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Combined Effects of Synaptic and Axonal Integrity on Longitudinal Gray Matter Atrophy in Cognitively Unimpaired Adults

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Abstract

Background and Objectives: Synaptic dysfunction and degeneration is a predominant feature of brain aging and synaptic preservation buffers against Alzheimer's disease (AD) protein-related brain atrophy. We tested whether cerebrospinal fluid (CSF) synaptic protein concentrations similarly moderate the effects of axonal injury, indexed via CSF neurofilament light [NfL], on brain atrophy in clinically normal adults.

Methods: Clinically normal older adults enrolled in the observational Hillblom Aging Network study at the UCSF Memory and Aging Center completed baseline lumbar puncture and longitudinal brain MRI (Mean scan [follow-up]=2.6 [3.7 years]). CSF was assayed for synaptic proteins (synaptotagmin-1, synaptosomal-associated protein 2 [SNAP-25], neurogranin, growth associated protein 43 [GAP-43]), axonal injury (NfL), and core AD biomarkers ($ptau_{181}/A\beta_{42}$ ratio, reflecting AD proteinopathy). Ten bilateral temporo-parietal gray matter ROIs shown to be sensitive to clinical AD were summed to generate a composite temporo-parietal ROI. Linear mixed-effects models tested statistical moderation of baseline synaptic proteins on baseline NfL-related temporo-parietal trajectories, controlling for $ptau_{181}/A\beta_{42}$ ratios.

Results: Forty-six clinically normal older adults (Mean age=70; 43% female) were included. Synaptic proteins exhibited small to medium correlations with NfL (*r* range: .10 to .36). Higher baseline NfL, but not $ptau_{181}/A\beta_{42}$ ratios, predicted steeper temporo-parietal atrophy (NfL x time: β=-0.08, p<.001; $ptau_{181}/A\beta_{42}$ x time: β=-0.02, p=.31). SNAP-25, neurogranin, and GAP-43 significantly moderated NfL-related atrophy trajectories ($-0.07 \leq \beta s \geq -0.06$, *p*s<.05) such that NfL was associated with temporo-parietal atrophy at high (more abnormal) but not low (more normal) synaptic protein concentrations. At high NfL concentrations, atrophy trajectories were 1.5 to 4.5 times weaker when synaptic protein concentrations were low (β range: -0.21 to -0.07) than high (β range: -0.33 to -0.30).

Conclusions: The association between baseline CSF NfL and longitudinal temporo-parietal atrophy is accelerated by synaptic dysfunction and buffered by synaptic integrity. Beyond AD proteins, concurrent examination of *in vivo* axonal and synaptic biomarkers may improve detection of neural alterations that precede overt structural changes in AD-sensitive brain regions.

Introduction

Neurodegeneration is commonly indexed using longitudinal brain atrophy on structural MRI, indicating a cumulative diminution of the neuropil[1]. However, dynamic molecular alterations in neural integrity and functioning occur prior to observable atrophy on MRI[2]. Cerebrospinal fluid (CSF) markers that capture discrete components of neural structures, particularly axonal and synaptic proteins, have enhanced the characterization of these dysregulated neural pathways. Neurofilament light (NfL) is a well-studied marker of degeneration in large-caliber myelinated axons[3]. More recent work highlights the important role of degeneration and dysfunction of the synapse through quantification of proteins reflecting presynaptic vesicular machinery (synaptosomal-associated protein-25 [SNAP-25] and synaptotagmin-1 [SYT-1]), postsynaptic calcium-mediated signaling pathway modulation (neurogranin), and axonal outgrowth regulation (growth associated protein 43 [GAP-43])[4-7]. As complementary markers of neural structure and function, joint modelling of NfL and synaptic proteins may facilitate our molecular understanding and detection of neural insults that precede overt structural brain changes in pathologies that target the axon and synapse.

CSF NfL and synaptic proteins are increasingly incorporated into brain aging and Alzheimer's disease (AD) biomarker models to improve the prognostication of brain regions vulnerable to future atrophy[8, 9]. Independent of AD proteinopathy (CSF $A\beta_{42}$ and $ptau_{181}$), higher concentrations of both CSF NfL and synaptic proteins (reflecting greater axonal and synaptic

damage, respectively) predict global and hippocampal volume loss in mild cognitive impairment (MCI)[10, 11], and steeper hippocampal atrophy, memory decline, and future conversion to MCI in cognitively unimpaired individuals[4, 11-16].

While synaptic dysfunction tracks with worsening progression of AD and other neurodegenerative diseases, mounting evidence highlights synaptic preservation as a core component of cognitive resilience, particularly against AD proteinopathy. In autopsy studies of individuals with pathological AD, those who exhibited more normal synaptic protein levels were more likely to be cognitively unimpaired at death than those with synaptic failure despite similar burden of AD pathology[17, 18]. We recently reported that the deleterious effects of abnormal CSF AD proteins on medial temporal lobe and total gray matter volumes were detected only in those with high (more abnormal) CSF synaptic protein levels, even in clinically normal adults[19]. A longitudinal analysis similarly demonstrated that the association between low baseline CSF $A\beta_{42}$ on temporal lobe atrophy and cortical thinning was moderated by baseline CSF neurogranin[20], a postsynaptic protein involved in hippocampal and neocortical plasticity.

Although maintained synaptic integrity may attenuate adverse associations of $A\beta_{42}$ and $ptau_{181}$ with structural neuroimaging, how synaptic processes potentially buffer and/or contribute to early axonal changes and atrophy rates is unclear. In a cohort of cognitively unimpaired adults, we examined the relationship between baseline CSF NfL and longitudinal gray matter atrophy. We then evaluated the moderating role of synaptic integrity on this relationship, using a CSF panel of pre- and post-synaptic proteins. We hypothesized that NfL-related declines in gray matter volumes would be attenuated at lower levels of synaptic dysfunction, even when accounting for AD pathology (CSF $ptau_{181}/A\beta_{42}$ ratio).

Methods

Participants

Participants were 46 community-dwelling older adults enrolled in the observational Hillblom Longitudinal Aging Network study at the UCSF Memory and Aging Center. Participants underwent comprehensive neurological and neuropsychological evaluations, as well as a study partner interview to determine neurobehavioral status. All participants were reviewed and classified as clinically normal per consensus case conference with a board-certified neurologist and board-certified neuropsychologist, and were deemed functionally intact based on structured clinical interview with a study partner (Clinical Dementia Rating global score = 0). Participants were included in the present analysis if they completed a baseline lumbar puncture and underwent neuroimaging visits, which occurred roughly 15-18 months apart. Study visits included in the present analysis took place between October of 2010 and January of 2019. Of the 587 actively enrolled Hillblom Aging Network participants during this period, 68 completed a baseline lumbar puncture, 54 of those 68 had complete CSF biomarker data available for primary variables used in the current analysis, and 46 of those 54 underwent neuroimaging visits. Given that the Hillblom Aging Network is an active longitudinal study with rolling enrollment, the number of neuroimaging visits per participant included in the present analysis ranged from one (baseline only) to six, with a mean number of 2.7 study visits per participant. Similarly, the duration between baseline lumbar puncture and final neuroimaging visit ranged from zero years (baseline only; n=5) to 8.7 years, with a mean duration between baseline lumbar puncture and final neuroimaging visit of 3.7 years.

Standard Protocols, Approvals, Registrations, and Patient Consents

The study protocol was approved by the UCSF Committee on Human Research. All participants provided written informed consent to study procedures.

CSF Assays

Lumbar punctures were performed in the morning following a 12-hour fast, and CSF was collected, processed, and stored according to standard protocols[4]. CSF $A\beta_{42}$, $A\beta_{40}$, and $ptau_{181}$ were assayed via the Lumipulse platform and the $ptau_{181}/A\beta_{42}$ ratio was selected to model AD-related proteinopathy in analyses[21]. Assay methods for quantification of synaptic proteins (neurogranin, GAP-43, SNAP-25, and SYT-1) are described in detail elsewhere[19]. CSF NfL was assayed via an in-house enzyme-linked immunosorbent assay (ELISA), also described elsewhere[22]. For analysis, synaptic markers and $ptau_{181}/A\beta_{42}$ ratios were $\log_{10}$-transformed to improve distributions and all CSF markers were standardized into z-scores to facilitate interpretation of results.

Neuroimaging

Participants underwent longitudinal structural magnetic resonance imaging (MRI) at the UCSF Neuroscience Imaging Center using either a Siemens Trio Tim or Prisma Fit 3T scanner. Magnetization prepared rapid gradient-echo (MPRAGE) sequences were used to obtain whole brain T1-weighted images sagittally using the following parameters: repetition time (TR) = 2300 ms, inversion time (TI) = 900 ms, echo time (TE) = 2.98 ms, flip angle = 9°, field-of-view (FOV) = 240×256 mm with 1×1 mm in-plane resolution and 1 mm slice thickness. Parameters for both Trio and Prisma scanners had nearly identical parameters but slightly different echo times (Trio: 2.98 ms; Prisma: 2.9 ms).

Before processing, all T1-weighted images were visually inspected for quality control and those with excessive motion or image artifact were excluded. Magnetic field bias was

corrected using the N3 algorithm[23]. Tissue segmentation was performed using unified segmentation in SPM12[24]. All segmentations were carefully inspected to ensure robustness of the process. Each participant's native space gray matter segmentation was normalized and modulated, via nonlinear and rigid-body transformations, to study-specific template space using DARTEL (Diffeomorphic Anatomical Registration using Exponentiated Lie algebra[25]). A Gaussian kernel of 4-mm full width half maximum was applied for smoothing of images. Transformations (linear and nonlinear) between DARTEL's space and ICBM space were conducted to enable statistical comparisons[26]. Finally, brain volumes of interest were quantified by translating a standard parcellation atlas[27] into ICBM space and summing the gray matter within each region of interest (ROI).

For our primary outcome, we computed a composite ROI that summed bilateral gray matter volumes of the 10 atlas ROIs involving temporo-parietal structures previously reported to be vulnerable to AD[28]: hippocampus, entorhinal cortex, parahippocampal gyrus, amygdala, fusiform gyrus, middle temporal, inferior temporal, temporal pole, precuneus, inferior parietal. Total intracranial volume was also computed for each participant as the sum of total gray matter, white matter, and cerebrospinal fluid volumes.

Statistical Analysis

We first examined Pearson's correlations among $A\beta_{42/40}$, $ptau_{181}$, NfL, and synaptic proteins for descriptive purposes. Next, a series of linear mixed effects (LME) models with random slopes and intercepts analyzed longitudinal temporo-parietal volumetric changes by including years since baseline lumbar puncture visit (time) as a fixed and random effect, covarying for baseline age, sex, *APOE* status, intracranial volume, and scanner. To determine the relative contributions of baseline NfL and AD proteinopathy to temporo-parietal trajectories, we

tested interactions of NfL and $ptau_{181}/A\beta_{42}$ ratios with time (NfL x time, $ptau_{181}/A\beta_{42}$ x time), both separately and in a combined model. To address our primary aim of examining the moderating role of synaptic integrity in NfL-related temporo-parietal volumetric change, separate models entered each synaptic protein as a moderator of the NfL x time interaction on temporo-parietal trajectories (i.e., SNAP-25 x NfL x time, SYT-1 x NfL x time, neurogranin x NfL x time, GAP-43 x NfL x time), controlling for $ptau_{181}/A\beta_{42}$ ratios. The false discovery rate was set to 5% to account for multiple comparisons in these primary analyses examining synaptic protein moderation of NfL-related temporo-parietal trajectories. To probe significant moderation effects of synaptic proteins, we calculated the effects of time on temporo-parietal volumes when synaptic protein and NfL levels were one standard deviation above (z=1) and below (z=-1) sample mean levels. To mitigate potential instability of model estimates due to our relatively small sample, we estimated 95% confidence intervals of standardized model coefficients using bootstrapping with 1000 samples. All LME models were conducted using the lme4 package in *R*.

Data Availability Statement

Anonymized, de-identified data from this report will be made available upon request from any qualified investigator. UCSF MAC data requests can be sent to the corresponding author.

RESULTS

Table 1 presents descriptive statistics for the study sample at baseline. Similar to our cross-sectional report[19], participants were on average 70 years-old (baseline age range = 53 – 86 years) with 17.3 years of education, 57% male, 39% *APOE* ε4+, and cognitively unimpaired (Mini Mental State Exam [MMSE]; mean = 29/30). The present sample was comparable to individuals in the larger Hillblom cohort who were not included in the present analysis (n=541)

with respect to age, education, and MMSE (all *p*s >.05), but did have a higher proportion of men (57% vs. 41%, *p*=.030) and *APOE* ε4+ (39% vs. 22%, *p*=.001).

NfL correlations with CSF synaptic and AD proteins

Figure 1 presents NfL correlations with synaptic proteins. The associations between NfL and synaptic proteins ranged from small, positive, and statistically non-significant (SYT-1: r = .10, p = .507; Ng: r = .23, p = .120) to medium, positive, and statistically significant (SNAP-25: r = .32, p = .030; GAP-43: r = .36, p = .015). With respect to AD proteins, NfL exhibited a medium, positive, and statistically significant association with $ptau_{181}/A\beta_{42}$ ratios (r = .35, p = .016). Our recent report extensively characterized the associations between AD and synaptic proteins in this cohort[19]. Briefly, synaptic and AD proteins exhibited significant associations whereby the highest levels of AD proteinopathy (low $A\beta_{42/40}$ and high $ptau_{181}$) were observed at the highest levels of synaptic dysfunction.

Baseline NfL and AD proteinopathy effects on gray matter trajectories

Time was significantly associated with declining temporo-parietal volumes (β [95% CI] = -0.20 [-0.25, -0.15]); p < .001). Figure 2 displays the effects of baseline NfL and $ptau_{181}/A\beta_{42}$ ratios on temporo-parietal trajectories. The negative slope of time on temporo-parietal volumes was significantly moderated by both baseline NfL (p < .001) and baseline $ptau_{181}/A\beta_{42}$ (p = .028) in separate models, however only NfL remained a significant moderator of temporo-parietal trajectories when both terms were entered in a combined model (NfL x time: β [95% CI] = -0.08 [-0.12, -0.04]; p < .001; $ptau_{181}/A\beta_{42}$ x time: β [95% CI] = -0.02 [-0.06, 0.01]; p = .252). Specifically, the negative slope of time on temporo-parietal volumes was steeper at higher (+1SD) levels of NfL (time: β [95% CI] = -0.26 [-0.31, -0.21]) compared to lower (-1SD) levels of NfL (time: β [95% CI] = -0.11 [-0.17, -0.05]). In contrast, the slope of time on temporo-

parietal volumes did not differ when $ptau_{181}/A\beta_{42}$ was high (time: β [95% CI] = -0.20 [-0.27, -0.15]) versus low (time: β [95% CI] = -0.16 [-0.22, -0.11]).

Synaptic moderation of NfL-related gray matter ROI trajectories

Controlling for $ptau_{181}/A\beta_{42}$ ratios, primary analyses entered synaptic proteins as moderators of NfL-related temporo-parietal trajectories (see Figures 3 and 4). The NfL x time interaction on temporo-parietal volumes was significantly moderated by SNAP-25 (NfL x SNAP-25 x time: β [95% CI] = -0.06 [-0.10, -0.02]; p = .011, FDR-adjusted p = .015), neurogranin (NfL x neurogranin x time: β [95% CI] = -0.06 [-0.10, -0.02]; p = .010, FDR-adjusted p = .015), and GAP-43 (NfL x GAP-43 x time: β [95% CI] = -0.07 [-0.12, -0.03]; p = .001, FDR-adjusted p = .004). Specifically, the deleterious effect of NfL on temporo-parietal trajectories was observed at higher (more abnormal) synaptic protein levels, but not at lower (more normal) synaptic protein levels. The moderating effect of SYT-1 on the NfL x time interaction was not statistically significant (NfL x SYT-1 x time: β [95% CI] = -0.03 [-0.07, -0.02]; p = .258), although a similar pattern was observed. As displayed in Figure 3, the magnitude of the slope of time on temporo-parietal volumes was steepest when NfL and synaptic levels were high (β range: -0.33 to -0.30), whereas slopes were approximately 1.5 to 4.5 times weaker when NfL and/or synaptic levels were low (β range: -0.21 to -0.07).

Discussion

In our cohort of clinically normal older adults, higher baseline CSF NfL levels were associated with faster longitudinal atrophy in temporo-parietal regions, but this relationship depended upon synaptic state. The combination of axonal injury (higher CSF NfL) and synaptic dysfunction (higher CSF SNAP-25, neurogranin, or GAP-43) predicted the steepest atrophy rates, whereas the association between baseline axonal injury and temporo-parietal atrophy was

markedly attenuated at low baseline levels of synaptic dysfunction. This pattern of synaptic moderation was statistically robust to AD proteinopathy (CSF $ptau_{181}/A\beta_{42}$ ratio) and was detected across presynaptic and postsynaptic proteins. Correlations between synaptic proteins and NfL were relatively modest, suggesting that synaptic dysfunction and axonal injury are dissociable yet complementary aspects of neurodegeneration. Axonal breakdown in the absence of synaptic dysfunction may not strongly relate to brain atrophy; or framed differently, maintenance of synaptic integrity may buffer neurodegenerative effects of axonal breakdown. Beyond traditional AD proteins, concurrent examination of axonal and synaptic markers informs the prediction of structural decline in AD-vulnerable brain regions in clinically normal adults.

Our observation that baseline NfL was more strongly linked to temporo-parietal atrophy than $ptau_{181}/A\beta_{42}$ parallels a prior study reporting CSF NfL as a stronger predictor of hippocampal atrophy than CSF $A\beta_{42}$ and $ptau_{181}$ in a similar cohort solely comprised of cognitively unimpaired older adults[12]. Studies across the clinical AD spectrum show that NfL concentrations are associated with neurodegeneration in brain networks vulnerable to AD, including temporo-parietal cortical thinning[29], declining cingulum microstructural integrity[20], and hippocampal atrophy with ventricular expansion[30]. These studies also demonstrate that NfL predicts structural changes irrespective of Aβ burden, supporting conclusions that NfL associations with neurodegeneration are likely non-specific to AD pathology. However, a recent translational study reported Aβ-induced increases in CSF NfL corresponded to reduced parietotemporal cortex and hippocampal density in an early-stage AD animal model. The Aβ-specific effects of NfL in the same brain regions were replicated with neuroimaging data in humans[31]. Thus, axonal injury as indexed by CSF NfL, though likely not AD-specific, prognosticates atrophy in brain regions sensitive to AD.

The “dying-back” hypothesis of neurodegeneration in AD posits that synaptic loss and axonal disconnection precede somatic cell death[32]. Compatible with this notion, our data demonstrate synergistic effects of baseline synaptic proteins and NfL on longitudinal temporo-parietal atrophy. Furthermore, the adverse impact of axonal injury on gray matter volumetric change was substantially buffered at lower (more normal) concentrations of CSF synaptic proteins, which encompassed proteins from pre- and post-synaptic compartments. To date, neurogranin is the most widely studied synaptic protein in human CSF AD biomarker studies. Lower CSF neurogranin has been shown to attenuate Aβ-related structural brain changes[20, 29, 30], likely due to its critical involvement in synaptic plasticity and regeneration through modulation of calmodulin in dendritic spines[33]. GAP-43, although less studied than neurogranin, also facilitates long-term potentiation and synaptogenesis in medial temporal and neocortical structures[9, 34, 35]. GAP-43 is also enriched at axon terminals and supports axonal regeneration[36], which may underlie its moderation of NfL-related atrophy and explain its significant correlation with NfL. SNAP-25, in coordination with other SNARE complex proteins, governs presynaptic vesicular trafficking but also fast membrane transport to the axonal growth cone[37], a process that is disrupted during dying-back neurodegeneration[32, 38]. As a presynaptic vesicle cargo molecule, SYT-1 mediates neurotransmitter release in the hippocampus[39, 40]. In addition to supporting presynaptic homeostasis, some data suggest SYT-1 facilitates axonal growth processes during neurodevelopment[41, 42], although its role in axonal degeneration is unclear. Collectively, the observed synaptic-dependent associations between NfL and gray matter volumes are consistent with the known role of synaptic signaling in axonal regeneration and importantly add to the growing literature highlighting the synapse as a salient indicator of risk/resilience to neuropathology[19, 43].

In addition to synaptic-related effects on axonal function, NfL may also have a direct influence on synaptic function. Neurofilaments are predominantly involved in axonal scaffolding, however they also support dendritic branching[44]. Mouse knockout models show NfL-dependent modulation of synaptic neurotransmission and long-term potentiation in the hippocampus[45]. Thus, the utility of joint elevations in CSF NfL and synaptic proteins in prognosticating gray matter atrophy could reflect multiple aspects of early synaptic dysfunction, in addition to axonal degradation, that ultimately progress toward gross atrophy. Future cellular work aimed at dissecting the differential roles of neurofilaments at synapses and axons, in the context of both AD and non-AD pathology, may help clarify the mechanisms by which synaptic proteins mitigate NfL-related neurodegeneration.

Our findings should be interpreted in light of several limitations. Although our longitudinal study design and implementation of bootstrapped confidence intervals helps mitigate statistical power issues related to our relatively small study sample, we are still limited in our ability to estimate more complex model terms (e.g., 4-way interactions: AD proteins x NfL x synaptic proteins x time). Our longitudinal MRI data allowed modeling of atrophy trajectories but lumbar punctures were only performed cross-sectionally. Repeated CSF collection would facilitate the temporal characterization of NfL and synaptic protein trajectories, alongside AD protein accumulation and structural MRI changes. Although temporo-parietal volumes capture a classic neuroanatomical signature particularly vulnerable to AD pathology and CSF synaptic proteins are sensitive to AD, variance in these biomarkers in cognitively unimpaired adults may not exclusively signal AD-specific mechanisms, particularly when considering their dynamic interplay with NfL. Thus, our findings may reflect processes relevant to an AD neurobiological phenotype, but we cannot definitively rule out a role for non-AD

pathologies contributing to observed volumetric changes. Although the higher proportion of men and *APOE* 4-carriers in our CSF subcohort, relative to the larger Hillblom cohort, may reflect increased motivation for these individuals to participate in lumbar puncture, our study data cannot definitively address this hypothesis. Furthermore, our study sample represents a relatively homogenous cohort, which necessitates replication across more demographically and socioeconomically diverse individuals that may possess different risk and resilience factors for brain health.

Prior studies demonstrate synaptic moderation of the negative effects of CSF AD proteins on brain health[19, 20, 30]. Our results build on this finding and provide novel evidence that synaptic processes further moderate the relationship between CSF NfL and longitudinal brain atrophy in cognitively unimpaired adults. Taken together, our findings further implicate synaptic dysfunction as an accelerant of brain atrophy and synaptic integrity as a buffer against neurodegeneration in temporo-parietal structures commonly targeted by AD. Future behavioral and/or pharmacological interventions may consider inclusion of axonal and synaptic functioning markers that reflect relevant biological processes underlying neurodegenerative changes and possible treatment responsiveness.

Appendix 1: Author Contributions

Name	Location	Contribution
Rowan Saloner, MS	UCSF, San Francisco	Design and conceptualized study; analyzed data; drafted the manuscript for intellectual content
Corrina Fonseca, BS	UCSF, San Francisco	Major role in acquisition and coordination of data (MRI acquisition and analysis)
Emily W. Paolillo, PhD	UCSF, San Francisco	Revised manuscript for intellectual content
Breton M. Asken, PhD	UCSF, San Francisco	Revised manuscript for intellectual content
Nina Djukic, BS	UCSF, San Francisco	Major role in acquisition and coordination of data (participant acquisition and sample coordination)
Shannon Lee, BS	UCSF, San Francisco	Major role in acquisition and coordination of data (participant acquisition and sample coordination)
Johanna Nilsson, MSc	University of Gothenburg, Sweden	Major role in acquisition of data (CSF marker quantification); revised manuscript for intellectual

		content
Ann Brinkmalm, PhD	University of Gothenburg, Sweden	Major role in acquisition of data (CSF marker quantification); revised manuscript for intellectual content
Kaj Blennow, MD, PhD	University of Gothenburg, Sweden	Major role in acquisition of data (CSF marker quantification); revised manuscript for intellectual content
Henrik Zetterberg, MD, PhD	University of Gothenburg, Sweden	Major role in acquisition of data (CSF marker quantification); interpretation of data; revised manuscript for intellectual content
Joel H. Kramer, PsyD	UCSF, San Francisco	Major role in study conceptualization and manuscript revision for intellectual content
Kaitlin B. Casaletto, PhD	UCSF, San Francisco	Major role in study conceptualization; interpretation of data; manuscript revision for intellectual content

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Figure Legends

Figure 1. Cerebrospinal fluid (CSF) NfL correlations with CSF synaptic proteins

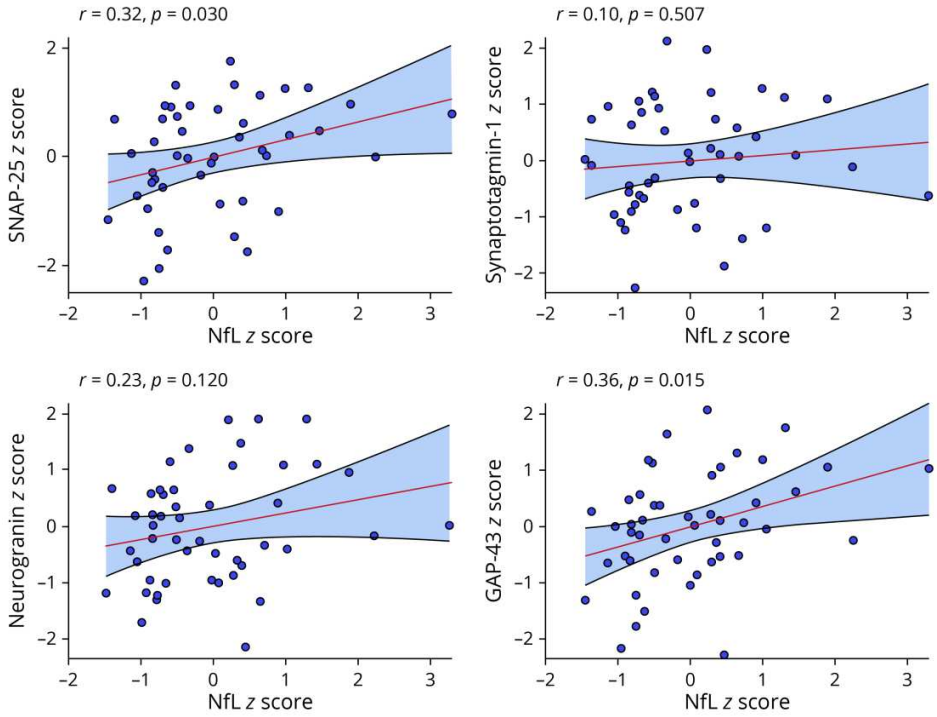
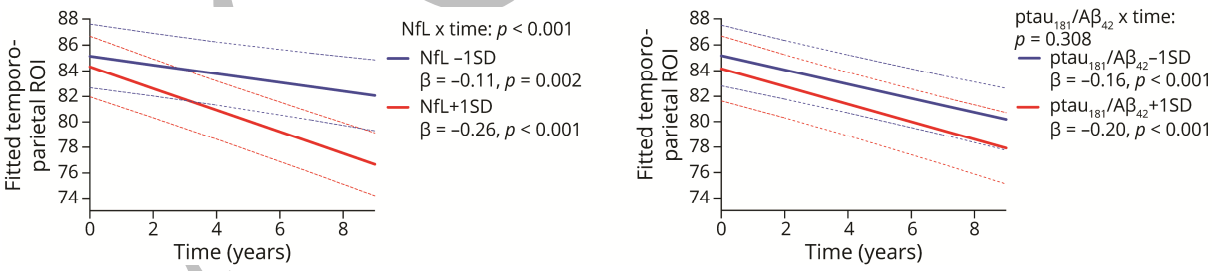


Figure 2. Cerebrospinal fluid (CSF) NfL and $ptau_{181}/A\beta_{42}$ effects on gray matter (temporo-parietal) trajectories



Legend. *All interactions were modeled continuously - biomarker levels split into +/- 1 standard deviation for illustration purposes.*

Figure 3. Cerebrospinal fluid (CSF) NfL effect on gray matter (temporo-parietal) trajectories stratified by CSF SNAP-25 (A) and synaptotagmin-1 (B)

Legend. *All interactions were modeled continuously - biomarker levels split into +/- 1 standard deviation for illustration purposes.*

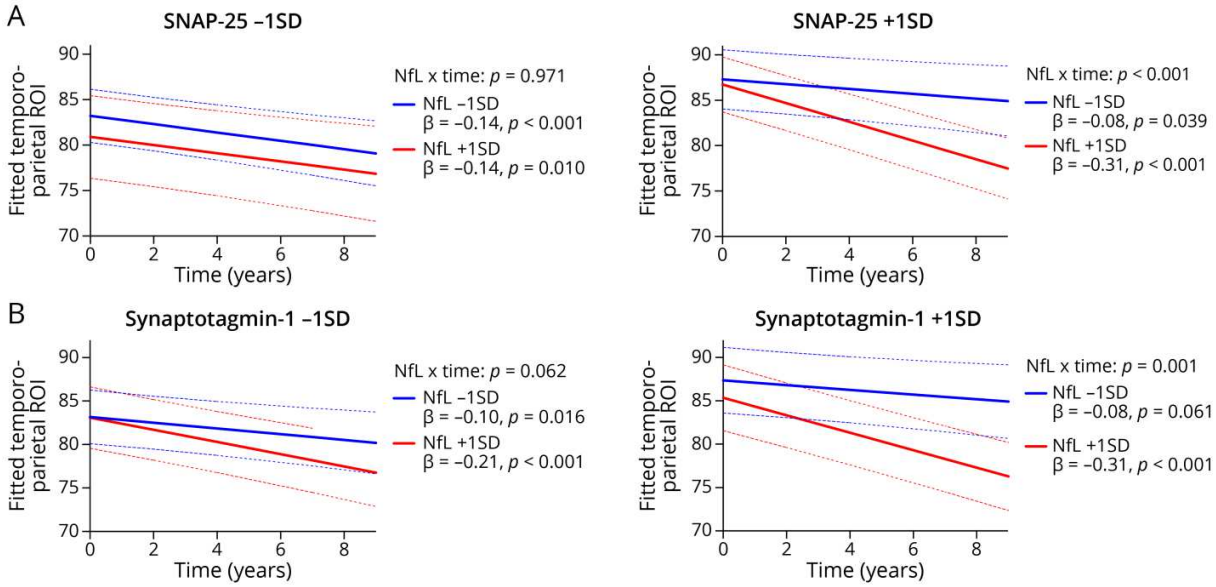


Figure 4. Cerebrospinal fluid (CSF) NfL effect on gray matter (temporo-parietal) trajectories stratified by CSF neurogranin (A) and GAP-43 (B)

Legend. *All interactions were modeled continuously - biomarker levels split into +/- 1 standard deviation for illustration purposes.*

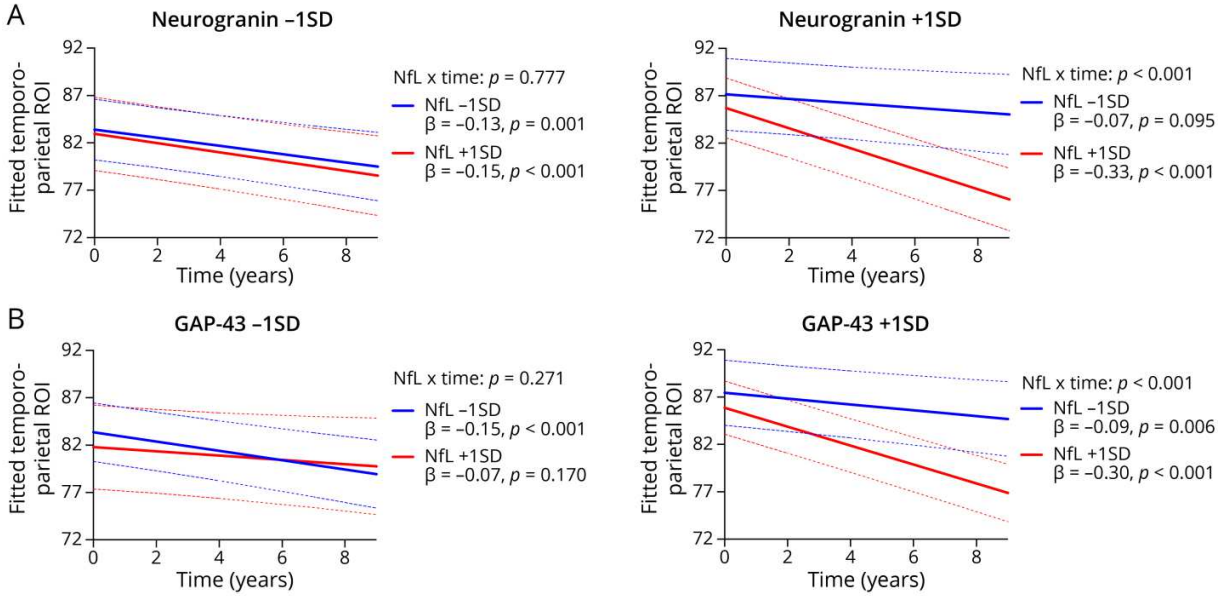


Table 1. Baseline characteristics (N=46)

	Mean (SD), median [IQR] or n (%)	**Range**
Age	69.5 (7.00)	53.4 - 86.6
Sex (% male)	26 (56.5%)	
Education (years)	17.3 (2.21)	12 - 20
Race		
White	41 (85.4%)	
Asian	3 (6.3%)	
Black	1 (2.1%)	
Not specified	1 (2.1%)	
MMSE	29.2 (1.03)	26 - 30
APOE (% ε4+)	18 (39.1%)	
Hypertension	13 (28.3%)	
Hypercholesterolemia	15 (32.6%)	
Diabetes	0 (0%)	
NfL (pg/mL)	868.0 (333.47)	13 - 1610
SNAP-25 (pM)	17.1 (6.24)	6.4 - 31.4
Synaptotagmin-1 (pM)	27.6 (12.49)	9.0 - 64.4
Neurogranin (pg/mL)	222.0 (85.64)	88.5 - 415.8
GAP-43 (pg/mL)	3239.4 (1092.2)	1358.9 - 6354.7
$ptau_{181}/A\beta_{42}$	0.032 [0.027 – 0.046]	0.022 – 0.152
% Abnormal (>0.068)	6 (13.0%)	
$A\beta_{42/40}$ (pg/mL)	0.08 (0.01)	0.04 – 0.11
% Abnormal (<0.061)	8 (17.4%)	
$ptau_{181}$ (pg/mL)	42.7 (18.1)	17.4 – 102.3
% Abnormal (>61)	7 (15.2%)	