

Amyloid-beta aggregates at cribriform plate driving lymphatic dysfunction in Alzheimer's Disease

Sophia M. Vrba^{1,2,3}, Collin J. Laaker^{2,4}, Ajinkya R. Limkar^{1,5,6}, Dhruv Bansal², Avery R. Holtman², Abigail M. Sehmer², Melinda Herbath², Thanthrige Thiunuwan Priyathilaka², Jenna M. Port^{2,3}, Martin Hsu⁷, William A. Riche⁶, Tyler K. Ulland^{2,8}, Erin G. Brooks², Jeffrey Helgager², Jeffrey Nirschl^{2,8}, Mariana Pehar^{9,10}, Matyas Sandor², Zsuzsanna Fabry²

¹Medical Scientist Training Program, University of Wisconsin Madison, Madison WI
²Department of Pathology and Laboratory Medicine, University of Wisconsin Madison, Madison WI
³Cellular and Molecular Pathology Training Program, University of Wisconsin Madison, Madison WI
⁴Neuroscience Training Program, University of Wisconsin Madison, Madison WI
⁵Cellular and Molecular Pharmacology Training Program, University of Wisconsin Madison, Madison WI

⁶Department of Urology, Carver Cancer Center, University of Wisconsin Madison, Madison WI
⁷Department of Genetics, University of North Carolina Chapel Hill, Chapel Hill NC
⁸Wisconsin Alzheimer's Disease Research Center, University of Wisconsin Madison, Madison WI
⁹Division of Geriatrics and Gerontology, Department of Medicine, University of Wisconsin Madison, Madison WI
¹⁰Geriatrics Research Education Clinical Center, William S. Middleton Memorial Veterans Hospital, Madison WI

Introduction to Alzheimer's Disease

- Alzheimer's Disease (AD) is the **most common** form of dementia¹, affecting 60 million people worldwide, and **>90% of patients** can have olfactory dysfunction².
- AD is a progressive, neurodegenerative disease characterized by excess deposition and **impaired clearance of amyloid beta (Aβ)** and neurofibrillary tangles of hyperphosphorylated tau.
- There are **no curative treatments** for AD. Current treatments include acetylcholinesterase inhibitors, which do not slow disease progression but improve symptoms³, and N-methyl-D-aspartate receptor antagonists⁴. Monoclonal antibodies such as lecanemab have been approved with a 25% slower cognitive decline in patients, but there are concerns with their side effect profile⁵.
- Research has shown that **impaired cerebrospinal fluid (CSF) drainage** is seen in patients in AD, and it has been postulated that **improving lymphatic drainage** may improve Aβ clearance^{6,9}.
- CSF is reabsorbed through 1) arachnoid villi and granulations, 2) perivascular channels termed the "glymphatic system," 3) dural lymphatic vessels, and 4) lymphatic pathways along cranial nerves. One important cranial nerve pathway is the cribriform plate lymphatics (cPLVs), which exist between the olfactory bulb and along the olfactory nerves (ON). The contribution of each to CSF clearance remains under debate.⁹⁻¹³

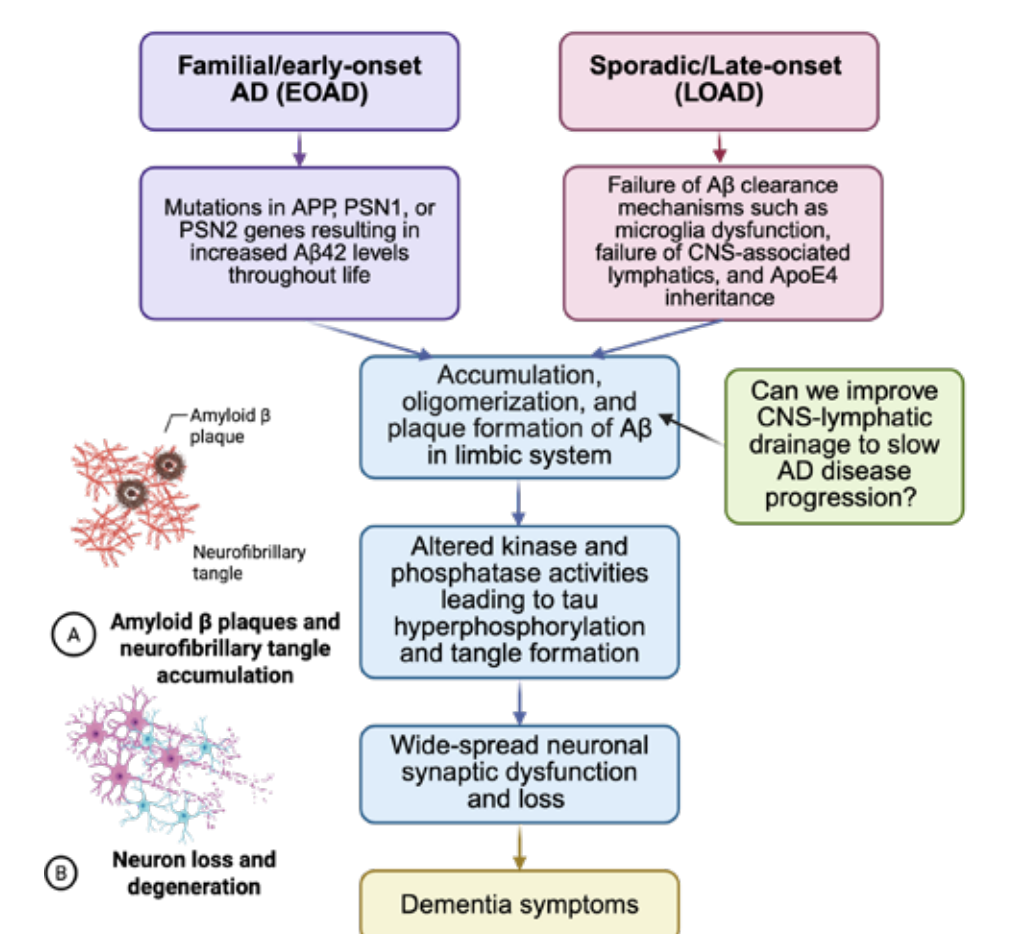


Figure 1: Diagram of Alzheimer's disease (AD) disease progression. Patients will present with EOAD or LOAD with both resulting in accumulation of Aβ in the limbic system. Aβ will result in tau hyperphosphorylation leading to neurofibrillary tangle formation. Patients will have neuronal loss and dysfunction presenting as dementia. Made with Biorender.

Despite important work describing the cribriform plate lymphatic endothelial cells (cPLECs) and associated cPLVs as an important drainage site and hotspot for immunosurveillance of CSF in models of infection, autoimmunity, and stroke, **the role of the cribriform plate (CP) in AD has not been described**^{10,13-16}. Moreover, PET-measured CSF clearance in humans has shown reduced tracer clearance in the nasal turbinates suggesting **impaired clearance at the CP**^{8,17}. We utilized APP/PS1 mice, which carry mutations in amyloid precursor protein (APP) and processing proteins, PSEN1, to model AD disease progression.

Research Question

In AD patients and in APP/PS1 mice, is the cribriform plate a site of Aβ deposition? Does it contribute to decreased CSF clearance and functional deficits?

Aβ deposits at the cribriform plate in a post-mortem AD human samples

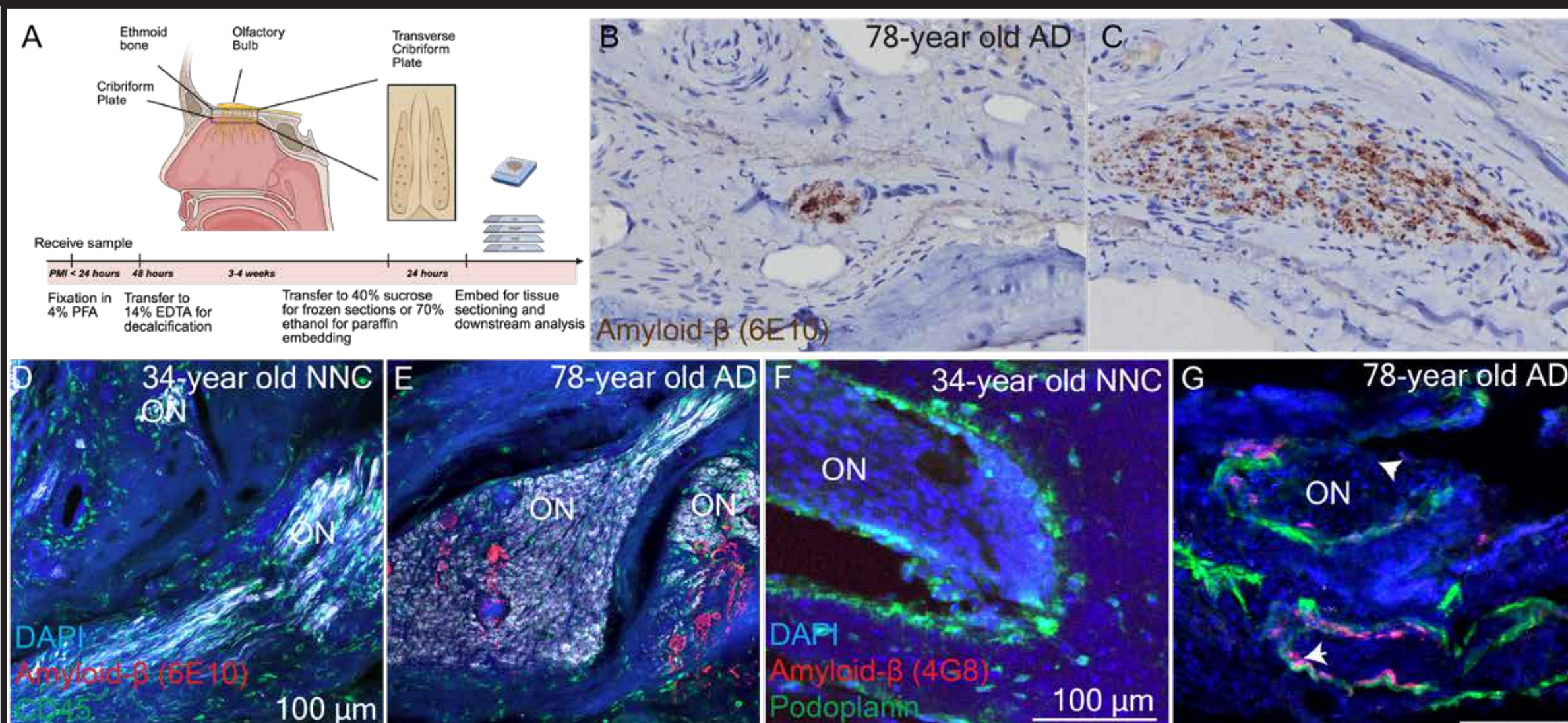


Figure 3: Aβ deposits in the cribriform plate lymphatics (cPLVs) and in olfactory nerves in confirmed AD patient. (A) Experimental schematic of processing of human cribriform plate (CP) samples. (B-C) Representative image of a decalcified post-mortem cribriform plate from a 78-year old patient with confirmed AD stained with Amyloid-β (4G8) showing Amyloid-β at olfactory nerves (ON). (D-E) Representative image from a non-neurologic control (NNC) 34-year old CP (D) and a 78-year old confirmed AD CP (E) stained for Amyloid-β (6E10), CD45 (YAML501.4), and DAPI. (F-G) Representative image from a non-neurologic control (NNC) 34-year old CP (F) and a 78-year old confirmed AD CP (G) stained for Amyloid-β (4G8), Podoplanin (MZ-1.3), and DAPI. AD = Alzheimer's Disease, NNC = Non-neurologic control, ON = olfactory nerve.

Aged APP/PS1 mice recapitulate olfactory dysfunction seen in AD patients

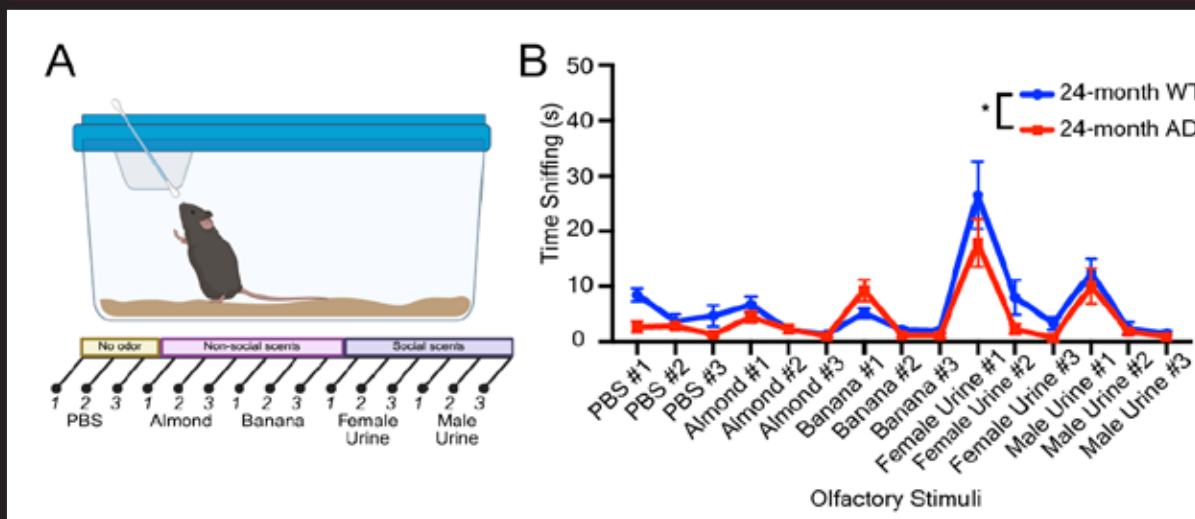


Figure 4: Aged APP/PS1 mice show olfactory dysfunction that worsens with age and disease status. (A) Experimental schematic of olfaction habituation dishabituation testing. (B) Results from olfaction habituation dishabituation testing on 24-month old male and female mice littermate and APP/PS1 mice (n=10). Two-way ANOVA was performed for (B-C) with * p-value < 0.05 and *** p-value < 0.001. Results shown are data +/- S.E.M.

Aβ coats olfactory nerves in APP/PS1 mice resulting in decreased activation

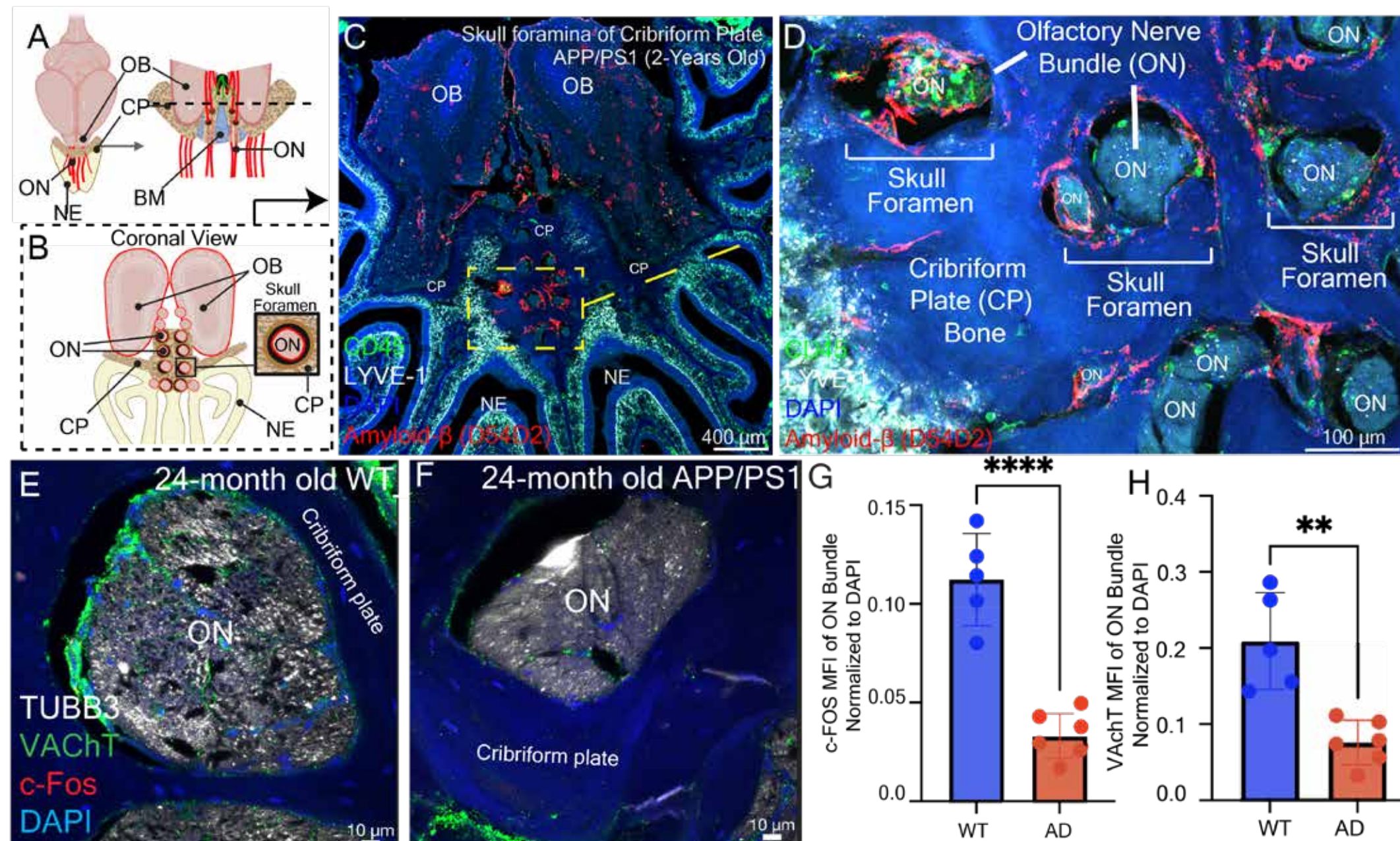


Figure 5: Aβ coats olfactory nerves in 24-month old APP/PS1 mice and is associated with decreased c-FOS activation and decreased VachT of ON. (A-B) Anatomical schematic of cribriform plate with transverse view (A) displaying olfactory nerves (ON) transversing cribriform plate (CP) and (B) coronal view. (C-D) Representative image of a decalcified 24-month old APP/PS1 CP stained for CD45 (30-F11), LYVE-1 (ALY7), and Amyloid-β (D54D2). (E-G) Representative image of decalcified 24-month old littermate (E) and 24-month old APP/PS1 mice (F) olfactory nerves stained for TUBB3, VachT, c-Fos (9F6), and DAPI. (G-H) Quantification of olfactory nerve area normalized to DAPI (n=5-6) with average of 5 ON areas using ImageJ. Data is shown as +/- S.D. A Student's t-test was performed on GraphPad Prism with with (G) p-value < 0.0001 and (H) 0.0013. OB = olfactory bulbs, ON = olfactory nerve, CP = cribriform plate, NE = nasal epithelium.

Aβ deposits in cribriform plate lymphatics in APP/PS1 mice resulting in atrophy

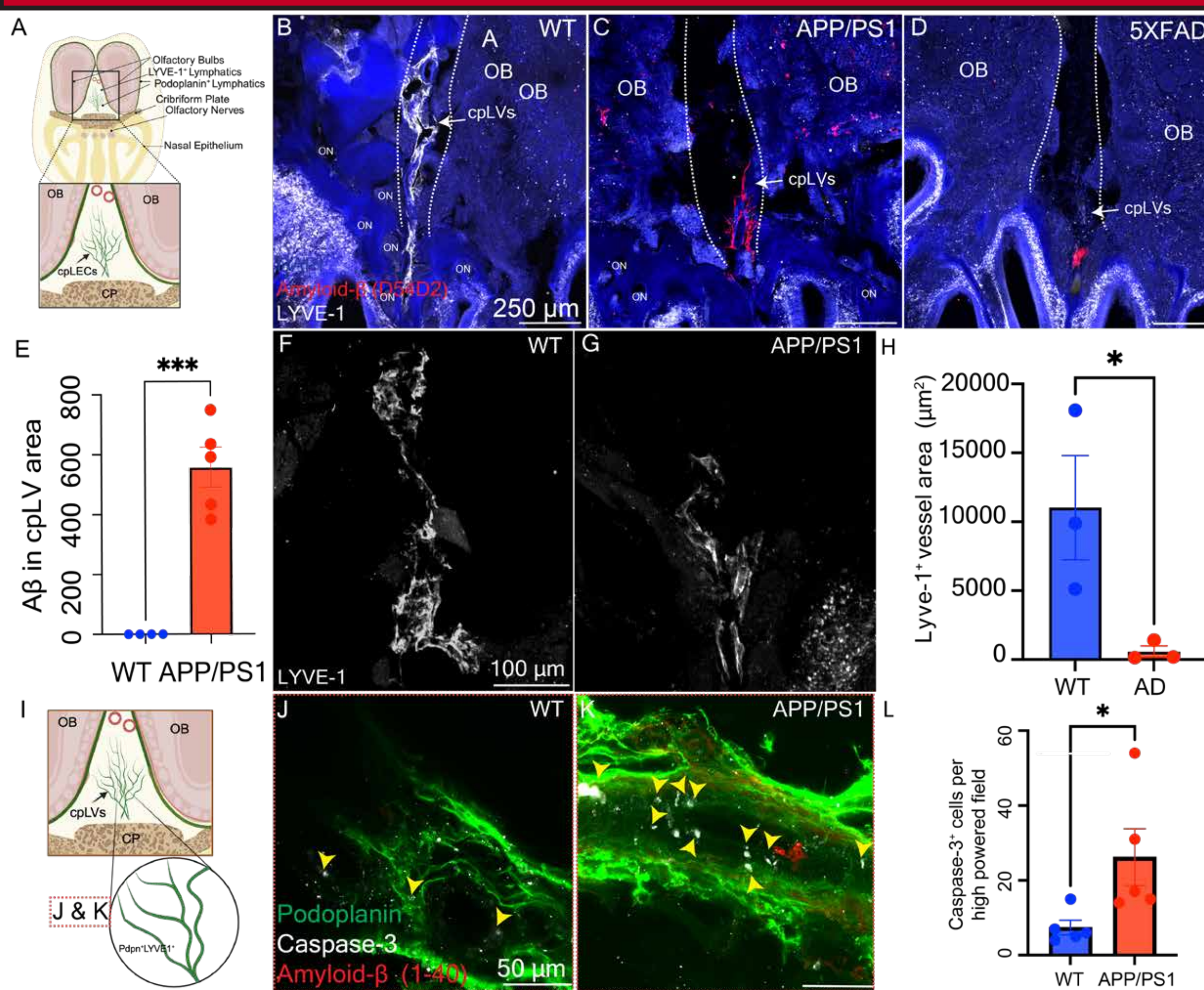


Figure 6: Aβ deposits in the cribriform plate lymphatics (cPLVs) in APP/PS1 and 5XFAD mice resulting in decreased LYVE-1+ vessel area and increased cleaved caspase-3. (A) Anatomical schematic of cribriform plate area displaying the olfactory bulbs (OB) and the cribriform plate (CP) with the cribriform plate lymphatic area (cPLV) between the OBs. (B-D) Representative images of whole-head decalcified cribriform plates of 24-month old littermate (B), 24-month old APP/PS1 (C), and 17-month old 5XFAD (D) stained for LYVE-1 (ALY7), Amyloid-β (D54D2), and DAPI. (E) Quantification of Amyloid-β at the cPLV area in 24-month old APP/PS1 mice (n=5) using ImageJ. (F-G) Single-channel image of LYVE-1 area of 24-month old littermate (F) and 24-month old APP/PS1 mice (G). (H) Quantification of cribriform plate lymphatic vessel (cPLV) LYVE-1+ vessel area (n=3) using ImageJ. (I) Anatomical scheme of cPLV area with red box indicating where (J) and (K) were taken on 60X magnification. (F) Representative image of 24-month old littermate (J) and 24-month old APP/PS1 (K) stained for Podoplanin (8.1.1), cleaved caspase-3 (D175), and Amyloid-β (1-40). Data is shown as +/- S.E.M. A Student's t-test was performed on GraphPad Prism with with p-value = =0.0002 (E), 0.0259 (H), and 0.0226 (L). OB = olfactory bulbs, ON = olfactory nerves, WT = wild-type, cp = cribriform plate lymphatic vessels.

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APP/PS1 mice have decreased CSF efflux at the cribriform plate area

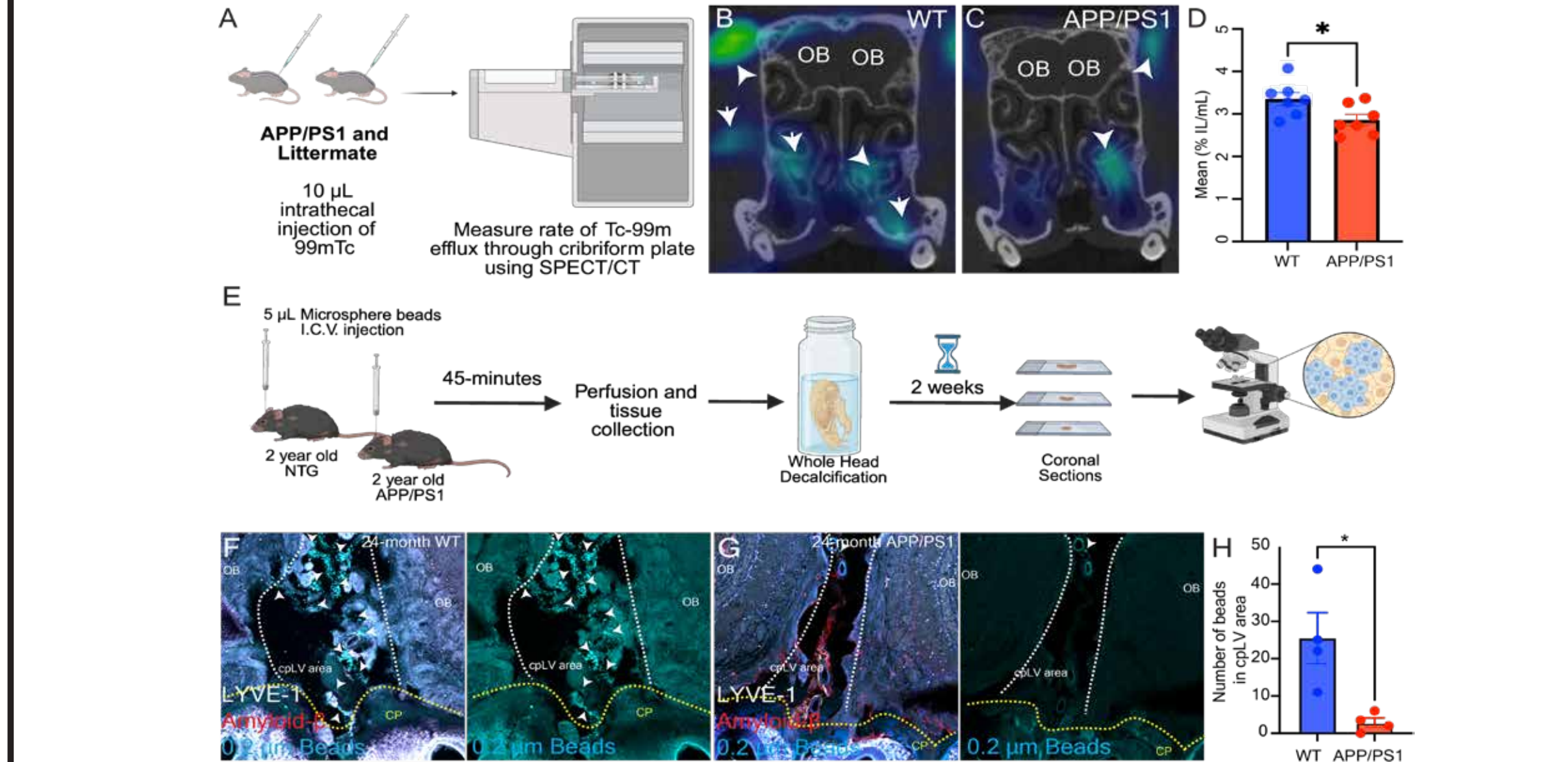


Figure 7: Aged APP/PS1 mice display decreased CSF efflux measured through serial SPECT imaging and clearance of microsphere beads. (A) Experimental schematic of technetium-99m (Tc-99m or 99mTc) clearance. 99mTc was administered through an intrathecal injection into 24-month old APP/PS1 mice and littermate controls and serial SPECT images were taken. (B-C) Representative CT images at the cribriform plate with SPECT data overlaid using ImageJ software of 24-month old littermate (B) and 24-month old APP/PS1 mice (C). (D) Quantification of 99mTc in the CP area at five-minutes post-injection through ImageJ software. (E) Experimental schematic of intraventricular 0.2 μm Microsphere bead injection. (F-G) Representative images of decalcified 24-month old littermate (F) and 24-month old APP/PS1 (G) injected with 0.2 μm Microsphere beads stained with LYVE-1 (ALY7), Amyloid-β (D54D2), and DAPI adjacent to single-channel image of 0.2 μm Microsphere beads. (H) Quantification of 0.2 μm Microsphere beads within the cPLV area (F-G). Data is shown as +/- S.E.M. Results of a two-sided Student's unpaired t-test (* P<0.05). OB = olfactory bulbs, CP = cribriform plate, cPLV area = cribriform plate lymphatic area.

APP/PS1 have increased immune cells and decreased CD11C^{hi} cells at cPLV area

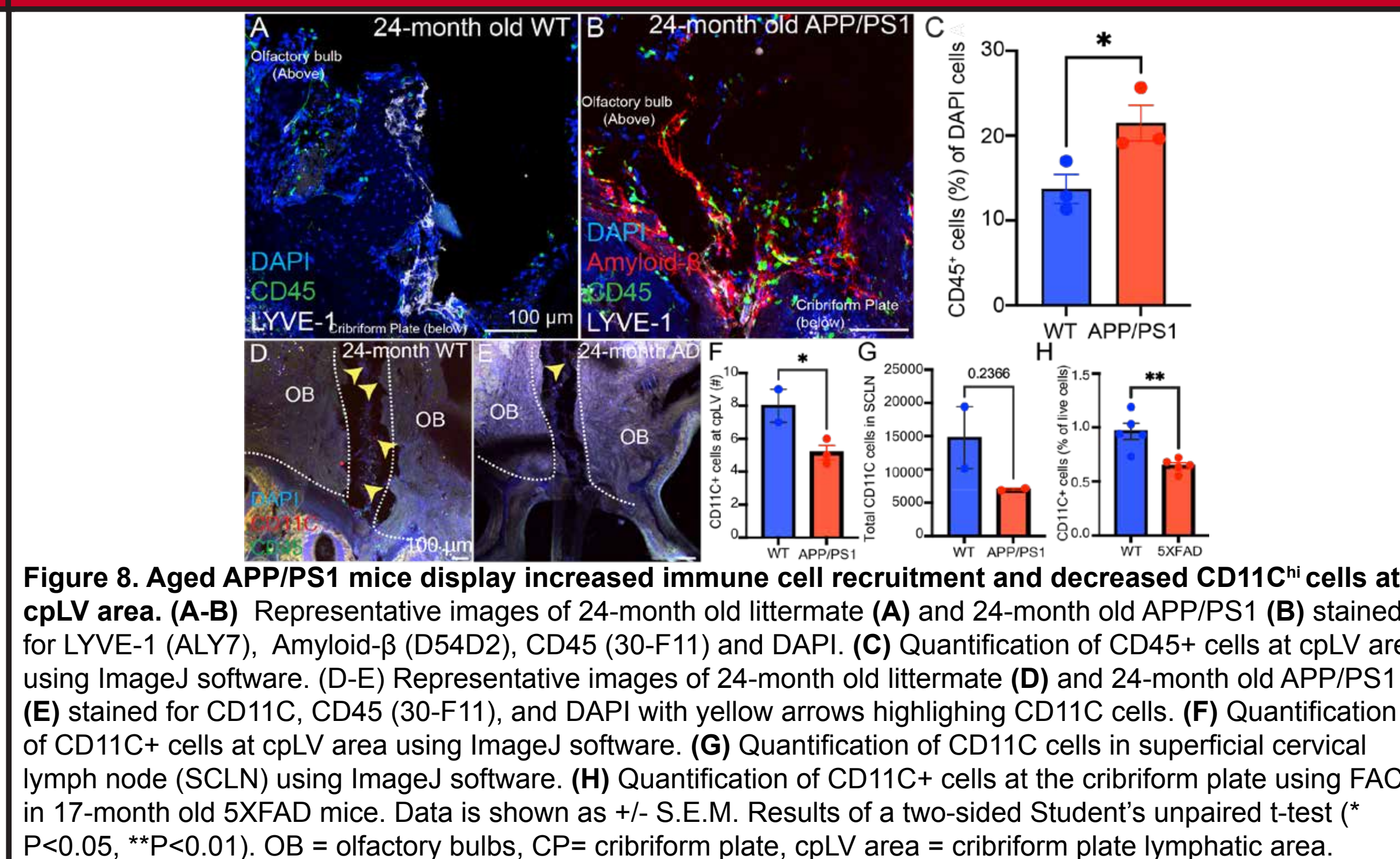


Figure 8: Aged APP/PS1 mice display increased immune cell recruitment and decreased CD11C^{hi} cells at cPLV area. (A-B) Representative images of 24-month old littermate (A) and 24-month old APP/PS1 (B) stained for LYVE-1 (ALY7), Amyloid-β (D54D2), CD45 (30-F11), and DAPI. (C) Quantification of CD45+ cells at cPLV area using ImageJ software. (D-E) Representative images of 24-month old littermate (D) and 24-month old APP/PS1 (E) stained for CD11C, CD45 (30-F11), and DAPI with yellow arrows highlighting CD11C cells. (F) Quantification of CD11C+ cells at cPLV area using ImageJ software. (G) Quantification of CD11C cells in superficial cervical lymph node (SCLN) using ImageJ software. (H) Quantification of CD11C+ cells at the cribriform plate using FACS in 17-month old 5XFAD mice. Data is shown as +/- S.E.M. Results of a two-sided Student's unpaired t-test (* P<0.05, ** P<0.01). OB = olfactory bulbs, CP = cribriform plate, cPLV area = cribriform plate lymphatic area.

Summary

- The contribution of CNS-associated lymphatics to the development of AD remains debated. The cribriform plate lymphatics have not been studied in the context of AD, and **we show that the cribriform plate is an active area of Aβ accumulation**, warranting further investigation in both humans and mice.
- We show that in 24-month-old APP/PS1 mice, there is **decreased CSF drainage** at the cribriform plate through multiple modalities, recapitulating clinical findings.
- The restricted CSF drainage correlates with substantial Aβ deposition in the **cribriform plate foramina, perineural accumulation** along olfactory nerves, and close association with the cribriform plate **LYVE-1⁺ lymphatic vessels**.
- The deposition results in pathology such as altered **olfaction**, **decreased c-Fos** and **VachT of ON** and **decreased lymphatic vessel area**. Interestingly, despite the decrease in vessel area, there is an increase in **CD45⁺ immune cell** recruitment and elevated **caspase-3⁺ expression** suggesting an inflammatory response. We demonstrate **decreased CD11C^{hi} cell** recruitment at cPLV area suggesting impaired dendritic cell trafficking, likely impacting antigen priming. Future experiments will involve delineating this relationship.
- This study highlights the cribriform plate as a **previously unknown site of Aβ pathology** and supports further investigation into CNS-associated lymphatics as **potential modulators and therapeutic targets** in AD. Future experiments include utilizing lymphangiogenic agents to see if CSF efflux can be restored.

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