



Age- and sex-dependent effects of DNA glycosylase Neil3 on amyloid pathology, adult neurogenesis, and memory in a mouse model of Alzheimer's disease

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ARTICLE INFO

Keywords:

Alzheimer's disease
Neural stem cells
Adult neurogenesis
Hippocampus
DNA glycosylase
Neil3
Oxidative stress

ABSTRACT

Oxidative stress generating DNA damage has been shown to be a key characteristic in Alzheimer's disease (AD). However, how it affects the pathogenesis of AD is not yet fully understood. Neil3 is a DNA glycosylase initiating repair of oxidative DNA base lesions and with a distinct expression pattern in proliferating cells. In brain, its function has been linked to hippocampal-dependent memory and to induction of neurogenesis after stroke and in prion disease. Here, we generated a novel AD mouse model deficient for Neil3 to study the impact of impaired oxidative base lesion repair on the pathogenesis of AD. Our results demonstrate an age-dependent decrease in amyloid- β (A β) plaque deposition in female Neil3-deficient AD mice, whereas no significant difference was observed in male mice. Furthermore, male but not female Neil3-deficient AD mice show reduced neural stem cell proliferation in the adult hippocampus and impaired working memory compared to controls. These effects seem to be independent of DNA repair as both sexes show increased level of oxidative base lesions in the hippocampus upon loss of Neil3. Thus, our findings suggest an age- and sex-dependent role of Neil3 in the progression of AD by altering cerebral A β accumulation and promoting adult hippocampal neurogenesis to maintain cognitive function.

1. Introduction

Alzheimer's disease (AD) is the most common cause of dementia with a complex pathogenesis that is not yet fully understood. Microscopically, it is characterized by extracellular amyloid plaques (A β), derived from amyloid precursor protein (APP), and intracellular neurofibrillary tangles (NFTs). Interestingly, AD is affecting more women than men, not only in prevalence, but also in severity [45]. Female patients exhibit a faster clinical decline than men [24,37]. Mouse models of AD show a greater amyloid load in female mice [7]. Thus, investigating sex differences is of importance to better understand AD pathogenesis, as well as to improve diagnostics and treatment options.

Progressive memory impairment is associated with degeneration of the hippocampus, an area of the brain that is severely affected in AD [43,

44]. The dentate gyrus (DG) of the hippocampus is critical for learning and memory functions and harbors neural stem cells with the capacity to generate new neurons throughout life, a process termed neurogenesis. While the majority of neural stem cells remain quiescent in the adulthood, some contribute to the formation of new neurons by giving rise to transit-amplifying progenitor cells [29]. The decline of adult hippocampal neurogenesis found in patients with AD [43], might contribute to the impaired memory and cognitive dysfunction in AD patients [25, 63].

Oxidative stress is an integral part early in AD pathogenesis and has been shown to negatively affect adult neurogenesis [26,33,69]. Elevated levels of oxidative DNA damage have been found in post-mortem brains of AD patients, and in patients with mild cognitive impairment [40,53]. Oxidative stress causing DNA damage is a result of an imbalance

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between free radicals and antioxidative detoxification. A β has been proposed to stimulate production of reactive oxygen species (ROS) by interacting directly with mitochondria, promoting oxidative stress and leakage of ROS [5,20,41,60]. On the contrary, studies observed that oxidative damage anticipated A β accumulation and even triggered its production [2,50].

Oxidative DNA damage is recognized by DNA glycosylases and repaired through the base excision repair (BER) pathway. The Neil-family glycosylases Neil1, Neil2 and Neil3 express overlapping substrate specificity, recognizing oxidized pyrimidines and purines [30]. Neil3 is expressed in specific cell cycle stages, peaking at S/G2 with a preference of repairing base lesions in single-stranded DNA (ssDNA) [21, 46]. Increased levels of 5OH-dC, a substrate of NEIL3, has been found in the parietal and temporal lobe of AD patients [38,40,42,66]. In contrast to the other Neil-family members, Neil3 shows a cell-specific expression in proliferative cells of the testis, thymus and hematopoietic tissues, as well as the neurogenic niches of the brain [22,39,55,57,64]. Its function in the brain has been associated with learning and memory, anxiety-like behavior, and neural regeneration after hypoxic-ischemic injury. Interestingly, Jalland et al. found a protective function of Neil3 during prion disease as a result of induced neurogenesis [28]. Altogether, these studies indicate a role for Neil3 in neurological diseases involving dysregulated neurogenesis such as AD.

This study aimed to investigate the impact of Neil3 on the progression of AD by generating and characterizing a novel Neil3-deficient AD mouse model.

2. Materials and methods

2.1. Mouse models

All experiments were approved by the Norwegian Animal Research Authority and conducted in accordance with the laws and regulations controlling experimental procedures in live animals in Norway and the European Union's Directive 2010/63/EU.

All mice used in our study were on a C57BL/6J background. APP/PS1 mice previously described by Radde et al. [51] which were generally bred heterozygous and *Neil3*^{-/-} mice previously described by Sejersted et al. [56] were used to generate APP/PS1x*Neil3*^{-/-} mice. All experiments were performed with mice at 1 and 6 months of age. The mice were housed at ambient temperature on a 12:12 h light:dark cycle with free access to water and food.

2.2. Immunohistochemical (IHC) and -fluorescent (IF) staining

Mice were transcardially perfused with PBS before brain dissection. For IF, mice were additionally injected with 100 mg/kg bodyweight BrdU (Sigma) 24 h prior to dissection. Brains were embedded in paraffin and sliced into 4 μ m thick coronal sections. Sections were rehydrated in Xylol 2 \times 5 min followed by ethanol 100% 2 \times 3 min, 96% 1 min and 70% 1 min. For A β -plaque staining, the HRP/DAB-kit (Abcam) was used. Sections were first blocked in H₂O₂ for 10 min, and antigen retrieval was performed by incubating sections at 100 °C citrate buffer (100 mM, pH 6.0) for 5 min (IHC) or 3 min (IF) under pressure. For IHC sections were blocked with Protein block for 10 min and incubated with primary antibodies against A β (6F3D Amyloid β , Thermo Fisher) diluted 1:100 in PBS + 0.5% goat serum + 0.5% BSA for 1 h at room temperature. Sections were thereafter incubated in Biotinylated Goat Anti-Polyvalent and Streptavidin Peroxidase for 10 min each and treated with DAB for 1–10 min. Sections were washed three times with washing buffer (PBS + 0.2% Tween 20) and incubated in hematoxylin (Sigma Aldrich) for 3 min, 98% ethanol + 2% NH₄OH for 1 min, Eosin Y (Sigma Aldrich) for 15 s before dehydration followed by mounting (Entellan mounting medium, Sigma Aldrich). Sections were scanned by the Cellular and Molecular Imaging Core facility (CMIC, NTNU) using an OlympusVS120S5 with 20 \times objective, scanned on one Z-plane with low focus grid density.

Plaque quantification was done using ImageJ. Color-presets were chosen to minimize background to count and measure number of A β -plaques. For the final quantification, the average of at least two sections per animal stained in independent runs were used.

For IF staining, sections were blocked in blocking solution (5% BSA + 5% goat serum in PBS) for 1 h and incubated with rat anti-bromodeoxyuridine (BrdU) 1:400 diluted in PBS + 0.5% BSA + 0.5% goat serum. Sections were washed in wash buffer (PBS + 0.2% Tween 20) and incubated with DAPI staining solution (1 μ g/mL, Invitrogen) for 10 min before mounted in Vectashield mounting medium (Vector Laboratories). Microscopy was carried out using a Zeiss LSM 510 Meta confocal laser scanning microscope equipped with a 40X oil immersion lens, and quantifications were performed manually and blinded. For the final quantification, the average of at least two sections per animal stained in independent runs were used.

2.3. Extraction of nucleic acids

Extraction of total DNA and RNA was performed using the Allprep DNA/RNA/Protein kit (Qiagen) according to the manufacturers protocol. Initially, 350 μ L Buffer RLT + β -ME was added to the brain tissue and samples were homogenized with MagNA Lyser for 10 s at 5000 speed followed by centrifugation for 3 min at 16 400 rpm before proceeding according to the kits protocol. DNA and RNA concentrations were measured using NanoDrop (Thermo Fisher).

2.4. RNA sequencing

Total RNA from whole brain of one male and one female APP/PS1 and APP/PS1x*Neil3*^{-/-} mice, respectively per age group was sent to BGI Tech Solutions Co., Hong Kong, for RNA sequencing on an Illumina HiSeq4000. Unpaired reads were trimmed and filtered for quality. Quality of the trimmed reads were assessed using FastQC (v0.11.9) and FastQ-Screen (v0.14.1). Reads were mapped to the GRCh38 transcriptome using Hisat2 and reads per gene were counted using the Rsubread package in R (3.6.3). EdgeR (3.32.1) was used to generate a log₂ CPM matrix which was then exported to Perseus (v.1.6.14.0) to generate heatmaps. Male and female counts were separated, Z-scored by row and clustered using Euclidean hierarchical clustering. Gene lists from clusters were exported and analyzed using PantherDB for an overrepresentation analysis looking at Gene Ontology (GO) biological pathways.

2.5. Quantification of oxidative DNA damage by mass spectrometry

DNA samples were digested by incubation with a mixture of nuclease P1 from Penicillium citrinum (Sigma), DNaseI (Roche) and ALP from E. coli (Sigma) in 10 mM ammonium acetate buffer pH 5.3, 5 mM MgCl₂ and 1 mM CaCl₂ for 30 min at 40 °C. The samples were methanol precipitated, supernatants were vacuum centrifuged at room temperature until dry, and dissolved in 50 μ L of water for LC/MS/MS analysis. Quantification was performed with the use of an LC-20AD HPLC system (Shimadzu) coupled to an API 5000 triple quadrupole (ABSciex) operating in positive electrospray ionization mode. The chromatographic separation was performed with the use of an Ascentis Express C18 2.7 μ m 150 \times 2.1 mm i.d. column protected with an Ascentis Express Cartridge Guard Column (Supelco Analytical) with an Exp Titanium Hybrid Ferrule (Optimize Technologies Inc.). The mobile phase consisted of A (water, 0.1% formic acid) and B (methanol, 0.1% formic acid) solutions. The following conditions were employed for chromatography: For unmodified nucleosides – 0.13 mL/min flow, starting at 10% B for 0.1 min, ramping to 60% B over 2.4 min and re-equilibrating with 10% B for 4.5 min; For 5-OH(dC) - 0.14 mL/min flow, starting at 5% B for 0.1 min, ramping to 70% B over 2.7 min and re-equilibrating with 5% B for 5.2 min; For 8-oxo(dG) - 0.14 mL/min flow, starting at 5% B for 0.5 min, ramping to 45% B over 8 min and re-equilibrating with 5% B for 5.5 min.

For mass spectrometry detection the multiple reaction monitoring (MRM) was implemented using the following mass transitions: 252.2/136.1 (dA), 228.2/112.1 (dC), 268.2/152.1 (dG), 243.2/127.0 (dT), 244.1/128 [5-OH(dC)], 284.1/168.1 [8-oxo(dG)].

2.6. Human A β 42 and A β 40 measurement by ELISA

Whole brain samples were homogenized in carbonate buffer (pH 11.5, 100 mM Na₂CO₃, 50 mM NaCl, protease inhibitor) using the MagNA Lyser (Roche) for 5 s at 5000 speed. Samples were centrifuged for 90 min at 16400 rpm. The supernatant was diluted in 8 M guanidine buffer (pH 8, guanidine/HCl 8.2 M, Tris/HCl 83 mM) to obtain soluble A β 42 fraction. For the insoluble A β , the remaining pellets were resuspended in 5 M guanidine buffer (pH 8, guanidine/HCl 5 M, Tris/HCl 50 mM) and shaken at room temperature for 3 h. Samples were centrifuged for 20 min at 16 400 rpm. Protein concentration was measured with NanoDrop (Thermo Fisher). A β 42 and A β 40 concentrations were measured using the Human A β 42 or Human A β 40 ELISA kit (Thermo Fisher), respectively according to the manufacturer's protocol. Absorbance was read at 450 nm with FLUOstar Omega (BMG Labtech).

2.7. Behavioral analysis

The Elevated Zero Maze (EZM) assessing activity and anxiety behavior was performed on a circular runway 60 cm above the floor with four alternating open and closed areas. The mice were placed on the maze facing a closed area and allowed 5 min for free exploration of the apparatus. The T-Maze was used to measure working memory by assessing spontaneous alternation in a T-shaped arena according to the protocol described by Deacon and Rawlins [14]. A total of 6 training and test runs, distributed across two days with 1 h in between was performed per animal. During the training, mice were placed in the start arm and allowed to choose a goal arm within a maximum of 90 s. Mice were confined for 30 s in the chosen arm and then removed from the arena for 60 s. In the test run, mice were placed back into the start arm and allowed to choose between the two goal arms within a maximum of 90 s. A successful alternation was scored if mice choose to enter the previously non-visited goal arm in the training. During the behavioral tasks mice were monitored and analyzed by using ANY-maze video tracking system (Stoelting).

2.8. Statistics

Statistical analyses were done with GraphPad Prism software version 8, using 2-way ANOVA with Tukey's multiple comparisons test. P-values

< 0.05 were considered significant (*p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001). Figures are shown with mean + standard deviation.

3. Results

3.1. Reduced plaque deposition upon loss of Neil3 in female AD mice

We generated a novel APP/PS1xNeil3^{-/-} mouse model to study how Neil3 affects AD pathology. The mice were viable and fertile, and showed no differences in weight compared to the APP/PS1 mice (Suppl Fig. 1). To assess whether Neil3 has an impact on amyloidogenesis we first stained for A β plaques in the brain of the AD mice strains during disease progression (Fig. 1A). To exclude region-specific plaque accumulation the cortex and hippocampus were assessed separately but no region-specific differences were found, and the data were combined in the following analyses. Quantification of plaque numbers demonstrated an age-dependent increase from 1 to 6 months in all strains (Fig. 1B). Female APP/PS1 mice had higher plaque numbers at 6 months compared to its male counterparts. In contrast, Neil3-deficient AD mice showed no sex-specific change in A β deposition, thus female APP/PS1xNeil3^{-/-} mice exhibited a significantly lower number of A β plaques compared to female APP/PS1. However, this finding was not reflected measuring A β 42 concentrations in whole brain by ELISA (Suppl. Fig. 2). Although female APP/PS1 mice showed a tendency to higher guanidine-soluble (mostly fibrillar material) A β 42 compared to male mice, no significant difference was found upon loss of Neil3 (Suppl. Fig. 2A). Similarly, buffer-soluble (mostly monomers and small oligomers) level of A β 42 was comparable between the mouse strains at the age groups analyzed (Suppl. Fig. 2B). Furthermore, guanidine- and buffer-soluble level of A β 40 showed a significant age-dependent increase but no significant differences between the genotypes at any age analyzed (Suppl. Fig. 2C and D). This suggest that Neil3 is involved in plaque pathology in a sex-dependent manner, but not in cerebral A β 42 or A β 40 accumulation.

3.2. No differences in levels of global oxidative DNA damage

Elevated levels of 5OH-dC, a known substrate of NEIL3 has been found in post-mortem brains of AD patients [18,39,40,65,66]; thus, we quantified the level by LC-MS in the AD mouse models (Fig. 2A). We found no differences in cerebral 5OH-dC levels neither between genotypes, nor age. In addition, levels of the common marker of DNA oxidation 8oxo-dG that was shown to accumulate during AD progression [18,47,58] were comparable between the mice strains and age groups

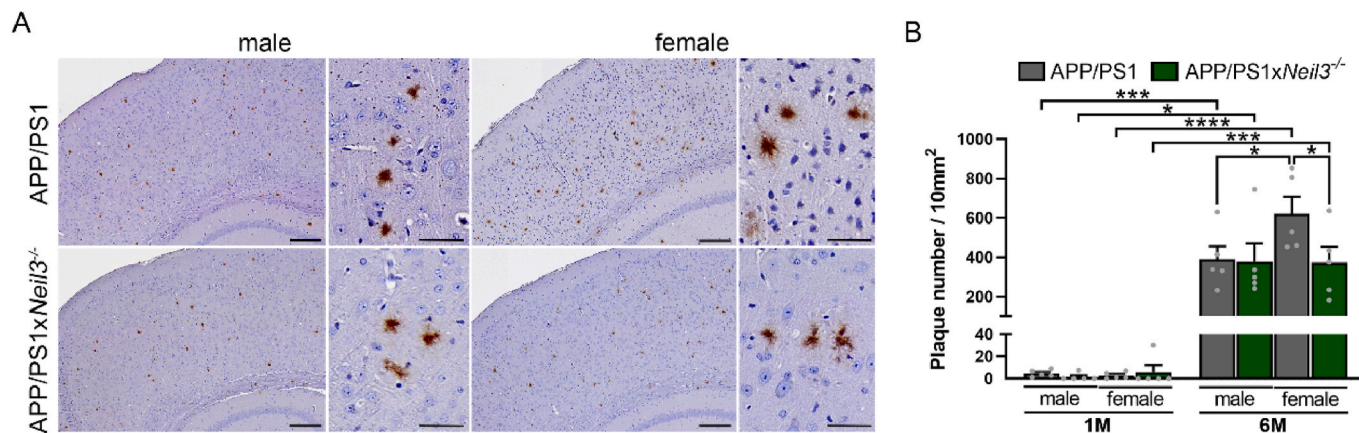


Fig. 1. Loss of Neil3 decreases cerebral plaque deposition in female APP/PS1 mice with age. (A) Representative images of immunohistochemically stained amyloid- β plaques in the cortex and (B) quantification of plaque number in the brain of APP/PS1 and APP/PS1xNeil3^{-/-} mice at 1 month (1 M) and 6 months (6 M) of age. Data are shown as mean + SD; n = 5 per group. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

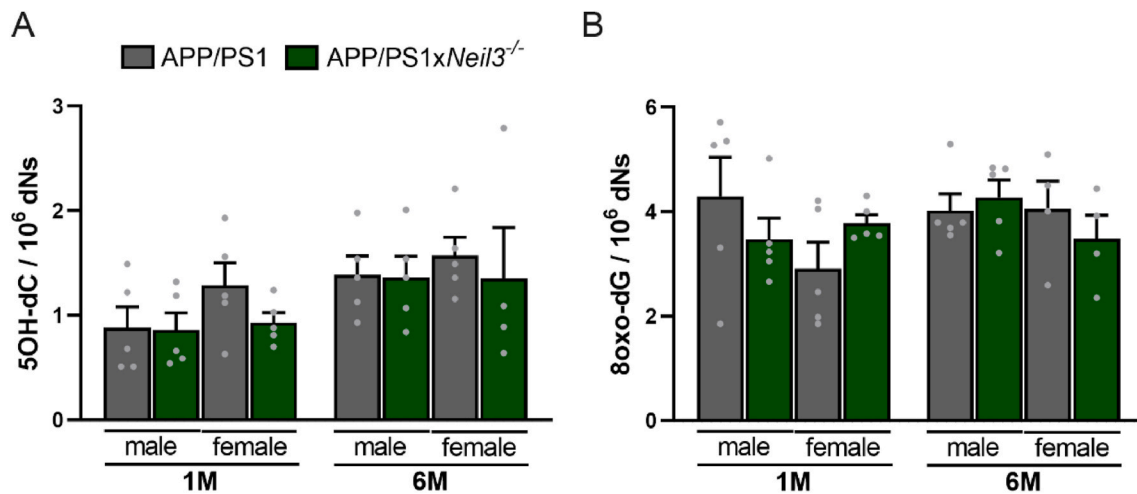


Fig. 2. No differences in cerebral accumulation of oxidative base lesions in the Neil3-deficient AD mouse model. (A) Levels of oxidized base lesions 5-hydroxycytosine (5OH-dC) and (B) 8-oxoguanine (8oxo-dG) in brain samples of APP/PS1 and APP/PS1xNeil3^{-/-} mice at 1 month (1 M) and 6 months (6 M) of age measured by using mass spectrometry. Data are shown as mean + SD; n = 4–5 per group.

(Fig. 2B). This indicates that Neil3 is dispensable for global oxidative DNA base damage repair in the brain of AD mice at the time points analyzed. Moreover, increased oxidative DNA damage seems not to be a hallmark in our AD mouse model until 6 months of age.

3.3. Age-dependent impairment of working memory upon loss of Neil3 in male AD mice

As Neil3 was shown to affect anxiety, learning and memory in adult mice [52], we investigated whether Neil3-deficiency also affects behavior in the AD mouse model. The Elevated Zero Maze (EZM) was used to examine activity measured as distance traveled and anxiety levels measured by the time spent in the open arm. We found no major differences in activity and anxiety behavior between genotypes and age groups (Fig. 3A and B). Only Neil3-deficient male AD mice were significantly less active from 1 month to 6 months of age (Fig. 3A). The alternation rate as a measure of working memory was assessed in a T-Maze. Whereas AD progression from 1 month to 6 months did not affect working memory, loss of Neil3 led to a significant age-dependent decrease of working memory exclusively in male AD mice (Fig. 3C). In contrast, wildtype (WT) and Neil3^{-/-} male mice demonstrated similar working memory at 6 months of age (Suppl. Fig. 3). This indicates that the effect of Neil3 on working memory is age- and sex-dependent and specific to AD.

3.4. Impaired adult neurogenesis upon loss of Neil3 in male AD mice

Adult neurogenesis in the hippocampus has been shown to be important for cognitive functions [1,15,59] and Neil3 is expressed in stem cells of the hippocampus. To address whether impaired adult hippocampal neurogenesis may contribute to memory impairment in Neil3-deficient mice during AD progression, we used BrdU injection to stain proliferating cells in the DG (Fig. 4A). We observed significant fewer dividing cells in Neil3-deficient male AD mice at 1 month of age compared to male controls (Fig. 4B). Additionally, number of BrdU⁺ cells dropped drastically from 1 to 6 months of age in all AD mouse strains, suggesting an age-dependent decrease in proliferation capacity of DG cells during AD progression.

3.5. Hippocampal oxidative DNA damage is increased in Neil3-deficient female mice early in AD

Since Neil3 is expressed in the hippocampus [52] and oxidative stress-induced DNA damage occurs brain region-specific [8], we examined level of oxidative DNA base lesions in hippocampal tissue of the AD mouse strains. Interestingly, measurement of 5OH-dC (Fig. 5A) and 8oxo-dG (Fig. 5B) showed a significantly higher amount of oxidative DNA damage in female AD mice upon loss of Neil3 at 1 month compared to female APP/PS1 mice. Male Neil3-deficient AD mice showed a similar trend to increased DNA damage level at 1 month, however, the results were not statistically significant. Similar to the DNA damage level in

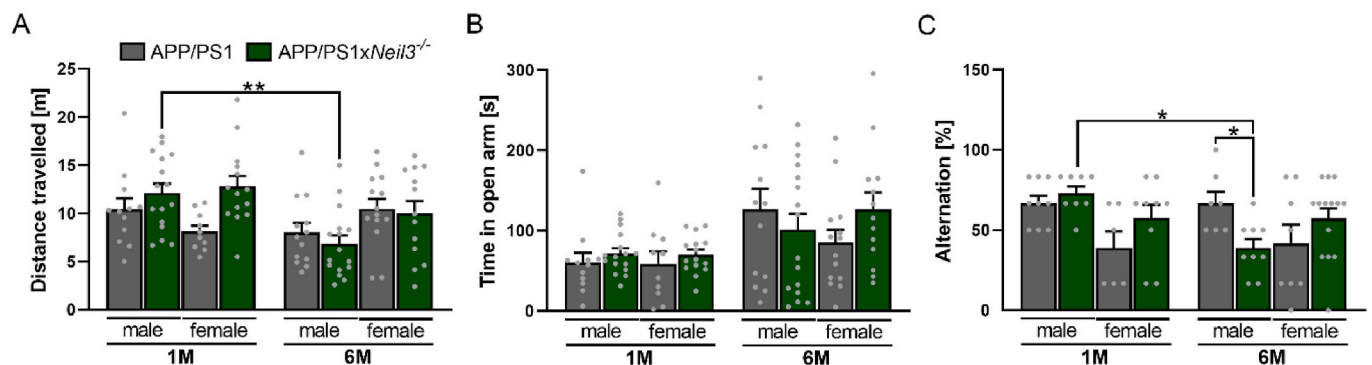


Fig. 3. Behavioral testing reveals impaired working memory with age upon loss of Neil3 in male AD mice. (A) Distance traveled and (B) Time spent in the open arm assessed by using an elevated zero maze, and (C) Alternation rate assessed in a T-maze of APP/PS1 and APP/PS1xNeil3^{-/-} mice at 1 month (1 M) and 6 months (6 M) of age. Data are shown as mean + SD; n = 6–16 per group. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

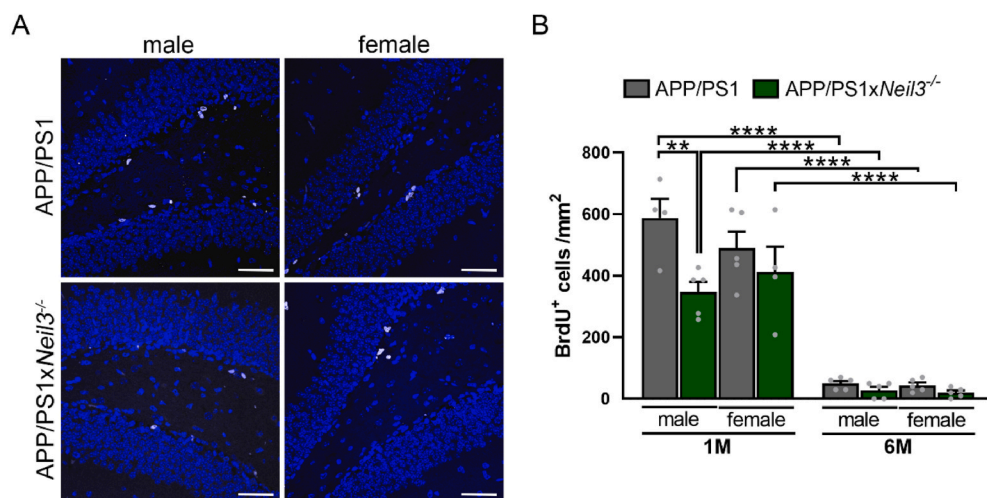


Fig. 4. Lack of Neil3 leads to reduced stem cell proliferation in the dentate gyrus (DG) of young male AD mice. (A) Representative images of BrdU-stained cells (white) and DAPI-stained nuclei (blue) in the DG of 1 month old APP/PS1 and APP/PS1xNeil3^{-/-} mice. Scale bar 50 μ m. (B) Quantification of BrdU-positive cells in the DG of APP/PS1 and APP/PS1xNeil3^{-/-} mice at 1 month (1M) and 6 months (6M) of age. Data are shown as mean + SD; n = 4–5 per group. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

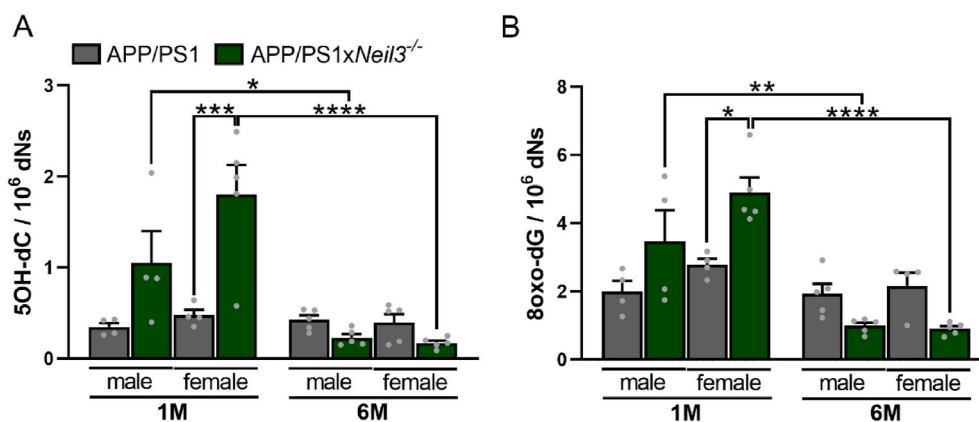


Fig. 5. Increased accumulation of oxidative base lesions in the hippocampus of young Neil3-deficient AD mice. (A) Levels of oxidized base lesions 5-hydroxycytosine (5OH-dC) and (B) 8-oxoguanine (8oxo-dG) in hippocampal samples of APP/PS1 and APP/PS1xNeil3^{-/-} mice at 1 month (1M) and 6 months (6M) of age measured by using mass spectrometry. Data are shown as mean + SD; n = 4–5 per group. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

whole brain, we found no accumulation of oxidative base lesions in APP/PS1 mice with age. However, Neil3-deficient AD mice demonstrated a significant decrease in oxidative DNA base lesions from 1 month to 6 months of age (Fig. 5). These data indicate that Neil3 is important for repair of oxidative DNA base lesions or regulation of oxidative stress-induced DNA damage in the hippocampus early in AD pathogenesis.

3.6. RNA sequencing reveals sex-specific differences in expression of genes involved in synaptic processes

To identify potential molecular pathways that could explain the effect of Neil3 in AD pathogenesis, we analyzed the transcriptome in the whole brain of the AD mouse strains at 1 month and 6 months of age by RNA sequencing. Hierarchical clustering with GO pathway analysis revealed sex-specific differences in AD-related processes (Fig. 6, and Suppl. Tables 1 and 2). Male AD mice showed a significant enrichment of genes in inflammatory processes that increased in expression (cluster 1) and in postsynaptic processes that decreased in expression (cluster 10) from 1 month to 6 months of age (Fig. 6A). Interestingly in cluster 9, we found an age-dependent decreased expression of genes involved in regulation of nervous system processes and synaptic organization and plasticity distinctly in Neil3-deficient AD mice. In contrast, female AD mice presented less differences on the transcriptome level with fewer significant GO processes identified (Fig. 6B). Cluster 1 showed an age-

dependent decrease of genes involved in receptor signaling and activity regulation whereas in cluster 5 we found a similar increase in inflammation-related processes as for male AD mice. However, no differences in gene expression upon loss of Neil3 in these clusters in female AD mice were identified. This indicates that Neil3 induces sex-dependent changes in AD on a transcriptional level.

4. Discussion

In the present study we generated a novel mouse model deficient for Neil3 to study the role of impaired oxidative base lesion repair in AD pathogenesis. We found that Neil3 altered AD-relevant processes such as amyloidosis, adult neurogenesis, memory and oxidative stress in an age and sex-specific manner. Whereas female Neil3-deficient mice showed significantly less A β plaque deposits and increased oxidative DNA damage, male Neil3-deficient mice presented with reduced number of proliferating neural stem cells and impaired working memory during AD progression.

Sex differences in AD are well known but poorly considered when studying AD pathogenesis in animal research [3,16]. Women have faster cognitive deterioration in MCI and AD [19,24,37,62]. Several studies have shown higher amyloid load in female AD mice [10,23,32], which is in line with our findings in the APP/PS1 mice. Interestingly, loss of Neil3 prevented this sex-dependent increase in A β accumulation in female mice. Plaque deposition can be altered through estrogen receptor (ER)

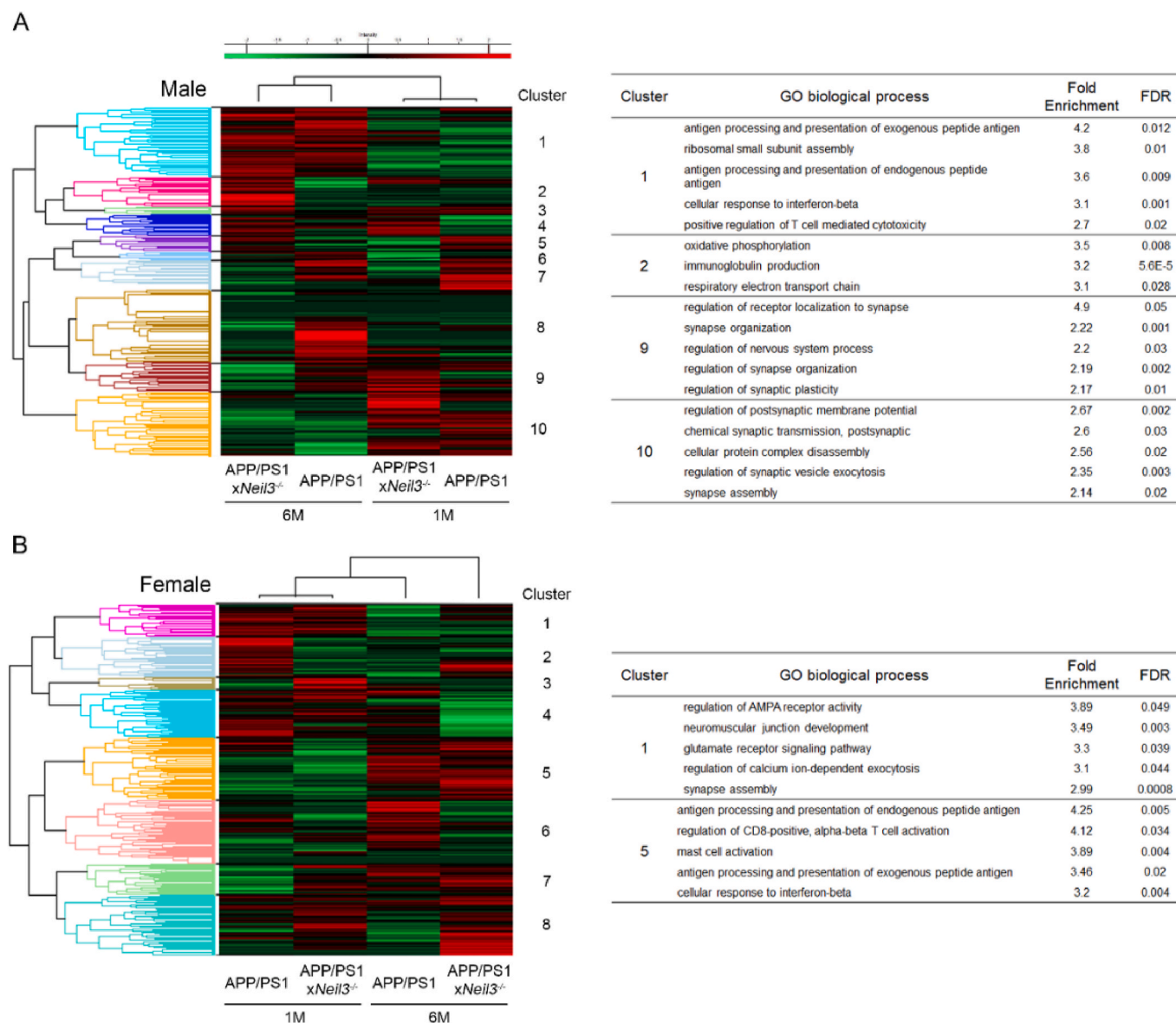


Fig. 6. RNA sequencing reveals differences in nervous system processes with age and upon loss of Neil3 in male AD mice. Heatmap with hierarchical clustering and associated maximum top five GO biological processes in brain of (A) male and (B) female APP/PS1 and APP/PS1xNeil3^{-/-} mice at 1 month (1 M) and 6 months (6 M) of age.

activation [9,68,70]. Notably, DNA glycosylases OGG1 and MUTYH have been shown to affect ER signaling in brain [4] and DNA glycosylases MPG and TDG directly interact with ER to alter transcription [12, 34]. Hence, Neil3 may have a similar function preventing amyloidogenesis. Together, our data suggest that Neil3 affects plaque burden in a sex-dependent manner potentially by altering hormone signaling in AD mice.

Neil3 is a DNA glycosylase known to repair oxidative base lesions. However, previous studies found no differences in levels of oxidative base lesions after myocardial infarction or during prion disease upon loss of Neil3 [28,48]. Similarly, we found no accumulation of the NEIL3 substrate 5OH-dC in the whole brain of Neil3-deficient AD mice. This indicates no correlation between A β toxicity and oxidative stress in our mouse model, although a delayed increase of oxidative base lesions due to amyloid toxicity at later stages of AD cannot be excluded.

Neil3 was shown to affect learning and memory in aged mice [52]. Here, we find that loss of Neil3 in AD leads to age-dependent impaired working memory in male but not female mice at 6 months of age whereas Neil3-deficient mice in a non-AD background demonstrated

similar working memory as their respective controls. The study by Regnell et al. [52] however found impaired memory in 18 months old male Neil3^{-/-} mice. Thus, our results indicate an age-dependent and AD-specific impact of Neil3 on cognitive function. Gene expression of Neil3 was previously found most prominent during early stages of postnatal development and to decrease with age [54], thus Neil3 must alter pathways important for memory formation early during AD pathogenesis or in distinct cells of the adult brain. It is believed that amyloid plaques are associated with cognitive decline in AD [35,36]. We see no correlation of plaque number and memory impairment in our AD model as female AD mice with the highest plaque burden show similar working memory as male AD mice.

Adult neurogenesis is important for cognitive function. In fact, Hollands et al. [25] were the first to demonstrate that reduction of neurogenesis induced memory and learning deficits in a mouse model of AD. Tobin et al. [63] showed that neurogenesis in humans declines with age, and that was associated with cognitive dysfunction in MCI and AD patients. Neil3 is expressed in the neurogenic niches of the brain [22, 55], and has been shown to contribute to neurogenesis after brain injury

in postnatal mice [52,56]. Here, we demonstrate that depletion of Neil3 in AD results in lower number of proliferating stem cells in the hippocampus of male mice indicating that Neil3 contributes *in vivo* to adult hippocampal neurogenesis during AD. This is in line with a previous study that demonstrated failure of *in vitro* expansion of adult hippocampal neural stem cells upon loss of Neil3 [52]. However, the impact of Neil3 on adult neurogenesis in AD was only evident in young mice as proliferation capacity of neural stem cells was almost completely lost in aged AD mice. It has previously been demonstrated that adult neurogenesis decreases with age and during AD progression [43,67]. Interestingly, estrogens show a neuroprotective role, increasing neurogenesis in rodent studies [6,61]. Thus, Neil3-dependent hormonal alterations might contribute to promote neurogenesis in the AD mouse model. Interestingly, microglia are known to alter neurogenesis [49], and a reduced number of microglia was found in Neil3-deficient mice after prenatal hypoxic-ischemic injury [56]. However, whether this contributes to the sex-dependent differences in hippocampal adult neurogenesis in AD requires more investigations in the future.

In mouse models of AD, impaired hippocampus-dependent learning and memory was correlated with decreased neurogenesis in the DG [27, 67]. It is tempting to speculate that Neil3 contributes to cognitive function by maintaining adult neurogenesis as we found reduced stem cell proliferation exclusively in Neil3-deficient male AD mice that also presented with impaired working memory. DNA damage accumulation in the hippocampus seems neither contributing to impaired adult neurogenesis nor memory as we found increased level of oxidative base lesion particularly in female AD mice lacking Neil3. Surprisingly, not only did the NEIL3 substrate 5OH-dC accumulate in Neil3-deficient AD hippocampus but also 8-oxodG, a common marker for oxidative stress. NEIL3 has been shown to excise oxidation products of 8-oxodG but not 8-oxodG itself [71], suggesting that loss of Neil3 might increase oxidative stress in general in the AD mouse model. Moreover, the level of oxidative base lesions in the hippocampus decreased during AD progression in the Neil3-deficient mice. This might be explained by an active removal of cells with a high oxidative stress burden in the hippocampus during AD. This is in line with previous studies showing that excessive DNA damage leads to cell elimination through apoptosis as a protective mechanism [11,13,17,31].

5. Conclusion

We present for the first time a role of DNA glycosylase Neil3 in AD pathogenesis. We show that Neil3 aggravates amyloid pathology, promotes adult hippocampal neurogenesis, and maintains memory function in a sex-and age-dependent manner but independent of global oxidative base lesion repair in a mouse model of AD. Our findings further confirm that impaired adult neurogenesis is an early event in AD that correlates with memory formation. Moreover, our study underlines the importance of considering sex differences when studying mouse models of AD.

Author contributions statement

M.A.E. and S.S. contributed equally to experiments and analysis, while M.A.E. and K.S. were major contributors in writing the manuscript. A.B.N.N contributed to IHC staining and analysis, T.S. performed the RNA sequencing analysis, while N.B. and K.S. conducted behavioral studies. M.B. and K.S. designed and supervised the project. All authors read and approved the final manuscript.

Availability of data and material

The RNA sequencing dataset supporting the conclusions of this article is available in the NCBI's Gene Expression and Omnibus repository, accession number GSE197199 and <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE197199>.

Funding

This work was supported by The Norwegian University of Science and Technology (NTNU), the Research Council of Norway grant 275777, the Central Norway Regional Health Authority of Norway grants 90172200 and 90369200.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We want to thank the Proteomics and Modomics Experimental Core Facility (PROMEC) at NTNU for the help with mass spectrometry analysis, as well as the Cellular and Molecular Imaging Core Facility (CMIC) at NTNU for the help with scanning mouse brain sections.

List of abbreviations

A β	amyloid beta
A β 40	amyloid beta peptide 40
A β 42	amyloid beta peptide 42
AD	Alzheimer's disease
APP	amyloid precursor protein
BER	base excision repair
DG	dentate gyrus
ER	estrogen receptor
EZM	elevated zero maze
IF	immunofluorescence
IHC	immunohistochemistry
MCI	mild cognitive impairment
MS	mass spectrometry
NEIL3	Nei endonuclease VIII-like 3
NFTs	neurofibrillary tangles
ROS	reactive oxygen species
ssDNA	single-stranded DNA
WT	wild type
5OH-dC	5-hydroxy-deoxycytosine
8oxo-dG	8-oxo-deoxyguanosine

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.freeradbiomed.2022.11.020>.

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