Accelerated dystrophy and decay of oligodendrocyte precursor cells in the APP/PS1 model of Alzheimer's-like pathology

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Author contribution statement

IC-D-L-R: Formal Analysis; Investigation; Methodology; Writing - original draft.
GF: Formal Analysis; Investigation; Methodology; Validation.
ADR: Investigation.
AV: Conceptualiztion; Writing - review & editing.
OR: Resources; Writing – review & Editing
DG-N: Conceptualization; Data curation; Formal analysis; Funding acquisition; Project administration; Resources; Supervision; Validation; Visualization; Writing - review & editing.
AMB: Conceptualization; Data curation; Formal analysis; Funding acquisition; Project administration; Resources; Supervision; Validation; Visualization; Writing - original draft; Writing - review & editing.

Keywords

Hippocampus, myelin, OPC, Oligodendrocyte progenitor cell, Alzheimer"s disease

Abstract

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Myelin disruption is a feature of natural aging and of Alzheimer´s disease (AD). In the CNS, myelin is produced by oligodendrocytes, which are generated throughout life by oligodendrocyte progenitor cells (OPCs). Here, we examined age-related changes in OPCs in APP/PS1 mice, a model for AD-like pathology, compared with non-transgenic (Tg) age-matched controls. Analysis was performed in the CA1 area of the hippocampus following immunolabelling for NG2 with the nuclear dye Hoescht, to identify OPC and OPC sister cells, a measure of OPC replication. The results indicate a significant decrease in the number of OPCs at 9 months in APP/PS1 mice, compared to age-matched controls, without further decline at 14 months. In addition, the number of OPC sister cells declined significantly at 14-months in APP/PS1 mice, which was not observed in age-matched controls. Notably, OPCs also displayed marked morphological changes at 14 months in APP/PS1 mice, characterized by an overall shrinkage of OPC process domains and increased process branching. The results indicate that OPC disruption is a pathological sign in the APP/PS1 mouse model of AD.

Contribution to the field

There is increasing recognition that glial cells are important in the pathogenesis of Alzheimer´s disease (AD). In recent years, evidence has accumulated that myelin loss occurs at an early stage of AD, but the reasons are unknown. In this study, we describe age-dependent changes in oligodendrocyte progenitor cells (OPC) in the APP/PS1 mouse model of AD. Our results demonstrate that OPC disruption is a pathological sign in this mouse model and is a potential factor in accelerated myelin loss and cognitive decline.

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Keywords: oligodendrocyte progenitor cell, OPC, myelin, hippocampus, Alzheimer's disease

Abstract

Myelin disruption is a feature of natural aging and of Alzheimer´s disease (AD). In the CNS, myelin is produced by oligodendrocytes, which are generated throughout life by oligodendrocyte progenitor cells (OPCs). Here, we examined age-related changes in OPCs in APP/PS1 mice, a model for AD-like pathology, compared with non-transgenic (Tg) age-matched controls. Analysis was performed in the CA1 area of the hippocampus following immunolabelling for NG2 with the nuclear dye Hoescht, to identify OPC and OPC sister cells, a measure of OPC replication. The results indicate a significant

decrease in the number of OPCs at 9 months in APP/PS1 mice, compared to age-matched controls, without further decline at 14 months. In addition, the number of OPC sister cells declined significantly at 14-months in APP/PS1 mice, which was not observed in age-matched controls. Notably, OPCs also displayed marked morphological changes at 14 months in APP/PS1 mice, characterized by an overall shrinkage of OPC process domains and increased process branching. The results indicate that OPC disruption is a pathological sign in the APP/PS1 mouse model of AD.

Introduction

Alzheimer's disease (AD) is the most common type of dementia and it is characterized by the formation of intracellular neurofibrillary tangles (NFTs) and extracellular amyloid-β (Aβ) plaques (Braak and Braak, 1991). White matter disruption is present at an early stage of AD pathology (Bartzokis, 2011, Ihara et al., 2010), and post-mortem analyses indicate that a loss of oligodendrocytes in AD could serve as a diagnostic tool for differentiating white matter pathologies in dementia (Sjöbeck and Englund, 2003, Brickman et al., 2015). Studies in human AD and mouse models indicate loss of oligodendrocytes and demyelination is most pronounced at the core of Aβ plaques (Mitew et al., 2010). Hence, myelin loss is a feature of human AD and mouse models (Desai et al., 2009), but the underlying causes are unresolved.

In the adult brain, oligodendrocyte progenitor cells (OPCs) are responsible for the life-long generation of oligodendrocytes, required to myelinate new connections formed in response to new life experiences, and to replace myelin lost in pathology (Xiao et al., 2016, McKenzie et al., 2014, Young et al., 2013, Hughes et al., 2018). OPCs are identified by their expression of the NG2 proteoglycan and are sometimes known as NG2-cells or NG2-glia (Butt et al., 2002). Prior to differentiating into mature myelinating oligodendrocytes, OPCs transition through an intermediate phase identified by expression

of the G-protein coupled receptor GPR17 (Viganò et al., 2016). Notably, early changes in OPCs may be a pathological sign and underlie myelin loss in mouse models of AD-like pathology (Rivera et al., 2016, Mitew et al., 2010, Vanzulli et al., 2020). This possibility is supported by immunostaining of post-mortem AD brain showing reduced NG2 immunoreactivity in individuals with high Aβ plaque load (Nielsen et al., 2013b).

The APP/PS1 transgenic mouse expresses familial AD-causing mutated forms of human APP (APPswe, Swedish familial AD-causing mutation) and presenilin1 (PS1dE9) and is used extensively as a model for AD-like pathology (Borchelt et al., 1997). The APP/PS1 mouse presents early Aβ plaque deposition in the hippocampus at 4-5 months of age and extensively throughout the forebrain by 8 months (Borchelt et al., 1997), which is linked to greatly impaired synaptic long-term potentiation (LTP) after 8 months of age in the CA1 area of the hippocampus in APP/PS1 (Gengler et al., 2010). Furthermore, several studies provide evidence that white matter and myelin disruption are early clinical signs of APP/PS1 mice (Dong et al., 2018, Wu et al., 2017, Shu et al., 2013, Chao et al., 2018), with evidence that myelin disruption in APP/PS1 mice aged 6 months is accompanied by decreased learning and spatial behavior performance (Dong et al., 2018, Chao et al., 2018). In addition, there is evidence of increased NG2 cell numbers in the temporal lobe of 6 months old APP/PS1 mice (Dong et al., 2018), and clustering of hypertrophic NG2 cells around Aβ plaques in the cortex of 14 month old APP/PS1 (Li et al., 2013). Here, we examined changes in OPCs in 9 and 14 months old APP/PS1 mice, compared to age-matched non-transgenic controls, and focused on the AD-relevant CA1 area of the hippocampus. Our results indicate a premature decline in OPC numbers at 9 months in APP/PS1, whilst at 14 months OPCs displayed cellular shrinkage and increased process branching in APP/PS1, characteristic of reactive changes in response to pathology (Ong and Levine, 1999, Butt et al., 2002). This study identifies pathological changes in OPCs in the APP/PS1 mouse model of AD.

MATERIAL AND METHODS

Ethics

The animal study was reviewed and approved by the University of Southampton Animal Welfare Ethical Review Body (AWERB). All procedures were carried out in accordance with the Animals (Scientific Procedures) Act 1986 of the UK.

Animals and tissue

Transgenic APP/PS1 mice were used that contain human transgenes for both APP (KM670/671NL, Swedish) and PSEN1 (L166P). APPswe/PSEN1dE9 mice (APP/PS1) on a C57BL/6 background were originally obtained from the Jackson Laboratory and heterozygous males were bred at our local facilities with wild-type female C57BL/6J (Harlan). Offspring were ear punched and genotyped using PCR with primers specific for the APP-sequence (forward: GAATTCCGACATGA CTCAGG, reverse: GTTCTGCTGCATCTTGGACA). Mice not expressing the transgene were used as non-transgenic wild-type littermate controls. Mice were housed in groups of 4 to 10, under a 12-h light/12h dark cycle at 21oC, with food and water ad libitum. No mice were excluded and experimental groups contained a spread of sexes. Mice weight was monitored throughout the experiment. APP/PS1 mice and age matched non-transgenic controls aged 9 and 14 months old were perfusion fixed intracardially under terminal anaesthesia with 4% paraformaldehyde (PFA), then post-fixed for 2 hours with 4% PFA. Sections were cut on a vibratome (Leica) at a thickness of 35 μm then stored in cryoprotectant at -70°C until use.

Immunohistochemistry

Sections were treated for a blocking stage of either 10-20% normal goat serum (NGS) or normal donkey serum (NDS) or 0.5% bovine serum albumin (BSA) for 1-2 h, depending on the primary antibodies to be used. Sections were washed 3 times in PBS, and incubated overnight in primary antibody diluted in blocking solution containing 0.25% Triton-X: rabbit anti-NG2, 1:500 (Millipore); rabbit anti-Olig2, 1:500 (Millipore); rabbit anti-GPR17, 1:100 (Cayman Labs); rat anti-MBP, 1:300 (Millipore). Sections were washed 3 times in PBS, and incubated overnight in primary antibody diluted in blocking solution containing 0.25% Triton-X: rabbit anti-NG2, 1:500 (Millipore); rabbit anti-Olig2, 1:500 (Millipore); rabbit anti-GPR17, 1:100 (Cayman Labs); rat anti-MBP, 1:300 (Millipore). Tissues were then washed 3 times in PBS and incubated with appropriate fluorochrome secondary antibody (AlexaFluor® 488, AlexaFluor® 568, 1:400, Life Technologies), or biotinylated secondary antibody (Vector Labs) diluted in blocking solution for 1-2h. Finally, sections were washed 3 times with PBS before being mounted on glass slides and covered with mounting medium and glass coverslips ready for imaging.

Imaging and Analysis

Immunofluoresecence images were captured using a Zeiss Axiovert LSM 710 VIS40S confocal microscope and maintaining the acquisition parameters constant to allow comparison between samples within the same experiment. Acquisition of images for cell counts was done with x20 objective. Images for OPC reconstruction were taken using x100 objective and capturing z-stacks formed by 80-100 single plains with an interval of 0.3 µm. Cell counts were performed in the CA1 area in projected flattened images from z-stacks formed by 10 or 15 *z*-single plain images with 1µm interval between them, and cell density was calculated as the total number of cells per unit area expressed as cells per mm^2. The relative density of MBP immunolabelling was measured within a constant field of view (FOV) using ImageJ. For DAB immunostaining of Olig2+ oligodendrocytes, sections were examined

on an Olympus dotSlide digital slide scanning system based on a BX51 microscope stand with integrated scanning stage and Olympus CC12 colour camera. The cell coverage of OPCs was measured using ImageJ by drawing a line around the cell processes and measuring the area enclosed within the line and expressing the data relative to the area of the CA1 in each section. For morphological analysis of single OPCs, cells were drawn using Neurolucida 360 and their morphology was analysed using Neurolucida 360 explorer for measurements of the number of processes per cell, number of process terminals (end-points), number of nodes (branch points) and cell complexity; OPC cell complexity refers to the normalization and comparison of processes derived from the dendritic complexity index described (Pillai et al., 2012), whereby Neurolucida 360 Explorer calculated cell *complexity* from the sum of [*terminal orders* + *number of terminals*] multiplied by the [*total dendritic length* / *number of primary dendrites*], where *terminal* is defined as a process ending and *terminal order* is the number of branches along a process, between the cell body and the terminal (calculated for each terminal). For Sholl analysis, the interval between Sholl shells was 5µm. Data were expressed as Mean±SEM and tested for significance by ANOVA followed by Tukey's post-hoc test for cell numbers, myelin immunostaining, OPC cell domains and neurolucida analyses of OPCs, and Sidak's multiple comparisons test for Sholl analysis, using GraphPad Prism 6.0.

RESULTS

Premature decline of OPCs in the hippocampus of APP/PS1 mice

The hippocampus displays a high degree of adult oligodendrogenesis, which is important for learning and plasticity (Steadman et al., 2020). Here, we used NG2 immunolabelling to identify adult OPCs (Nishiyama et al., 2016) in the CA1 area of the hippocampus (Fig. 1); NG2 is also expressed by pericytes, which are directly applied to blood vessels and readily distinguished from OPCs, which are distinguished by their complex process bearing morphology (Hamilton et al., 2010). OPCs are

uniformly distributed throughout the hippocampus at both 9 and 14 months, in APP/PS1 mice and age-matched controls (Fig. 1A, B). NG2+ OPCs are often observed as duplets or triplets (some indicated by arrows in Fig. 1A, B, and at higher magnification in the inset in Fig. 1A). OPC duplets have been shown to be recently divided sister cells and their frequency is a measure of OPC cell division (Boda et al., 2014), confirming previous studies that adult OPCs continue to divide slowly in old age (Young et al., 2013, Psachoulia et al., 2009). Quantification confirmed a significant difference in the numerical density of NG2+ OPCs in APP/PS1 at 9 months compared to age-matched controls (Fig. 1C; two-way ANOVA $p \leq 0.05$, followed by Tukey's post hoc test). The data indicated a 50% decrease in NG2+ OPCs at 9 months in APP/PS1 to a level observed at 14 months in natural ageing (Fig. 1C); there was no further decline in OPC numbers between 9 and 14 months APP/PS1 mice, which were the same as age-matched controls (Fig. 1C). In addition, there was a significant decrease in the numerical density of OPC sister cells at 14 months in APP/PS1 mice (Fig. 1D; two-way ANOVA $p \leq 0.05$, followed by Tukey's post hoc test, $p \leq 0.05$). Overall, the results indicate a premature decline in OPC numbers at 9 months in APP/PS1 mice.

Decline in myelination in the hippocampus of APP/PS1 mice

The hippocampus displays a high degree of myelination, which is essential for cognitive function (Abraham et al., 2010), and myelination has been shown to be disrupted in this area in APP/PS1 mice and it is relevant to AD pathology (Ota et al., 2019, Chao et al., 2018, Dong et al., 2018). Immunolabelling for MBP is prominent in the CA1 area at both 9- and 14-months in controls and in APP/PS1 (Fig. 2A, B), as are GPR17+ cells, which are an intermediate stage between OPCs and myelinating oligodendrocytes (upper insets, Fig. 2A, B), and Olig2+ cells, which is expressed by all oligodendroglial cells (lower insets, Fig. 2A, B). Between 9- and 14- months of age, we observed no significant changes in the numerical density of GPR17+ and Olig2+ oligodendrocytes in controls or

APP/PS1 (Fig. 2C, D), and so we did not analyse oligodendrocyte cell numbers further; it should be noted there was wide variability in GPR17+ cells at 14-months in controls, but overall there was difference in the number of GPR17+ cells in APP/PS1 between 9- and 14-months in the CA1 region of the hippocampus. Significant age-related changes in MBP immunostaining were detected in the CA1 region and this was not observed in APP/PS1 mice (Fig. 2E; ANOVA, $p \leq 0.01$, followed by Tukey's post hoc tests). Overall, the results indicate MBP immunostaining is retarded at later stages of pathology in APP/PS1 mice.

OPC exhibit cellular shrinkage at 14 months in APP/PS1 mice

The results above indicate OPC are disrupted in APP/PS1 mice, which is often associated with changes in OPC morphology in AD and other pathologies (Butt et al., 2019a, Butt et al., 2019b, Vanzulli et al., 2020). We therefore examined OPC morphology in depth, using high magnification confocal images and measuring the process domains of individual cells and the total coverage of NG2 cells within the CA1 (Fig. 3). Significant differences were detected in the size of OPC process domains in 14 month APP/PS1 (Fig. 3Biii; ANOVA, $p \leq 0.001$, followed by Tukey's post hoc test, $p \leq 0.001$); no differences were observed in OPCs at 9-months in APP/PS1 compared to controls. The results indicate that at 14-months OPCs display a significant shrinkage in APP/PS1.

OPC exhibit increased process branching and cellular complexity at 14 months in APP/PS1 mice

The underlying morphological changes resulting in OPC shrinkage in APP/PS1 mice were examined in further detail using Neurolucida cell tracing. Confocal images of 80-100 *z*-sections, each of 0.3µm thickness, were captured using a x100 oil objective and reconstructed and analyzed using Neurolucida 360 and Neurolucida 360 Explorer (Fig. 4A, B; n=9 cells from 3 animals in each group). Consistent with the results above, OPC morphology was significantly altered at 14-months in APP/PS1 compared

to age-matched controls, with the average number of processes per cell being unaltered (Fig. 4C), whereas processes displayed increased branching, with a significantly greater number of process terminals or end points (Fig. 4D; ANOVA $p \leq 0.01$, followed by Tukey's post hoc test, $p \leq 0.05$) and number of branch points or nodes (Fig. 4E; ANOVA $p \leq 0.01$, followed by Tukey's post hoc test, $p \leq 0.01$), with a consequent 3-fold increase in the Neurolucida measurement of cell complexity in 14-month APP/PS1 compared to age-match controls (Fig. 4F; ANOVA $p \leq 0.01$, followed by Tukeys post hoc test, $p \leq 0.01$). In contrast, no changes in the morphological parameters of OPCs were detected between 9- and 14-months in wild-type mice (Fig. 4C-F) or in 9-month APP/PS1 OPC compared to age-matched controls (Fig. 4C-F). The age-related changes in OPC complexity in APP/PS1 mice was examined further using Sholl analysis (Fig. 5A; n=9 cells for each group, ANOVA followed by Sidak's multiple comparisons test). Sholl analysis confirmed significant differences in OPC morphology in APP/PS1 mice between 9 and 14 months, with significant increases in the number of end points (Fig. 5B), the number of nodes (Fig. 5C), and in process lengths (Fig. 5D). In addition, analysis of processes length in the different branch orders identified that OPCs displayed increased process length in the distal branches (Fig. 5E). In contrast to these changes in APP/PS1, no significant differences were found in OPC morphology in natural aging (Fig. 5, insets); at 14 months, OPCs displayed a decrease in process lengths in the proximal branches, whereas this parameter was increased in APP/PS1 at 14 months (Fig. 5E, inset). It is important to note that the small number of cells analysed by Neurolucida and Sholl may have introduced the possibility of bias. Nonetheless, the measurements of OPC process domains, together with Neurolucida and Sholl analyses, all indicate that OPC shrinkage is a key feature in APP/PS1 at 14-months and is associated with increased process branching, giving OPCs a more fibrous appearance that is similar to 'reactive' NG2 cells reported in human AD and AD models (Li et al., 2013, Nielsen et al., 2013a, Vanzulli et al., 2020), as well as injury models (Jin et al., 2018, Butt et al., 2005, Ong and Levine, 1999), and this was not observed in age-matched controls

Discussion

Age-related loss of myelin has been shown to be a pathological feature of human AD (Bartzokis, 2011, Brickman et al., 2015) and in animal models of AD (Dong et al., 2018, Desai et al., 2009, Mitew et al., 2010, Vanzulli et al., 2020). We observed a decrease in MBP immunostaining at 14 months in the hippocampus of APP/PS1 mice, consistent with evidence that myelination is disrupted in this model of AD (Dong et al., 2018, Wu et al., 2017, Shu et al., 2013, Chao et al., 2018). The key findings of the present study are that there is a premature decrease in OPC density at 9-months in APP/PS1 mice, and that at 14-months OPC displayed a shrunken and fibrous morphology, indicative of morphological dystrophy. These findings indicate that changes in OPCs are potential factors in the progression of AD pathology.

Our data support previous studies that there is a decline in the number of OPCs in natural ageing (Young et al., 2013). Notably, this age-related loss of OPCs occurred at 9 months of age in APP/PS1, indicating a premature loss of OPCs in this model of AD. The reduction in OPCs numbers at any point is a measure of changes in cell proliferation and/or death at earlier points, hence the reduction in OPC numbers at 9 months in APP/PS1 mice reflects an acceleration of the age-related loss of OPCs, which in natural aging occurs at later ages. The decrease in OPCs at 9 months in APP/PS1 indicates their capacity for self-renewal, defined as maintaining OPC numbers relatively constant over time, was reduced at a point prior to this age, which is consistent with evidence of advanced OPC senescence in 7.5-month-old APP/PS1 mice (Zhang et al., 2019). We observed a reduction in OPC sister cells at 14 months in APP/PS1, which is a measure of recently divided OPCs (Boda et al., 2014), suggesting that OPC self-renewal may be compromised at later ages in APP/PS1, although further studies are required to confirm this, for example using multiple injections of BrdU. The changes in OPCs were associated with a reduction in MBP immunostaining at 14-months in APP/PS1 mice compared to controls. MBP

immunostaining, taken as a measure of the overall extent of myelination, was increased between 9- and 14-months in wild-type controls, but not in APP/PS1 mice, consistent with multiple lines of evidence that myelination is disrupted in AD-like pathology (Dong et al., 2018, Wu et al., 2017, Shu et al., 2013, Chao et al., 2018, Desai et al., 2009, Mitew et al., 2010, Vanzulli et al., 2020). We did not detect evident changes in GPR17+ and Olig2+ oligodendrocytes, and no conclusions can be drawn on the overall numbers of oligodendrocytes at this time. The decrease in MBP immunostaining at 14-months in APP/PS1 mice may reflect changes in the number and lengths of myelin sheaths, which has been reported in aging (Hughes et al., 2018, Hill et al., 2018). Myelin remodelling is important for nervous system plasticity and repair (Ortiz et al., 2019, Chorghay et al., 2018, Williamson and Lyons, 2018, Foster et al., 2019), and the decline in myelination in APP/PS1 may be related to neuronal loss and learning dysfunction in these mice (Chao et al., 2018). The results provide evidence of OPC and myelin disruption in the hippocampus of APP/PS1 mice, suggesting key features of human AD are replicated in this mouse model.

Notably, the early loss of OPCs at 9-months in APP/PS1 hippocampus is followed at 14-months by a more fibrous appearance of NG2+ OPCs due to cell shrinkage and increased branching, similar to the fibrous morphology of ‘reactive’ NG2-glia (Butt et al., 2002, Ong and Levine, 1999). Notably, fibrous or reactive NG2-glia have been reported to be associated with amyloid-β plaques in human AD and mouse models (Nielsen et al., 2013b, Li et al., 2013, Vanzulli et al., 2020, Zhang et al., 2019), and further studies are required to determine whether OPC morphological changes depend on their relation to amyloid-β plaques, as has been reported for astrocytes (Rodríguez et al., 2016). Since OPCs are the source of new myelinating oligodendrocytes in the adult brain (Rivers et al., 2008, Dimou et al., 2008, Zhu et al., 2008, Kang et al., 2010), it is possible their dystrophy in AD-like pathology may be a causative factor in myelin loss, but this will require comprehensive analyses to verify, such as fate-

mapping and live-cell imaging. Furthermore, the underlying causes of OPC shrinkage in APP/PS1 are unresolved, but OPC are known to contact synapses in the hippocampus (Bergles et al., 2000), and reduced synaptic activity is an important feature in APP/PS1 mice (Gengler et al., 2010), which could result in retraction of OPC processes (Chacon-De-La-Rocha et al., 2020). In addition, neuronal activity regulates myelination and myelin repair (Gibson et al., 2014, Wake et al., 2011, Ortiz et al., 2019), and the observed disruption of OPCs suggests this may be an important factor in myelin loss in AD-like pathology.

Conclusions

Our findings demonstrate that OPCs undergo complex age-related changes in the hippocampus of the APP/PS1 mouse model of AD-like pathology. We conclude that OPC disruption is a pathological sign in AD and is a potential factor in accelerated myelin loss and cognitive decline.

Competing interests:
AMB and ADR declare they are share-holders and co-founders of the company GliaGenesis Ltd. All the authors declare that they have no other competing interests.

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Authors' contributions:

IC-D-L-R: Formal Analysis; Investigation; Methodology; Writing - original draft.

GF: Formal Analysis; Investigation; Methodology; Validation.

ADR: Investigation.

AV: Conceptualiztion; Writing - review & editing.

OR: Resources; Writing – review & Editing

DG-N: Conceptualization; Data curation; Formal analysis; Funding acquisition; Project administration; Resources; Supervision; Validation; Visualization; Writing - review & editing.

AMB: Conceptualization; Data curation; Formal analysis; Funding acquisition; Project administration; Resources; Supervision; Validation; Visualization; Writing - original draft; Writing - review & editing.

Data Availability Statement

All data generated or analysed during this study are included in this published article.

Figure Legends

Figure 1 Changes in OPCs in the CA1 area of the hippocampus of APP/PS1 mice. Hippocampi of 9months old and 14months old APP/PS1 mice were compared to age-matched controls. (A, B) Representative confocal images of immunofluorescence labelling for NG2 (green) to identify OPCs and counterstaining with Hoechst (blue) for nuclei, to identify OPC sister cells (some indicated by arrows), as illustrated at higher magnification (inset, Ai), from non-transgenic controls (Ai, Bi) and APP/PS1 mice (Aii, Bii), aged 9 months (Ai, Aii) and 14 months (Bi, Bii); scale bars = 50µm in main panels and 10 mm in inset. (C, D) Bar graphs of the numerical density of NG2+ OPCs (C) and OPC sister cells (D). Data are expressed as Mean ± SEM; *p<0.05, ANOVA followed by Tukey's post hoc test, n= 3 animals for each group.

Figure 2 Changes in oligodendrocytes and myelin in the CA1 area of the hippocampus of APP/PS1 mice. Hippocampi of 9months old and 14months old APP/PS1 mice were compared to age-matched controls. (A, B) Representative photomicrographs of immunolabelling for MBP (red) to identify the extent of myelination, together with GPR17 for immature oligodendrocytes (upper insets, green) and Olig2 for total number of oligodenrocyte lineage cells (lower panels, brown); scale bars = 50µm, except upper insets = 20 mm. (C-E) Bar graphs of numerical density of GPR17+ cells (C) and Olig2+ cells (D), together with MBP immunofluorescence density (E); data are expressed as Mean ± SEM; *p<0.05 ANOVA followed by Tukey's post hoc test, n= 3 animals for each group.

Figure 3 OPC process domains in the CA1 area of the hippocampus of APP/PS1 mice. Hippocampi of 9 months old and 14 months old APP/PS1 mice were examined, compared to age-matched controls, using immunofluorescence labelling for NG2 (green) to identify OPCs. High magnification confocal projections of OPCs and their process domains (indicated by broken white lines) in the 9 months old hippocampus (Ai, Aii), and the 14 months old hippocampus (Bi, Bii), in controls (Ai, Bi) and APP/PS1 (Aii, Bii). Scale bars = 20µm. (Aiii-Biii) Box-Whisker plots of the total area of OPC process domains. Data are Mean ± SEM, ***p<0.001, ANOVA, followed by Tukey's post hoc test, n=10 cells for WT-9mo and APP-9mo, n=13 cells for WT-14mo and n=17 cells for APP-14mo, from 3 animals in each group.

Figure 4. OPC morphological changes in the CA1 of the 14 months old APP/PS1 mouse model compared to an aged-matched control. Data were generated by Neurolucida 360 analysis of cells. Box-whisker plots of (A) cell body area, (B) cell body volume, (C) process volume, (D) total cell volume, (E) cell complexity, (F) ramification index. Data expressed as Mean±SEM. ANOVA, followed by Tukey's post hoc test, *$p \leq 0.05$, **$p \leq 0.01$; n= 9 cells from 3 animals in each group.

Figure 5. Sholl analysis of age-related changes in OPC morphology in APP/PS1 and age-matched controls. (A) 3D morphology of NG2 immunolabelled OPC in the CA1 area of the hippocampus (generated using isosurface rendering with Volocity software, PerkinElmer), illustrating Sholl shells (concentric circles, 5 mm apart, with the cell body in the middle), and the morphological parameters measured; the points of process branching are termed nodes (blue dots), the points where the processes intersect the Sholl shells are termed intersections (yellow dots), the number of process terminals or end points, and the process branch order, with 1st order closest to the cell body (adapted from Sholl 1953 and Rietveld et al. 2015). (**B-E**) Graphs comparing OPC morphological parameters in AAP/PS1 mice aged 9 months (-●-) and 14 months (-■-), together with age-matched controls (insets); two-way ANOVA followed by Sidak's multiple comparisons test. *p<0.05, **p<0.01, ***p<0.001, p<0.0001. n = 9 cells from 3 animals in each group.

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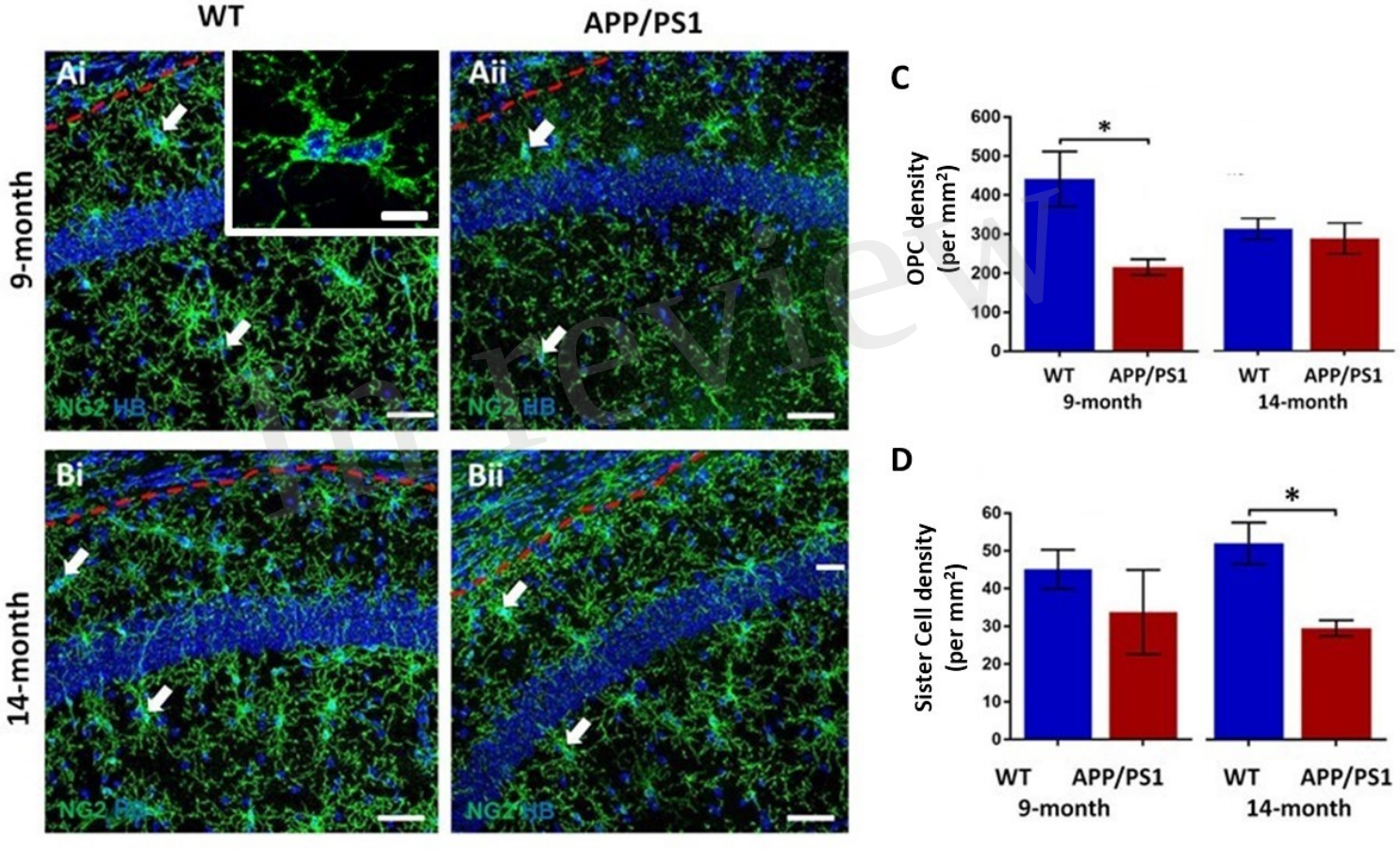
Figure 1.JPEG



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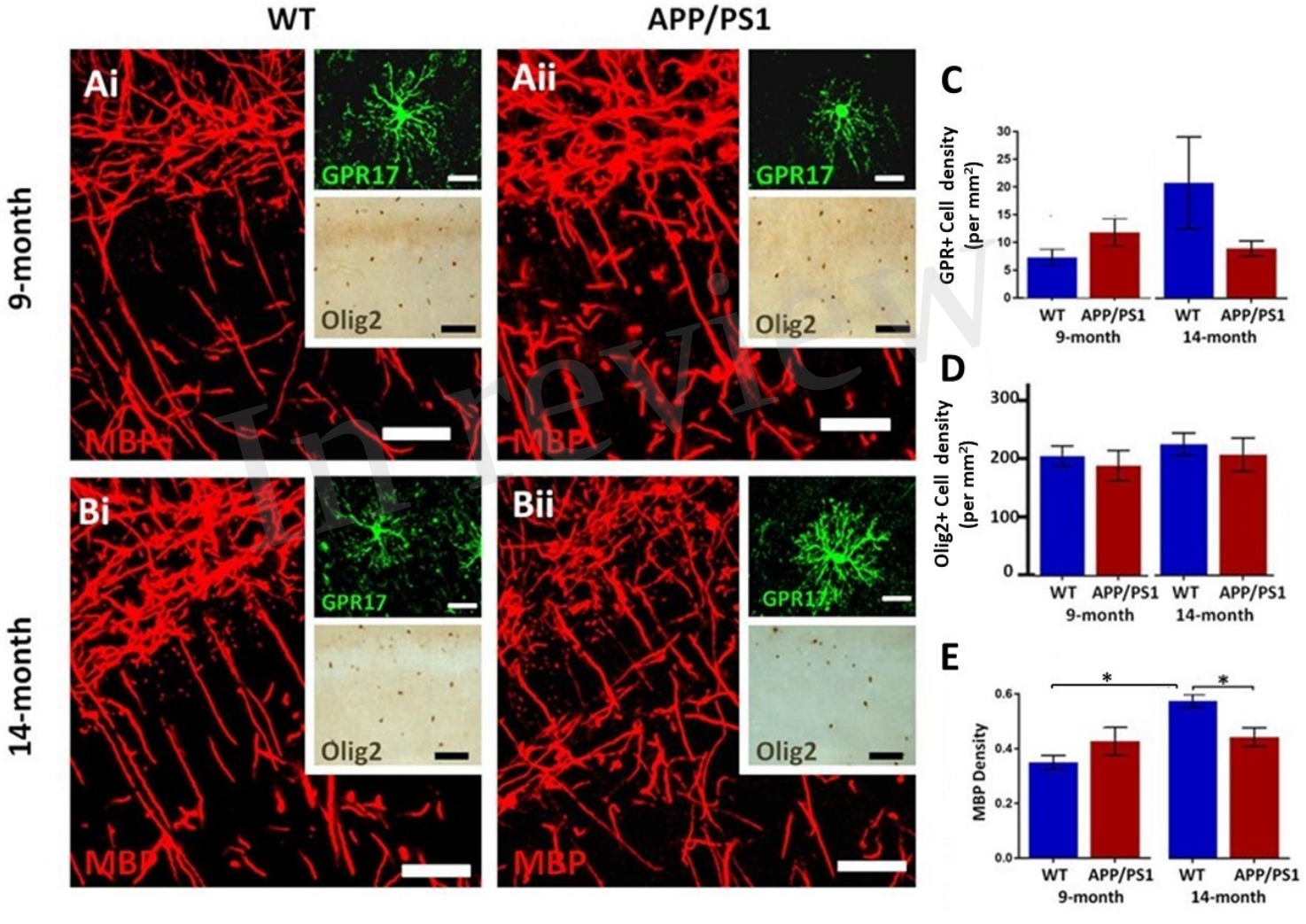

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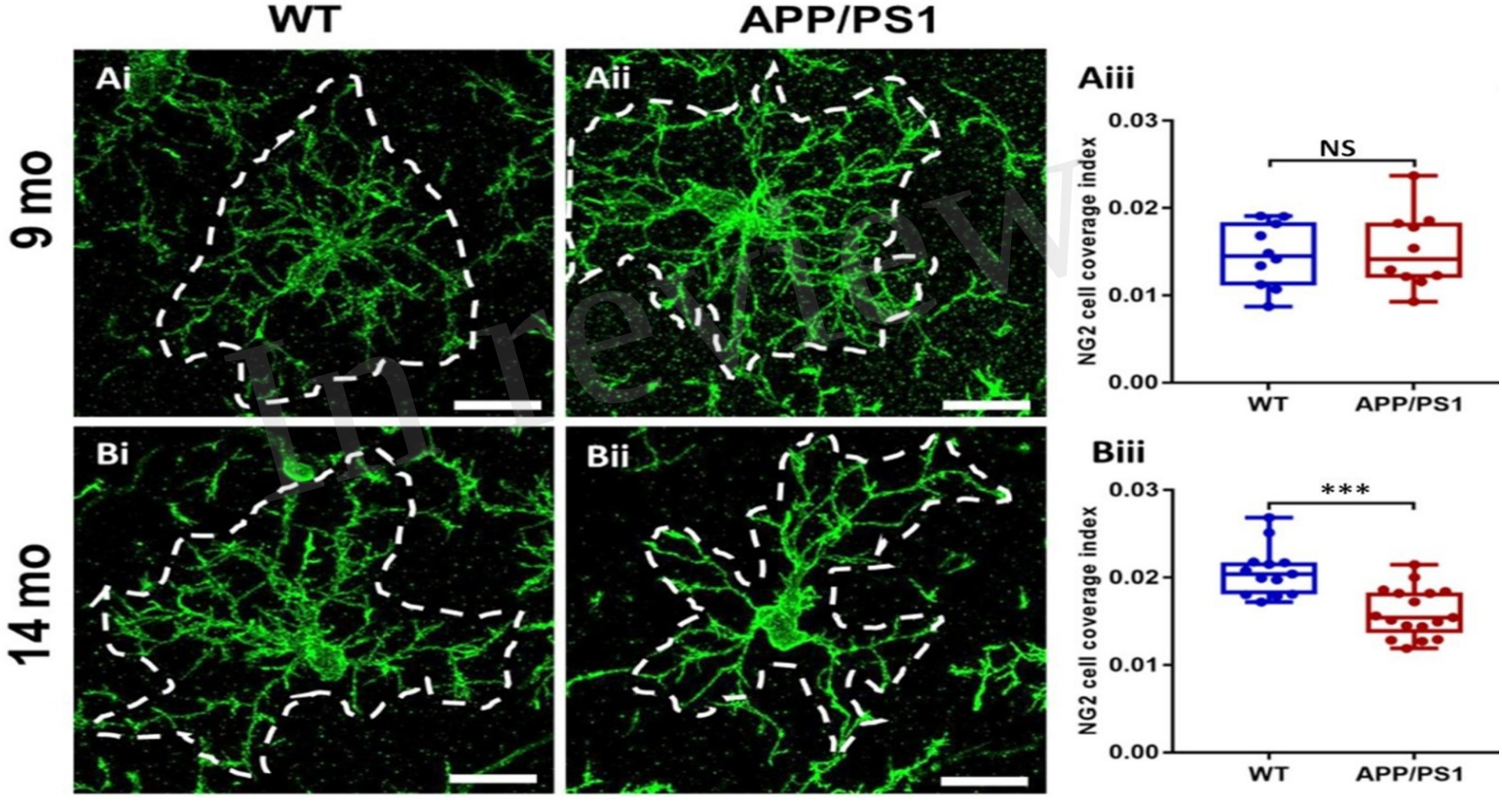

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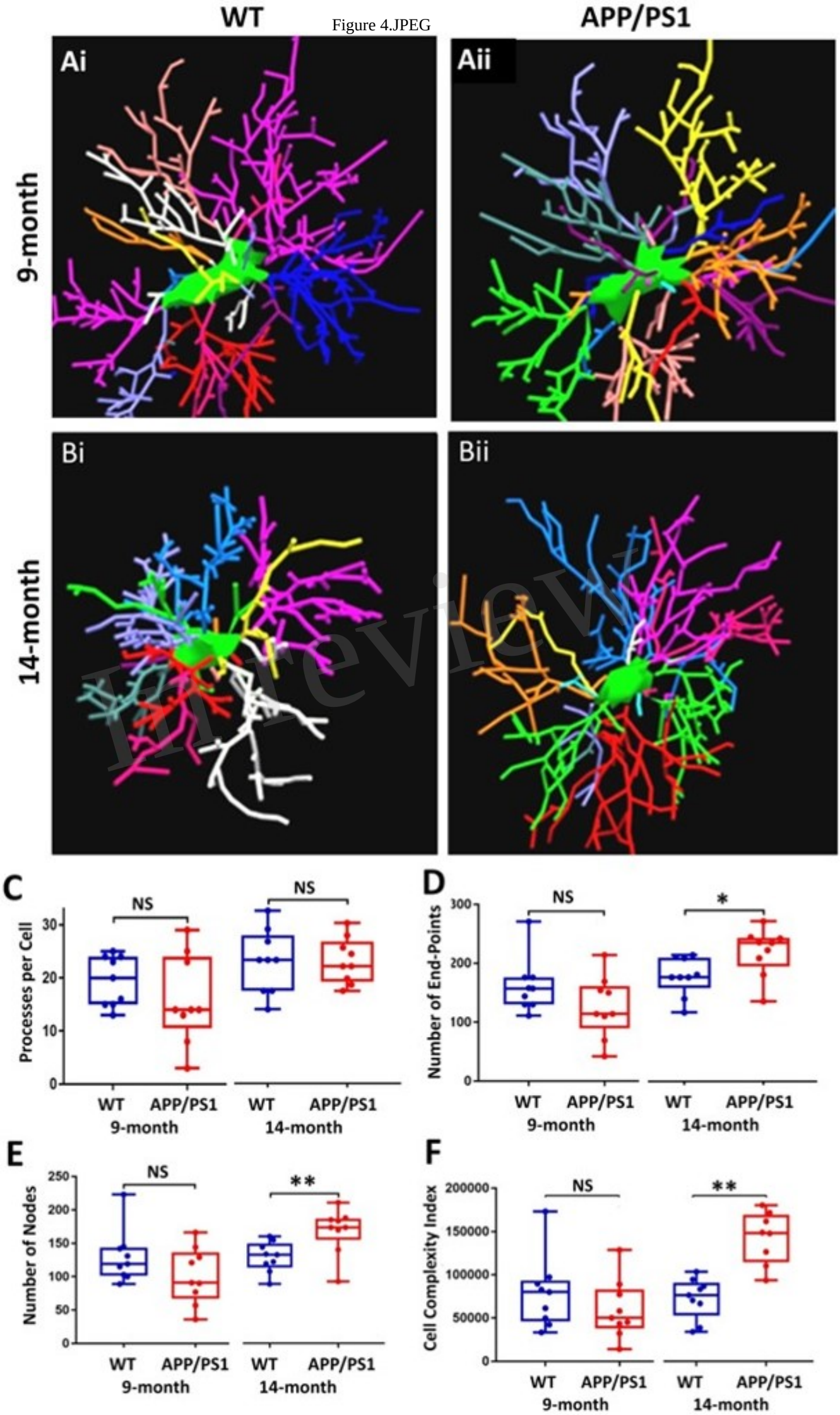

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