Tau oligomer-containing synapses are engulfed by microglia and astrocytes and correlate with cognition in Alzheimer disease

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Key points

Question: Are synapses containing tau oligomers excessively eliminated by glia in brains of demented individuals at early clinical stages of AD?

Findings: Brains from individuals with dementia but not those without dementia (‘resilient’) and identical intermediate Braak III-IV stages at autopsy have significant loss of synapses ahead of neurofibrillary tangle deposition and increased engulfment of tau oligomer-containing synapses by microglia and astrocytes.

Meaning: Early synapse loss in AD may be driven by glial-mediated enhanced engulfment of synapses containing tau oligomers suggesting a potential key role of microglia, astrocytes, and synaptic tau oligomers in development of early cognitive impairment in AD.

Abstract

Importance: Identifying potential drivers of synapse loss that, beyond Aβ-plaques and neurofibrillary tangles, may more closely correlate with emergence of cognitive deficits in Alzheimer disease (AD) and be relevant for early therapeutic intervention.

Objective: Investigate whether accumulation of tau oligomers in synapses is associated with excessive synapse elimination by microglia and/or astrocytes and with cognitive outcomes (demented vs. non-demented 'resilient') of individuals with equal burdens of AD neuropathologic changes (ADNC) at autopsy.

Design: Cross-sectional postmortem study of 40 human brains from the MADRC brain-bank with Braak III-IV stages of tau pathology but divergent antemortem cognition (demented vs. resilient) and cognitively normal with negligible ADNC. We conducted our analyses 02/2022 through 05/2023.

Setting: Massachusetts Alzheimer Disease Research Center (MADRC) Brain Bank. We assessed the visual cortex – a region not yet impacted by tau tangle deposition at Braak III-IV stages – and used expansion microscopy (ExM) to achieve nanoscale resolution for visualization of synapses and tau oligomers, and to analyze their spatial relationships with microglia and astrocytes.

Participants: 19 early-stage dementia ('demented') and 13 non-demented ('resilient') with identical Braak III-IV, and 8 non-demented with Braak 0-II ('controls') were included (age at death 88±8.1 years, 48% female). They were matched for age, sex, and ApoE. Evidence of Lewy bodies, TDP-43 aggregates, or other lesions different to AD neuropathology were exclusion criteria. Brains that did not meet those criteria, such as

those with evidence of Lewy bodies, TDP-43 aggregates, or other brain lesions different to AD neuropathology, were not included.

Main Outcomes and Measures: We quantified Aβ-plaque and tau neuropil thread burdens, synapse density, tau oligomers in synapses, and internalization of tau oligomer-tagged synapses by microglia and astrocytes, and investigated their relationships with cognitive outcomes.

Results: In 40 participants, (age at death age at death 88±8.1 years, 48% female) demented but not resilient had significant loss of presynaptic, postsynaptic, and colocalized mature synaptic elements (43%, 33%, and 38% respectively) compared to controls and higher proportions of microglia- and astrocyte-internalized synapses. In demented but not in resilient, tau oligomers more often colocalized with synapses, and the proportion of tau oligomer-containing synapses inside microglia and astrocytes was significantly increased compared to controls. These brain changes in demented preceded tau tangle deposition.

Conclusion and Relevance: Our findings suggest that microglia and astrocytes excessively engulf synapses in AD brains of demented individuals, and that the abnormal presence of tau oligomers in synapses may serve as a signal for increased glial-mediated synapse elimination and early loss of brain function in AD.

Introduction

Loss of synapses is one of the earliest hallmarks of neurodegeneration in Alzheimer disease (AD) and the closest correlate of dementia severity [1–3] but the underlying mechanisms remain largely unclear. Clinicopathological correlation studies suggest that not everyone who harbors amyloid beta (Aβ)-plaques and neurofibrillary tangles (NFTs) in the brain will inevitably develop synaptic and neuronal loss and symptoms of dementia during life [4–7]; we refer to this phenomenon as brain 'resilience' to AD neuropathological changes (ADNC). Resilient brains exhibit preservation of synaptic markers and less inflammatory microglial and astrocyte changes compared to demented brains with equivalent loads of Aβ-plaques and NFTs [8-11]. Thus, the aberrant response of microglia and/or astrocytes may be the primary contributor to synaptic loss and clinical disease expression in AD. Neuroinflammation in AD has also gained considerable interest supported by the identification of several risk-factor genes expressed in microglia [2] and novel insights into microglial-mediated synapse elimination [12–16]. Additionally, studies suggest that tau oligomers can disrupt synaptic function [17–20] and closely associate with cognitive deficits in AD [20–26], and we and others have shown that tau oligomers abnormally accumulate in synapses of demented but not resilient and control brains [8,27,28]. Recent in-vitro data also suggest that accumulation of tau oligomers in synapses triggers release of neurotransmitters that cause aberrant glial responses with glial-mediated internalization of synapses [3,29] and subsequent glial dysfunction that results in further accumulation and spreading of toxic tau [30–32]. These emerging associations between glial phenotypic changes, tau oligomers in synapses, and loss of synaptic integrity in AD favor a disease model beyond Aβ-

plaques and NFTs, in which early accumulation of tau oligomers in synapses could serve as a signal for microglia and/or astrocytes to engulf and eliminate synapses. The temporospatial relationships of the potential drivers of synaptic loss and dementia in AD remain unknown. For years, we lacked techniques with enough resolution to allow quantitative detection and study of individual synapses. Here, we took advantage of expansion microscopy (ExM), a recently developed and well-validated technique that achieves nanoscale imaging of 3D tissue samples through physical magnification by polymer embedding and swelling [33–36] to evaluate the precise spatial relationships of microglia and astrocytes, tau oligomers, and synaptic elements in an informative cohort of age-matched symptomatic AD and resilient brains at equivalent intermediate stages of tau tangle pathology (Braak III-IV). The analysis of the visual cortex (a region not yet impacted by classic NFT deposition at those stages) enabled us to address the following key questions: 1) Does loss of synapses precede classic tau tangle appearance?; and if so 2) Are microglia and/or astrocytes responsible for synaptic engulfment?; 3) Are the synapses that contain oligomeric tau the ones that are preferentially internalized and eliminated by glia?; and most importantly 4) Is the presence or absence of this tissue injury response what determines whether an individual who harbors Aβ-plaques and NFTs in the brain will manifest or not clinical symptoms of disease during lifetime?

Material and Methods

Human brain samples

This cross-sectional postmortem study included 40 human brains from the Massachusetts Alzheimer Disease Research Center (MADRC) Brain Bank. After a participant's death, written consent is obtained from the legally authorized representative, in compliance with Massachusetts law. For deaths occurring outside of the hospital, a witnessed telephone conversation is held followed by completion of the written consent in keeping with hospital policy. Autopsies were performed according to standardized protocols [37] and tissue collection and use was approved by the local Institutional Review Boards. Brains were scored by Thal phase for Aβ deposition (0-5) [38], Braak stage for NFTs (0–VI) [39], and the Consortium to Establish a Registry for Alzheimer's Disease (CERAD) scale for neuritic plaques (A-C) [40], and divided into three groups: 1) cognitively normal whose post-mortem exam demonstrated a Braak stage 0-II ('controls') (N=8); 2) cognitively normal but whose post-mortem examination demonstrated a Braak stage III-IV ('resilient') (N=13); and 3) cognitively impaired (either at mild cognitive impairment (MCI) or mild dementia stages) whose postmortem examination demonstrated a Braak stage III-IV ('demented') (N=19). A subset of 5 demented and 3 resilient fulfilled criteria for primary age-related tauopathy (PART) (5 demented and 1 resilient were 'definite' PART and 2 resilient were 'possible' PART) [41]. Cases with evidence of Lewy body pathology, phospho-TDP-43 aggregates, or other lesions different to classic AD pathology were excluded. 26 cases (4 controls, 6 resilient and 16 demented) had undergone extensive antemortem cognitive assessments close to death as part of their longitudinal enrolment in the Uniform Data Set (UDS) of the NIA Alzheimer's Disease Centers program [42]. In the remaining 14 cases, the cognitive status was assessed by review of clinical records. The three groups were matched for age,

sex and ApoE status. Resilient and demented were also matched for Thal, Braak and CERAD scores. Quantitative assessments of Aβ-plaques, neuropil threads and ɣH2AX burdens in the visual cortex (BA17/18) were conducted [10] (eMethods and eFigure 1 in Supplement). Demographics, cognitive and neuropathological data are summarized in Table 1.

Expansion microscopy (ExM)

ExM enables to physically expand tissue specimens isotropically in the three dimensions to 4-5x their original size [43], allowing optical resolution of fine glial processes and individual synapses [43,44]. Previous studies validated the isotropy of the expansion achieved by the ExM protocol at the nanoscale in multiple tissue types including brain tissue [45]. We applied ExM following previously published protocols [43,46,47] with minor modifications (eMethods in Supplement) and attained an average tissue expansion factor of 4.6x in line with prior publications [43,46,47] (eFigure 2 in Supplement).

Confocal Imaging and 3D-image analyses

Expanded sections were imaged using an Olympus FV3000 confocal microscope at a resolution of 1024 x 1024 pixels. A z-stack of 0.46 µm was applied to optimize discrimination of true signal (synaptic puncta present in a minimum of two consecutive z-stack images) from artifacts [48]. For synapse density measures, 3-4 fields of view (FOV) per section were randomly selected in layer II of visual cortex in 2 non-adjacent sections (6-8 FOV per case). Synapsin1 and PSD95 immunostaining was used to identify pre- and postsynaptic elements, respectively (Figure 1). For microglia, IBA1+ ameboid-shaped cells [49] were selected. For astrocytes, GFAP+ cells with clearly visible bodies and processes were selected. 2-3 IBA1+ ameboid microglial cells and 3-4

GFAP+ astrocytes per section were imaged. Quantifications of TOC1+ tau oligomer-containing synapse densities and tau oligomer-containing synapses colocalized with IBA1+ microglia or GFAP+ astrocytes were conducted with Imaris software (BitPlane, South Windsor, CT, USA). Individual layers were created for each pre- and postsynaptic element selecting a diameter of largest sphere of 0.8 µm and a seed point diameter of 0.5 µm. To assess colocalizing pre-/postsynaptic 'mature' puncta, pre- and postsynaptic layers were masked and the setting voxels outside the surface distance was set to 0 µm. For colocalization analyses of synaptic puncta with microglia and astrocytes, cell bodies and processes were reconstructed in 3D and distances from puncta to cell surface were calculated; only negative distance values were considered 'puncta inside' a glial cell. Number of puncta internalized by each microglia and astrocyte was normalized to individual cell volume and total number of puncta in each section.

Western Blotting of Synaptosome and cytosol-enriched preparations

Synaptosome (SYN) and cytosolic (CYT)-enriched fractions were prepared from frozen blocks containing the visual cortex following previously published protocols [8,50,51] with minor modifications (eMethods in Supplement). Western blot (WB) analyses were conducted using reducing/denaturing and native conditions following previously published protocols [52,53] with minor modifications (eMethods in Supplement). The list of antibodies used for immunohistochemistry and WB can be found in eTable 1 in Supplement.

Statistical analyses

D'Agostino-Pearson normality test was applied to test for Gaussian distribution. Multiple group analyses were performed using one-way ANOVA for parametric variables, and

Kruskall-Wallis for non-parametric variables. Posthoc analyses to assess for between group differences were evaluated with Holm-Šídák's test. Correlation analyses were performed with Pearson test when both variables were normally distributed, and with Spearman test when at least one variable was not normally distributed. Significance level was set at $p < .05$.

All statistical analyses and graphs were generated using Graphpad Prism version 9.4.1 (Graphpad Software Inc, La Jolla, CA). Data are presented as mean ± standard deviation (SD) for normally distributed variables and median ± range for not normally distributed variables, as indicated. When applicable, confidence intervals (CI) are displayed.

Results

Synapses are lost ahead of tangle deposition in demented but not in resilient brains

Detailed quantifications of Aβ-plaque and NFT loads across multiple brain regions in the demented and resilient brains included here were previously published elsewhere [10]. Burdens of Aβ-plaque deposits (defined as percentage of cortex occupied by Aβ-plaques labelled by 4G8 antibody) and neuropil threads (defined as neurites labelled by AT8 antibody) in the visual cortex did not significantly differ between resilient and demented brains (1.5±1.7% vs. 2.9±3.7%, [p=.60] and 0.004±0.008% vs. 0.003±0.0025%, [p=.50], respectively) (eFigure 1 in Supplement). As expected at Braak III-IV stages, no NFTs were present in the visual cortex of either resilient or demented. In agreement with previous results [10], number of ɣH2AX positive cells per square

millimetre (mm^2) was significantly increased in demented compared to resilient and controls (508±461 vs.168±165 vs.76±87 [p=.001]) (eFigure 1 in Supplement). Densities of Synapsin1+ presynaptic, PSD95+ postsynaptic, and Synapsin1+/PSD95+ 'mature' puncta per cubic millimetre (mm^3) were significantly decreased by 43%, 33%, and 38%, respectively in demented compared to resilient and controls (Figure 1 and eFigure 3 in Supplement). Similar differences were found when analyses were limited to the smaller subset of individuals with PART (data not shown), indicating that early loss of synapses in visual cortex of demented brains occurs regardless of presence or absence of Aβ-plaques, and precedes NFT deposition. In agreement with prior studies, colocalized 'mature' puncta in control brains represented about 65% of all puncta [54,55]. Significant correlations were detected between 'mature' puncta densities and antemortem CDR-SoB (R= -0.62 [p=.003]), MMSE (R=-0.76 [p=.001]), WAIS-R scores (R=-0.85, [p=.001]), and γH2AX positive cells (R=-0.5 [p=.009]) (Figure 1 and eFigure 3 in Supplement). Brains were well-matched for postmortem intervals (PMIs) (Table 1). No correlation was found between PMIs and any of the variables studied here (not shown).

These results reinforce the close association between loss of synapses and decline in cognition at early AD clinical stages and point to the different tissue response (e.g. loss vs. preservation of synapses) between demented and resilient as the most likely anatomical basis for their widely divergent clinical phenotypes in the setting of equivalent burdens of Aβ-plaques and NFTs.

Synapse engulfment by microglia and astrocytes is enhanced in demented compared to resilient brains

Detailed analysis of pro-inflammatory and homeostatic markers of microglial cells and astrocytes in the demented and resilient brains included here were previously published elsewhere [10]. Quantification of synaptic puncta inside IBA1+ ameboid microglia and GFAP+ astrocytes revealed a significantly higher proportion of internalized Synapsin1+ presynaptic, PSD95+ postsynaptic, and Synapsin1+/PSD95+ 'mature' puncta by both microglia and astrocytes in demented compared to resilient and controls (in IBA1+ ameboid microglia: 7.7%±2.8% vs. 1.7%±1.2% vs. 1%±0.7% for presynaptic puncta [p<.0001]; 8.5%±0.7% vs. 1.9%±0.4% vs. 0.9%±0.2% for postsynaptic puncta [p<.0001]; 13.3%±3.9% vs. 2.6%±1.9% vs. 0.9%±0.5% for 'mature' puncta [p<.0001]; in GFAP+ astrocytes: 11.2%±6.4% vs. 2.8%±1.5% vs. 2.2%±1.9% for presynaptic puncta [p=.001]; 13.3%±7.6% vs. 1.8%±0.7% vs. 2.7%±2.8% for postsynaptic puncta [p=.001]; 17.2%±10.9% vs. 3.7%±4% vs. 2.7%±1.8% for 'mature' puncta [p=.001]) (Figure 2 and eFigure 4 in Supplement).

To rule out artefactual variations of the expansion factor within single astrocyte and microglial cells, we used the pan-astrocytic cytosolic marker ALDH1L1 combined with GFAP, and the lysosomal marker LAMP2 combined with PSD95. ALDH1L1 labelled the cytosol of GFAP+ and GFAP- astrocytes (eFigure 5 in Supplement). Double immunostaining with LAMP2 and either GFAP or IBA1 antibodies convincingly demonstrated the co-localization of engulfed synaptic puncta and lysosomes in the cytoplasm of GFAP+ astrocytes and IBA1+ microglial cells (eFigure 6 in Supplement).

These results demonstrate that not only microglia but also astrocytes are capable of engulfing synapses in the human brain, and that glia-mediated excessive internalization of synapses precedes overt NFT deposition likely contributing to early synaptic brain

function loss. The reduced microglial- and astrocyte-mediated engulfment of synapses in resilient brains may be directly responsible for the preserved cognition of these individuals.

Tau hyperphosphorylation and accumulation of tau oligomers in synapses precede tangle deposition in demented but not in resilient brains

We assessed early tau hyperphosphorylation sites (AT270/pTau(Thr181), pTau217(Thr217), and AT180/pTau(Thr231)), and levels of total tau (Tau5), N-terminal tau (Tau12), and C-terminal tau (Tau46) by WB in synaptosome-enriched fractions. Levels of pTau181 were significantly increased in synaptosomes of demented compared to resilient and controls (0.27 (0.14-0.4) vs. 0.06 (0.007-0.1) vs. 0.18 (0-0.5) [p=.02]) (Figure 3 and eFigure 7 in Supplement); no significant differences were detected in the levels of pTau217 or pTau231 (not shown). Tau oligomers were measured in whole tissue homogenates and synaptosome-enriched fractions using the well-characterized antibody TOC1 [56,57]. Levels of TOC1 in total homogenates did not significantly differ among the three groups but demented brains contained a significantly higher amount of TOC1 in synapses compared to resilient and controls (96147 (82963-109330) vs. 50834 (31077-70591) vs. 58112 (36361-79862) [p<0.0001]) (Figure 3).

These data suggest that tau hyperphosphorylation at Thr181 and accumulation of tau oligomers (TOC1) in synapses may be early key pathological modifications of tau that determine the very different fate of synapses and cognitive outcomes of demented vs. resilient individuals at Braak III-IV stages.

Tau oligomers are increased in pre- and postsynaptic compartments in demented compared to resilient brains

A significant increase of TOC1+ tau oligomers colocalizing with both Bassoon+ presynaptic and PSD95+ postsynaptic puncta was found in demented compared to resilient and controls (67.8%±6.6% vs. 16.3%±2.7% vs.18.2%±4.3% for TOC1+/Bassoon+ puncta [p<.0001]; 52.6%±2.9% vs.13.8±3.8% vs.15%±3.2% for TOC1+/PSD95+ puncta [p<.0001]) (eFigure 8 in Supplement). This agrees with the data above showing significantly higher levels of TOC1+ tau oligomers in synaptosome-enriched fractions of demented compared to resilient and controls. Importantly, TOC1+ tau oligomers in synapses (but not 4G8+ plaques or AT8+ neuropil burdens) negatively correlated with MMSE scores (R=-0.58, 95% CI -0.1/-0.85 [p=.03]).

Synapses containing tau oligomers are increasingly engulfed by microglia and astrocytes in demented compared to resilient brains

We quantified the proportion of internalized TOC1+/Bassoon+ presynaptic and TOC1+/PSD95+ postsynaptic puncta by IBA1+ ameboid microglia and GFAP+ astrocytes in a subset of 10 representative cases (4 demented and 4 resilient brains matched for Aβ-plaque, neuropil thread, and vascular burden, and 2 controls, see eTable 2 in Supplement). We found a significantly higher proportion of internalized tau oligomer-labelled presynaptic and postsynaptic puncta in both IBA1+ ameboid microglia and GFAP+ astrocytes in demented compared to resilient brains (demented vs. resilient vs. controls in microglia: 7.4%±1.8% vs. 5.1%±1.9% vs. 3.7%±0.8% internalized

TOC1+/Bassoon+ puncta [p=.006]; 11.6%±3.6% vs. 6.8%±1.3% vs. 7.4%±2.5% internalized TOC1+/PSD95+ puncta [p=.001]); in astrocytes:: 7%±2.1% vs. 4.3%±2.6% vs. 4%±0.7% internalized TOC1+/Bassoon+ puncta [p=.001]; 7.9%±2.2% vs. 5.3%±1.8% vs. 3%±1.5% internalized TOC1+/PSD95+ puncta [p=.001]) (Figure 4). Internalized tau oligomer-containing synaptic puncta represented a large percentage of total puncta engulfed by microglia and astrocytes in demented brains (91% of presynaptic and 93% of postsynaptic puncta in IBA1+ ameboid microglia, and 63% of presynaptic and 60% of postsynaptic puncta in GFAP+ astrocytes) indicating that tau oligomer-containing synaptic elements are the ones preferentially engulfed in those brains.

Discussion

We studied an informative cohort of human brains carefully matched for intermediate (Braak III-IV) stages of tau pathology at autopsy but widely diverging antemortem cognitive status (demented vs. resilient). Most of the demented were at an MCI or mild dementia stage (Table 1), giving us the opportunity to identify brain changes that, beyond Aβ-plaques and NFTs, could be more closely associated with cognition at these early disease stages. We found that synapse densities in the visual cortex (a brain region not yet affected by NFTs in Braak III-IV stages) were already significantly reduced in demented brains, and strongly correlated with markers of early cellular damage (γH2AX) and antemortem cognitive scores. Moreover, we found that synapses were excessively internalized by both microglia and astrocytes in demented compared to resilient brains, and that early aberrant accumulation of tau oligomers (TOC1) in

synapses was intimately associated with engulfment of synapses by microglia and astrocytes. To our knowledge, this is the first study to report evidence of astrocyte engulfment of synapses in human brains, and to suggest that tau oligomers may be driving glia-mediated synapse elimination in early AD.

Several in-vitro and in-vivo studies mimicking AD have shown that microglia and astrocytes can engulf synapses [13,58–61]. Emerging human studies also suggest that microglia may play a role in the excessive elimination of presynaptic elements at high Braak AD stages [14,16]. However, the potential involvement of astrocytes in synapse elimination in human AD has not yet been explored. Astrocytes are significantly more abundant than microglial cells, and most synapses are in close contact with astrocytes [62,63]. Thus, the potential contribution of astrocytes to early synaptic loss in AD could be even greater than that of microglia. To assess individual synaptic elements and overcome the optical resolution limitations of conventional imaging techniques, we applied expansion microscopy (ExM) [43,46,47]. Prior work on this emerging method has demonstrated that it provides isotropic expansion with maintenance of structural relationship which allows use of light microscopy to reveal spatial relationships that were not otherwise directly observable, and is critical when examining the interplay of glial cells with synapses. By using established methods for ExM we obtained an average expansion factor of 4.6x which allowed to attain an effective resolution of 25-30 nm, sufficient to study individual synaptic elements, tau oligomers, and their spatial relationships with microglia and astrocytes using confocal imaging.

We assessed synapse densities in layer II of the visual cortex, an integrating cortical layer for primary and higher order visual information that becomes consistently affected in AD by Aβ-plaques and tau tangle deposition when the pathology progresses to higher Braak stages (V-VI) [64-67] and evaluated synapse colocalization with IBA1+ ameboid-shaped microglia and GFAP+ astrocytes. Morphologic subtypes of microglia expressing similar phenotypic markers can display distinct neurobiological behaviors [68] and ameboid-shaped IBA1+ cells are presumably the more neurotoxic microglial subset [69,70]. Synapses were labelled using Synapsin1, a protein ubiquitously present on the surface of pre-synaptic vesicles [71], and PSD95, a protein found in the postsynaptic density of excitatory neurons [72]. This synaptic marker combination labels 80-90% of all synapses of the human brain [73,74]. We sampled a minimum of 6 FOV in each brain to account for interindividual variability and the possibility of increased synapse loss in the vicinity of Aβ-plaques [75]. Our results showed that demented but not resilient brains had a significant loss of presynaptic and postsynaptic elements and colocalized `mature' synapses - presumably required for neuronal signal transmission [76] - in the visual cortex. Importantly, our colocalized synapse measures (in the order of ~$5x10^9/mm^3$ in controls after accounting for the 4.6x volume expansion factor, Figure 1) were comparable with previously published work [73,77–79] including the use of electron microscopy (EM) [80] and synaptic biomarker measurements in early AD dementia stages [81,82]. The overall around 5x higher synapse densities observed here when compared with initial studies using EM [83] is likely due to the recently described 'decrowding' phenomenon of ExM and may account for a more accurate estimate of the total synapse densities in the human brain [46]. Of note, synapse densities in the subset of 8

PART brains were equivalent to those found in Aβ-plaque containing brains, pointing to common underlying mechanisms responsible for synaptic loss in AD and PART unrelated to Aβ.

We observed a significant increase in microglia- and astrocyte-engulfed synaptic elements including ‘mature’ synapses in demented compared to resilient brains. Importantly, we stringently considered only fully internalized synaptic elements as `engulfed synapses’, to unequivocally exclude the likely physiologic contacts between glial cells and synaptic elements. The excessive internalization and elimination of ‘mature’ and presumably still functioning synapses by microglia and astrocytes strongly suggests that aberrant glial responses are likely the primary drivers of synaptic loss and loss of brain function in demented. Contrary to prior hypotheses [60], we did not observe an accumulation of `single’ pre- or postsynapses in demented brains but rather an overall loss of these likely `non-functional’ synaptic elements, favoring the idea that the pathogenic role of glia in early disease stages may primarily involve active and excessive engulfment of synapses rather than defective elimination of dysfunctional synaptic elements.

Growing evidence suggests that tau oligomers may be earlier and better determinants of cognitive decline in AD than NFTs [84]. Recent human brain studies have demonstrated that tau oligomers are increased in synapses of demented brains [8,17,28,85–87] and that synaptic oligomers are particularly synaptotoxic and correlate with cognition in mouse models of tauopathy [20,88]. Here, we investigated the possibility that

accumulation of tau oligomers in synapses may trigger the enhanced elimination of synapses by microglia and astrocytes in human brains. In agreement with a recent study [17], we found that TOC1+ tau oligomers were significantly increased in both, presynapses and postsynapses in demented brains, and we observed the novel finding that tau oligomer-containing synapses are the ones preferentially engulfed by microglia and astrocytes in those brains. This could theoretically induce a self-perpetuating cycle of glial-mediated elimination of tau oligomer-containing synapses, chronic glial cell dysfunction, enhanced glial accumulation of tau oligomers, and subsequent release/propagation of toxic oligomers to new synapses, causing the slowly progressive dementia syndrome characteristic of AD. A negligible accumulation of tau oligomers in the synapses of resilient brains is associated with suppressed glial inflammatory responses and anatomic preservation of synapses, and thus likely determines preserved cognition.

Limitations

Autopsy studies do not allow to draw conclusions on specific mechanisms. We cannot rule out that tau oligomers directly damage synapses, and that glial engulfment of oligomer-containing synapses may be a mechanism to remove dysfunctional synaptic elements. Future studies are needed to better understand the specific pathways and molecular mechanisms that drive the interactions between synaptic tau oligomers and glial cells and their temporal relationship with brain function. Although brains were carefully matched for other coincident neuropathological changes, demented showed a higher vascular composite score than resilient and control brains; thus, we cannot

exclude with certainty a potential deleterious effect of vascular factors on synapse function and/or glia-mediated synapse elimination. Our study included brains from individuals who died at a relatively advanced age (>85 years) which may limit generalizability of the results to younger ages.

Conclusions

Synaptic loss in AD may be primarily driven by aberrant engulfment of synaptic elements by microglia and astrocytes, rather than by Aβ-plaques or NFTs, and abnormal accrual of tau oligomers in synapses could be the key signal targeting those synapses for elimination, leading to loss of brain function. These observations may be relevant for the development of in-vivo biomarkers that may more accurately determine the future of asymptomatic individuals who harbor Aβ-plaques and NFTs in their brains, and guide novel interventions that mimic resilient brains to prevent accumulation of tau oligomers in synapses and halt neurodegeneration (e.g. loss of synapses) and clinical symptoms of dementia.

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Disclosures

Teresa Gómez-Isla participated served in an Eli Lilly Data Monitoring Committee.

Access to Data and Data Analysis

Dr. Raquel N. Taddei (first author) and Dr. T. Gomez-Isla (corresponding author) had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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Legends

Table 1: Baseline sociodemographic and neuropathologic characteristics of the N=40 human brains studied.

Demographic, clinical, and neuropathologic features of the total N=40 subjects included. All antemortem cognitive measures were significantly worse in demented compared to resilient. Cognitive measures in resilient were not significantly different than in controls. Thal phase: No amyloid deposition (A0), amyloid in neocortex (A1), amyloid in allocortex/limbic region (A2), amyloid in diencephalon/basal ganglia (A3), amyloid in brainstem/midbrain (A4), amyloid in cerebellum (A5); CERAD score: No neuritic plaques (C0), sparse plaques (C1), moderate plaques (C2), frequent plaques (C3); Cerebrovascular composite score includes subscores for: hypertensive cerebrovascular, atherosclerosis, cerebral atherosclerosis, occlusive atherosclerosis and cerebral amyloid angiopathy score [89]; CDR-global: Clinical Dementia Rating global score; CDR-SoB: Clinical Dementia Rating Sum of Boxes score; MMSE: Mini-Mental State Examination score; N: number; NA: not applicable; PMI: Postmortem interval; SD: Standard deviation. Significance levels (*) indicate differences between demented and resilient brains with their respective p-value. ***p** <.05; ****p** <.01; *****p** <.0001.

Figure 1: **Synapse densities across groups and correlation analyses between synapse densities and cognitive measures.**

Synapse densities were significantly reduced in the visual cortex of demented compared to resilient and control brains when assessing Synapsin1+ presynapses, PSD95+ postsynapses, and colocalized mature Synapsin1+/PSD95+ synapses (**a**);

representative images showing Synapsin1+ presynapses in magenta, PSD95+ postsynapses in green, and colocalized Synapsin1+/PSD95+ synapses after expansion microscopy (ExM) with confocal imaging (**a**, first three columns) and in Imaris 3D reconstructed images (**a**, fourth column); loss of mature synapses (**b**) was significantly correlated with antemortem MMSE score (**c**) and CDR-SoB (**d**) in the visual cortex; Analyses shown were performed on 28 brains (6-8 FOV per case). Synapse densities represented in the graphs correspond to the values obtained in expanded tissue sections and must be multiplied by a factor of ~100 (=4.6^3) to account for the 4.6x volume expansion factor achieved by the ExM protocol used here in order to extrapolate them to pre-expanded tissue material. Light-gray circles = controls, medium-gray triangles = resilient, dark-gray squares = demented. *C* Control (Braak 0-II), N=6; *R* Resilient (Braak III/IV), N=8; *D* Demented (Braak III/IV), N=14; FOV: field of view. ***p** <.05; ****p** <.01. Scale bar 5 µm.

Figure 2: Analyses of engulfment of synaptic elements by microglia and astrocytes.

Microglia and astrocytes engulfed significantly more Synapsin1+ presynapses, PSD95+ postsynapses and Synapsin1+/PSD95+ colocalized synapses in demented compared to resilient and control brains. Example of Imaris 3D image reconstructions (**a**) showing internalized synaptic elements inside IBA1+ ameboid microglial cells and GFAP+ astrocytes, and quantifications of engulfed synaptic elements inside IBA1+ microglia and GFAP+ astrocytes, respectively (**b**). Reconstructed 3D Imaris images (**a**) showing glial cells in blue, Synapsin1+ presynapses in magenta (**a1, a5**), PSD95+ postsynapses

in green (**a1, a5**), and colocalized Synapsin1+/PSD95+ puncta in yellow (**a2-a4, a6-a8**), displaying colocalized synapses in yellow inside and outside of a microglial cell (**a3**) and an astrocyte (**a7**), and colocalized synapses in yellow only inside the microglia (**a4**) and astrocyte (**a8**). Scale bars 5 μm. ****p** <.01; *****p**<.001.

Figure 3: Measures of oligomeric and hyperphosphorylated tau species in total brain homogenates and in synaptosome fractions.

Synaptosomes of demented brains showed a significant increase of TOC1+ tau oligomers and of hyperphosphorylated AT270 (pThr 181)+ tau compared to resilient and controls. Representative gel images are displayed in the figure. Western Blot (WB) analysis of oligomeric tau (TOC1+) in total brain tissue homogenates (**a**) and synaptosome extractions (**b**) was conducted by quantifying the signal intensity of the full lane labelled as `oligomers' for each case in the native TOC1 WB. No significant differences were detected in demented compared to resilient and controls in total brain tissue homogenates (**a**); WB analyses of synaptosome-enriched fractions from the visual cortex (**b-d**) showed a significant increase in TOC1 in demented compared to resilient and controls (**b**) and a significant increase in hyperphosphorylated AT270 (pThr 181)+ tau in demented compared to resilient and controls (**c**), while there was no difference in hyperphosphorylated Tau217 (pThr 217)+ tau and/or AT180 (pThr 231)+ tau (not shown). Total tau, as measured with mid-domain total tau antibody (Tau5) (**d**) did not differ across demented, resilient, and control brains (**d**). Analyses were performed on N=31 total homogenates (6 controls, 10 resilient, 15 demented) and N=21 synaptosome extractions (4 controls, 5 resilient, 12 demented), respectively.

Arrowheads in black indicate the band width quantified for each of the WB analyses and respective antibodies; *C/Ctrl* Control (Braak 0-II); *R/Res* Resilient (Braak III/IV); *D/Dem* Demented (Braak III/IV); WB: Western Blot. ***p** <.05; ****p** <.01.

Figure 4: Glial-mediated engulfment of oligomer-tagged synapses.

TOC1+ tau oligomers were significantly increased in Bassoon+ presynapses and PSD95+ postsynapses of demented compared to resilient and control brains and TOC1+ pre-/postsynapses were increased inside IBA1+ ameboid microglia and GFAP+ astrocytes in demented compared to resilient and controls (a, b). Representative Imaris 3D reconstructed images with white arrowheads indicating engulfed, and white arrows indicating non-engulfed PSD95+/TOC1+ synapses inside an IBA1+ ameboid microglia before (**a1**) and after (**a2**) making the cell body transparent, and Bassoon+/TOC1+ synapses inside an GFAP+ astrocyte before (**a3**) and after (**a4**) making the cell body transparent; assessments of proportion of internalized TOC1+presynapses and TOC1+postsynapses inside IBA1+ ameboid microglia and GFAP+ astrocytes, respectively (**b**). Analyses were performed on N=20 IBA1+ and N=30 GFAP+ cells from 10 brains (2 controls, 4 resilient, 4 demented); *C* Control (Braak 0-II); *R* Resilient (Braak III/IV); *D* Demented (Braak III/IV). Scale bars 5 µm. ***p** <.05; ****p** <.01; *****p** <.001;

Table 1

Cognitive status	Control	Resilient	Demented
Number of subjects, total N=40	8	13	19
Cognitive scores			
MMSE (median, range)	29 (1)	29.5 (3)	26.5 (19)*
CDR-global (median, range)	0.25 (0.5)	0 (0.5)	1 (2.5)****
CDR-SoB (median, range)	0.5 (2)	0.(0.5)	6 (16.5)****
WAIS-R subscore (median, range)	40.5 (12)	46.5 (18)	33 (28)**
Boston naming test (median, range)	29 (2)	27 (3)	23 (22)**
Trail making Test A (median, range)	35 (6)	30 (18)	46 (97)*
Verbal fluency (animals) (median, range)	18.5 (11)	17.5 (7)	11 (21)*
Verbal fluency (vegetables) (median, range)	16 (13)	15 (8)	7 (10)**
Age (years)			
Mean (SD)	86.9 (7.4)	86.2 (10.6)	89.6 (6.3)
Years of education			
Mean (SD)	17.5 (1.7)	14.4 (1.7)	17.4 (1.9)
Sex			
Female N (%)	4 (50%)	7 (54%)	8 (42%)
Male N (%)	4 (50%)	6 (46%)	11 (58%)
ApoE allele status			
ApoE2 allele (%)	1 (8%)	1 (7%)	3 (13%)
ApoE3 allele (%)	11 (92%)	12 (86%)	19 (79%)
ApoE4 allele (%)	0 (0%)	1 (7%)	2 (8%)
Brain weight (grams)			
Mean (SD)	1269 (224)	1247 (162)	1224 (123)
Neuropathology ('ABC' score)			
A-Thal phase, median (range)	0 (4)	2 (4)	3 (5)
B-Braak stage, median (range)	1 (1)	3 (1)	4 (1)
C-CERAD score, median (range)	0 (0)	1 (2)	1 (3)
Vascular score			
Composite score, mean (SD)	4.1 (1.7)	2.8 (2)	5.6 (2.5)**
PMI (hours)			
Mean (SD)	23.5 (15.2)	18 (14.2)	19.8 (6.7)
Last clinical visit prior to death			
Years (mean, SD)	1.5 (2.2)	0.6 (0.4)	0.5 (0.4)
Duration of symptoms (years)			
Mean (SD)	NA	NA	14.7 (6.5)