Tripartite Relationship Among Synaptic, Amyloid, and Tau Proteins: An In Vivo and Postmortem Study

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Abstract.

Objective: To test the hypothesis that fundamental relationships along the amyloid, tau, and neurodegeneration (A/T/N) cascade depend on synaptic integrity in older adults in-vivo and postmortem.

Methods: Two independent observational, cross-sectional cohorts: 1) in-vivo community-dwelling, clinically normal adults from the UCSF Memory and Aging Center completed lumbar puncture and MRI (exclusion criteria, CDR>0), and 2) postmortem decedents from the Rush Memory and Aging Project (exclusion criteria, inability to sign informed consent). In-vivo measures included cerebrospinal fluid (CSF) synaptic proteins (synaptotagmin-1, SNAP-25, neurogranin, and GAP-43), Aβ42/40, ptau181, and MRI gray matter volume (GMV). Postmortem measures captured brain tissue levels of presynaptic proteins (complexin-I, complexin-II, VAMP, and SNARE complex), and neuritic plaque and neurofibrillary tangle (NFT) counts. Regression models tested statistical moderation of synaptic protein levels along the A/T/N cascade (synaptic proteins*amyloid on tau, and synaptic proteins*tau on GMV).

Results: 68 in-vivo older adults (age=71y, 43%F) and 633 decedents (age=90y, 68%F, 34% clinically normal) were included. Each in-vivo CSF synaptic protein moderated the relationship between Aβ42/40 and ptau181 ($-0.23<\beta s<-0.12$, $ps<0.05$) and the relationship between ptau and GMV ($-0.49<\beta s<-0.32$, $ps<0.05$). Individuals with more abnormal CSF synaptic protein demonstrated expected relationships between Aβ-ptau and ptau-brain volume, effects that were absent or reversed in those with more normal CSF synaptic protein. Postmortem analyses recapitulated CSF models. More normal brain tissue levels of complexin-I, VAMP, and SNARE moderated the adverse relationship between neuritic plaque and NFT counts ($-0.10<\beta s<-0.08$, $ps<0.05$).

Conclusions: Pathogenic relationships of Aβ and tau may depend on synaptic state. Synaptic markers may help identify risk and/or resilience to AD proteinopathy.

Introduction.

Synaptic failure is a core feature of Alzheimer's disease (AD), often tracking better with cognition than proteinopathy or even neuronal cell loss[1,2]. The "synaptic hypothesis" of AD posits that synaptic signaling is disrupted prior to deposition of neurofibrillar amyloid plaques or tau tangles in the context of soluble amyloid beta (Aβ) oligomers and may contribute to AD pathogenesis and spread[3–7]. In a positive feedback loop, low Aβ *promote*s synaptic firing and synaptic firing results in further release of APP and Aβ. In the setting of chronic aberrant synaptic activity and/or poor clearance mechanisms, excessive Aβ aggregations then leads to postsynaptic internalization of NMDA receptors and synaptic loss[8–11]. Indeed, synaptic loss and dysfunction appears to be most pronounced surrounding the Aβ plaque penumbra, and synaptic proteins appear to colocalize within Aβ plaques[12–14]. Additionally, tau hyperphosphorylation may be propagated by and further propagate the severity and spread of aberrant synaptic firing and ultimately lead to widespread neuronal cell loss[15–17]. Given its apparent early and intricately interweaved involvement in AD pathogenesis, disentangling the tripartite relationship among the synapse, Aβ, and tau in humans may open critical therapeutic windows for prevention and treatment of AD.

Of high clinical relevance, preserved synaptic integrity may in fact protect cognitive functioning, potentially regardless of AD proteinopathy. In experimental rodent models of human APP or tauopathies, restoration of synaptic functioning or even select synaptic pathways (e.g., KIBRA, EPH2B) can rescue cognition despite ongoing production of amyloid or tau[18–23]. Careful comparison of autopsied adults showing cognitive resilience to neuropathologic AD evidence uniquely increased abundances of pre- and post-synaptic density proteins (synaptophysin, PSD-95),[24,25] and structurally, greater spine count[25] and length and proportions of plasticity-related spines (e.g., thin vs. stubby)[26] compared to non-resilient AD peers. Further, autopsy studies modeling all available neuropathological lesions demonstrate that synaptic protein abundances[27–31] and gene expression[32] in brain tissue account for significant cognitive variance beyond pathologic burden. Together, these data underscore the potential of the synapse as a therapeutic target to help prevent and/or reverse AD-related cognitive impairment.

While most evidence for the role of the synapse in AD come from animal or human autopsy studies, increasingly advanced technologies are emerging to capture *in-vivo* human synaptic integrity. Molecular quantification studies using PET imaging of the presynaptic SV2A receptor[33,34] or cerebrospinal fluid (CSF)

synaptic proteins are beginning to further corroborate synaptic pathways as important differentiators in AD compared to typical aging. Of relevance to the current study, dysfunctional levels of CSF pre- (SNAP-25, synaptotagmin-1) and postsynaptic (neurogranin, neuronal pentraxin-2) proteins and proteins that localize to both compartments (GAP-43) have previously demonstrated sensitivity differentiating adults who are cognitively normal from those with a clinical AD syndrome, predict risk for future cognitive decline, brain hypometabolism, and amyloid PET, and differentiate autosomal dominant AD mutation carriers >10 years prior to estimated symptom onset[35–42]. Our prior work also suggests that CSF neurogranin levels relate to memory performance more strongly and independently from traditional AD biomarkers, even among clinically normal older adults[43]. Simultaneous capture of the *in-vivo* dynamics among age-related Aβ, tau, and synaptic integrity in humans is increasingly available and has potential to advance our fundamental understanding of how cognitive aging and AD unfolds.

In our cross-sectional study, we aimed to evaluate how synaptic integrity markers may modulate the neurotoxic effects of AD proteinopathy. We quantified synaptic proteins in CSF reflecting presynaptic vesicle machinery (SNAP-25, synaptotagmin-1), postsynaptic calcium modulation (neurogranin), and growth factor regulation in both pre- and postsynaptic compartments (GAP-43). We followed the A/T/N cascade framework[44,45] to test the moderating effect of synaptic proteins on the relationship between amyloid and phosphorylated tau (ptau), ptau and gray matter, and finally, AD proteinopathy on cognitive functioning. Given that synaptic dysfunction may be a very early phenomenon in AD pathogenesis, we evaluated these relationships in a cohort of clinically normative older adults. We hypothesized that markers of better synaptic integrity would buffer the adverse effects of CSF $AB_{42/40}$ and $ptau_{181}$ both on each other and on brain structural and functional outcomes.

Methods.

Participants. Sixty-eight participants who completed a baseline lumbar puncture were drawn from the Hillblom Aging Network at University of California, San Francisco Memory and Aging Center, an ongoing study characterizing neurobehavior of typical aging. Participants completed comprehensive neurological and neuropsychological evaluation, and study partner interview, and were reviewed determined to be within

normative standards at interdisciplinary case conferences by board certified neurologists and neuropsychologists (further cohort descriptions see[46–49] and Supplemental Methods).

<Table 1 here>

Cerebrospinal fluid (CSF) Analytics. CSF was collected in the morning after a 12-hour fast, processed, and stored following to standard procedures (see Supplemental Methods).

AD proteins. Samples were analyzed for the 42 and 40 amino acid forms of beta amyloid ($A\beta_{1\text{-}40}$, $A\beta_{1\text{-}42}$,) and 181 amino acid form of phosphorylated tau ($ptau_{181}$) via Lumipulse[50–52].

Synaptic proteins. CSF neurogranin (Ng) concentration was measured using an in house sandwich ELISA as previously described (ref: Kvartsberg H, Lashley T, Murray CE, Brinkmalm G, Cullen NC, Hoglund K, et al. The intact postsynaptic protein neurogranin is reduced in brain tissue from patients with familial and sporadic alzheimer's disease. Acta neuropathologica. 2019; 137: 89-102). CSF growth associated protein-43 (GAP-43; aka, neuromodulin) concentration was measured using an in house sandwich ELISA as previously described (ref: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6333489/). Synaptosome associated protein-25 (SNAP-25) and synaptotagmin-1 (STY1) were analyzed via simultaneous immunoprecipitation assays using monoclonal antibodies. Quantification was performed by mass spectrometry…. For SNAP-25, we quantified the N-terminal amino acids 32-40, which are present in only the longest soluble forms of SNAP-25. Previous works have demonstrated increased sensitivity of these peptides (compared to total N-terminal levels) differentiating cognitive impairment(refs).

Neuroimaging. A subset (n=51) of participants also completed a 3T brain magnetic resonance imaging (MRI) within 180 days of lumbar puncture (see Supplemental Methods)[53]. Given its high relevance as an AD biomarker, we selected bilateral substructures of the medial temporal lobe volumes (hippocampus, parahippocampus, entorhinal), as well as total gray matter volume as an indicator of global neurodegeneration. Total intracranial volume was statistically regressed out of each ROI prior to analyses.

Neuropsychological evaluation. We selected measures of episodic memory, executive functions, and processing speed, given their sensitivity to aging and AD using a standardized battery previously described[47,49,54]. Episodic memory and executive functions measures were available for all participants, while

the computerized processing speed metrics were available in a subset (n=39). See Supplemental Methods for additional measure information.

Statistical Analyses.

First, we evaluated univariate correlations among AD and synaptic CSF proteins, including visual investigation for nonlinear trends. Nonlinear trends were statistically tested via regression models entering both the linear (X) and nonlinear (X^2) terms. Effects with α<0.05 were considered statistically significant.

Current A/T/N framework posits the temporal sequence of AD pathogenesis initiates with beta-amyloid accumulation followed by tau hyperphosphorylation and then neurodegeneration[44,45]. We therefore aimed to determine the modifying role of synaptic integrity markers at each of these pathogenic junctures. To do so, we conducted a series of parallel linear regression interaction models, all of which covaried for age, sex, and education. First, we tested the interaction between each CSF synaptic protein and $A\beta_{42/40}$ (synaptic protein*$A\beta_{42/40}$) on $ptau_{181}$ levels. Next, we examined the interaction between each CSF synaptic protein and $ptau_{181}$ (synaptic protein*$ptau_{181}$) on gray matter volumes. Finally, we aimed to evaluate the modifying effect of synaptic proteins on the relationship between $A\beta_{42/40}$ or $ptau_{181}$ and cognition (memory, processing speed, or executive functions). To follow-up a trend observed in models examining individual presynaptic CSF proteins and cognition, we conducted post-hoc models testing the effect of a presynaptic protein ratio (SNAP-25/STY1), following similar previous protein-protein interaction approaches[28,55]. The presynaptic ratio aimed to capture relative SNARE membrane to vesicle protein-protein interaction, with higher levels indicating more optimal integrity.

For illustration of continuous interaction models, figures plot synaptic integrity by tertiles. To estimate effect sizes of interaction models, we calculated bivariate correlations showing the relationship of interest in participants with low vs. high synaptic integrity markers (CSF upper vs. lower tertile). To provide clinically relevant estimates, we selected Aβ [$A\beta_{42/40}$= 0.061 (Aβ positivity)] and $ptau_{181}$ [$ptau_{181}$= 61 ($ptau_{181}$ positivity)] values at the threshold of positivity based on estimates for clinical AD. Marginal means were then estimated at these parameters in participants with high vs. low synaptic integrity (CSF upper vs. lower tertile) and effect size (fold differences) were calculated.

All analyses were conducted using JMP15 and figures created using Stata15 software packages.

Results.

Associations among synaptic proteins and AD biomarkers. On average, participants were 71-years-old and showed high global cognitive screening (MMSE M=29); 18% demonstrated elevated CSF $A\beta_{42/40}$ and 13% demonstrated elevated $ptau_{181}$. Each of the synaptic proteins demonstrated a linear, positive association with $ptau_{181}$ (r range= 0.61 to 0.88, all p-values <0.001; Figure 1B), as well as among each other (r range= 0.62 to 0.89, all p-values <0.001). The relationship between CSF synaptic proteins and $A\beta_{42/40}$ was negative but modest in size (r range= -0.28 to 0.39, all p-values <0.03); however, upon visualization and further evaluation, a quadratic relationship was present between synaptic proteins Ng (Ng^2 B=-1.2, p=0.03) and GAP-43 ($GAP\text{-}43^2$ B=-1.7, p=0.003) with $A\beta_{42/40}$ such that low-to-medium synaptic protein concentrations were associated with the highest CSF $A\beta_{42/40}$ levels (inverted-U; see Figure 1A). Presynaptic proteins, SNAP-25 and SYT-1, did not show a statistically significant quadratic relationship with $A\beta_{42/40}$ (p values >0.10). Models examining the quadratic relationship between $ptau_{181}$ and synaptic proteins did not suggest superior fit (all quadratic parameter p-values >0.12). $A\beta_{42/40}$ and $ptau_{181}$ demonstrated a relatively modest, linear correlation (r= -0.49, p<0.001).

<Figure 1 here >

Synaptic proteins buffer the relationship between $A\beta_{42/40}$ and $ptau_{181}$. Adjusting for demographics (age, sex, education), there was a significant interaction with each of the synaptic proteins on the relationship between $A\beta_{42/40}$ and $ptau_{181}$ (Ng B= -0.23, p<0.001; GAP-43 B= -0.12, p=0.03; SYT-1 B= -0.18, p=0.049; SNAP-25 B=- 0.22, p=0.01; Figure 2, Supplementary Table 1). Participants with low synaptic integrity markers (CSF upper tertile) showed the expected strong, negative relationship between CSF $A\beta_{42/40}$ and $ptau_{181}$ [r= -0.67 (GAP-43), r= -0.71 (Ng), r= -0.66 (SYT-1), r= -0.70 (SNAP-25)], while participants with high synaptic integrity markers (CSF lower tertile) showed small to even *positive* associations between CSF $A\beta_{42/40}$ and $ptau_{181}$ [r= 0.01 (Ng), r= -0.18 (GAP-43), r= 0.48 (SYT-1), r= 0.57 (SNAP-25)]. Estimated using CSF $A\beta_{42/40}$ and $ptau_{181}$ cut-offs for clinical AD, Aβ+ adults with high synaptic integrity markers demonstrated 1.25 (SYT-1), 1.35 (SNAP-25), 1.30 (GAP-43), and 1.43 (Ng) fold lower $ptau_{181}$ compared to their Aβ+ low synaptic integrity peers.

<Figure 2 here >

Synaptic proteins buffer the relationship between ptau$_{181}$ and gray matter volume. Adjusting for demographics, CSF ptau$_{181}$ or A$\beta_{42/40}$ were not strongly associated with medial temporal (ptau$_{181}$: B=-0.08, p=0.57; A$\beta_{42/40}$ B=0.24, p=0.09) or total gray matter (ptau$_{181}$ B=0.15, p=0.26; A$\beta_{42/40}$: B=0.09, p=0.52) volumes. However, when entered into the model, each of the synaptic proteins demonstrated a significant interaction on the relationship between ptau$_{181}$ and medial temporal (Ng B=-0.44, p=0.025; SYT-1 B=-0.45, p=0.01; SNAP-25 B=-0.40, p=0.034; GAP-43 B=-0.45, p=0.02) or total gray matter volumes (Ng B=-0.42, p=0.03; SYT-1 B=-0.49, p=0.01; SNAP-25 B=-0.32, p=0.10; GAP-43 B=-0.43, p=0.03)(Supplementary Table 2). An inverse-U relationship between ptau$_{181}$ and gray matter emerged that was dependent on synaptic marker levels (Figure 3). Only participants with low synaptic integrity markers demonstrated the expected negative association between ptau$_{181}$ and gray matter volume (r range= -0.59 to -0.37); an effect that reversed and showed *positive* associations between ptau$_{181}$ and gray matter volume in those with high synaptic integrity markers (r range= 0.06 to 0.54).

There was also a moderating effect of synaptic proteins on the relationship between A$\beta_{42/40}$ and gray matter volumes, but the effect only neared or reached statistical significance for presynaptic proteins SYT-1 (MTL: B=0.35, p=0.044; total GMV B=0.34, p=0.06) and SNAP-25 (MTL: B=0.39, p=0.02; total GMV B=0.49, p=0.005) (Ng and GAP-43 p-values <0.12). Again, comparing lower vs. upper CSF tertiles, participants with worse SYT-1 and SNAP-25 markers demonstrated a stronger link between A$\beta_{42/40}$ and gray matter volumes (r range= 0.25 to 0.46), while their peers with high presynaptic integrity markers showed small relationships between A$\beta_{42/40}$ and gray matter (r range= 0.15 to 0.19).

<Figure 3 here >

Presynaptic proteins buffer the relationship between A$\beta_{42/40}$ and cognition. Adjusting for demographics, A$\beta_{42/40}$ or ptau$_{181}$ showed generally small and non-statistically significant relationships with cognition in our sample of clinically normal older adults (A$\beta_{42/40}$: B range=-0.01 to -0.18, all p-values > 0.90; ptau$_{181}$: B range= -0.26 to 0.16, all p-values>0.60). Each of the individual synaptic proteins did not reach statistical significance moderating the relationship between A$\beta_{42/40}$ and cognition, though presynaptic proteins (SNAP-25 and SYT-1) demonstrated modest effect sizes (SYT-1 executive functions B=0.24, p=0.13; SNAP-25 processing speed B=0.29, p=0.32). When considered together as a ratio (SNAP-25/SYT-1), these latter effects strengthened and the SNAP-25/SYT1 ratio showed a significant interaction on the relationship between A$\beta_{42/40}$ and executive

functions (B=0.32, p=0.039), as well as processing speed (B=-0.49, p=0.039), but not memory (B=0.12, p=0.54)(Figure 4). Participants with low presynaptic integrity ratios demonstrated expected, positive associations between CSF $A\beta_{42/40}$ and cognition [r= 0.55 (speed), r= 0.49 (executive functions)], while those with high presynaptic integrity ratios demonstrated smaller and *inverse* relationships between CSF $A\beta_{42/40}$ and cognition [r= -0.25 (speed), r= -0.38 (executive functions)]. Estimated using CSF $A\beta_{42/40}$ cut-offs for clinical AD, Aβ+ adults with high presynaptic integrity ratios demonstrated 1.34-fold better processing speed and 1.58-fold better executive functions compared to their Aβ+ low presynaptic integrity peers.

Ng (all p-values>0.83) and GAP-43 (all p-values >0.47) did not show a statistical interaction between $A\beta_{42/40}$ and cognition. None of the individual proteins, nor presynaptic protein ratio significantly moderated the effect of $ptau_{181}$ on cognition (all p-values >0.12).

<Figure 4 here>

Discussion

Using *in-vivo* CSF markers of synaptic integrity and AD proteinopathy in cognitively normal adults, we demonstrate that the neurotoxic effects of Aβ and ptau may be highly dependent on synaptic state. Namely, the negative relationship between the two canonical AD proteins was only present among individuals who also demonstrated poor synaptic integrity markers. Further, older adults with maintained markers of synaptic integrity demonstrated disproportionately (>1.3-fold) less tau hyperphosphorylation and cognitive dysfunction for their Aβ levels, and less cortical atrophy for their ptau levels. Interestingly, AD proteinopathy levels evidenced some *positive* associations with outcomes in adults with the highest synaptic integrity markers (Aβ positively related with ptau, and ptau positively related to gray matter), perhaps suggesting initial recruitment (e.g., spread) and/or compensatory processes involving the synapse. Not everyone with Aβ or ptau have adverse clinical sequalae. Our results suggest that patients with AD proteinopathy *and* uniquely elevated markers of synaptic dysfunction may be at the most elevated risk. This work further extends the growing animal and human autopsy literature *into living humans with subclinical disease* suggesting that the synapse may be 1) at the center of AD pathogenesis and may need to be considered to fully capture AD development, 2) involved in AD proteinopathy development prior to overt cognitive dysfunction, and 3) a target to support preserved resilience to AD neuropathology.

Aβ and tau play important roles regulating synaptic functioning under normal physiologic conditions in animal and *in-vitro* models, which these data begin to recapitulate in humans. While Aβ potentiates neuronal activity at low levels, elevated levels depress synaptic firing and become neurotoxic[3–7]. Our data (Figure 1) are the first to show this curvilinear relationship between Aβ and synaptic integrity in humans, an effect particularly prominent on proteins both present in the postsynaptic compartment involved in calcium and dendritic growth regulation (neurogranin and GAP-43, respectively). Presynaptic Aβ may enhance the release probability of synaptic vesicles, leading to potentiated synaptic firing and excessive release of extracellular Aβ at the synapse[3–7]. Postsynaptically, high Aβ levels promote internalization of AMPA and NMDA receptors[8–11] and excess extracellular glutamate, ultimately leading to desensitization of the postsynaptic membrane and long-term depression (LTD)[56]. Our data appear to reflect this delicate balance between Aβ and synaptic integrity even in subclinical older adults who have overall low levels of Aβ aggregation. The presynaptic vesicle protein markers, SYT-1 and SNAP-25, demonstrated generally linear relationships with Aβ and attenuated the effects of Aβ on gray matter and cognition, while neurogranin and GAP-43, postsynaptic calcium signaling and neural growth cone regulation proteins, showed an inverse-U relationship with Aβ and did not moderate Aβ effects on cognition. These data suggest that the synaptotoxic effects of Aβ may differ depending on the synaptic pathway and/or compartment involved. Synaptic vesicle proteins may be sensitive to the direct effects of Aβ (one-to-one relationship) and regulate its impact on functioning (cognition), while downstream in the postsynaptic compartment, the adverse effect of Aβ may differ depending on severity of Aβ aggregation. Interestingly, the relative ratio of presynaptic proteins buffered the relationship between Aβ and speeded executive functions greater than episodic memory. While memory deficits are considered the early clinical hallmark of AD, executive functions and especially processing speed are domains with greatest sensitivity to (nonspecific) brain insult and may suggest a broader disrupted network associated with synaptic vesicle imbalance very early in disease development, more consistent with the nonlocalizing spread of Aβ[57].

On other hand, tau is a microtubule stabilizing protein essential for bidirectional transport of cellular cargo between the cell body and synapse. Though relatively less well understood, the predominating view is that misfolding of tau may lead to both a loss of microtubule structure and/or gain of toxic function via “blocked” axonal transport and signaling[58] -- both functionally cutting off the synapse from essential cellular cargo (e.g., synaptic receptors, mitochondrial proteins). At the synapse, tau can also bind to presynaptic vesicles, inhibiting

their mobility and release rate[17], and postsynaptically hyperphophorylated tau can mislocalize to dendritic spines disrupting glutamate receptor anchoring[16]. Of note, synaptic activity itself appears to potentiate tau release from neurons both *in-vivo* and *in-vitro*, which may contribute to its prion-like spread along synaptically-connected neighboring cells[59–61]. Together, these converging works suggest a close, bidirectional relationship between tau and synaptic functioning. Our data are consistent with these animal models and suggest that synaptic integrity tracks closely and linearly with tau phosphorylation levels, potentially regardless of synaptic compartment and/or posited pathway. Interestingly, ptau demonstrated a nonlinear relationship with cortical volume, and each synaptic marker closely tracked along this relationship. Those with high synaptic integrity showed *positive* associations between ptau and cortical volume, an effect that reversed in adults with low synaptic integrity markers. This finding converges well with a recent study in rodents demonstrating rapid, peak sheading of neurogranin in CSF during early phases of induced neurodegeneration that then level off[62], and human studies mirroring a similar shaped curve with CSF $ptau_{181}$ peaking early and then declining after estimated disease onset in autosomal dominant AD[63]. Together, these data suggest $ptau_{181}$ and synaptic dysfunction ramp up concurrently in the earliest phases of pathogenesis and potentially initiate a neurodegenerative process that is then sustained. Of note, few participants in our study had *both* high $ptau_{181}$ and high synaptic integrity (CSF lower tertile), suggesting that perhaps ptau is a hallmark of a nonfunctional synaptic connection.

Further, our data indicate that the Aβ-ptau relationship itself may depend on synaptic integrity, such that synaptic dysfunction may be a necessary component in order for an adverse Aβ and ptau relationship to occur. This finding potentially places the synapse at the center of AD pathogenesis. Although observational, this finding suggests that synaptic processes may be intimately linked to Aβ-induced tau hyperphosphorylation and/or aggregation of abnormal Aβ/tau may propagate synaptic dysfunction and degeneration. Indeed, synaptic dysfunction appears to be present before formation of amyloid plaques or tau tangles[3–7], suggesting that it may be a necessary driver for fulminant of AD pathogenesis. Zempel and colleagues further demonstrated that spine loss induced by exogenous Aβ only occurs in dendritic regions in which tau has been missorted and microtubules are disrupted[64,65], highlighting the dependent relationship among these three processes. Importantly, synapses may also play a role propagating disease spread. Injection of brain extract from animals expressing either abnormal Aβ or tau seeds local plaque or tangle formation that spreads to

neighboring, axonally-connected cells and regions[66–68]. Though not spatially localizing, the interactive relationship observed among CSF markers of Aβ, ptau, and synaptic integrity may reflect these exponentially propagating processes.

Lastly, these findings converge closely with both animal and human studies implicating the synapse in cognitive resilience to AD pathology. Amyloid and tau accumulation are common features of the aging brain[69,70] that do not necessitate clinical impairment; over a third of older adults (65+) show fulminant plaques and tangles at death without ever evidencing cognitive impairment in life[30,71]. Converging human autopsy studies have identified unique preservation of synaptic structure,[24–26] protein levels,[24,25] and gene expression[32] in adults showing cognitive resilience to neuropathologic AD compared to non-resilient AD peers. Our data extend these findings into living humans suggesting that Aβ and ptau may not exert adverse effects on each other or on brain structure and function when synaptic integrity is maintained. This growing body of literature indicates that synaptic preservation may sit at the crux of cognitive resilience and therapies that directly support synaptic functioning may be high potency targets to stave off clinical manifestations of age-related neuropathologies. Additionally, pioneering but decades-old studies led by Marian Diamond established that enriching lifestyle behaviors (e.g., physical or cognitive activity) are synaptogenic in animal models[72,73], an effect consistently replicated[74–79]. The synapse may therefore be a particularly amenable target for powerful multimodal interventions that combine behavioral and pharmacologic therapies to prevent ADRDs.

Our study has several important limitations. First, we evaluated traditional markers of AD proteinopathy, $A\beta_{42/40}$ and $ptau_{181}$. Increasing data suggest that *soluble* Aβ and tau (oligomers, dimers) and other specific isoforms (e.g., $ptau_{217}$) may be differentially neuro- and synaptotoxic; future works evaluating specificity of Aβ and tau isoform are highly warranted. Additionally, integration of *in-vivo* molecular markers reflecting other highly implicated pathways (e.g., glial, vascular), as well as spatially rich PET synaptic ligands would provide important context to these likely highly dynamic processes. Of note, we evaluated these relationships in a cohort of cognitively normal, high-functioning older adults. Although we demonstrate presence of these relationships in subclinical disease states, it is certainly possible that our sample of cognitively sustained older adults represent a selection bias (e.g., survivor effect) that does not generalize to all cognitive aging trajectories. Further exploration of how these markers covary together across disease state and stage, as well

as other culturally distinct backgrounds (e.g., lower socioeconomic status, educational diversity, health access disparity groups) will be critically informative.

Our study demonstrates very early dependence of AD proteinopathy on synaptic integrity in living, clinically normal older adults without overt cognitive dysfunction. As molecular biofluid and imaging markers advance, we suggest a critical need to concurrently capture these discrete molecular processes in clinic to better diagnose, prognosticate, and understand AD pathophysiology *in-vivo* in humans. Given the high positivity rate particularly for Aβ at older ages[57], markers of synaptic integrity may provide much needed specificity to indicate those most at risk of developing AD dementia. These findings further support efforts to preserve synaptic integrity via behavioral (e.g., exercise, cognitive stimulation) and/or pharmacological approaches to buffer the negative effects of common age-related proteinopathies.

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Conflicts of interest

HZ has served at scientific advisory boards for Denali, Roche Diagnostics, Wave, Samumed, Siemens Healthineers, Pinteon Therapeutics and CogRx, has given lectures in symposia sponsored by Fujirebio, Alzecure and Biogen, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program (outside submitted work).

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Figure 1. A) Relationship between CSF synaptic proteins and $A\beta_{42/40}$; B) Relationship between CSF synaptic proteins and $ptau_{181}$.

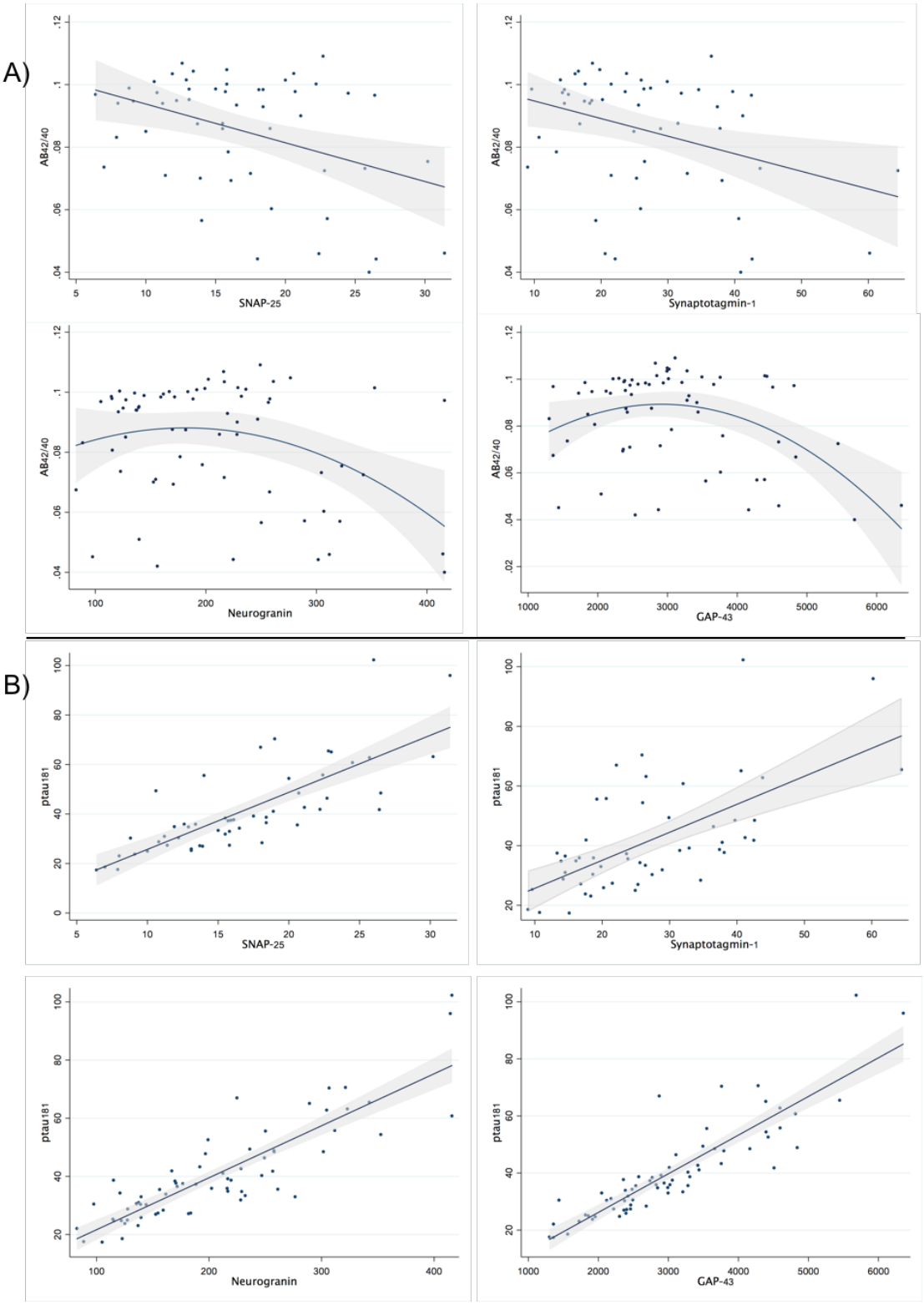

Figure 2. Markers of synaptic integrity attenuate the relationship between $AB_{42/40}$ and $ptau_{181}$. *Note. Synaptic integrity levels illustrated in tertiles.*

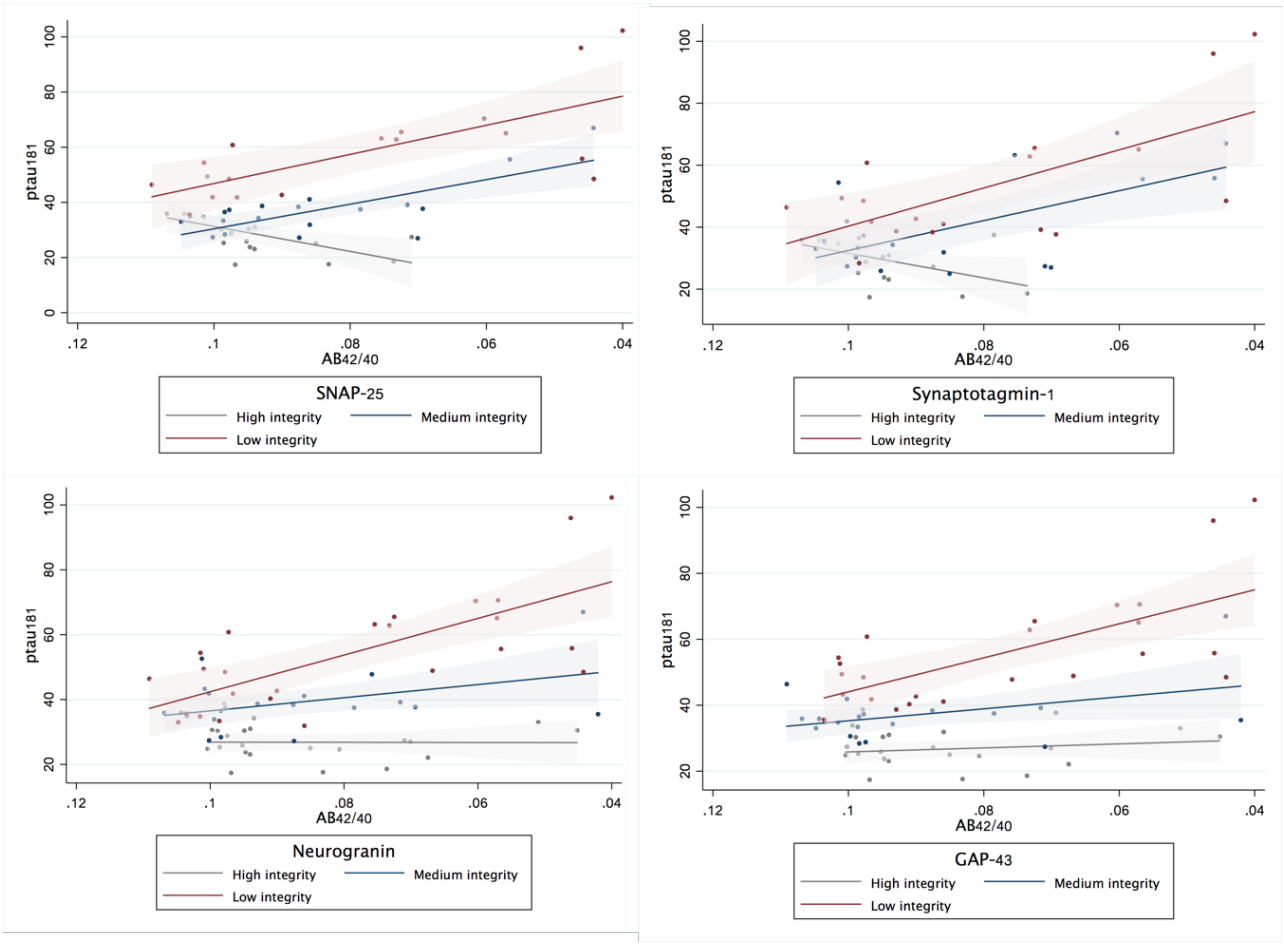

Figure 3. Markers of synaptic integrity attenuate the relationship between ptau$_{181}$ and medial temporal lobe atrophy. *Note. Synaptic integrity levels illustrated in tertiles.*

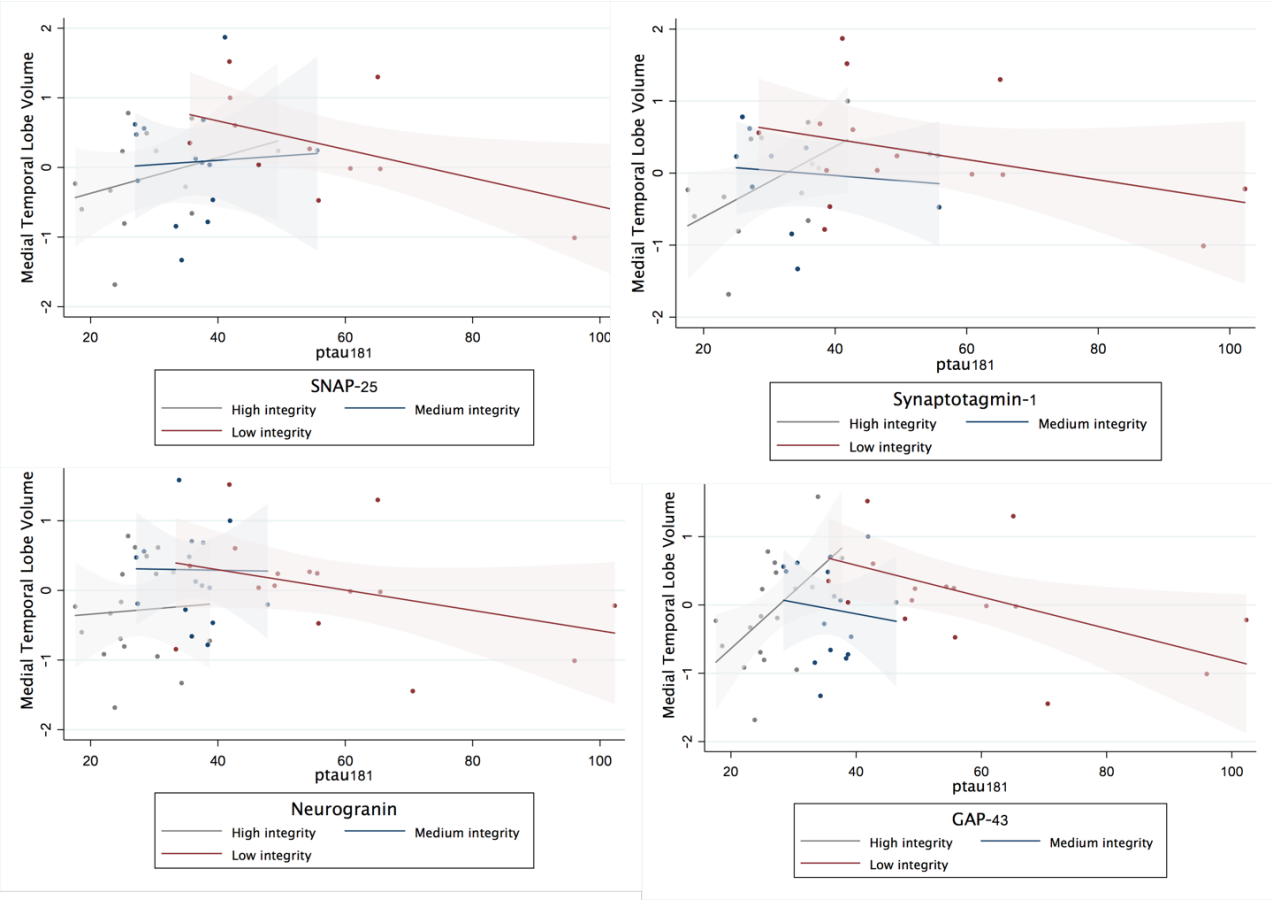

Figure 4. Markers of presynaptic integrity (SNAP-25/SYT-1) attenuate the relationship between $AB_{42/40}$ and cognition. *Note. Synaptic integrity levels illustrated in tertiles.*

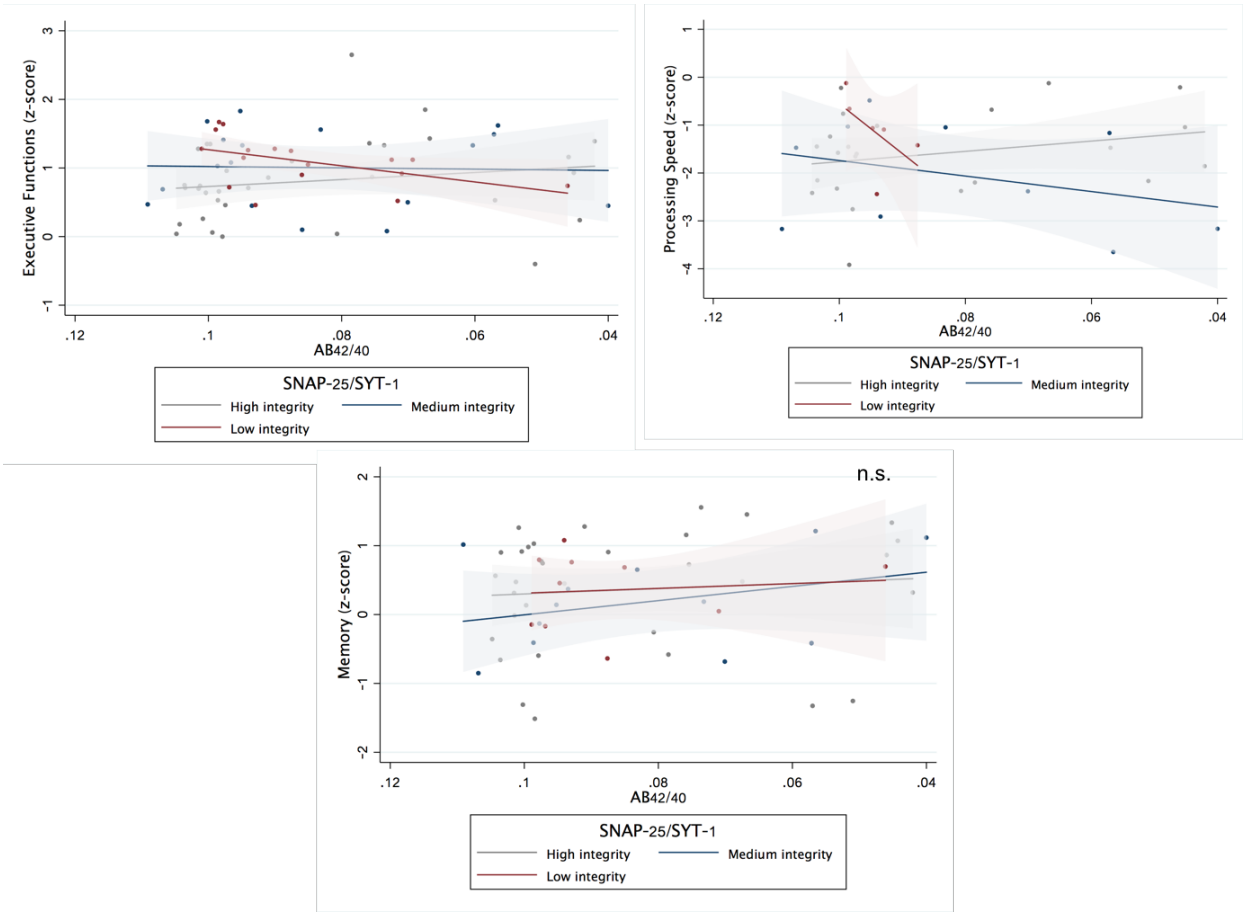

Table 1. Clinical and demographic characteristics of study sample (n=68).

	Mean (SD)	**Range**
Age, y	70.7 (7.1)	53, 83
Sex (%F, n)	42.6% (29)	---
Education, y	17.2 (2.2)	12, 20
Race (%, n) White Asian Black Unknown	 86.7% (59) 8.8% (6) 1.5% (1) 2.9% (2)	
Mini Mental Status Examination (MMSE)	29.1 (1.2)	25, 30
CVLT-II Long Delay Free Recall (16-item)	12.5 (3.0)	5, 16
Benson Figure Delayed Recall (17-item)	12.7 (2.5)	7, 17
NIH EXAMINER Executive Composite (z-score)	0.93 (0.56)	-0.45, 2.7
Processing speed (z-score)[†]	-1.6 (0.97)	-3.9, -0.12
Cerebrospinal Fluid (CSF) Markers		
Synaptotagmin-1 (pM)	26.6 (12.0)	9, 64.4
SNAP-25 (pM)	16.8 (6.0)	6.4, 31.4
Neurogranin (pg/mL)	206.4 (80.9)	82.6, 415.8
GAP-43 (pg/mL)	3045.4 (1086.8)	1301.7, 6354.7
$A\beta_{42/40}$ (pg/mL) % Elevated (<0.61) (%, n)	0.08 (0.02) 17.6% (12)	0.04, 0.11
$ptau_{181}$ (pg/mL) % Elevated (>61) (%, n)	40.8 (16.8) 13.2% (9)	17.4, 102.3

[†]Compared to 20-30 year old adults.

Supplementary Tables

Supplementary Table 1. Regression interaction models showing CSF synaptic proteins moderate the relationship between CSF $A\beta_{42/40}$ and $ptau_{181}$. *Each column represents individual model.*

Outcome: $ptau_{181}$																
	beta				**95% CI**				**Standardized B**				**p-value**			
Synaptic Model	*SYT-1*	*SNAP-25*	*Ng*	*GAP-43*	*SYT*	*SNAP-25*	*Ng*	*GAP-43*	*SYT*	*SNAP-25*	*Ng*	*GAP-43*	*SYT*	*SNAP-25*	*Ng*	*GAP-43*
Age	0.97	0.32	0.27	0.22	0.54, 1.4	-0.09, 0.72	0.07, 0.48	-0.01, 0.44	0.36	0.12	0.12	0.09	<.001	0.12	0.01	0.06
Sex	-4.3	-3.97	-3.90	-1.43	-9.9, 1.3	-9.2, 1.2	-6.9, -0.90	-4.89, 2.02	-0.12	-0.11	-0.12	-0.04	0.13	0.13	0.01	0.40
Education	-0.23	-0.39	0.51	0.33	-1.5, 1.0	-1.5, 0.77	-0.16, 1.2	-0.42, 1.08	-0.03	-0.05	0.07	0.04	0.71	0.50	0.13	0.38
$A\beta_{42/40}$	-359.5	-254.0	-153.5	-193.4	-533.9, -196.1	-424.3, -83.6	-233.9, -73.1	-280.15, -106.59	-0.38	-0.27	-0.18	-0.23	<.001	0.004	<0.001	<.001
SYT-1	0.69				0.42, 0.96				0.47				<.001			
SNAP-25		1.65				1.2, 2.1				0.55				<0.001		
Ng			0.15				0.13, 0.17				0.72				<0.001	
GAP-43				0.011				0.010, 0.013				0.75				<0.001
$A\beta_{42/40}$*SYT-1	-12.38				-24.7, -0.06				-0.18				**0.049***			
$A\beta_{42/40}$* SNAP-25		-30.81				-55.0, -6.7				-0.22				**0.01**		
$A\beta_{42/40}$*Ng			-2.0				-2.8, -1.1				-0.23				**<0.001**	
$A\beta_{42/40}$*GAP-43				-0.076				-0.15, -0.004				-0.12				**0.03**

Note. Sex: Male= 1, Female= 2.

Supplementary Table 2. Regression interaction models showing CSF synaptic proteins moderate the relationship between CSF ptau$_{181}$ and total gray matter volumes. *Each column represents individual model.*

Outcome: Total Gray Matter Volume (residual L^3)																
	beta				**95% CI**				**Standardized B**				**p-value**			
Synaptic Model	*SYT-1*	*SNAP-25*	*Ng*	*GAP-43*	*SYT-1*	*SNAP-25*	*Ng*	*GAP-43*	*SYT-1*	*SNAP-25*	*Ng*	*GAP-43*	*SYT-1*	*SNAP-25*	*Ng*	*GAP-43*
Age	-0.002	-0.002	-0.002	-0.002	-0.004, -0.0004	-0.004, -0.001	-0.003, -0.001	-0.004, -0.001	-0.41	-0.48	-0.44	-0.48	0.02	0.003	0.001	<0.001
Sex	-0.01	-0.012	-0.013	-0.008	-0.033, 0.008	-0.031, 0.01	-0.03, 0.008	-0.03, 0.01	-0.18	-0.16	-0.17	-0.11	0.24	0.28	0.22	0.44
Education	-0.001	-0.001	0.0007	0.0004	-0.006, 0.003	-0.006, 0.003	-0.004, 0.005	-0.004, 0.005	-0.11	-0.09	0.04	0.03	0.45	0.53	0.76	0.84
ptau$_{181}$	0.0005	-0.0001	-0.0002	0.0001	-0.0004, 0.001	-0.001, 0.001	-0.002, 0.001	-0.002, 0.002	0.29	-0.03	-0.08	0.07	0.25	0.92	0.82	0.87
SYT-1	0.0006				-0.0005, 0.002				0.24				0.27			
SNAP-25		0.003				0.0003, 0.006				0.52				.03		
Ng			0.0003				-1.6e-6, 0.001				0.60				0.051	
GAP-43				1.5e-5				-7.0e-6, 3.7e-5				0.45				0.18
ptau$_{181}$* SYT-1	-4.657e-5				-8.3e-5, 1.06e-5				-0.49				**0.012**			
ptau$_{181}$* SNAP-25		-6.634e-5				-0.0001, 1.4e-5				-0.32				**0.10**		
ptau$_{181}$*Ng			-6.2e-6				-1.1e-5, -5.2e-7				-0.42				**0.031**	
ptau$_{181}$* GAP-43				-4.7e-7				-8.9e-7, -5.1e-8				-0.43				**0.029**

Note. Sex: Male=1, Female=2.

Supplementary Table 3. Interaction models demonstrating the specific protective effect of presynaptic proteins on the relationship between AB and cognition.

	beta	**95% CI**	**Standardized Beta**	**p-value**
Outcome: Processing Speed				
Age	-0.05	-0.11, 0.019	-0.31	0.16
Sex	-0.20	-1.06, 0.667	-0.10	0.64
Education	-0.05	-0.22, 0.12	-0.11	0.57
$A\beta_{42/40}$	8.46	-13.67, 30.60	0.16	0.89
SNAP-25/SYT-1	-0.11	-1.69, 1.48	-0.029	0.44
$A\beta_{42/40}$(SNAP-25/SYT-1)*	*-119.90*	-223.35, -16.44	*-0.49*	*0.03*
Outcome: Executive Functions				
Age	-0.028	-0.05, -0.004	-0.36	0.02
Sex	-0.24	-0.52, 0.05	-0.23	0.10
Education	0.032	-0.03, 0.09	0.14	0.31
$A\beta_{42/40}$	0.53	-0.12, 1.17	0.25	0.11
SNAP-25/SYT-1	2.65	-5.18, 10.48	0.092	0.50
$A\beta_{42/40}$(SNAP-25/SYT-1)*	*-52.97*	*-92.39, -13.55*	*-0.39*	*0.0096*
Outcome: Episodic Memory				
Age	-0.002	-0.05, 0.04	-0.015	0.93
Sex	0.39	-0.12, 0.89	0.27	0.13
Education	0.034	-0.06, 0.13	0.11	0.49
$A\beta_{42/40}$	-0.31	-1.32, 0.71	-0.11	0.54
SNAP-25/SYT-1	-13.14	-25.64, -0.65	-0.34	0.04
$A\beta_{42/40}$*(SNAP-25/SYT-1)	-24.26	-90.40, 41.88	-0.13	0.46

Supplemental Methods.

Participants. Participants represent a convenience sample of community-dwelling older adults in the Bay Area. Inclusion criteria: 1) no diagnosed memory or neurological condition (e.g., epilepsy, large vessel stroke), 2) no major medical (e.g., active neoplasm, HIV, dialysis), psychiatric disorder (e.g., schizophrenia), or active substance use disorder, and 2) no functional decline operationalized as Clinical Dementia Rating (CDR) of 0 via study partner interviews.

Cerebrospinal fluid (CSF) Analytics. CSF was collected in the morning after a 12-hour fast. to minimize possible confounding effects (e.g., circadian rhythm, metabolic). Approximately 22mL of CSF were tapped and centrifuged at 2000g for 10 minutes before being frozen in 0.5 mL aliquots and continuously stored at -80°C. Board certified laboratory technicians blinded to participant clinical history performed analyses per protocols approved by the Swedish Board of Accreditation and Conformity Assessment using one batch of reagents. Samples were run in duplicate with coefficients of variance >10% or extreme outliers (>3x outer quartile range) removed from analyses.

Neuroimaging. Participants completed 3T Magnetom Vision TIM Siemens Trio brain magnetic resonance imaging (MRI). T1-weighted magnetization prepared rapid acquisition GRE structural scan was acquired (acquisition time 8 minutes, 53 seconds), sagittal orientation, field of view 160 x 240 x 256 mm and isotropic voxel resolution of 1 mm^3 (repetition time = 2300ms, echo time = 2.98 ms, time inversion = 900 ms, and flip angle = 9). Before processing, all images were visually inspected for quality and those with excessive motion or other image artifact excluded. Magnetic field bias was corrected using the N3 algorithm[80]. Tissue segmentation was performed using the unified segmentation procedure in SPM12[81]. Each participant's T1-weighted image was warped to create a study-specific template using Diffeomorphic Anatomical Registration using Exponentiated Lie algebra (DARTEL)[82]; subsequently, the images were normalized and modulated in the study-specific template space using nonlinear and rigid-body registration. Images were smoothed using an 8-mm Gaussian kernel with 8-mm full width half maximum. For registration with a brain parcellation atlas, linear and nonlinear transformations between DARTEL's space and ICBM space were applied[83]. Quantification of volumes in specific brain regions at each time point was accomplished by transforming a standard parcellation

atlas into ICBM space and summing all modulated gray matter within each parcellated region[84]. Total intracranial volume was estimated for each subject in MNI space.

Neuropsychological evaluation. *Episodic memory* was measured via the California Verbal Learning Test, second edition[85] (immediate recall total, long delay (20-30 minute) free recall, and recognition discriminability) and the Benson Figure (delayed free recall) performances; scores were blom transformed and combined onto a z-score metric. *Executive functions* were quantified via the Executive Composite index of the NIH EXAMINER. The NIH EXAMINER is a computerized battery comprised of six subtests tapping into various executive functioning skills (e.g., working memory, cognitive control, generativity). The Executive Composite is calculated using item response theory; composite scores were only calculated for individuals with standard error of measurement <0.75, consistent with the normative study[86]. *Processing speed* was measured using a validated, experimental computerized battery of verbally mediated reaction-time tasks (e.g., Rhyme, Word Judgement, Pronounce)[87]. Tasks performances are averaged into a composite z-score reflecting reaction time of participant performance compared to a young adult normative sample; z-scores were transformed such that higher values indicate better performances.