Cholinergic synaptic plasticity shapes resilience and vulnerability to tau

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Abstract

Synaptic dysfunction is a hallmark of Alzheimer's disease (AD). Yet due to their plasticity, synapses may also adapt to early AD pathology. Using within-subject positron emission tomography scans targeting the vesicular acetylcholine transporter (VAChT) protein, tau, and amyloid in healthy older adults at risk for AD, we show that cholinergic neurons increase presynaptic VAChT levels when colocalized to tau, but not amyloid, with higher responses predicting resilience to cognitive decline over ~10 years. In mice lacking forebrain VAChT, we demonstrate that cholinergic synaptic plasticity plays a causal role in sustaining cognitive flexibility and hippocampal structural integrity. Whole-brain single-nucleus RNA sequencing atlases reveal that cholinergic neurons upregulate a gene-network enriched for synaptic plasticity, with high centrality for the microtubule-associated protein tau (*MAPT*) gene. Our findings identify cholinergic synaptic plasticity as a critical mediator of resilience and vulnerability to tau in presymptomatic AD.

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Main

Synaptic dysfunction is among the first detectable features of Alzheimer's disease (AD)[1,2], and the strongest correlate of early cognitive decline[3–7]. However, synapses are also among the brain's most dynamic structures. Synapses can rebalance their strength and connectivity through homeostatic plasticity, a form of cellular adaptation that is thought to underlie the brain's resilience in the face of sustained stress[8–11]. It may even explain how many individuals maintain cognitive function despite years of accumulating AD pathology[12].

Yet whether synapses mount such compensatory responses to specific AD pathologies in the living human brain remains unknown. Prevailing models of AD assume a passive trajectory: β-amyloid (Aβ) and tau pathologies accumulate, circuits fail, and cognition declines. But if synaptic adaptation occurs before neurodegeneration, then it may not only delay decline, but also determine which individuals resist it[13]. The challenge is to capture these early synaptic changes *in vivo*, and disentangle whether they reflect compensation, vulnerability, or both.

Here we leverage molecular imaging to quantify cell-type-specific presynaptic function alongside regional tau and Aβ pathology in healthy older adults at elevated risk for AD. We show that basal forebrain (BF) cholinergic synapses mount an adaptive response to early tau pathology, but not Aβ. We demonstrate that this response predicts longitudinal cognitive resilience, modulates tau spreading, and breaks down in individuals who later decline. Supported by transcriptomic and multimodal neuroimaging work across species, our results position cholinergic synaptic plasticity as a homeostatic buffer to tau, and its failure as a tipping point toward neurodegeneration.

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Results

Extensive neuropathological staging and neuroimaging work points to the cholinergic BF as one of the first sites of pathology and neurodegeneration in the progression of AD[14–17]. More recent studies have demonstrated that longitudinal BF atrophy in at-risk older adults predicts the progression of cortical neurodegeneration, which reflects the spatial profile of cholinergic presynaptic loss observed in AD patients[18–20]. Parallel lines of evidence suggest that BF cholinergic synapses are essential for promoting Hebbian plasticity[21–26] and stabilizing cellular homeostasis[27–32] in adult neural circuits. Together, these findings suggest that the selective vulnerability of the cholinergic BF projection system may in part be due to early presynaptic adaptations to sustain plasticity and network function under increasing pathological load.

To explore BF cholinergic homeostatic synaptic plasticity in healthy older adults at elevated risk for AD, we collected [^{18}F]-fluoroethoxy-benzovesamicol ([^{18}F]-FEOBV) positron emission tomography (PET) data in individuals enrolled in the PRe-symptomatic EValuation of Experimental or Novel Treatments for Alzheimer's Disease (PREVENT-AD) program (**Table 1**)[33]. [^{18}F]-FEOBV is one of a limited number of PET tracers that permits direct *in vivo* imaging of synapses in humans[34]. It selectively binds to the vesicular acetylcholine transporter (VAChT), a protein exclusively produced by cholinergic neurons and most abundantly expressed within the presynaptic nerve terminals[35]. Because VAChT is responsible for packaging acetylcholine into synaptic vesicles to facilitate cholinergic neurotransmission[36], [^{18}F]-FEOBV uptake provides a cell-type specific measure of cholinergic presynaptic function. To investigate whether adaptations in cholinergic presynaptic function track with early pathological load, we obtained tau ([^{18}F]-AV1451) and β-amyloid (Aβ; [^{18}F]-NAV4694) PET data within ~1.5 years of each participant's VAChT-PET scan (**Supplemental Fig. 1**).

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Table 1 PREVENT-AD Demographic Information

Demographics	**PREVENT-AD**
n	64
Age (at VAChT-PET, Years)	70.53(0.6243)
Females (*n*) %	44(68.75)
APOε4 Carriers (*n*) %	23(35.94)
Education (Years)	15.31(0.3952)
Baseline CDR	0(0)
Baseline MoCA	27.92(0.1867)
Baseline RBANS (Total Score)	101.9(1.154)
Follow-Up RBANS (Total Score)	98.78(1.218)
Baseline to Follow-Up RBANS (Days)	2783(63.22)
RBANS APC (Total Score)	-0.4872(0.1393)
VAChT- to Tau-PET ITI (Days)	517.5(32.18)
VAChT- to Aβ-PET ITI (Days)	508(30.83)

Continuous data are presented as mean(SEM); categorical data are presented as the number of participants(percentage). APC=Annualized Percent Change; APOε4=apolipoprotein-E ε4; CDR=clinical dementia rating; ITI=inter-scan time interval; MoCA=Montreal Cognitive Assessment; PET=positron emission tomography; RBANS=Repeatable Battery for the Assessment of Neuropsychological Status; VAChT=vesicular acetylcholine transporter.

Mapping personalized brain profiles of tau and Aβ

We anticipated that the spatial profiles of tau and Aβ pathology, as well as their potential interdependencies with VAChT, would likely exhibit considerable variation across individuals in the presymptomatic stage of AD[37–40]. We therefore adapted a normative deviation mapping approach[41,42] to capture each participant's unique pattern of tau and Aβ pathology in the brain (see *Methods*). Using an iterative, leave-one-out strategy, we generated voxel-wise population median (*M-i*) and scaled median absolute deviation (*MAD-i*) reference images across the cohort, ensuring statistical independence between each participant's PET images and their corresponding reference images. Supporting the robustness of this strategy, we observed consistently high correlations across all pairwise combinations of *M-i* or *MAD-i* reference images (**Supplemental Fig. 2**). For each participant, we identified voxels with tau- or Aβ-PET uptake values falling above or below the 95% confidence interval (CI) of their respective *M-i*

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and *MAD-i* reference voxels. These voxels were classified as regions of high (i.e., >U95) or low (i.e., <L95) pathology, while voxels within the 95% CIs were assigned to intermediate load regions (i.e., L95–M and M–U95) (**Fig. 1a**). Overall, our normative deviation mapping approach identifies individualized profiles of tau and Aβ which are significantly higher or lower than expected in a population of healthy older adults at elevated risk for AD (**Supplemental Fig. 3,4**).

Tau and Aβ may act both independently and synergistically to alter synaptic function[43–46]. We therefore computed the spatial overlap of tau and Aβ pathology within each individual's four pathological load intervals (i.e., <L95, L95–M, M–U95, >U95) to generate three unique profiles of pathology: regions with increasing tau but not Aβ (i.e., Tau~Aβ), regions with increasing Aβ but not tau (i.e., Aβ~Tau), and regions with increasing co-localized pathology (i.e., Tau∩Aβ) (**Fig. 1b**). We then investigated the anatomical distribution and relative concentration of these distinct pathological profiles across individuals, with a focus on regions with high pathology (>U95) that intersect the BF projection zone (BFpz), including the BF, hippocampus, amygdala, and neocortex. Aligning with known early-stage anatomical patterns of pathology[47,48], high Tau~Aβ profiles were found most abundantly in medial and lateral temporal regions (**Fig. 1c**), while high Aβ~Tau profiles were enriched in default mode network hubs (**Fig. 1d**). Profiles of high Tau∩Aβ most frequently appeared in the posterior cingulate and occipitotemporal cortex (**Fig. 1e**). Consistent with prior normative mapping frameworks[41], we also observed that regions of high and low pathology were spatially smaller than intermediate pathology regions (**Fig. 1f–h**). We also verified that tau- and Aβ-PET uptake selectively increased across Tau~Aβ and Aβ~Tau regions, respectively, and that both tracers showed progressive increases moving from low to high Tau∩Aβ profiles (**Fig. 1f–h**).

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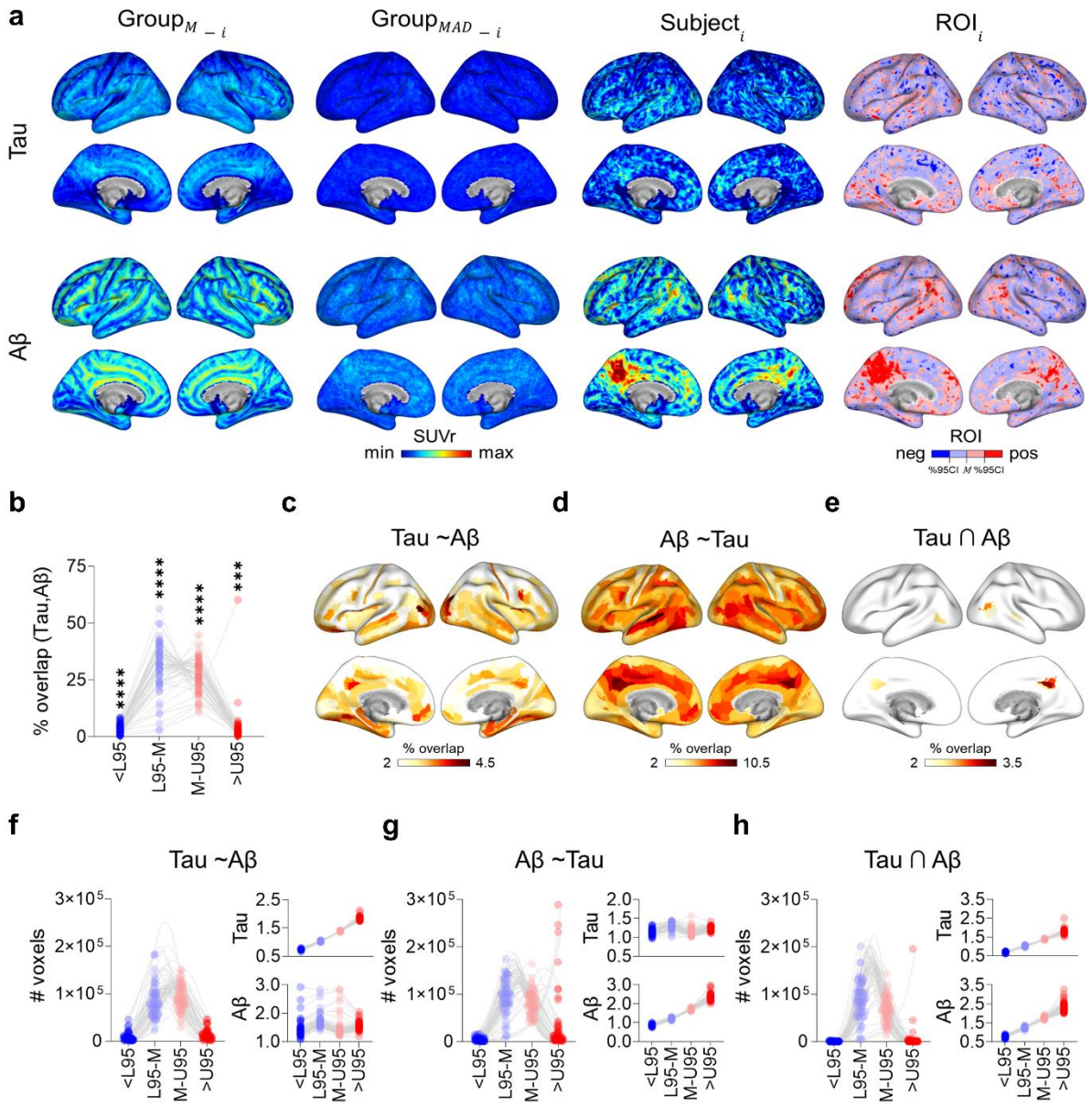

Fig. 1 Individualized mapping of tau and Aβ in healthy older adults at elevated risk for Alzheimer's disease.

a, Representative normative median deviation mapping steps for standardized uptake value ratio (SUVr) Tau-PET (*top*) and Aβ-PET (*bottom*) images. From left to right, showing (1) population median reference images (Tau$_{M\text{-}i}$=0.9–2.4; Aβ$_{M\text{-}i}$=0.9–3), (2) population median absolute deviation images (*MAD-i*=0–1), (3) corresponding example subject images (Tau=0.9–2.4; Aβ=0.9–3), and (4) corresponding example subject segmented CI regions of interest (ROI) (<L95=dark blue; L95–M=light blue; M–U95=light red; >U95=dark red). **b,** Spaghetti plots of

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Jaccard index values (percentage) for Tau and Aβ across segmented CI ROIs (One sample Wilcoxon signed-rank test). Colour coding is the same as the ROI_i map in **a**. **c-e,** Mean percentage overlap across subjects for Tau~Aβ (**c**), Aβ~Tau (**d**), and Tau∩Aβ (**e**) >U95 ROIs in each parcel of the BFpz, projected on medial and lateral brain surfaces. Parcels are derived from the Human Connectome Project Multi-Modal Parcellation-extended atlas[49]. Darker colours indicate greater overlap of >U95 ROIs with a parcel across subjects. **f-h**, Spaghetti plots showing the number of voxels (*left*) and mean SUVrs (*right*) across Tau~Aβ (**f**), Aβ~Tau (**g**), and Tau∩Aβ (**h**) ROIs for each individual (Friedman test followed by Dunn's post-hoc test). Colour coding is the same as the ROI_i map in **a**. Statistical details in **Supplemental Information** file. ***$P \leqq 0.001$,****$P \leqq 0.0001$.

Cholinergic synaptic function adapts to tau, not Aβ

We first tested whether BF cholinergic neurons mount a presynaptic response to increasing tau pathology, Aβ pathology, or both, in healthy older adults at elevated risk for AD. On one hand, an increasing burden of tau, Aβ, or their combination may damage BF cholinergic projections, resulting in a loss of cholinergic synaptic terminals or downregulation of synaptic VAChT. On the other hand, if cholinergic synapses adaptively respond to early pathology, VAChT levels should increase, rather than decrease, moving from areas of lower to higher pathological burden. Consistent with the adaptive response framework, we found a significant main effect of Tau~Aβ region on VAChT, where within-subject VAChT levels significantly increased with increasing tau load (**Fig. 2a**). By contrast, regions with increasing Aβ but not tau failed to differentiate within-subject levels of VAChT (**Fig. 2b**). In areas with co-localized tau and Aβ, we again observed a significant main effect of region on VAChT levels. However, this effect was driven by an increase in VAChT in areas with low to intermediate levels of co-localized pathology, and was abolished when comparing regions with intermediate to high levels of joint

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tau and Aβ aggregation (**Fig. 2c**). Together, these findings suggest that tau pathology is a primary driver of an adaptive cholinergic presynaptic response in older adults at elevated risk for AD.

To determine whether the observed increases in [^{18}F]-FEOBV uptake reflect true adaptive changes in VAChT levels, we performed [^{18}F]-FEOBV microPET imaging in a *BAC*-transgenic mouse line with global overexpression of synaptic VAChT (HYP[50]) and wild-type (WT) controls (**Supplemental Fig. 5a**). We found that HYP mice exhibited consistently higher [^{18}F]-FEOBV uptake throughout the BFpz relative to WT mice (**Supplemental Fig. 5b**) and closely tracked with VAChT protein levels in cortical and hippocampal tissue (**Supplemental Fig. 5c**). These findings validate [^{18}F]-FEOBV as a cell-type-specific marker of cholinergic presynaptic function, capable of detecting endogenous upregulation of VAChT *in vivo*.

Cholinergic synaptic adaptation to tau preserves cognition

If tau-mediated increases in synaptic VAChT reflect a compensatory cholinergic mechanism for sustaining neuronal function, then individuals who mount such responses should exhibit preserved cognition over time. To test this, we integrated longitudinal neuropsychological data spanning up to 10 years preceding the multi-tracer PET scanning period (Repeatable Battery for the Assessment of Neuropsychological Status (RBANS), see *Methods*) and generated an annualized percent change (APC) score for each participant (0=stable, positive=improve, negative=decline, see *Methods*). Across participants, we then computed the linear relationships between RBANS APC scores and VAChT levels in each Tau~Aβ region separately. In regions where individualized tau burden exceeded the median (M–U95 and >U95, **Fig. 2d**), we found that VAChT levels increased with a shift from more negative to more positive RBANS APC scores.

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These findings imply a potential interdependence, where VAChT increases across Tau~Aβ regions within some individuals, but not in others. If positively trending profiles of VAChT reflect adaptive synaptic responses to increasing tau, then they may also predict cognitive resilience. To quantify each individual's adaptive cholinergic response to tau, we calculated the linear slope between their mean tau and VAChT levels across the Tau~Aβ regions (**Fig. 2e**). We then examined the association between participants' adaptive response slopes and RBANS APC scores. We found that the sign of tau-mediated adaptive response slopes shifted from negative (i.e., declining VAChT) to positive (i.e., increasing VAChT) with a concurrent shift in the sign of RBANS APC scores from negative to positive (**Fig. 2f**).

The heterogeneity in adaptive response slopes across individuals suggests that the relationship between VAChT and tau varies in healthy older adults at elevated risk of AD (**Fig. 2e,f**), hinting at the presence of biologically meaningful subgroups. To explore this possibility, we ranked individuals according to their adaptive response slopes (**Fig. 2g**) and assigned the top 25% to the resilient group (R), the middle 50% to the intermediate group (I), and the bottom 25% to the vulnerable group (V)[51]. Across the testing window, we found that the resilient group maintained their performance on the RBANS, whereas the vulnerable group exhibited a significant drop of ~1% per year (**Fig. 2h**). No significant differences were detected between vulnerable and resilient subgroups in terms of age, biological sex, or apolipoprotein-ε4 (*APOε4*) carriership. We also did not detect significant between-group differences in baseline cognition or years of education (**Table 2**).

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Table 2 Resilient and Vulnerable Group Demographic Comparisons

Demographics	**Resilient**	**Vulnerable**	**Statistic**	***P*-value**
n	16	16		
Age (at VAChT-PET, Years)	68.99(65.33-75.12)	69(67.62-73.3)	*U*=118	0.7240
Females (*n*) %	10(62.5)	10(62.5)		>0.9999
APOε4 Carriers (*n*) %	5(45.5)	5(45.5)		>0.9999
Education (Years)	14.75(0.829)	15.56(0.926)	*t*=0.6535	0.5184
Baseline RBANS (Total Score)	99.88(2.234)	99.56(1.411)	*t*=0.1183	0.9066

Continuous data are presented as mean(SEM), except age which is presented as median(95CI). Categorical data are shown as the number of participants(percentage). Fisher's exact test was used to assess differences in categorical variables and two-sided independent samples *t*-tests were used for continuous variables, except for age which was assessed with a two-sided Mann-Whitney U test. APOε4=apolipoprotein-E ε4; PET=positron emission tomography; RBANS=Repeatable Battery for the Assessment of Neuropsychological Status; VAChT=vesicular acetylcholine transporter.

Cholinergic synaptic adaptation reduces tau spreading

Our findings thus far suggest that cholinergic synaptic adaptation is a compensatory mechanism to tau pathology that confers cognitive resilience. If true, adaptive synaptic increases in VAChT may also act to contain the spatial extent of tau pathology in the brain. Consistent with this prediction, we observed that the total number of voxels in high Tau~Aβ regions were significantly larger in vulnerable compared to resilient individuals (**Fig. 2i**). To visualize this effect, we examined the average anatomical distribution of significant tau pathology in each group by computing the percentage overlap of high Tau~Aβ regions with parcels in the BFpz. Moving from resilient, to intermediate, to vulnerable subgroups, we found that tau pathology becomes increasingly more distributed, extending from the basolateral strip to the neocortex (**Fig. 2j**). In a direct comparison between resilient and vulnerable subgroups, we identified that vulnerable individuals exhibit high tau pathology in a significantly larger network of brain regions (**Fig. 2k**).

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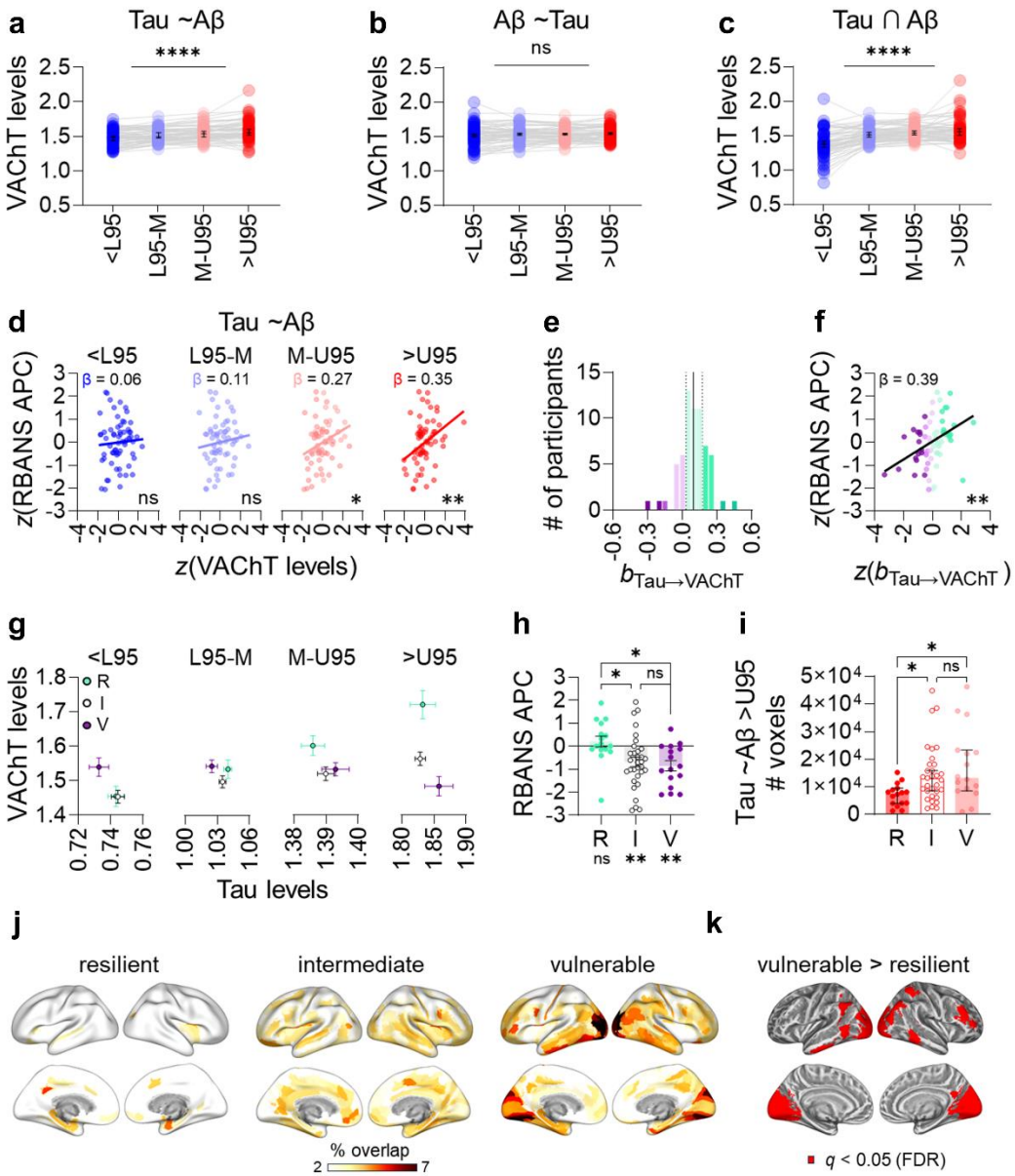

Fig. 2 Cholinergic synaptic adaptation to tau confers cognitive resilience and contains tau spreading.

a–c, Spaghetti plots showing within-subject VAChT-PET standardized uptake value ratios (SUVrs) across (**a**) Tau~Aβ, (**b**) Aβ~Tau, and (**c**) Tau∩Aβ regions (x-axes; <L95=dark blue; L95–M=light blue; M–U95=light red; >U95=dark red). Asterisks denote the main effect of region on within-subject VAChT levels (median±95%CI; Friedman test followed by Dunn's post-hoc test, or mean±SEM; Repeated-Measures One-Way ANOVA). **d,** Scatter plots showing the relationship between VAChT SUVrs inside each Tau~Aβ region and RBANS

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APC total scores across participants (linear models with robust regression). Colour coding is the same as in **a**. **e,** Histogram showing the distribution of regression coefficients from the linear relationship between mean tau and VAChT SUVrs across Tau~Aβ regions for each participant (linear models with robust regression). **f,** Scatter plot showing regression coefficients from **e** and RBANS APC total scores (linear model with robust regression). **g,** Plots showing average tau and VAChT SUVrs across each Tau∼Aβ region, split by resilient (R: *n*=16, aqua), intermediate (I: *n*=32, white), and vulnerable (V: *n*=16, purple) subgroups (mean±SEM; mixed effects model with Tukey's post-hoc test). **h,** RBANS APC total scores by subgroup (mean±SEM; One-way ANOVA with Tukey's post-hoc test; One sample *t*-test). Colour coding is the same as **g**. **i,** Tau∼Aβ >U95 ROI sizes by subgroup (median±95%CI; Kruskal-Wallis test with Dunn's post-hoc test). **j,** Mean percentage overlap of >U95 Tau∼Aβ regions with BFpz parcels in resilient (*left*), intermediate (*middle*), and vulnerable (*right*) subgroups, projected on medial and lateral brain surfaces. Parcels are derived from the Human Connectome Project Multi-Modal Parcellation-extended atlas[49]. Darker colours indicate greater overlap of >U95 profiles with a parcel in a subgroup. **k,** Subgroup comparison (resilient versus vulnerable) of >U95 Tau∼Aβ mean percentage overlap with BFpz parcels, projected on medial and lateral brain surfaces (two-sided Wilcoxon rank-sum test; adjusted for the false-discovery rate (FDR) at 5%). Statistical details in **Supplemental Information** file. *$P \leqq 0.05$, **$P \leqq 0.01$,****$P \leqq 0.0001$, ns,not significant.

Cholinergic deficits impair cognition and brain structure

Our findings thus far indicate that cholinergic synapses selectively adapt to increasing tau pathology, and that a lack of cholinergic synaptic responsivity is associated with a profile of longitudinal cognitive decline and increased vulnerability to tau spreading. To more directly investigate whether a constraint on cholinergic presynaptic function causally affects brain

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structure and function, we performed *in vivo* multimodal imaging and cognitive profiling in adult (6–7-month-old) WT mice and mice with a conditional knock-out (KO) of forebrain VAChT[27,52].

In KO mice, VAChT protein expression is selectively eliminated from forebrain cholinergic neurons through *Cre*-dependent recombination. Biochemical analyses confirmed that VAChT protein levels were severely reduced in KO mice in cortical and hippocampal targets of the cholinergic BF, as well as striatal cholinergic interneurons, whereas regions innervated by brainstem cholinergic neurons remained intact (**Fig. 3a**). To extend these findings *in vivo*, we performed [^{18}F]-FEOBV microPET imaging in both KO and WT mice. Consistent with the selective elimination of VAChT in forebrain cholinergic neurons, KO mice exhibited reduced forebrain [^{18}F]-FEOBV uptake relative to WT mice, with no corresponding differences observed in the brainstem (**Fig. 3b**).

To assess cognitive function under normal or reduced forebrain VAChT conditions, WT and KO mice performed a pairwise visual discrimination and reversal (PVD-PVR) touch screen task of cognitive-flexibility (**Fig. 3c**, see *Methods*). During PVD testing, both WT and KO mice performed significantly above chance (>50% correct) at target detection. However, consistent with a causal role for cholinergic synaptic plasticity in updating learned associations[53–55], forebrain VAChT KO mice exhibited a significant and sustained impairment during reversal testing (PVR), whereas WT mice successfully achieved reversal learning (**Fig. 3d**). We further demonstrate that partial restoration of acetylcholine bioavailability in KO mice via continuous administration of an acetylcholinesterase inhibitor (donepezil; 1.5 mg kg^{-1}) significantly improved PVR task performance to above chance levels (**Fig. 3d**).

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Does a targeted disruption of forebrain cholinergic presynaptic function impact neuronal integrity in downstream targets? To address this question, we performed whole-brain *in vivo* 9.4T structural magnetic resonance imaging (MRI) in KO and WT mice. We then applied voxel-based morphometry to quantify grey matter volume differences between the groups. Compared to WT mice, we observed significantly lower grey matter volume across multiple BFpz regions in KO mice, with the most pronounced reductions observed in the hippocampus (**Fig. 3e**). We additionally confirmed in age-matched *Cre*-only expressing mice that the observed changes in hippocampal structural integrity were not due to off-target recombination events (**Supplemental Table 1**). These findings further reinforce the critical contributions of BF cholinergic synaptic function to the mechanisms supporting both cognition and neuronal health.

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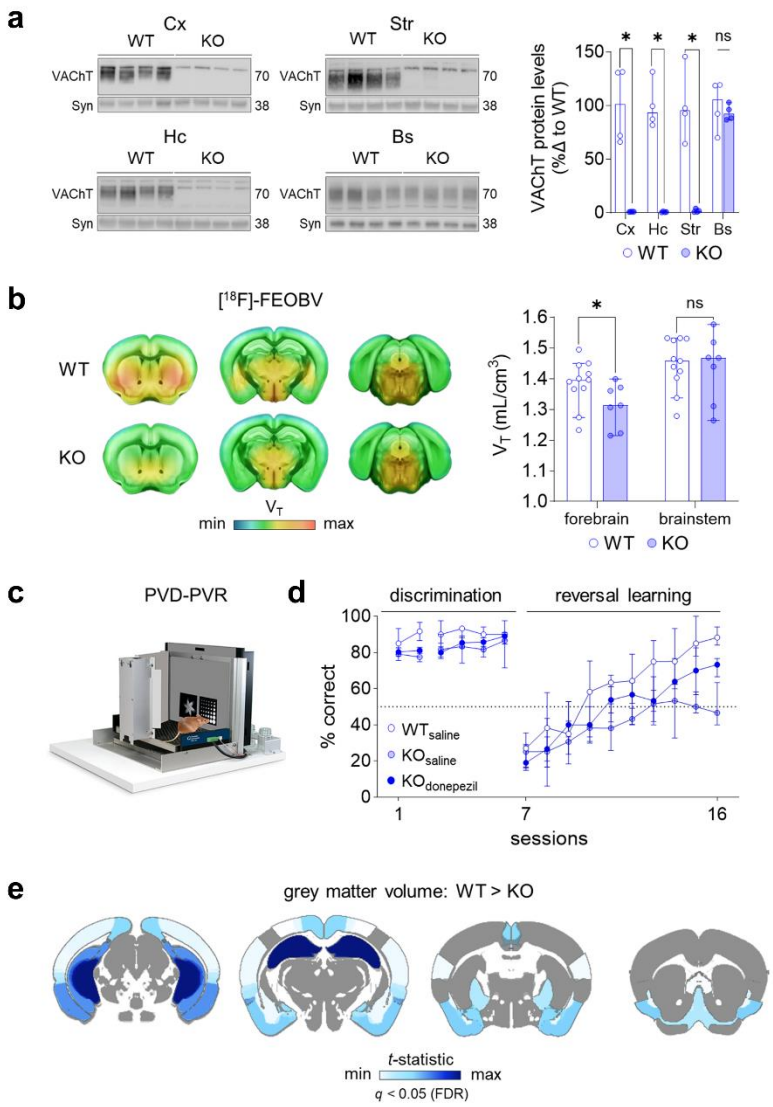

Fig. 3 Loss of forebrain VAChT impairs cognitive flexibility and induces hippocampal neurodegeneration in mice.

a, VAChT and synaptophysin (Syn) protein levels in WT (n=4) and KO (n=4) mice from cortex (Cx), hippocampus (Hc), striatum (Str), and brainstem (Bs) tissue samples (median±95%CI; two-sided Mann-Whitney U test). **b,** Average [^{18}F]-FEOBV total volume of distribution (V_T) brain profiles in WT (n=11) and KO (n=7) mice, overlaid on coronal sections of the mouse brain (min-max: 0.3–2.1). Forebrain and brainstem [^{18}F]-FEOBV V_T values in WT and KO mice (median±95%CI; two-sided Mann-Whitney U test). **c,** Schematic of mouse pairwise-

visual discrimination/reversal (PVD-PVR) touchscreen task. **d,** PVD-PVR task performance (% correct trials) in saline-treated WT (*n*=6) and KO (*n*=8) mice, and donepezil-treated KO mice (*n*=11). PVD (Sessions 1-6) and PVR (Sessions 7-16) testing sessions shown, and the dotted line indicates chance (50%) performance (median±IQR; One sample Wilcoxon signed-rank test). **e,** Between-group comparisons of regional grey matter volume in the BFpz (WT: *n*=14, KO: *n*=10). Regions from the Allen Mouse Brain Atlas common coordinate framework version 3[56] overlaid on coronal sections of the mouse brain, thresholded and colour-coded by *t*-statistic (two-sided independent samples *t*-test; adjusted for the false-discovery rate (FDR) at 5%). Statistical details in **Supplemental Information** file. Example mouse data displayed using MRIcroGL: https://www.nitrc.org/projects/mricrogl. *$P \leqq 0.05$, ns,not significant.

Cholinergic synaptic plasticity is *MAPT*-centric

The selective profile of grey matter volume loss observed in forebrain VAChT KO mice led us to hypothesize that this pattern of structural vulnerability may reflect region-region variation in the expression of gene-networks prone to dysregulation in AD. To interrogate this possibility, we examined the spatial correlation between the profile of regional grey matter volume differences in WT and KO mice and gene expression in the healthy adult mouse brain (**Fig. 4a**)[57]. We leveraged an *a priori* gene-network enriched for presynaptic functions, such as neurotransmitter secretion and plasticity, that is differentially expressed in cortical neurons vulnerable to pathology and neurodegeneration in AD[58]. We found that regions exhibiting greater grey matter loss in KO mice also showed higher intrinsic expression of the plasticity gene-network (**Fig. 4b**), an association that exceeded expectations under spatially constrained null models (**Fig. 4c**)[59].

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To better understand the cellular basis of this relationship, we cross-referenced the expression of the plasticity gene-network in single-nucleus RNA sequencing (snRNAseq) atlases derived from the healthy adult mouse[60] and healthy adult human brain[61]. This strategy enabled us to systematically survey the network's expression in virtually every class of neurons in the telencephalon. Spatial clustering of transcriptionally distinct neuronal groups in the telencephalon are shown in **Fig. 4d** (mouse) and **Fig. 4e** (human), colour coded according to their dominant neurochemical profile (i.e., glutamatergic, GABAergic, cholinergic, other). For each neuronal cluster, we then computed a network expression score using the differentially expressed genes that overlapped with the network. Consistent with a convergence between synaptic plasticity and vulnerability, we found that cholinergic neurons in both the adult mouse (**Fig. 4f**) and human (**Fig. 4g**) brain ranked in the top 25% of neuronal clusters exhibiting significant network upregulation. We further confirmed that these cholinergic neurons most likely originated from the BF based on their unique transcriptional profiles. Other neurons which highly upregulated this gene-network include BF GABAergic neurons and long-projection glutamatergic neurons from cortical, hippocampal and amygdalar areas (**Fig. 4f,g**).

We next explored which genes are most central to this network. To do so, we re-weighted the significant edges from the original functional network[58] by the expression values of overlapping genes in BF cholinergic neurons (see *Methods*). In both mice and humans, we found that the microtubule-associated protein tau (*MAPT*) gene exhibited a high degree of network centrality (**Supplemental Fig. 6**). To illustrate the gradient of *MAPT* expression across all telencephalic neuronal clusters, we mapped the raw *MAPT* expression values to the same low dimensional spaces as in (**Fig. 4d,e**). We identified that BF cholinergic neurons are among the neuronal groups with the highest *MAPT* expression in both the adult mouse (**Fig. 4h**) and human (**Fig. 4i**) telencephalon.

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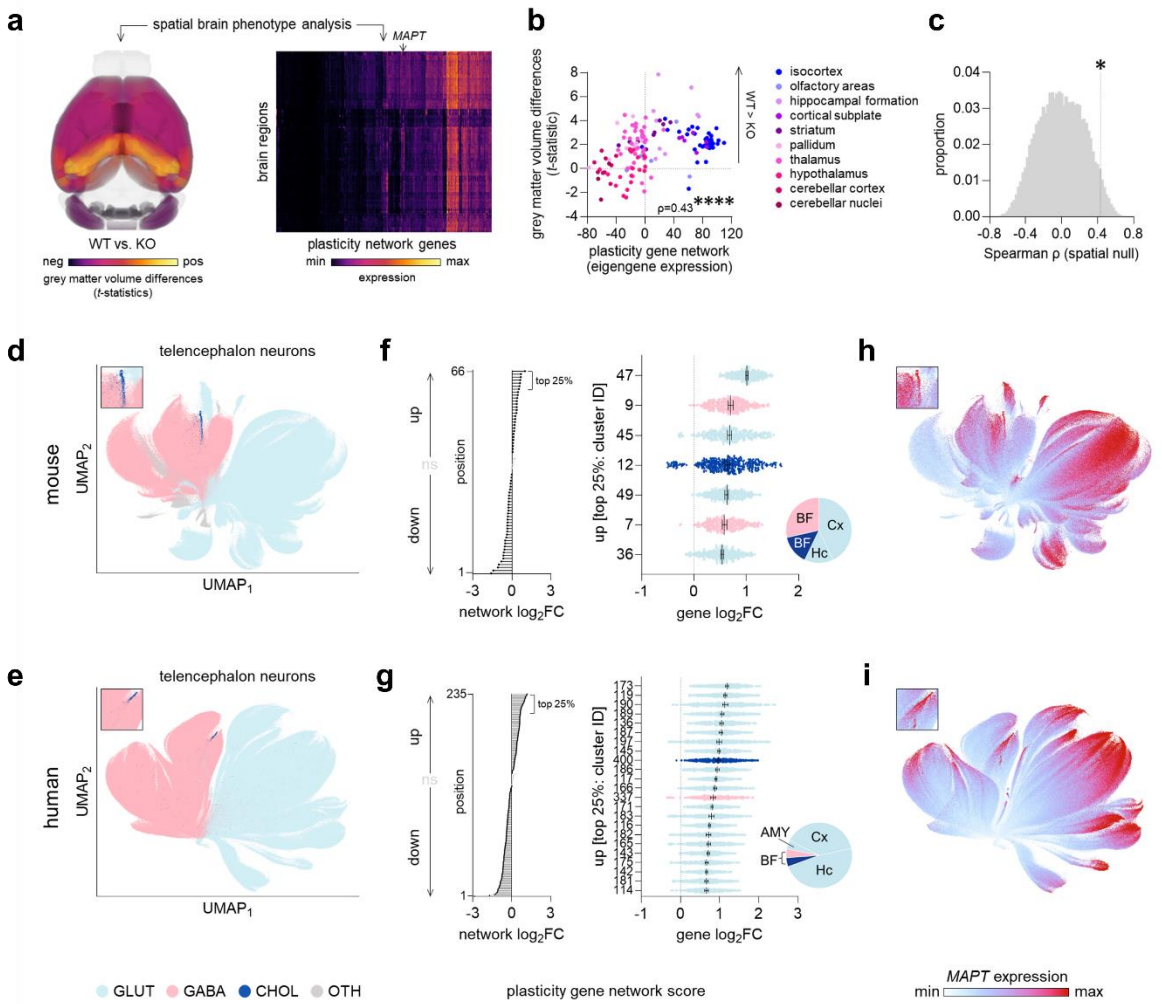

Fig. 4 Plasticity and vulnerability converge in basal forebrain cholinergic neurons and cholinoreceptive targets.

a, Schematic of spatial brain phenotyping analysis: (*left*) regional grey matter volume differences (*t*-statistics; neg-pos: -3–8) between VAChT KO and WT mice across parcels from the Allen Mouse Brian Atlas[62]; (*right*) heatmap displaying normalized expression levels of plasticity network genes from Roussarie *et al.*[58] across parcels from the Allen Mouse Brian Atlas, ordered using hierarchical clustering (expression; min-max: 0–50). **b,** Scatter plot showing the relationship between the spatial profile of grey matter volume differences in WT

versus KO mice and the first principal component (eigengene) of plasticity network expression, colour coded according to each parcel's region of origin (two-sided Spearman rank correlation). **c,** Histogram showing the distribution of 40,000 surrogate null Spearman ρ correlations computed from spatially autocorrelated random maps. The dotted line marks the observed correlation. **d,e,** Uniform manifold approximation and projection (UMAP) plots, colour-coded by major neurochemical class, showing nuclei isolated from telencephalic neurons in the healthy adult mouse[60] (**d**) and human[61] (**e**) brain. See *Methods* for details on selected snRNAseq atlases. **f,g,** (*left*) Bar plots show the median of $\log_2$FC values for differentially expressed plasticity network genes[58] across neuronal clusters in the mouse (**f**) and human (**g**) telencephalon, positioned according to the sign and significance of their network score. (*right*) Scatter plots illustrate the top 25% of neuronal clusters with significant positive gene-network expression scores (mice: n=7, humans: n=23) (median±95%CI; One sample Wilcoxon signed-rank test). Scatter plots are colour-coded according to the neurochemical annotation legend in (**d**,**e**). Pie charts show the distribution of the top 25% of neuronal clusters by major neurochemical class and suspected region of origin (AMY=amygdala, BF=basal forebrain, Cx=cortex, Hc=hippocampus). **h,i,** UMAP plots highlighting raw *MAPT* expression values for each neuronal nuclei in the telencephalon of mice (**h**, min-max: 0–18) and humans (**i**, min-max: 0–30). Statistical details in **Supplemental Information** file. Example mouse data displayed using MRIcroGL: https://www.nitrc.org/projects/mricrogl. *P≦0.05, ****P≦0.0001, ns,not significant.

Discussion

We demonstrate that homeostatic synaptic plasticity serves as a common mechanism through which BF cholinergic neurons both adapt and contribute to early tau dysregulation, increasing their vulnerability to disease. Under physiological conditions, VAChT regulates cholinergic

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synaptic output in response to local network activity, while a *MAPT*-associated gene-network supports the presynaptic structural and molecular adaptations required to sustain this plasticity. Among the at-risk older adults studied here, our identification of both resilient and vulnerable subgroups suggests that there are distinct profiles of cholinergic synaptic adaptation to tau pathology in the aging brain. In resilient individuals, tau-induced synaptotoxicity was partially mitigated by a robust presynaptic VAChT response. In vulnerable individuals, a reduced capacity to mount this adaptive response was associated with tau propagation and cognitive decline.

The fundamental relationship between cholinergic presynaptic function and tau aligns with theoretical models of homeostatic synaptic plasticity[8–11,63], which posit that neurons adaptively regulate synaptic efficacy to preserve stability under sustained perturbation. Our data offers one of the first *in vivo* demonstrations of this mechanism in humans, wherein a graded presynaptic cholinergic response to tau pathology predicts long-term cognitive function and modulates tau spreading. Importantly, the failure of homeostatic synaptic plasticity in some individuals may explain the inflection point between normal brain aging and the AD trajectory. Such a progression echoes broader models of cellular aging, in which early transcriptomic, epigenetic, or metabolic disruptions trigger compensatory cellular adaptations that ultimately fail, leading to disease phenotypes[65].

Although we cannot exclude the contribution of soluble or intracellular Aβ to early changes in cholinergic synaptic function[66–71], particularly during the initial stages of AD, the insoluble Aβ pathology quantified by Aβ-PET[72] in this study did not colocalize with adaptive VAChT upregulation. Recent studies combining tau- and Aβ-PET imaging with cortical gene expression profiles[73,74] have shown that these proteinopathies propagate along distinct

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transcriptional pathways, with tau propagation aligning to axon-related genes, including *MAPT*, and Aβ propagation associating with dendrite-related profiles. These findings, together with our observations, suggest that while Aβ-related mechanisms may contribute to early neuronal dysfunction, the transcriptional programs governing tau-mediated cholinergic synaptic adaptation and vulnerability operate through at least partially independent pathways.

Our findings in forebrain VAChT KO mice demonstrate causal interrelationships among cholinergic signalling, synaptic plasticity and the structural integrity of the BFpz. When the dynamic range of cholinergic neurotransmission is chronically constrained[75], as in VAChT KO mice, plasticity-dependent reversal learning is impaired. These deficits are partially rescued by enhancing acetylcholine availability, further underscoring the functional dependence of cognitive flexibility on cholinergic synaptic plasticity[53–55]. Forebrain VAChT KO mice also exhibited reductions in grey matter volume in brain regions densely innervated by BF cholinergic projections, including the hippocampus. We found that this profile of neurodegeneration spatially covaried with regional enrichment for *MAPT* and its associated gene-network supporting plasticity[58] in the healthy adult mouse brain. Deprivation of VAChT in cholinergic synapses may therefore disrupt cholinergic surveillance of transcriptional programs involved in plasticity, including tau proteostasis, initiating a cascade of cellular dysfunctions resembling early AD pathology[27,52].

Although *MAPT* is traditionally associated with microtubule stabilization, it exhibits strong co-expression with genes involved in presynaptic plasticity, vesicular transport, and neurotransmitter release[58,76,77]. Complementary experimental evidence suggests that one of the primary loci of tau pathology is in presynaptic terminals[78,79], where its mislocalization disrupts axonal transport, neurotransmitter release probability, and vesicle recycling through aberrant

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interactions with synaptic proteins and trafficking complexes. These dysfunctions are exacerbated by the unique morphofunctional properties of vulnerable neurons, including highly branched, lightly myelinated axons[80], and a sustained propensity for activity-dependent remodelling in adulthood[81]. Using snRNAseq atlases to survey neurons in the healthy adult mouse[60] and human[61] telencephalon, we show that *MAPT* and its associated plasticity gene-network are highly enriched in specific types of neurons which populate vulnerable brain regions, including BF cholinergic and GABAergic neurons and glutamatergic projection neurons of the hippocampus, cortex and amygdala. Together, our findings support the hypothesis that intrinsic, plasticity-linked transcriptional profiles predispose specific neuron types to early tau dysregulation[81–85]. Consistent with this idea, prior neuropathological work has shown that tau neurofibrillary tangles are present in virtually all older adults' brains by the age of 60[17,86], and tend to colocalize areas densely innervated by BF cholinergic projections[81,83,87].

The mechanistic link between VAChT and tau helps articulate new directions for future research. Do adaptive synaptic responses to AD pathology exist in other neuronal populations? Why do these responses fail in some before others? Can this mechanism be modulated? Answers to these questions may inform therapeutic strategies that complement or preclude monoclonal antibodies targeting Aβ or tau[88].

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Methods

Human Ethics Statement

All participants provided written informed consent at baseline (BL) and all follow-up (FL-U) visits. The consent forms along with study protocols for the data acquisition procedures (i.e., imaging, neuropsychological testing) were approved by the Institutional Review Board at McGill University and the CIUSSS de l'Ouest de l'Ile de Montréal Research Ethics Board (Montreal, QC, Canada).

Participants

Participants (n=64) were recruited from the PREVENT-AD program. A detailed description of the PREVENT-AD initiative and specific inclusion/exclusion criteria for participant recruitment and enrollment can be found in Tremblay-Mercier *et al.*[33]. PREVENT-AD is an ongoing, longitudinal observational study of older adults with a first-degree family history of AD (i.e., parental or multiple-siblings). At the time of enrollment, participants (≥55 years old) were screened to rule-out cognitive impairment using the Clinical Dementia Rating (CDR) scale and the Montreal Cognitive Assessment (MoCA) test. Information on participants' biological sex, number of years of education, and *APOE* genotype were collected at BL.

RBANS and Longitudinal Cognition Scores

All participants completed RBANS testing at BL and all FL-U visits[89]. The RBANS includes 12 subtests (i.e., list learning, story learning, figure copy, line orientation, picture naming, semantic fluency, digit span, coding, list recall, list recognition, story recall, and figure recall) which generate five index scores (i.e., immediate memory, delayed memory, language,

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attention, and visuospatial abilities) along with a total score. To minimize practice effects in longitudinal assessments, the battery was administered in four equivalent versions across visits.

Individual longitudinal changes in RBANS performance were quantified using the following formula:

$$APC = \left[\left(\frac{FL-U}{BL}\right)^{\frac{365}{\Delta DAYS}} - 1\right] \times 100$$

where ΔDAYS is given by the number of days between the most recent FL-U and BL visits of cognitive testing. APC was used to account for inter-individual differences in the time interval between BL and most recent FL-U visits (**Supplemental Fig. 1**), as well as to investigate the relative change in RBANS total scores each year for every participant.

Human Imaging Timeline

A summary of the human imaging data timeline is depicted in **Supplemental Fig. 1**. Structural MRI (sMRI) brain images were acquired starting from each participant's BL visit (2012-2017), up to and including their most recent FL-U visit (2018-2023). Participants returned for VAChT-PET ([^{18}F]-FEOBV) imaging within ~1 year of their most recent FL-U sMRI scan. Due to the nature of the COVID-19 pandemic, FL-U timepoint sMRI data for 11 participants were acquired ~1.5-2 years after their VAChT-PET scan. VAChT-PET scans were acquired within ~1.5 years of each participant's Tau-PET ([^{18}F]-AV1451) and Aβ-PET ([^{18}F]-NAV) scans (**Table 1**).

Human MRI Scanner Hardware and sMRI Data Acquisition

sMRI data acquisition took place at the Cerebral Imaging Center of the Douglas Mental Health University Institute Research Centre (Montreal, QC, Canada) between 2012-2023. For all

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participants, BL sMRI brain images from 2012 to 2017 were performed on a Siemens MAGNETOM 3T Tim Trio system. Starting in 2018, sMRI data were collected on an upgraded version of the same system (Siemens MAGNETOM 3T Prisma Fit). Both hardware (i.e., gradient, radiofrequency (RF), shimming) and software components of the system were included in this upgrade.

For sMRI data collected on the Siemens MAGNETOM 3T Tim Trio system, T1-weighted (T1w) images were acquired with a 3D magnetization prepared rapid gradient echo (MP-RAGE) sequence with the following parameters: matrix dimensions = 256 x 240 x 176; voxel size = 1 mm x 1 mm x 1 mm; repetition time (TR) = 2300 ms; echo time (TE) = 2.98 ms; inversion time (TI) = 900 ms; flip angle (FA) = 9º; phase encode = anterior-posterior (A>>P); Bandwidth (BW) = 240 Hertz/pixel; GeneRalized Autocalibrating Partially Parallel Acquisitions (GRAPPA) 2; acquisition time (TA) = 5.12 min. For FL-U MRI data collected on the upgraded system (Siemens MAGNETOM 3T Prisma Fit), T1w images were acquired with a 3D MP-RAGE sequence encoding similar parameters: matrix dimensions = 256 x 256 x 192; voxel size = 1 mm x 1 mm x 1 mm; TR = 2300 ms; TE = 2.96 ms; TI = 900 ms; FA = 9º; phase encode = A>>P; BW = 240 Hertz/pixel; slice thickness = 1 mm; GRAPPA 2; TA = 5.30 min.

Longitudinal sMRI Data Processing

Preprocessing of human sMRI data was performed using the SPM12 toolbox (https://www.fil.ion.ucl.ac.uk/spm/software/spm12/) in MATLAB 2021b. A two-step method for longitudinal registration was implemented using the *serial longitudinal pipeline* from the SPM12 toolbox[90], as described previously[91,92]. First, the number of T1w scans acquired for each participant was determined. In the case of multiple T1w images within a single time point, the serial longitudinal pipeline was used to create an *intra-scan* average of the corresponding

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images. Next, an *inter-scan* midpoint average image from the collection of single or averaged T1w images was computed for each subject. The midpoint average T1w images were then segmented with the Computational Anatomy Toolbox's (CAT12[93]) *segmentation* module using tissue priors enhanced to improve the classification of subcortical grey matter[94]. The imported grey and white matter segments from the midpoint average T1w images were used to create a custom, age-appropriate cohort template by implementing diffeomorphic registration using geodesic shooting[95]. A deformation field between the cohort-specific template and each participant's midpoint average T1w image was also estimated during this step and then applied without modulation to the midpoint average T1w images to warp them into the cohort-specific template space.

To ensure the integrity of all midpoint average T1w images for intermodal coregistration, a weighted-average Image Quality Rating (IQR) score was computed in CAT12[93]. Images with a CAT12 IQR below 70% were flagged for manual inspection. Finally, the cohort-specific template was nonlinearly registered to the Human Connectome Project Multi-Modal Parcellation-extended (HCP MMPex) atlas space[49].

Human PET Radiotracer Synthesis

Radiotracer synthesis for human PET imaging took place on each scan date in the McConnell Brain Imaging Center's (BIC) Cyclotron Facility at McGill University (Montreal, QC, Canada). [^{18}F]-FEOBV was synthesized using a pure *levo* precursor (ABX advanced biochemical compounds, Radberg, DE) to obtain the (−)-FEOBV enantiomer, which is the only stereochemical configuration that exhibits binding affinity to VAChT[96]. ^{18}F labeling of (−)-FEOBV was performed in accordance with a previous study at the same site[20]. [^{18}F]-NAV4694 (Navidea Biopharmaceuticals, Dublin, OH) and [^{18}F]-AV1451 (Eli Lilly & Co, Indianapolis,

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IN) were synthesized and labelled in the same facility as previously described[97].

Human PET Scanner Hardware and Data Acquisition

All PET imaging took place in the BIC PET unit (Montreal, QC, Canada) on an ECAT High Resolution Research Tomograph (HRRT, CTI/Siemens) PET scanner. Prior to each participant's PET scans, a time-standardized transmission scan was acquired. For all participants, [^{18}F]-FEOBV brain PET imaging was conducted across two 30-minute imaging sessions, performed with a timing window of 6 ns and a 400-650 keV discrimination energy range[98]. The first session began following the intravenous injection of [^{18}F]-FEOBV and continued for 30 minutes (0-30 minutes; data not used in this study). Participants were then removed from the scanner before undergoing a second imaging session. This second session consisted of a 30-minute PET scan (180-210 minutes post-injection; 6 frames; 5 minutes/frame), corresponding to the established time period for [^{18}F]-FEOBV data acquisition in AD patients[20]. [^{18}F]-NAV4694 and [^{18}F]-AV1451 PET scans were acquired 40-70 minutes (6 frames; 5 minutes/frame) and 80-100 minutes (4 frames; 5 minutes/frame) post-injection, respectively. All PET images were reconstructed using an iterative, 3D ordered subset expectation maximization (OSEM3D) algorithm with 10 iterations and 16 subsets and included corrections for 3D scattering[99]. Each frame was reconstructed to a final dimension of 256 x 256 x 207 and a voxel size of 1.22 mm x 1.22 mm x 1.22 mm.

Human PET Data Processing

Human VAChT ([^{18}F]-FEOBV), tau ([^{18}F]-AV1451) and Aβ ([^{18}F]-NAV) brain PET data were processed using the SPM12 toolbox (https://www.fil.ion.ucl.ac.uk/spm/software/spm12/) in MATLAB 2021b, as described previously[91,92]. Briefly, images were visually inspected to ensure proper orientation and centered to the anterior commissure. For each participant, an

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intra-tracer average of frames was computed, and the average images were coregistered to the corresponding native space midpoint average T1w image. The coregistered average PET images were then normalized to each subject's mean uptake within previously validated tracer-dependent reference regions (VAChT: supratentorial-supraventricular white matter[20,100]; Aβ: cerebellar grey matter[97]; tau: inferior cerebellar grey matter[97]) to produce SUVr images. The deformation fields created during the custom sMRI template building were used to warp the images without modulation into the cohort-specific template space.

Normative Deviation Mapping

To characterize subject-specific variations in tracer uptake, a normative deviation technique was adapted from prior work[41,42]. This method involves two key steps: (1) the computation of population median and deviation reference images and (2) the segmentation of individual voxel-wise data into discrete regions of interest (ROI).

Reference Image Computation: To compute reference images, the preprocessed PET data for each participant was taken as input. Given the age-range and unequal proportion of males and females in the cohort (**Table 1**), a general linear model with age and sex as covariates was used to adjust each individual's preprocessed PET images for these sources of variation.

Additional preprocessing steps performed for normative deviation mapping included masking the PET data by a BFpz ROI. The BFpz ROI was created by combining the HCP MMPex[49] atlas parcels for all cortical, hippocampal, amygdalar, and BF regions into a binary mask, non-linearly warping this mask to the respective population template space and taking the logical overlap of this mask with the population template grey matter segmentation. The masked PET data were then smoothed using a 2 mm full-width at half-maximum (FWHM) Gaussian kernel.

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For each tracer, reference images were separately generated in a leave-one-out cross-validation framework. Voxel-wise group medians were calculated across all subjects, excluding the individual being processed in the current iteration to ensure independence:

$$M\text{-}i = \text{median}(n\text{-}i)$$

where i is the subject being processed, $n\text{-}i$ is the set of PET data excluding the left-out subject, and $M\text{-}i$ is the resulting reference median image excluding the left-out subject.

Robust variability estimates were computed using the median absolute deviation (MAD). The MAD is computed as the median of the absolute deviations from the reference median image:

$$MAD\text{-}i = \text{median}(|n\text{-}i - M\text{-}i|) \times S$$

where $MAD\text{-}i$ is the resulting reference MAD image excluding the left-out subject. For normally distributed data, the MAD systematically underestimates the standard deviation. To correct for this, a scaling factor (S) of ~1.4826 was applied to the MAD, making it a consistent estimator of the standard deviation under the assumption of normality[101]. Finally, for each subject, both the $M\text{-}i$ and $MAD\text{-}i$ reference images were stored as 3D-volumetric images.

CI-Based Voxel Segmentation: Using the reference images ($M\text{-}i$ and scaled $MAD\text{-}i$), each subject's PET data were segmented into four non-overlapping ROIs based on voxel-wise deviations from the reference images. The median deviation ROIs are: (1) below the lower 95% CI (<L95), (2) between the lower 95% CI and median (L95–M), (3) between the median and upper 95% CI (M–U95), and (4) above the upper 95% CI (>U95), given by the following equations:

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I. <L95: $i < (M\text{-}i - 1.96 \times MAD\text{-}i)$

II. L95–M: $(M\text{-}i - 1.96 \times MAD\text{-}i) < i < M\text{-}i$

III. M–U95: $M\text{-}i < i < (M\text{-}i + 1.96 \times MAD\text{-}i)$

IV. >U95: $i > (M\text{-}i + 1.96 \times MAD\text{-}i)$

For each segmentation step, continuous and binarized voxel masks were generated. Continuous maps retained the original voxel intensities, while binarized maps indicated voxel inclusion/exclusion. All SUVr extractions from normative deviation ROIs were performed using the binarized maps on the unsmoothed, adjusted PET images.

Reference Image Stability Analysis

To assess the stability of the population reference images (*M-i* or *MAD-i*) and independence from individuals characterized by strong deviations from the rest of the population, correlations were performed for each pair of the computed *M-i* or *MAD-i* maps. This led to a distribution of Pearson's and Spearman's correlation coefficients based on (n x n-1)/2 = 2016 comparisons, given n=64 individuals.

Mouse Ethics Statement

All small-animal imaging procedures were conducted in accordance with the Canadian Council of Animal Care's current policies and were approved by the University of Western Ontario's Animal Care Committee (Animal Use Protocols: 2020-163) and the Lawson Health Research Institute's Health and Safety board. Mice were housed as pairs in standard plexiglass cages in temperature- and humidity-controlled rooms (22–25 °C, 40%–60%) on 12-h light/12-h dark cycles, where food (regular chow) and water were made available *ad libitum*. Imaging and touch screen experiments were performed during the light cycle (7:00 AM-7:00 PM). All

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efforts were made to maximize animal welfare before, during, and after experiments.

Mouse Multimodal Imaging Experimental Overview

High-resolution *in vivo* 9.4 T sMRI and [^{18}F]-FEOBV microPET data were acquired at the Center of Functional and Metabolic Mapping (Agilent Animal MRI Scanner, Bruker) and the Lawson Health Research Institute's Preclinical Imaging Facility (Inveon DPET, Siemens Healthineers), respectively. All imaging data were acquired in mice at 6-7 months of age. Data were acquired in WT mice (WT: MRI = 8 male, 6 female; PET = 5 male, 6 female), either C57BL/6 J or VAChT$^{flox/flox}$, VAChT$^{Nk2x.1\text{-}Cre\text{-}flox/flox}$ mice (KO: MRI = 5 male, 5 female; PET = 4 male, 3 female), Nkx2.1-Cre mice (CRE: MRI = 3 male, 3 female), and ChAT-ChR2-eYFP mice (HYP: MRI/PET = 4 male, 3 female). A different analysis of the [^{18}F]-FEOBV microPET data for the WT mice (*n*=11) can be found in the reporting laboratories' previously published study[102]. VAChT$^{flox/flox}$ and VAChT$^{Nk2x.1\text{-}Cre\text{-}flox/flox}$ mice were generated on a C57BL/6 J background as previously described[27,103]. Nkx2.1-Cre (C57BL/6J-Tg(Nkx2-1-cre)2Sand/J) and ChAT-ChR2-eYFP mice (B6.Cg-Tg(Chat-COP4*H134R/EYFP)6Gfng/J) were purchased from The Jackson Laboratory (JAX: stock no. 008661, stock no. 014546), with the latter maintained as hemizygous.

Mouse sMRI Data Acquisition

Mice were anesthetized with 1.5–2.0% isoflurane in 1.5 L min^{-1} O_2 (g) and then moved to the center of a 30 mm RF volume coil (Bruker). Four iterations of magnetization transfer (MT)-weighted spoiled gradient-recalled echo (GRE) images were acquired using a fast low-angle shot 3D pulse sequence with the following parameters: FOV = 16 mm x 16 mm x 10 mm; matrix dimensions: 128 x 128 x 8; voxel size = 0.125 mm x 0.125 mm x 0.125 mm, slice thickness = 10 µm; TR = 30 ms; TE = 2.77 ms; FA = 9°; off-resonance saturation = 4.5 kHz

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(Gaussian pulse = 10 ms duration); effective FA = 900° [104].

Mouse sMRI Data Processing

Preprocessing of mouse anatomical brain images was performed using the SPM12 toolbox (https://www.fil.ion.ucl.ac.uk/spm/software/spm12/) in MATLAB 2020b as described previously[102]. Anatomical images (*n*=4) were averaged, resized, centered, and re-oriented to follow the right-hand coordinate system (RAS). Images were then coregistered to the mouse brain template from Hikishima *et al.*[105], bias-corrected using MICO[106], and segmented using tissue priors for grey matter, white matter, and cerebrospinal fluid tissue compartments[105]. The grey and white matter segmentations were then used to create nonlinear transformations for diffeomorphic registration to a study-specific average template using geodesic shooting[95]. Finally, the deformation fields estimated during the template-building step were applied with modulation to each mouse's grey matter segment to warp the images to template space. To parcellate mouse anatomical (and [^{18}F]-FEOBV, see below) brain images, the study-specific mouse brain template was non-linearly registered to the Allen Mouse Brain Atlas (ABA) common coordinate framework version 3 (ABAccfv3)[56]. For WT, KO, and CRE mice, brain regions for grey matter volume extractions were defined according to the symmetrized, course annotated ABAccfv3[56]. Extractions were restricted to voxels within each mouse's grey matter tissue segment that had a probability of at least 0.1 of belonging to the grey matter. Grey matter volume extractions were performed on the unsmoothed, modulated-warped grey matter segments, adjusted for each mouse's total intracranial volume (TIV), which was calculated by summing the grey matter, white matter and cerebrospinal fluid volumes in the *seg8.mat file created during segmentation[107].

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Mouse [^{18}F]-FEOBV microPET Data Acquisition

In vivo, dynamic [^{18}F]-FEOBV microPET data were acquired in WT, KO, and HYP mice, as previously described[102]. All [^{18}F]-FEOBV microPET imaging sessions were performed as 150-minute list-mode emission scans, with a timing window of 3.432 ns and a 350–640 keV discrimination energy range. Mice were anesthetized with 1.5–2.0% isoflurane in 1.0 L min^{-1} O_2 (g) and then injected intravenously with ~20 Megabecquerel (MBq) of [^{18}F]-FEOBV. Data were binned into 15 frames of 40 seconds, followed by 28 frames of 300 seconds. An OSEM3D algorithm with two iterations and 18 subsets was used to reconstruct [^{18}F]-FEOBV microPET images with the following parameters: matrix dimensions: 128 × 128 × 159; voxel size: 0.776 mm × 0.776 mm × 0.796 mm.

Mouse [^{18}F]-FEOBV microPET Data Processing

Parametric total volume of distribution (V_T) images for each mouse's [^{18}F]-FEOBV data were estimated as described previously[102]. Whole-brain [^{18}F]-FEOBV time-activity curves were fit according to Logan *et al.*[108], where an image-derived input function (IDIF) from the lumen of the left ventricle of the heart served as the reference[102]. Preprocessing of mouse [^{18}F]-FEOBV V_T images was performed using the SPM12 toolbox (https://www.fil.ion.ucl.ac.uk/spm/software/spm12/) in MATLAB 2020b. Preparation of [^{18}F]-FEOBV V_T images included resizing, centering, and re-orienting the images to follow the RAS. Each V_T image was then coregistered and cropped to the bounding box of its corresponding native space MRI image, and warped without modulation to the study-specific anatomical mouse brain template. For WT and HYP mice, extractions of average [^{18}F]-FEOBV uptake in the mouse BFpz, hippocampus and cortex were performed using region-specific masks based on the ABAccfv3[56]. A similar approach was also taken to create masks for extracting average [^{18}F]-FEOBV uptake in the forebrain (BFpz and striatum) and brainstem (midbrain, pons, and

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medulla) of WT and KO mice. In all cases, average uptake was calculated on the unsmoothed, warped [^{18}F]-FEOBV V_T brain images.

Western Blotting

Brain tissue samples for Western blotting were collected in a subset of WT, HYP, and KO mice following [^{18}F]-FEOBV microPET scans. Mice were anesthetized via intraperitoneal injection of a ketamine (100 mg kg^{-1}) - xylazine (25 mg kg^{-1}) 0.9% sodium chloride solution and then euthanized by cervical dislocation. Brain tissue was extracted, rapidly dissected on ice into appropriate tissue compartments (i.e., brainstem, striatum, hippocampus, cortex), snap-frozen on liquid nitrogen, and stored in 2 mL microcentrifuge tubes at -80ºC until needed. Protein isolation and immunoblotting were performed as previously described[109]. Briefly, tissue samples were thawed on ice and homogenized in RIPA buffer (50 mM Tris, 150 mM NaCl, 0.1% SDS, 0.5% sodium deoxycholate, 1% triton X-100, pH 8.0), supplemented with phosphatase inhibitors (1 mM NaF and 0.1 mM Na_3VO_4) and a protease inhibitor cocktail (diluted 1:100, Cat#539134-1SET; Calbiochem). Homogenates were sonicated, rocked for 20 minutes at 4ºC, and then centrifuged at 10,000g for 20 minutes at 4°C. Supernatants were collected and protein quantification was performed using the Pierce™ BCA Protein Assay Kit (Cat#23227; ThermoFisher Scientific). 20-25 µg of protein were loaded and separated on 4-12% Bis-Tris Protein Gels (Cat#NW04125BOX; Invitrogen) and then transferred onto PVDF membranes (Cat#IPVH00010; EMD Millipore) using the Trans-Blot Turbo system (Bio-Rad Laboratories, Hercules, CA, USA). Membranes were blocked for 60 minutes in 5% milk (for VAChT) or 5% BSA (for Synaptophysin) in 1xTBST. Primary antibodies included rabbit anti-VAChT (diluted 1:2000, Cat#139103; Synaptic Systems) and mouse anti-Synaptophysin (diluted 1:2000, Cat#ab8049; Abcam). Following 4ºC overnight primary antibody incubation in the respective blocking solutions, membranes were washed and then incubated for 60

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minutes at room temperature in corresponding horseradish peroxidase (HRP)-conjugated secondary antibodies. Bands were visualized using enhanced chemiluminescence (ECL) detection reagents in a ChemiDoc Imaging System (Bio-Rad Laboratories, Hercules, CA, USA). Densitometric analysis was performed in ImageLab (Bio-Rad Laboratories, Hercules, CA, USA).

PVD-PVR Touchscreen Testing

7 month-old WT (*n*=6) and KO (*n*=19) mice (male) were tested on the PVD-PVR task (SOP2; www.touchscreencognition.org) developed for the rodent automated Bussey-Saksida Touchscreen System (Model 81426, Campden Instruments, Lafayette, Indiana) with Abet II Touch Software Version 2.20 (Lafayette Instrument Company, Lafayette, Indiana). PVD-PVR is designed to measure the effects of pharmacological or genetic manipulation on visual learning and cognitive flexibility[110]. Prior to PVD-PVR testing, all mice completed behavioural habituation, pre-training stages and acquisition sessions in the touchscreen chamber[111]. Following meeting acquisition criteria, mice were subject to PVD. PVD requires mice to learn to associate a food reward (7 µL strawberry milkshake) with a nose-poke response to a correct image (S+ stimulus) in one touchscreen window, while simultaneously ignoring a second visually-distinct, incorrect image (S- stimulus) in the other touchscreen window. After completing six PVD sessions (two pre-pump implantation, four post-pump implantation), mice were tested on PVR learning across 10 sessions. In PVR, the strawberry milkshake food reward becomes linked to the previously incorrect image (S- stimulus), while nose poke responses to the former correct image (S+ stimulus) are no longer reinforced.

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Subcutaneous Osmotic Pump Implantation and Pharmacological Manipulation Paradigm

During PVD-PVR testing, a subset of KO mice underwent pharmacological manipulation by the continuous administration of the acetylcholinesterase inhibitor, donepezil, via subcutaneous osmotic pumps. Donepezil hydrochloride (C6821-50mg) monohydrate was obtained from Sigma-Aldrich (Oakville, Ontario) and rehydrated using saline and 10% dimethyl sulfoxide (DMSO) to a dose of 1.5 mg kg^{-1}. Dosage was determined using the Alzet drug concentration calculator to account for pump model and corresponding flow rate as well as the weight of the mice. Implantation of subcutaneous osmotic pumps (model 1004, Alzet, Durect Corporation, Cupertino, California) was performed in WT and KO mice following completion of PVD session two. Pumps were designed to deliver continuous treatment (WT (n=6) or KO (n=8): saline + 10% DMSO; KO (n=11): 1.5 mg kg^{-1} of donepezil) at a fixed rate of 0.11µL/hr for 28 days. Preparation for pump insertion included loading each pump with a 1 mL syringe, capping the pumps with a pin, and soaking them in saline with <1 hour to implantation. Prior to pump insertion, mice were anesthetized with isoflurane (4%, initial; 1.5%, maintenance) in 1 L min^{-1} O_2 (g) and put in a recumbent prone position. A small incision was made in the upper-right quadrant of each mouse's back, followed by the insertion of a pump and sealing of the incision site with a wound clip. Mice were given two days to recover from the surgeries before beginning session three of PVD.

Spatial Brain Phenotype Analysis

Regional t-statistics representing grey matter volume differences between forebrain VAChT KO and WT mice were computed for 212 anatomical parcels from the ABA, based on the parcellation scheme from Oh *et al.*[62]. Parcels in the brainstem (i.e., midbrain, pons, medulla) and low-intensity olfactory bulb areas were excluded, yielding 144 parcels for analysis.

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Corresponding regional *in situ* hybridization (ISH) data were obtained from the ABA[57], preprocessed as described previously[59]. For each parcel, gene expression was represented as "energy" scores, reflecting expression levels weighted by hybridization intensity. To perform gene-module based analyses, a gene-network enriched for presynaptic functions vulnerable to dysregulation in AD was obtained from Supplemental Table 5 in Roussarie *et al*.[58] ('Module C', n=702). Genes from this network that lacked expression data across an entire parcel were removed. After merging and cleaning, 401 of the 702 genes in the original module remained. A principal-component analysis was then applied to the cleaned gene-by-region matrix, and the first principal component was saved. The resulting eigengene score captured the dominant axis of co-expression within the plasticity gene module. Spearman rank correlation coefficients were computed between the region-wise profile of grey matter volume differences and the selected transcriptional profile (i.e., the module eigengene). To assess statistical significance while accounting for spatial autocorrelation, the observed correlation was compared to a null distribution generated from 40,000 spatial surrogate maps[112,113]. These surrogate maps preserved the intrinsic spatial autocorrelation structure of gene expression but randomized regional assignments[59,113]. A one-tailed P-value was computed as the proportion of surrogate correlations greater than the observed correlation.

Whole-Brain snRNAseq Analysis

Whole-brain adult human (~3 million nuclei) and mouse (~6 million nuclei) snRNAseq atlases were downloaded from Siletti *et al*.[61] and Langlieb *et al*.[60], respectively. Metadata provided with each dataset were used to annotate clusters according to cell-type (i.e., neuron, glia) and developmental compartment (i.e., telencephalon, diencephalon, mesencephalon, rhombencephalon). Only clusters annotated as neuronal and telencephalic were used.

Cluster-wise Differential Expression Analysis: Gene-sets for each telencephalic neuron cluster

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were filtered to include only genes which had non-zero expression values in >25% of the cluster's representative nuclei[114]. For each gene in a cluster's filtered gene-set, the arithmetic mean of the gene's expression within the cluster was computed (target mean), as well as the arithmetic mean of its expression across all other clusters, excluding the target cluster (reference mean). The $\log_2$ fold-change ($\log_2$FC) value for each gene in a target-reference pair was then calculated using these means. Differential expression (DE) of genes was assessed between all target-reference pairs with two sample Wilcoxon rank-sum tests. To adjust for multiple testing across clusters, false-discovery rate (FDR)-correction (5% threshold) was applied to the raw *P*-values generated from the rank-sum tests using the Benjamini–Hochberg procedure.

Gene-Network Expression Analysis and Centrality: The plasticity gene-network list ('Module C') was obtained from Roussarie *et al.*[58]. For each cluster, the median and 95% CIs of the $\log_2$FC values for all DE genes (after FDR-adjustment) participating in the gene-network were computed. Median values were then ranked and filtered to the top 25% of clusters expressing significant positive median gene-network scores in the mouse (n=7) and human (n=23) datasets. To determine the genes central to the gene-network as expressed by BF cholinergic neurons in the adult mouse (MC_12) and human (SPLAT_400) brain, the significant edges from the functional graph in Roussarie *et al.*[58] were obtained. All edges within the graph were then reweighted by the expression level of the genes at either end of each edge in BF cholinergic neurons using the geometric mean:

$$W_{ij} = \sqrt[3]{p_{ij} * \hat{e}_i * \hat{e}_j}$$

where:

$$\hat{e}_i = \frac{e_i}{max_{k \in M} e_k}$$

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And p_{ij} is the posterior probability of an edge in the original network, M is the set of all genes in the gene-network, e_i is the expression of gene i in BF cholinergic neurons. For both mouse and human BF cholinergic neurons, only overlapping genes with positive $\log_2$FC values were included in centrality analyses. Degree centrality was calculated on the reweighted, undirected network as the sum of edges connected to each gene.

Statistical Analysis

Statistical analyses were conducted using GraphPad Prism (version 10.4.1; GraphPad Software, La Jolla, CA), except for robust regressions and imaging analyses which were performed in MATLAB 2021b, and spatial brain phenotyping and snRNAseq analyses, which were carried out in Python v3.13.2. Sample sizes were determined based on previous studies in the field. Parametric tests and their corresponding post hoc comparisons were applied to groups with a sample size of at least 10 that met assumptions of normality (Kolmogorov–Smirnov test for n>50 or Shapiro-Wilk test for n<50) and homoscedasticity (Bartlett test). When these assumptions were not met, non-parametric tests with appropriate post hoc analyses were used. Statistical test results are provided in the figure legends and corresponding **Supplemental Information** files. All statistical analyses were performed at a P_{crit}=0.05, with adjustments for multiple comparisons or controlling for the FDR (5%), as needed.

Code and Data Availability

Human data in this article are from the PREVENT-AD program, which is promoting data access for the scientific community via their Canadian Alzheimer Platform (Brain Canada Foundation). For more information regarding PREVENT-AD data usage and availability, please refer to https://www.centre-stopad.com/en/plateforme-cap. The ABA ISH data is publicly available for download (https://community.brain-map.org/t/in-situ-hybridization-ish-

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data/2762). The mouse[60] and human[61] snRNAseq atlases leveraged in the article are publicly available for download and can be accessed through the corresponding published articles. The list of genes used to compute plasticity gene-network scores ('Module C') can be found in Table S5 from Roussarie *et al*.[58]. All other source data and code will be made available upon publication of the manuscript.

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Ethics Declaration

Competing Interests

J.P. serves as a scientific advisor to the Alzheimer Foundation of France. T.J.B and L.M.S have established a series of targeted cognitive tests for animals, administered via touchscreen with a custom environment known as the Bussey-Saksida touchscreen chamber. Cambridge Enterprise, the technology transfer office of the University of Cambridge, supported commercialization of the Bussey-Saksida chamber, culminating in a license to Campden Instruments. Any financial compensation received from commercialization of the technology is fully invested in further touchscreen development and/or maintenance. All other authors declare no competing interests.

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