden unexpected death in epilepsy (SUDEP) is a rare, but serious, potential outcome in people with epilepsy. SUDEP occurs most commonly in adults with epilepsy [10], with an incidence of 0.22-1.11 per 1000 person-years in children versus 0.35 to 6 per 1000 person-year in adults [10]. SUDEP accounts for up to 50% of deaths in individuals with treatment refractory epilepsy, and up to 17% of deaths in all individuals with epilepsy [10-12]. Primary SUDEP risk factors include frequent generalized tonic-clonic seizures (GTCS), polytherapy, and early onset, long duration of epilepsy history [10-13]. The etiology of SUDEP is poorly understood but may involve dysregulation of the cardiac, respiratory, and autonomic nervous systems because of chronic seizures[10,14-16]. Traditional preclinical SUDEP models reflect genetic risk-factors associated with epilepsy, including *Scn1a*$^{+/-}$ or *KCN1a null* models [16]. However, evidence from a diversity of SUDEP models consistently suggests that a genetic or acquired defect in the serotonin (5-HT) system increases SUDEP risk [14,16]. 5-HT neurons project to all the major respiratory nuclei [17,18]. They regulate many brain functions critical to post-ictal respiratory control, such as stimulating respiratory output [16], by acting as chemoreceptors that respond to an increase in systemic CO2 levels [16], and by enabling plasticity of the respiratory network in response to conditions such as by intermittent hypoxia [15,16,19]. Lesions of 5-HT neurons can decrease respiratory output. Thus, 5-HT dysregulation may increase propensity for SUDEP susceptibility secondary to GTCS due to decreased autoresuscitation and respiratory drive, leading to cardiorespiratory failure [15,16,19].

Postmortem AD studies also show reduced CNS 5-HT levels [20-23], including in the dorsal raphe nucleus and hippocampus (HPC) [23]. Advanced age (18-24-month-old) APP/PS1 mice have decreased serotonin transporter (SERT) density in the parietal and frontal cortex and Aβ40 itself can further reduce the activity of SERT [21]. Additionally, deletion of tryptophan hydroxylase 2 (TPH2) expression in the AD-associated APP/PS1 mouse disproportionally increases mortality, suggesting an understudied interaction between AD-related genotypes and dysregulation of 5-HT synthesis that increases the risk for premature mortality [20]. These prior clinical and preclinical studies have largely focused on older individuals at symptomatic stages, i.e., the time when cognitive symptoms are present and dense core Aβ plaques are evident. No study has yet assessed the seizure-induced changes in 5-HT system regulation prior to Aβ plaques deposition to further uncover the bidirectional interactions between seizures and AD risk factors on susceptibility to premature mortality.

While APP/PS1 models are frequently used to assess the functional and pathological impacts of spontaneous seizures in AD, PSEN2 variant models are also useful to assess the biological heterogeneity of AD pathology [24-26], including seizure risk [6]. Individuals with the most common PSEN2 variant, N141I, experience seizures and this variant may increase inflammatory response [24 25] that

could influence seizure susceptibility. PSEN2 variant models are also relevant to study the non-neuronal contributions to AD without Aβ plaque accumulation [24]. We have previously demonstrated that young APP/PS1 mice establish a hyperexcitable neuronal network faster than non-transgenic mice and that chronic seizures increase unintended mortality [27]. We thus quantified the seizure susceptibility and seizure-induced 5-HT system changes in young-adult (2-3 months-old) APP/PS1 and PSEN2-N141I EOAD mouse models to define whether chronic seizures evoked prior to potential neuropathological alterations conferred genotype-specific effects on seizure susceptibility, mortality, and 5-HT system regulation [24]. While APP/PS1 mice are known to demonstrate modest increases in premature mortality in later life [2928], we hypothesized that evoked seizures and accompanying accelerated mortality in pre-symptomatic stages reflected potential dysfunction in 5-HT system regulation in a manner consistent with that which is observed in genetic epilepsy models with a SUDEP phenotype. Our present study thus assessed the burden of chronic seizures and AD-associated genotypes on 5-HT system function and SUDEP risk. This study further reveals the functional and pathological burden of chronic hyperexcitability in the setting of AD-associated genotypes.

Material and Methods

Animals: Male APP/PS1 mice (strain 034832-JAX) were obtained from Dr. Gwenn Garden from stock originally purchased from the Jackson Laboratory. PCR-confirmed male APP/PS1 mice were bred to wild-type female C57Bl/6J mice. PSEN2-N141I transgenic mice and their non-excised transgene controls were obtained from Dr. Suman Jayadev, with breeding as previously described [24].

Mice were housed on a 14:10 light cycle (on at 6 h00; off at 20 h00) in ventilated cages with free access to food and water. Housing conditions conformed to 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 (protocol 4387-01) and conformed to ARRIVE guidelines. All behavioral testing was performed between 9 h00 and 17 h00 by an experimenter blinded to genotype.

Corneal kindling: Young adult male and female (2-months-old at kindling initiation) APP/PS1 and PSEN2-N141I versus their respective transgene negative (Tg-) control mice were corneal kindled with a 3 sec, 60 Hz 1.6-2.0 mA bilateral sinusoidal pulse twice per day for 2-3 weeks, consistent with our prior reports [6,29] and illustrated in Figure supplementary 1. Sham kindled mice of each genotype were similarly handled at each twice daily stimulation session, but no electrical current was delivered. Group sizes are stated in Table S1. Stimulation intensity was based on the subconvulsive minimal clonic seizure thresholds for age-, sex- and genotype-matched mice (*unpublished*).

Kindled seizure severity was scored by an investigator blinded to genotype according to the Racine scale [30]. The total number of mice for each group to achieve the fully kindling criterion of 5 consecutive Racine stage 5 seizures is detailed in Supplemental Table 1.

Preparation of biological samples: Mice were euthanized by live decapitation 24-72 hours after reaching corneal kindling criterion. Brain was rapidly excised and hemisectioned along the sagittal plane. HPC was microdissected and flash frozen to evaluate the protein changes secondary to AD genotype and/or corneal kindling via western blot (WB).

Western blot: For WB, samples were homogenized in Tissue Protein Extraction Reagent (TPER) [10 mL/g] and protease inhibitor cocktail (Millipore Sigma) [10 µL/mL]. The frequency used was 50 oscillations for 4 min, followed by centrifugation at 12 000× g for 10 min at 4°C. Total protein levels of

the area of interest were measured using the BCA method based on the principle of protein-dye binding. After adjusting protein levels following the BCA protocol, homogenates were mixed with Laemmli sample buffer (Bio-Rad®) containing β-mercaptoethanol (50 µL/mL of Laemmli), to get a final concentration of 1 mg/mL, and 20 uL was loaded into an electrophoresis gel. Proteins were blotted onto a nitrocellulose membrane (Bio-Rad®) with a dry transfer system (Bio-Rad), incubated with specific primary antibodies (Table S2). After that, each membrane was incubated with their respective secondary antibody and protein bands of interest revealed by use of the AP-conjugate colorimetric protocol. Blots were digitized and band intensities quantified by densitometric analysis using ImageJ (NIH ImageJ® software, National Biosciences, Lincoln, NE, USA). Raw densitometric data in different blots were transformed as fold change of the control mean, expressed in arbitrary units of OD, and Ponceau red was used as loading controls [31].

Statistical analysis: The sample sizes in the different experimental groups were always ≥5 (Supplemental Table 1). Mean seizure score during the corneal kindling acquisition period was assessed within APP/PS1 and PSEN2-N141I transgenic mice and matched Tg controls using Friedman's test. Latency to attain corneal kindling criterion and survival rate during kindling were assessed using a Mantel-Cox Log-Rank test and presented as a Kaplan-Meier curve. Seizure burden (the average of the seizure score until the end of the study) and the number of sessions to achieve the kindled criterion were assessed by a T-test and Welch correction. WB data was assessed using one-way or two-way ANOVA followed by the Student-Newman-Keuls test or the Bonferroni test, as appropriate, using GraphPad Prism, version 8.0 or later (GraphPad Software, San Diego, CA, USA). Statistical significance was defined as $p < 0.05$ for all tests.

Results

Young Female APP/PS1 mice are highly susceptible to 60 Hz kindled seizures

A primary goal of this study was to compare the 60 Hz kindled seizure susceptibility in two AD mouse models prior to the age when Aβ accumulation is widespread, thereby extending our earlier published works with PSEN2 null [6] and APP/PS1 [32] mice. Two-month-old APP/PS1 females kindled significantly faster compared to their respective Tg- littermates (Figure 1A, B). Moreover, APP/PS1 females also showed a 20% increase in seizure burden (Figure 1C) and needed significantly fewer sessions to achieve kindling criterion (Figure 1D). Young APP/PS1 males did not show accelerated kindling acquisition versus their respective Tg- littermates (Figure 1E, F). In accordance with this result, APP/PS1 males did not exhibit any differences in seizure burden (Figure 1G) and number of sessions to achieve criterion (Figure 1H). Thus, there were marked sex-related differences in corneal kindling rates in young APP/PS1 mice.

The PSEN2-N141I model of AD is associated with a pro-inflammatory microglial response following inflammatory stimulus, such as LPS challenge [24], causing us to hypothesize that these mice would have greater susceptibility to kindling due to the impacts of altered microglial phenotype and reactivity on seizure risk [33]. Contrary to our hypothesis, both male and female PSEN2-N141 mice did not show any significant variation in kindling acquisition rate versus their respective Tg- control mice (Figure 1I-J, M-N). There were also no significant changes in the seizure burden and stimulation sessions to achieve kindling criterion in either male or female PSEN2-N141I mice relative to Tg- control mice (Figure 1K-L, O-P). Thus, kindled seizure susceptibility is highly genotype-specific at pre-symptomatic stages and this

impact is particularly more evident in female APP/PS1 mice, aligning with our earlier published studies using the 6 Hz kindling protocol [27].

Young APP/PS1 mice are at higher mortality risk during and after corneal kindling acquisition

While assessing the genotype-related differences in kindling acquisition, we also encountered significant and unanticipated increases in mortality solely in young APP/PS1 mice (Figure 2). While it is well-documented that APP/PS1 mice can occasionally succumb to spontaneous seizure-induced mortality [4], prior reports only documented this mortality at ages when Aβ accumulation becomes evident after 4.5-months of age, suggesting that it is the plaque deposition leading to hyperexcitability that drives this premature mortality. Our present study demonstrated that evoked seizures in young APP/PS1 mice, at an age without Aβ accumulation, can alone provoke significant premature mortality (Figure 2A, D). Specifically, genotype and sex-dependent changes in mortality were detected. Nearly 70% of APP/PS1 females died during the kindling process, whereas no Tg- females died during this time (Figure 2A). APP/PS1 males also showed significantly increased mortality compared with their respective Tg- controls. In contrast with the females, this mortality occurred after male mice achieved the fully kindled state (Figure 2D). These data correlate with the seizure susceptibility explained above, where the combination of the APP/PS1 genotype and evoked seizures is sex- and time-dependent, being faster in females than in males. Further corroborating the hypothesis that hyperexcitability preceding aberrant Aβ processing can provoke mortality, we did not observe any significant differences in mortality in both male and female PSEN2-N141I variant versus Tg- control mice (Figure 2). These results reinforce the hypothesis that hyperexcitability in discrete AD-related genotypes adversely provokes premature mortality prior to Aβ accumulation.

Chronic evoked seizures are associated with 5-HT pathway dysregulation in young APP/PS1 mice

Considering the observations of extensive and inducible mortality in young APP/PS1 mice subjected to 60 Hz corneal kindling, as well as our earlier similar mortality findings with the 6 Hz kindling model [32], we hypothesized that this outcome could be linked to possible changes in molecular pathways associated with SUDEP risk in epilepsy. The 5-HT pathway may represent a critical component of the pathology of both epilepsy and AD, and 5-HT system dysfunction is heavily implicated in the pathobiology of SUDEP in epilepsy [15]. To understand the biological underpinnings of premature mortality in kindled APP/PS1 mice, we quantified the protein changes in several relevant components of the 5-HT pathway in HPC before (sham), and after corneal kindling acquisition (kindled). Young APP/PS1 males and females showed significant downregulation of TPH2 levels after they achieved the fully kindled state compared with the Tg- and the APP/PS1 sham group (Figure 3A). Furthermore, APP/PS1 fully kindled mice showed a reduction of MAOA expression compared to the sham group (Figure 3G). No protein changes were appreciable in the 5-HT1A and SERT markers in either sex (Figure 3C-F). Furthermore, PSEN2-N141I mice did not show any 5-HT related-protein changes compared with the Tg control (Figure 3). Thus, 5-HT expression in the HPC showed seizure-induced changes dependent on APP/PS1 genotype. These findings suggest that chronic seizures in the setting of the amyloidogenic model, APP/PS1, but not the accumulation of Aβ itself, drives a negative cascade of serotonergic system dysfunction to precipitate premature seizure-induced mortality, reflective of a potential non-canonical SUDEP model.

Presenilin 2 protein expression is downregulated in APP/PS1 mice and upregulated in PSEN2-N141I mice regardless of kindling status

Chronic seizures are known to negatively affect cognitive function and accelerate AD progression [1]. We therefore wanted to assess the impacts of evoked chronic seizures on AD-related protein changes in

young mice of both genotypes to ascertain the degree to which seizures differentially impact AD-associated pathology. Protein changes in HPC were genotype dependent. Although we did not observe significant changes in PS1 expression (Figure 4A-B), PS2 expression showed opposite seizure-independent changes within each mouse genotype. APP/PS1 mice showed a downregulation of the PS2 protein independent of corneal kindling (Figure 4C). Moreover, PS2 expression was increased in PSEN2-N141I mice regardless of kindling status (Figure 4D). No significant changes were appreciated in Aβ protein levels in any genotype relative to matched controls (Figure 4), confirming that our study was conducted prior to Aβ protein accumulation. Thus, these findings suggest that chronic seizures evoked in discrete AD-associated genotypes do not alone alter AD-related protein expression in young mice. However, we report differential and opposite genotype-specific changes in PS2 protein expression, suggesting that overexpression of this protein may protect against neuronal hyperexcitability that warrants further detailed study.

APP/PS1 young mice exhibit a decoupling of the serotonin pathway genotype-dependent

5-HT pathway has been involved in the pathology of epilepsy and SUDEP. Only the young APP/PS1 mice exhibited changes in the protein expression of the TPH2 and the MAOA in the isolated HPC. We evaluated if the changes in any of the measure elements of the 5-HT pathway were correlated (Figure 5). The sham kindled Tg- showed a direct correlation between the TPH2 and the MAOA protein expression (Figure 5A). However, neither of these proteins showed significant correlation with the SERT expression (Figure 5E, I). APP/PS1 mice did not show any correlation between any of the 5-HT proteins (Figure 5B, D, F, H, J, K). These results suggest that at pre-symptomatic stages, these animals exhibit a decoupling between 5-HT synthesis and metabolism dependent of the genotype.

Discussion

Although seizures can often present in people with AD, their causative mechanisms are still relatively unknown. It is likely that untreated focal seizures negatively affect the cognitive and neuropsychiatric burden of AD [1]. AD patients with seizures have elevated mortality compared to those without epilepsy [34]. Our present study further suggests that chronic seizures adversely affect survival in individuals with specific AD-associated risk factors. In this work, we quantified the susceptibility to seizures and their sequelae in two murine models of EOAD at an age well-before Aβ deposition [35]. APP/PS1 mice were highly susceptible to 60 Hz corneal kindling and mortality versus Tg- littermate mice. These effects were not observed in PSEN2-N141I mice when compared to their matched Tg controls. These present findings are reminiscent of earlier work demonstrating greater susceptibility of young APP/PS1 and 3xTg mice to 6 Hz kindling [27] and of aged Tg2576 mice to amygdala kindling [36], but further illustrates stark differences in kindling susceptibility of mice with PSEN2 variants or loss of normal function [6].Further, we herein illustrate that expression of an AD-associated genotype alone does not drive seizure-induced mortality risk and that this mortality can arise well-before Aβ deposition is detected. Interestingly, our present study demonstrated that kindled seizures greatly influenced the expression of 5-HT synthesis and degradation enzymes exclusively in the isolated HPC of APP/PS1 mice, suggesting possible deficits in 5-HT release as a result. One major critical finding of this work is thus that the pre-symptomatic APP/PS1 model subjected to an evoked chronic seizure presents a mortality phenotype and 5-HT release deficit reminiscent of canonical SUDEP models [37,38]. We lastly demonstrate that these two AD models showed innate differences in the expression of the PS2 protein, revealing a potentially novel modifier of vulnerability to seizures and formation of a hyperexcitable neuronal network that warrants further study. Altogether, our present study reveals substantial differences in the functional

and molecular impacts of kindled seizures in young mice with AD-associated genotypes to further expand on the understanding of biological impacts of seizures in AD.

Our present study reveals extensive seizure-induced mortality and dysregulation in 5-HT pathway protein expression exclusively in APP/PS1 mice and suggests a possible novel and inducible SUDEP model that is not associated with an epilepsy-linked ion channel variant (e.g., *KCN1a* null or *Scn1a*$^{+/-}$ mice [37,38]). 5-HT is one of the most promising targets driving SUDEP risk [15]. There are alterations in the 5-HT pathway in DBA1/2 mice, another established model of SUDEP [39]. Additionally, mice lacking the 5HT2C receptor have high mortality [14] and 5-HT agonists, such as fenfluramine, may prevent seizure-induced preclinical and clinical mortality [11,40]. Thus, 5-HT pathway disruptions confer mortality risk in epilepsy. Interestingly, APP/PS1 mice in our study did not die in the immediate 10-15 minute window after the transcorneal stimulus. All mice were found dead in their respective cages the following day, suggesting spontaneous nighttime mortality following a GTCS. Despite the SUDEP phenomenon usually happening during the night in people with epilepsy [15], this phenotype is not commonly described in animal studies. Numerous mechanisms may increase nocturnal SUDEP risk, including reduced nighttime supervision [15]. However, 5-HT levels are also reduced during the night in mice [41]. Additionally, the combination of corneal kindling and APP/PS1 spontaneous seizures may exacerbate the chronic and acute modulation of nocturnal 5-HT release. Our present study thus reproduces one of the most salient features of clinical SUDEP. As a result, it suggests this model may be a useful inducible platform on which to reliably expand our understanding of the overlapping chronic mechanisms that promote seizure-induced mortality in people with uncontrolled repeated seizures.

AD also promotes 5-HT pathway dysregulation, possibly due to excessive neuronal hyperexcitability. As in AD patients, spontaneous seizures occur in APP/PS1 mice [2,42]. Emerging clinical evidence would also suggest there is heightened mortality risk in people with dementia and AD and comorbid seizures [2,34]. Unlike prior preclinical studies in APP/PS1 mice, ours is the first to demonstrate such overt and inducible seizure-evoked mortality before Aβ deposition, in mice aged equivalent to a ~25 year-old human, when the incidence of SUDEP is highest [10]. 5-HT imbalance has been also reported in old APP/PS1 mice at symptomatic stages [22] and APP/PS1 mice lacking the TPH2 enzyme also demonstrate heightened mortality [20]. Tph2 inactivation influences APP processing, at least in the HPC [20], although levels of hippocampal Aβ were unchanged in our study. However, seizure-induced 5-HT pathway disruptions at pre-symptomatic stages have never been evaluated in AD-associated models. We found that reduced TPH2 expression coincided with chronic seizures and increased mortality exclusively in kindled APP/PS1 mice. TPH2 downregulation secondary to seizures has been previously reported in other epilepsy models characterized by a SUDEP phenotype, such as the DBA/1 mice [43]. Hippocampal MAOA protein expression was similarly affected by kindling only in APP/PS1 mice. MAOA downregulation may reflect a compensatory effect secondary to the reduction in 5-HT levels or seizure-induced inflammation leading to reduced MAOA levels [44]. Further, TPH2 and MAOA expression correlated to SERT expression only in control mice with seizures, suggesting a decoupling of 5-HT release, transport, and metabolism solely in kindled APP/PS1 mice (Figure 5). Cumulatively, our data indicate that the combination of APP/PS1 genotype and chronic seizures leads to significant reductions in 5-HT synthesis and metabolism enzymes prior to pathological Aβ deposition, pointing to a conserved role for seizures eliciting deficits in 5-HT release as a plausible mechanism underlying the seizure-associated mortality in young APP/PS1 mice.

Reduction of 5-HT release is usually followed by modifications of 5-HT-associated receptor and transporter proteins, which we expected to be affected by kindling. SERT downregulation is reported post-mortem in SUDEP decedents [45]. Interestingly, these SERT changes occurred in other brain

regions, such as the amygdala and raphe nuclei [45]. Mechanisms triggered by 5-HT tone reduction may also happen in the raphe nuclei, a region that is also under the control of the respiratory center, by the stimulation of their neurons [18]. SERT changes thus may be happening in regions other than the HPC associated with the 5-HT pathway, such as the raphe nuclei [45], for which our study was not designed to assess. MAOA can also be indirectly modified by cytokine release followed by an overexpression of SERT to avoid a 5-HT synaptic overload caused by MAOA deficiency [44]. Considering we did not presently observe changes in SERT protein expression, it is most likely that our observed reductions in MAOA expression led to a compensatory shift in 5-HT levels, which requires further study. We also expected similar disruptions in 5-HT1A receptor expression considering that 5-HT1A receptor expression changes have been observed in other TLE models. However, other 5-HT receptors such as the 5-HT2C and 5-HT2B have been postulated as potential drivers of cardiorespiratory arrest in the pathology of SUDEP [14,15,17]. Further studies are thus needed to confirm whether other 5-HT receptors are differentially expressed in young APP/PS1 mice following chronic seizure activity. Accordingly, PSEN2-N141I animals did not show any 5-HT pathway changes during corneal kindling, further illustrating stark genotype-related differences in the functional outcomes of seizures in the setting of AD-associated genetic risk factors.

Considering that PSEN2-N141I mice were largely protected from chronic seizure-induced mortality and disruptions in 5-HT synthesis and metabolism enzyme expression, another important novel aspect of this work is that PS2 protein overexpression may differentially influence susceptibility to neuronal hyperexcitability and accordingly, mortality risk. Indeed, PS2 protein expression was reduced in APP/PS1 mice relative to their Tg- controls, whereas it was increased in PSEN2-N141I mice relative to their Tg control mice, suggesting a critical contribution of this protein to seizure susceptibility that warrants further study. Presenilins, and PS2 in particular, trigger different mechanisms involved in AD-associated neurodegeneration independently of Aβ deposition through the modulation of calcium signaling at endoplasmic reticulum, mitochondrial function, and autophagy [46,47]. Calcium homeostasis plays a pivotal role in the generation and propagation of epileptiform events [48]. Neuronal hyperexcitability driven by disrupted calcium handling is a crucial factor in epilepsy and SUDEP [49]. Indeed, the antiseizure medicines levetiracetam and lamotrigine exert their anticonvulsant effect and preserve neuronal viability by restoring calcium homeostasis [48]. How presenilin proteins modulate calcium homeostasis is still largely unknown. Some studies suggest that PS1 and PS2 variations increase intracellular calcium, resulting in an increase in hyperexcitability and neurodegeneration at pre-symptomatic stages [50]. Moreover, animals with PS2 variants also have reduced calcium levels [50], suggesting inconclusive information about this phenomenon. Our data suggests that PSEN2-N141I and PSEN1-dE9 mutations trigger opposite presenilin-related mechanisms that exert different effects on seizure vulnerability. While it is well-known that loss of PSEN2 function can lead to a compensatory upregulation of PSEN1 levels [24,25], the behavioral outcomes of such impacts have yet to be functionally assessed in an intact organism. We herein demonstrate that increased expression of PS2 protein may be associated with an anticonvulsant effect, as evidenced by reduced seizure susceptibility in PSEN2-N141I mice (Figure 1). The PSEN2-N141I variant may be influencing the seizure-induced disruptions in calcium homeostasis that is commonly observed in the hyperexcitability of epilepsy. On the other hand, APP/PS1 mice demonstrated reduced PS2 protein expression, possibly translating to excess accumulation of intracellular calcium that ultimately increases seizure susceptibility in AD. Since PS2 may express in microglia and neurons, further studies are needed to determine if changes on the protein expression regarding the genotype occurs at glial or neuronal level.

Conclusions

Our present study reveals several critical novel findings of significant relevance to the study of seizures in AD, as well as to the understanding of seizure-induced mortality in epilepsy more broadly. First, we find substantial differences in seizure susceptibility in discrete AD-associated mouse models at an age well before pathological Aβ is known to accumulate. Second, we demonstrate marked differences in the susceptibility of APP/PS1 mice to mortality secondary to evoked seizures, producing a possible novel SUDEP model that is not associated with ion channel mutations of clinical epilepsy. Finally, we report that chronic kindled seizures elicit marked depletion of TPH2 and MAOA enzyme levels in APP/PS1, but not PSEN2-N141I, mice, further suggesting that seizure-induced mortality in the APP/PS1 model is due to deficits in 5-HT release. Considering that one of the most likely drivers of SUDEP risk is deficits in 5-HT release, these data cumulatively suggest a conserved pathological mechanism underlying chronic seizure-induced mortality in the absence of Aβ accumulation. Prior studies of seizures in AD mouse models had suggested that seizures secondary to Aβ accumulation were responsible for the precipitous declines in survival in APP/PS1 mice [20]. However, our present study now clearly demonstrates that seizures, and not Aβ accumulation, are the driving factor underlying mortality in this mouse model and that deficits in 5-HT release may be culpable. Future studies are needed to further characterize the cardiorespiratory and electrographic features associated with seizure-induced mortality in this model to further define whether it represents a novel and inducible, non-canonical model of SUDEP. Such studies would be critical to identify novel pathological features of SUDEP and seizure-associated mortality in people with AD.

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Conflict of Interest/Ethical Publication Statement

None of the authors has any conflict of interest to disclose. We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

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Figures and Figure Legends.

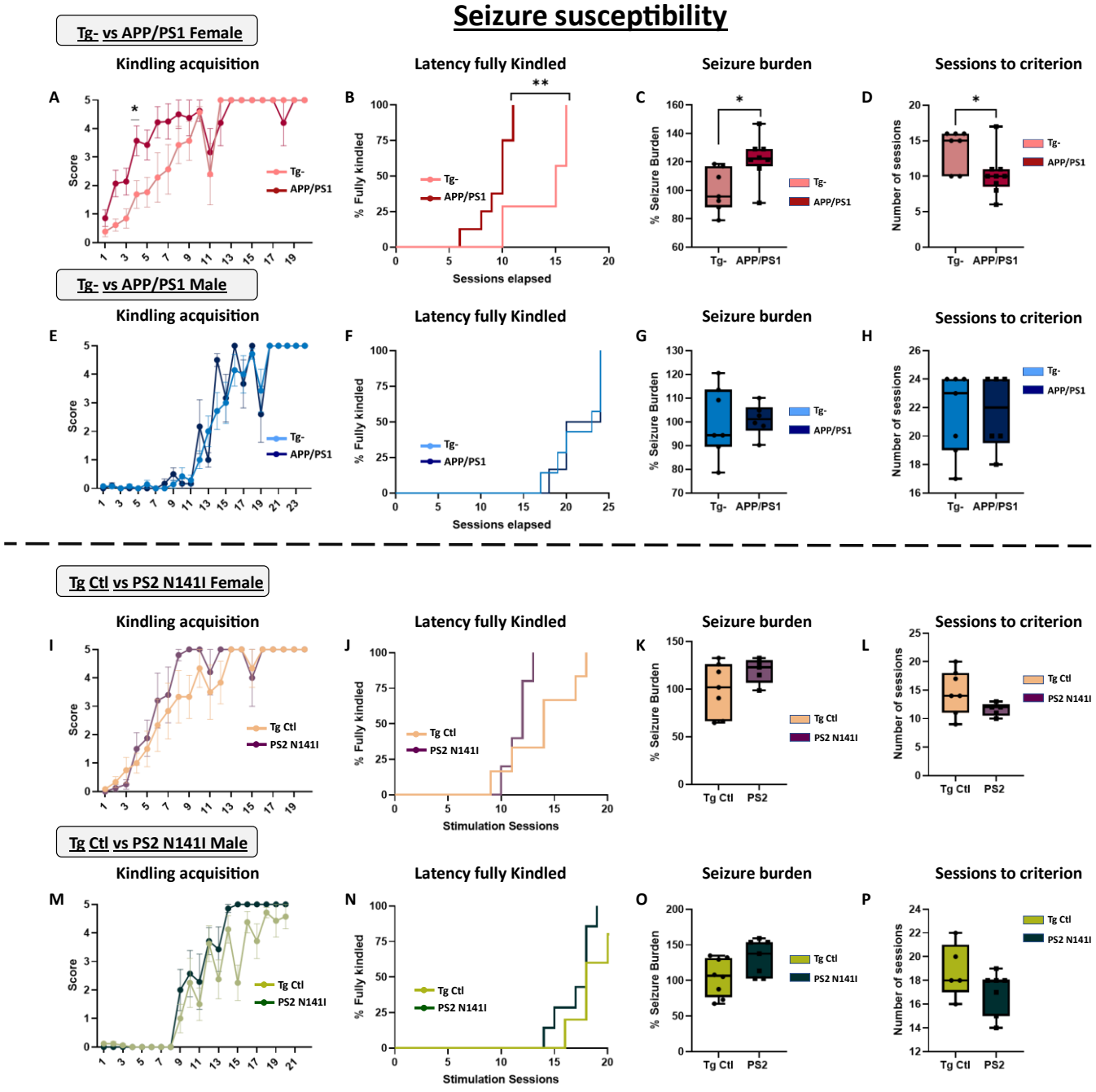
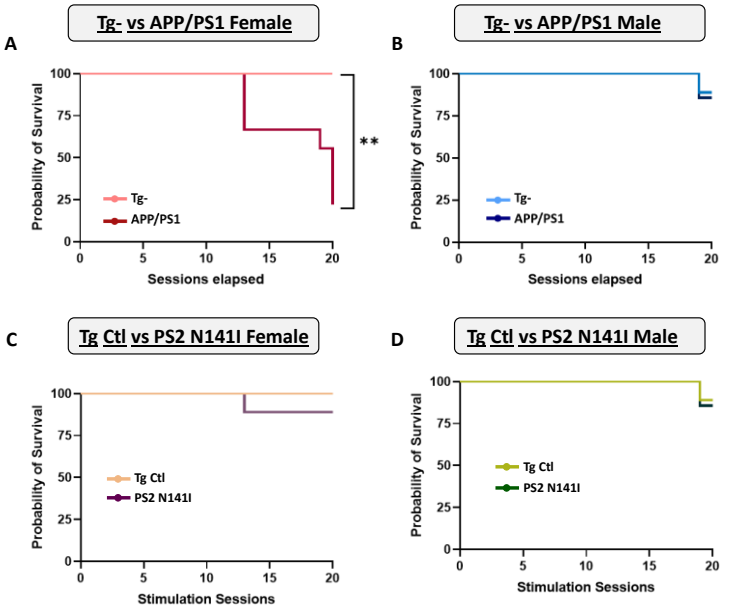


Figure 1: Seizure susceptibility and 60 Hz corneal kindling acquisition rate of 2-3-month-old male and female mice is highly influenced by AD-associated genotype. Young APP/PS1 females subjected to 60 Hz corneal kindling attained A-B) kindling criterion significantly faster than matched Tg- littermate females, C) higher seizure burden, and D) required fewer sessions to achieve the fully kindled criterion (five consecutive Racine stage 5 seizures). Young male APP/PS1 subjected to 60 Hz corneal kindling did not attain E-F) kindling criterion differences compared with matched Tg- littermate males.

They did not demonstrate significant differences in G) seizure burden, or H) sessions to achieve fully kindled criterion. Conversely, young PSEN2-N141I variant mice did not significantly differ from transgenic control mice across all outcome measures, reflecting substantial differences in seizure susceptibility with AD-associated genotypes (I-P). For the corneal kindling acquisition graphs, values are presented as mean seizure score ± SEM. Friedman's test was performed to evaluate differences between genotypes. (*) $p < 0.05$ *vs WT* (A, E, I, M). Data for latency were presented as a Kaplan-Meier plot and assessed by Log-Rank Mantel Cox test (B, F, J, N). For seizure burden (C, G, K, O) and sessions to achieve criterion (D, H, L, P), boxes represent the median and 95% CI; whiskers represent maximum and minimum values. Mann–Whitney test. (*) $p < 0.05$ *vs WT*. N= 8-12 per group.

Mortality during kindling



Mortality after kindling

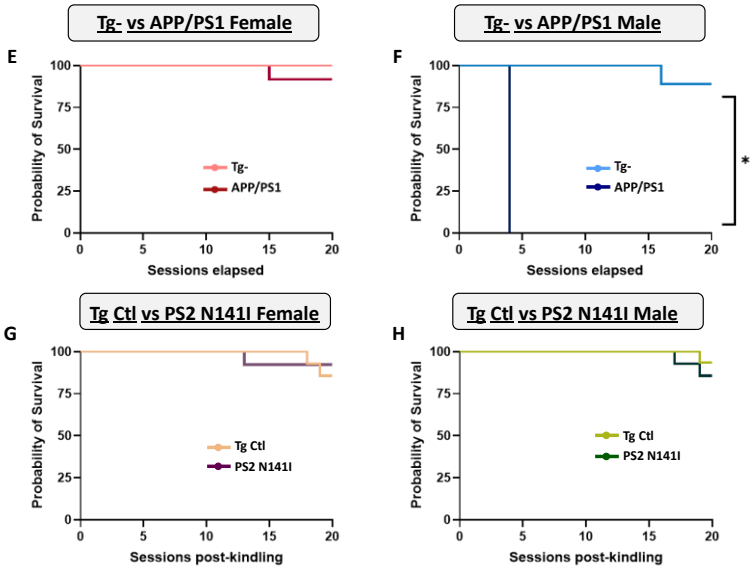


Figure 2. Corneal kindled APP/PS1 mice are highly susceptible to mortality, but survival of non-transgenic controls, PSEN2-N141I transgenic mice, nor transgenic control mice is not affected by chronic kindled seizures at the 2–3-month-old age. A) Young APP/PS1 females showed significant mortality compared with their respective WTs during the corneal kindling procedure. B) Survival of young male APP/PS1 mice was not significantly affected by corneal kindling. The survival of both C) male and D) female PSEN2-N141I and their respective transgenic control mice was not adversely affected by corneal kindling. E) Female APP/PS1 mice that survived the corneal kindling procedure and attained the fully kindled state did not demonstrate significant changes in survival relative to Tg- control littermates. However, F) young male APP/PS1 mice were most likely to die after attaining the fully kindled state. Post-kindling mortality was not observed in young PSEN2-N141I G) males nor H) female mice, suggesting that presence of the AD-associated PSEN2 variant does not adversely affect chronic seizure-induced adverse outcomes. Data for animal survival were presented as a Kaplan-Meier plot and assessed by Log-Rank Mantel Cox test, with * $p<0.05$ significant. N=8-12 per group.

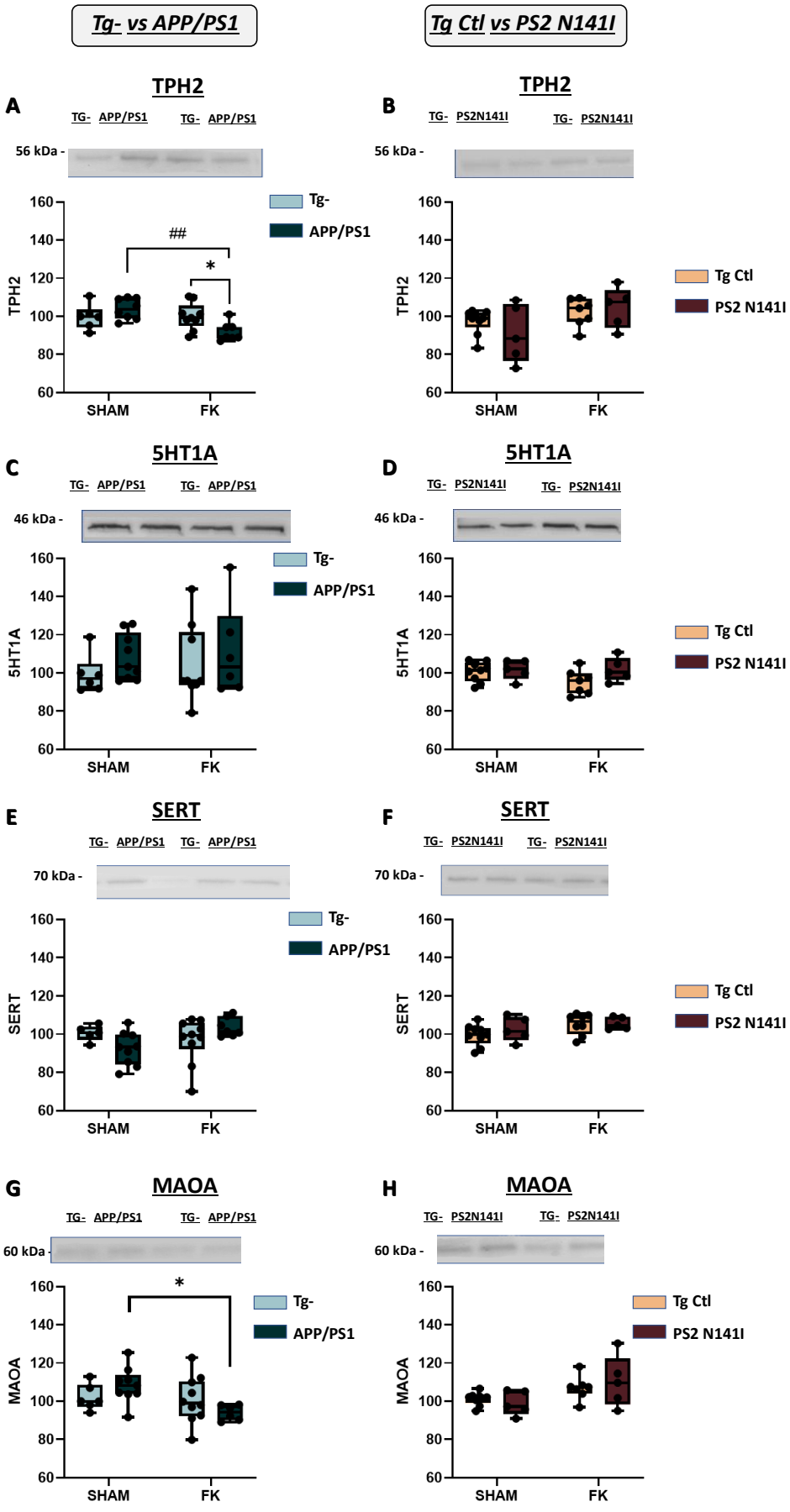


Figure 3. Chronic kindled seizures decrease serotonin synthesis and metabolism protein expression in isolated hippocampus in 2-month-old APP/PS1 mice. A) TPH2 protein levels in isolated hippocampal homogenates are reduced in kindled male and female APP/PS1 mice relative to their respective genotype-matched SHAM group and versus kindled Tg- group. B) These changes were not observed in kindled or sham PSEN2-N141I mice relative to Tg controls, suggesting that 5-HT synthesis may be reduced solely in APP/PS1 mice with kindled seizures. C-D) Expression of the 5-HT1A receptor in isolated hippocampal homogenates is not modified by kindling or genotype. E-F) SERT expression in isolated hippocampal homogenates is not influenced by genotype or kindling status. G) MAOA protein downregulation was observed between SHAM and kindled group only in isolated hippocampal homogenates in the APP/PS1 cohort H) but not in the PSEN2 N141I group, demonstrating that 5-HT release is possibly reduced exclusively in APP/PS1 mice with kindled seizures. Boxes represent the median and 95% CI; whiskers represent maximum and minimum values. Data were assessed by two-way analysis of variance followed by the Bonferroni test (*$p < 0.05$, **$p < 0.01$, ***$p < 0.005$). N= 5-8I.

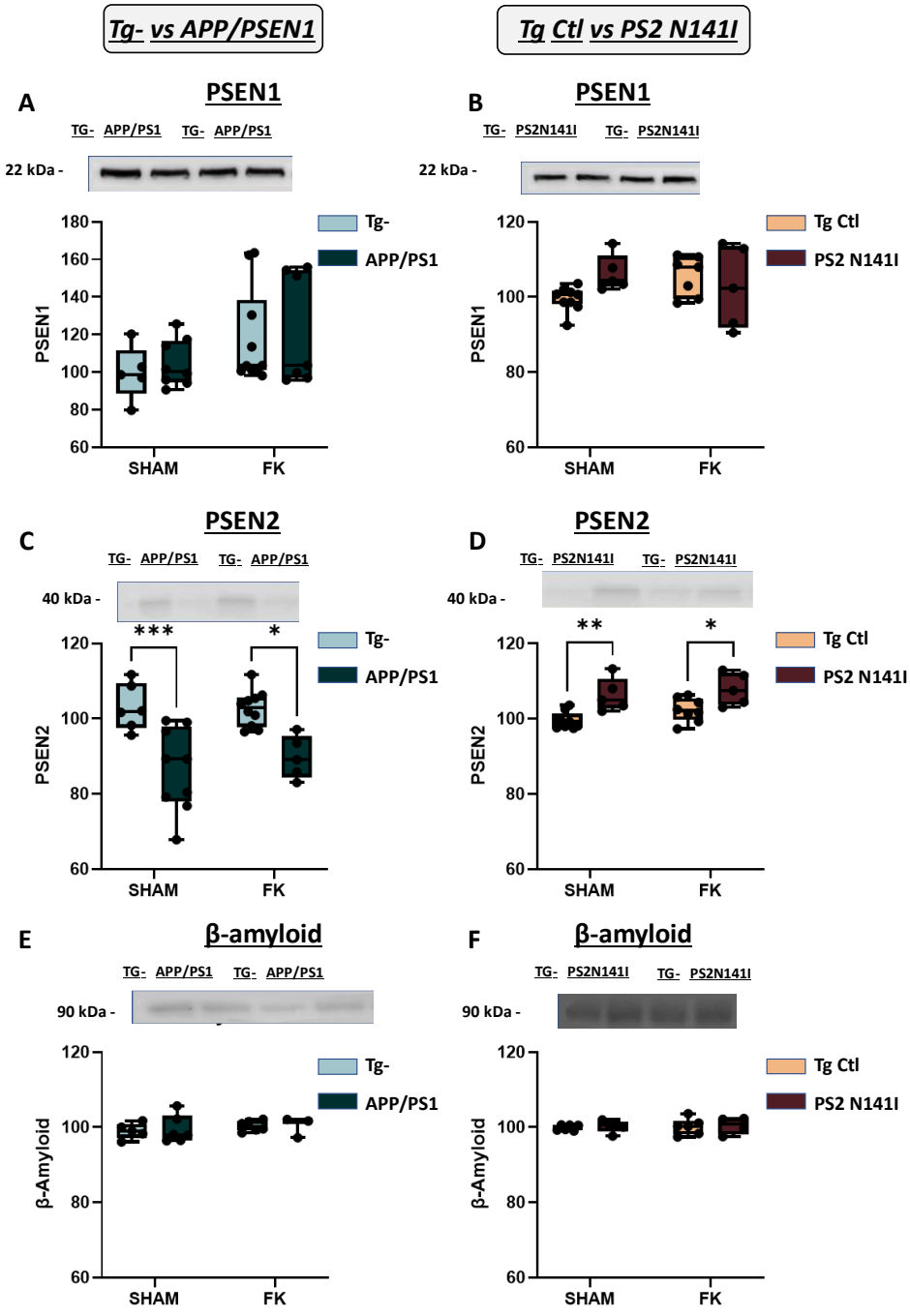


Figure 4. Expression of AD-related proteins in isolated hippocampus are genotype-dependent. PSEN1 expression in isolated hippocampal homogenates was not altered by kindled seizures in neither A) APP/PS1 nor B) PSEN2-N141I mouse models of AD relative to their respective control mice at age 2-3-months-old. C) APP/PS1 mice had reduced expression of the PS2 protein in isolated hippocampal homogenates relative to Tg- in both SHAM and kindled mice, suggesting that corneal kindled seizures do not alone modify expression of the PS2 protein at this age in mice of the APP/PS1 genotype. D) Conversely, PS2 protein expression was generally increased in PSEN2-NI141 mice relative to their matched Tg control, and this expression was not affected by kindled seizure history. There was no kindling-induced change in Aβ protein expression in isolated hippocampal homogenates in neither E)

APP/PS1 nor F) PSEN2-N141I mice relative to their matched controls. Boxes represent the median and 95% CI; whiskers represent maximum and minimum values. Data were assessed by two-way analysis of variance followed by the Bonferroni test (*$p < 0.05$, **$p < 0.01$, ***$p < 0.005$). N= 5-8.

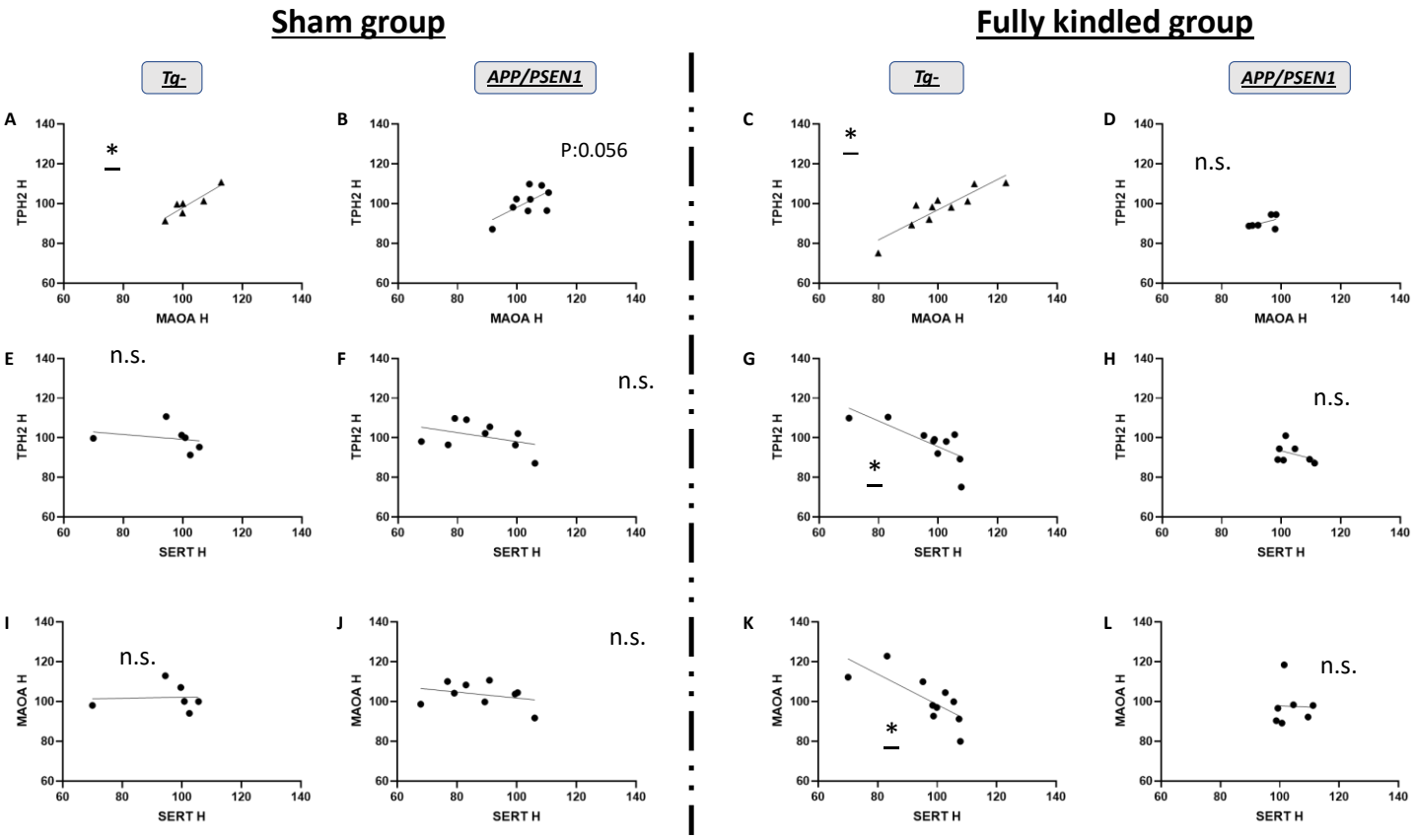


Figure 5: Expression of 5-HT synthesis and metabolism enzymes is tightly correlated to serotonin transporter expression in fully kindled Tg-, but not APP/PS1, mice in isolated HPC. Both A) Sham and C) Fully kindled Tg- group showed positive significative correlations between TPH2 and MAOA protein levels at HPC. No differences were observed between these two proteins in either of the APP/PS1 mice (B-D). G) Inverse correlation was observed in the fully kindled tg- group between TPH2 and SERT levels. E, F, H) No significant differences were observed in the tg- sham group and the APP/PS1 mice. K) Tg- fully kindled mice showed inverse correlation when compared MAOA and SERT protein expression. I, J, L) No differences were observed in the other experimental groups. Correlational studies were performed following Spearman correlation. *$p < 0.05$.