

Reviewed Preprint

v1 • July 16, 2026

Not revised

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Competing interests: No competing interests declared

Funding: See page 21

Reviewing editor: Amreen Mughal, National Institute of Neurological Disorders and Stroke, National Institute of Health, United States

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Connections across regional glymphatic clearance, neural activity and amyloid- β deposition in cortex

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eLife Assessment

The framework of this potentially **important** study – with the integration of multiple levels of analysis, glymphatic MRI, transcriptomics, functional MRI, and public amyloid maps, in one framework – is clever. The assertion that regional amyloid vulnerability may depend not just on neural activity alone, but on whether clearance is appropriately matched to activity, is an interesting and novel concept. However, the chosen approach to imaging glymphatic clearance relies on indirect inferences from a small subgroup. In its current form, the main conclusions of this study are therefore **incompletely** supported.

<https://doi.org/10.7554/eLife.111724.1.sa3>

Abstract

Neural activity inevitably produces waste, which promotes neurodegeneration with topographic features. The glymphatic system is important for waste clearance. However, the spatial characteristics of glymphatic clearance across cortex and whether it interplays with neural activity in contribution to amyloidosis in human remain unexplored. Here, by intrathecal administration of gadolinium-based contrast agents, glymphatic influx and clearance patterns across cortex in 96 participants are depicted via Glymphatic MRI. Analyses integrating post-mortem transcriptomic profiles from Allen Human Brain Atlas indicate that, genes related with excitatory and inhibitory neurons, and pathways engaging in synaptic function were enriched in regions with faster glymphatic clearance. FALFF was calculated from resting-state fMRI to represent neural activity. At the regional level, based on a subgroup with rs-fMRI (N = 15), regional glymphatic clearance was positively coupled with spontaneous neural activity. Mismatch index, reflecting decoupling between spontaneous neural activity and glymphatic clearance function, turned out to be positively associated with regional severity of amyloidosis using open-source 11C-PiB dataset. Together, this study for the first time demonstrates the intricate interplays between neural activity and glymphatic dynamics from transcriptional to physiological level. The mismatch between these two processes may serve as an undescribed comprehensive mechanism promoting regional vulnerability to proteopathy and subsequent neurodegeneration in cortex.

Introduction

The accumulation of metabolic waste serves as a leading cause in multiple neurological diseases¹. The brain's remarkable neurocomputational capacity, supported by synchronized neuronal activity, inherently generates substantial metabolic waste². In the last few decades, evidence suggested that higher regional activity is associated with increased susceptibility to pathological hallmarks such as amyloid- β (A β) deposition^{3,4}. While prevailing hypotheses predominantly

emphasize production side, waste deposition could equally be attributed to clearance side. The equilibrium disruption between waste generation and elimination may ultimately manifest as regional vulnerability and waste deposition in neurodegenerative diseases.

The landmark characterization of the glymphatic system has reshaped our understanding of cerebral waste clearance homeostasis. In this (re)circulation system, cerebrospinal fluid (CSF) convectively flows into the brain via perivascular space, enters the interstitial compartment through an astrocytic AQP4-dependent process, and finally flushes out metabolic waste, including A β and tau^{5,6}. Compelling experimental evidence demonstrates that glymphatic failure precedes and potentiates A β pathogenesis, with genetic ablation of AQP4 polarization exacerbating both amyloidosis and cognitive decline in AD models⁷. Crucially, the impairment of glymphatic clearance is not temporally and spatially uniform. In aging rodents, widespread astrogliosis and loss of perivascular AQP4 polarization is evident especially within the cortex⁸, but the regional glymphatic dynamics remain incompletely characterized. How and to what extent regional glymphatic dynamics contribute to A β accumulation in human is largely unknown.

Several evidence suggests neuronal activity as a dual-edged modulator of cerebral homeostasis. While driving metabolic waste production, it may simultaneously activate compensatory clearance. Better glymphatic clearance significantly correlated with higher FDG-PET uptake⁹, an indicator of metabolic demand and neural activity. Only recently, Jiang-Xie et al have demonstrated that rhythmic neuronal oscillations serve as the master organizer of CSF-to-interstitial fluid perfusion and brain clearance¹⁰. Together, these findings inspired us to systematically investigate the spatiotemporal coordination between glymphatic dynamics and brain physiological or molecular features, especially neural activity.

Topographically patterned vulnerability to waste deposition is a key feature in neurodegeneration. In this study, we investigated its neurobiological determinants, with a special focus on relationship between glymphatic clearance function and neural activity. Employing Glymphatic MRI by intrathecal administration of gadolinium-based contrast agents¹¹, we quantified spatiotemporally resolved CSF dynamics across cortex. To minimize individual variability and capture macroscale axis of variance in cortical glymphatic features, gradients of glymphatic influx and clearance were calculated, organizing regions along spatially continuous spectra^{12,13}. Integrating with post-mortem transcriptomic profiles¹⁴, we identified that transcriptomic features of glymphatic dynamics were related with components of neural activity. Then, in a subgroup with both resting-state functional MRI (rs-fMRI) and Glymphatic MRI, we examined whether glymphatic clearance coupled with regional neural activity and further defined activity-clearance mismatch regions. Leveraging open-source ¹¹C-PiB PET SUVR dataset¹⁵, we tested A β deposition vulnerability in mismatch regions. Study pipelines are shown in Fig. 1 [↗](#).

Materials and Methods

Patients

Written informed consent was obtained from all patients and the study was approved by the local ethic committees. Coherent with prior studies¹⁶, patients with indications for lumbar puncture and voluntary participation in this research were enrolled from July 2018 to December 2024. Exclusion criteria were history of a hypersensitivity reaction to contrast agents, history of severe allergic reactions in general, evidence of renal dysfunction, and pregnant or breastfeeding females. Additional exclusion criteria in the study were: (1) failure of T1 segmentation; (2) failure of signal normalization.

Participants with rs-fMRI were further included in fMRI-related analysis. Exclusion criteria for this subgroup included: (1) without fMRI; (2) excessive head motion (mean Power framewise displacement > 0.3); (3) with intracranial diseases proven to be associated with glymphatic dysfunction, including encephalitis, normal pressure hydrocephalus, and neurodegenerative diseases.

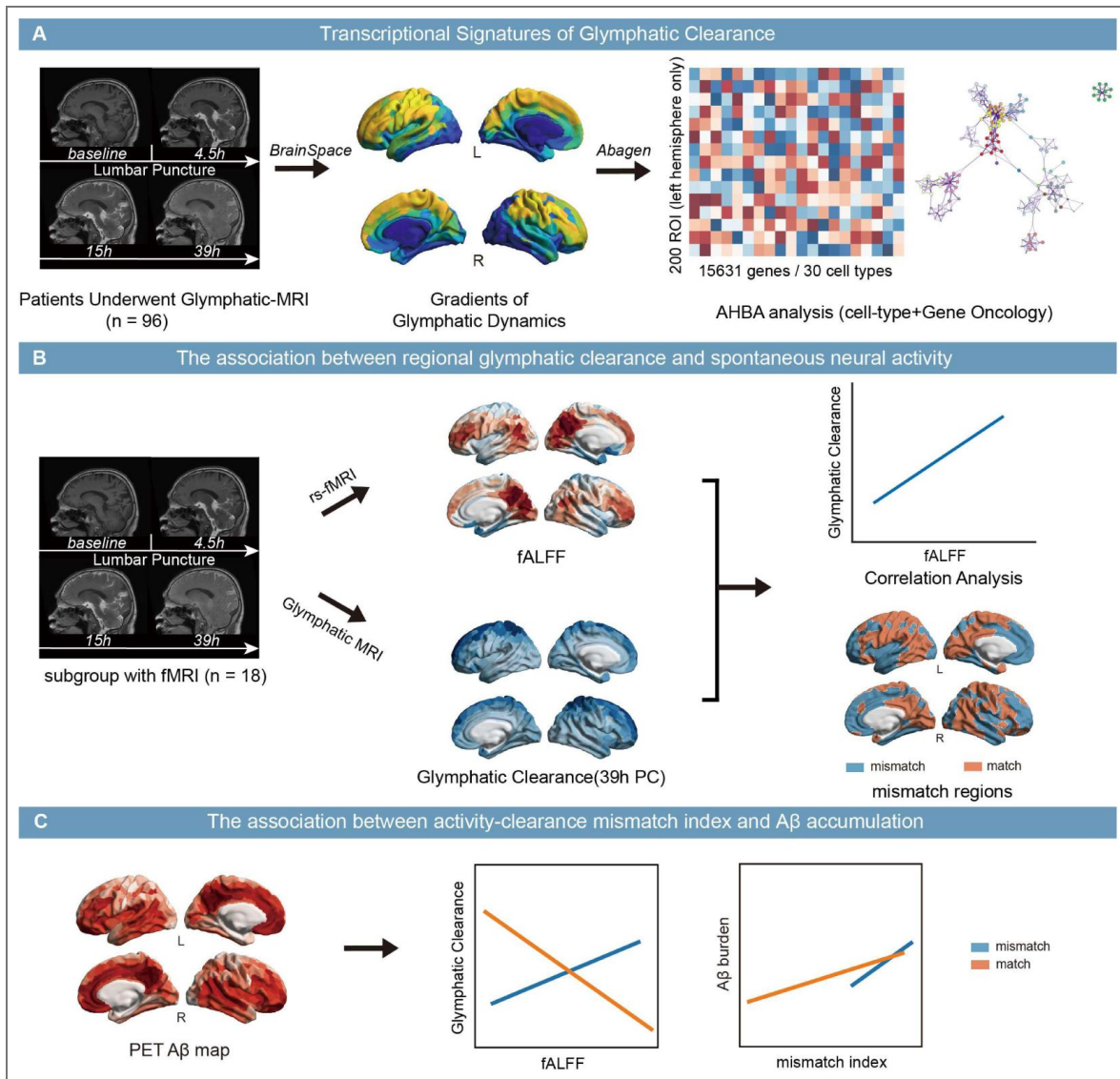


Fig. 1. Overview for analysis pipeline.

(A) The pipeline for computing glymphatic dynamics and transcriptional analysis. Gradients of cortical glymphatic influx and clearance were derived using BrainSpace. Utilizing Abagen toolbox, the glymphatic patterns were further analyzed with transcriptional data from Allen Human Brain Atlas (AHBA). Both gene ontological and cell type analyses were conducted. (B) The relationship between glymphatic clearance function and spontaneous neural activity measured as fALFF was explored in a subgroup with resting-state fMRI (n = 15). (C) The mismatch index between glymphatic clearance function and spontaneous neural activity was defined to investigate its association with amyloid- β (A β) using public dataset.

Considering diagnosis, neurodegenerative diseases with intracranial pathology included normal pressure hydrocephalus, Parkinson's disease, possible cerebral amyloid angiopathy, multiple system atrophy, Alzheimer's disease, and Lewy body dementia. All patients received an intrathecal injection of gadolinium at 3:30~4:00 pm on day 1, then underwent 3D-T1 at 4.5 hours, 15 hours and 39 hours after intrathecal injection of gadolinium (Glymphatic MRI). All patients slept (night sleep) as usual during the nights of days 1–3. All patients were admitted and observed in the ward for at least 39 hours after intrathecal injection.

Intrathecal administration of gadolinium

The site of intrathecal injection of the contrast agent was L3-4 or L4-5 lumbar intervertebral space. Intrathecal injection of 1ml of 0.5mmol/ml gadodiamide (Omniscan; GE Healthcare) was preceded by verifying the correct position of the syringe tip in subarachnoid space in terms of CSF backflow from the puncture needle. Following needle removal, the study subjects were instructed to rotate around the body's long axis twice and then remained in the supine position until 4 hours after intrathecal injection.

MRI protocol

All patients underwent MR by a 3.0T MR scanner using an 8-channel brain phased array coil. The imaging parameters of 3D-T1: repetition time = 7.3 ms, echo time = 3.0 ms, flip angle = 8°, thickness = 1 mm, field of view = 25 × 25 cm², matrix = 250 × 250. This sequence was acquired for four time points, i.e., before and 4.5, 15, and 39 hours after injection, to gain Glymphatic MRI. For rs-fMRI, it was acquired using a T2-weighted gradient echo-planar imaging sequence, time of repetition = 2000 ms, time of echo = 30 ms, flip angle = 77°, field of view = 240 × 240 mm², matrix = 64 × 64, slice thickness = 4.0 mm, slice number = 38.

Glymphatic MRI processing

Cortical glymphatic signal percentage change calculation

For Glymphatic MRI preprocessing, individual baseline T1 images were non-rigidly registered to ICBM template using FreeSurfer^{17,18} and further served as the reference. Serial T1 images were co-registered to this reference using SPM12. Mean signal for each region of interest (ROI) from 400-parcel Schaefer (Schaefer-400) atlas¹⁹ was extracted for serial T1 images and then normalized by dividing the corresponding time point of vitreous body signal according to previous studies²⁰, termed as signal ratio. Normalized T1 images were independently quality-checked by two neurologists (Y.L. and X.Z.). Signal percentage change (PC) for each ROI from baseline to 4.5, 15, or 39 hours was calculated according to equation (1) [1](#).

$$PC = \left(\text{Signal Ratio}_{4.5/15/39\text{ h}} - \text{Signal Ratio}_{\text{baseline}} \right) / \text{Signal Ratio}_{\text{baseline}} \times 100\% \quad (1)$$

Cortical glymphatic gradient calculation

Glymphatic gradients were calculated for both 4.5h and 39h. For each timepoint, PC map across cortex was used to generate a covariance matrix across participants. First, it involves z-scoring each participant's PC map followed by z-scoring the data across all participants for each ROI based on Schaefer-400 atlas. Second, the pairwise correlations between ROIs were calculated to construct the covariance matrix. Then, BrainSpace toolbox12 was applied to find the gradients for each PC map. A Diffusion mapping manifold algorithm (with default parameters) was used to derive the first gradient of the covariance matrix and used for further investigation.

Processing and analysis of functional MRI

Rs-fMRI was preprocessed by DPABI²¹. Preprocessing steps included: 1) convert the DICOM format to NIFTI format, 2) slice timing (removing the first 10 volumes), 3) realignment (to the middle volume), 4) nuisance regression (i.e., Friston-24 head motion parameters, white matter signal and cerebrospinal fluid signal which were extracted from individual T1 segmentation), 5) motion scrubbing (the volume of Power framewise displacement > 0.2 and its previous 1 and post 2 volumes were censored), 6) normalization (by DARTEL to Montreal Neurological Institute space

into 3×3×3 mm voxels), 7) smoothing (with a Gaussian kernel of 6×6×6 mm), and 8) band-pass filtering (0.01–0.1 Hz). After preprocessing, fractional amplitude of low-frequency fluctuation (fALFF) and regional homogeneity (ReHo) were calculated using DPABI. Briefly, power spectrum was computed for each ROI, the square root of which is the amplitude at each frequency. The fALFF map was calculated as the sum amplitude within 0.01–0.1 Hz divided by the sum of amplitudes over the whole frequency range for each ROI. ReHo was calculated for each voxel using Kendall's coefficient of concordance, assessing the temporal similarity with its 26 nearest voxels. The standardized ReHo was derived by normalizing each voxel's Kendall's coefficient of concordance against the whole brain's mean value, followed by smoothing with a 6mm FWHM Gaussian kernel. The z-score map of fALFF was used in formal analysis, with ReHo used in sensitivity analysis.

Transcriptomic analysis

Transcriptomic data

Regional microarray expression data were derived from Allen Human Brain Atlas (AHBA)²² based on six post-mortem brains (1 female, ages 24–57, 42.5 ± 13.38). Since the AHBA only included right hemisphere for two donors, we only considered left hemisphere for further analysis as published before¹⁴. Abagen toolbox was used to process and map the transcriptomic data onto the Schaefer-400 atlas based on previous studies^{14,23}. Briefly, it included pre-processing and assigning tissue samples to ROIs of atlas, which was based on guidance provided by Arnatkeviciute et al.¹⁴. As for pre-processing, it included gene information re-annotation, data filtering, and probe selection. This procedure retained 15631 probes, each representing a unique gene. As for assignment, sample-to-region matching was constrained by hemisphere and cortical-subcortical divisions. Due to the number and site mismatch of probes and ROIs, there would be regions failed to be assigned with a value. To ensure each ROI was assigned a value, the sample closest to the region was selected to represent it (i.e., “Centroid”). This procedure resulted in a 200 × 15631 matrix, denoting the expression of 15631 genes across 200 nodes.

Association between neuroimaging and transcriptomic data

We calculated partial least squares (PLS) regression between cortical glymphatic gradients (both 4.5h and 39h) and the expression of each gene across the 200 ROIs of the left brain based on Schaefer-400 atlas. The significance of the PLS was tested again using a permutation analysis (n = 10,000). It finally derived a vector of 15631 correlation coefficients representing the degree of certain gene spatially aligns with the pattern of glymphatic gradient.

Gene ontology (GO) analysis

GO analysis of significant positive or negative genes in PLS regression was performed by the Metascape platform²⁴ and corrected for false discovery rate (FDR), respectively.

Gene set enrichment analysis (GSEA)

We further performed GSEA on the whole gene list (i.e., the ordered set of 15631 genes from PLS analysis) using abagen^{14,23} to investigate the transcriptomic profile of 30 brain cell-types (astrocytes, endothelial cells, pericytes, microglia, oligodendrocytes, oligodendrocytes progenitor cells, 13 subtypes of excitatory neurons, and 11 subtypes of inhibitory neurons) related with glymphatic gradient. For each brain cell gene set, an enrichment score representing the level of enrichment was obtained. To correct for the size of different gene sets, the enrichment score was compared with those estimated from permutation tests (n = 10,000) and derived a normalized enrichment score (NES) for each gene set. The significance was corrected using FDR.

Cortical Aβ map

The Aβ map was originally collected by Ranasinghe et al.¹⁵ and was downloaded from the NeuroVault (<https://neurovault.org/>). Briefly, PET was acquired with PiB to visualize Aβ deposits in the brain of 32 cognitively impaired patients with Alzheimer's disease (mild cognitive impairment or dementia stage). Individual SUVR maps were computed in native space using data

acquired between 50 and 70 min after tracer injection, and using the cerebellum gray matter as a reference region (defined using FreeSurfer 5.3). The 32 SUVR maps were finally averaged across patients. The average SUVR map for PiB was parcellated based on the Schaefer-400 atlas.

Definition of fALFF- or ReHo-glymphatic clearance match-mismatch pattern

First, mean fALFF, ReHo and 39h PC maps were calculated across individuals. Then, the mean fALFF, ReHo and 39h PC across ROIs were transformed to z-scores, respectively. FALFF-glymphatic clearance match pattern was defined as ROIs with $z\text{-fALFF} > 0$ and $z\text{-39h PC} < 0$, or $z\text{-fALFF} < 0$ and $z\text{-39h PC} > 0$. FALFF-glymphatic clearance mismatch pattern was defined as ROIs with $z\text{-fALFF} > 0$ and $z\text{-39h PC} > 0$ (mismatch1), or $z\text{-fALFF} < 0$ and $z\text{-39h PC} < 0$ (mismatch2). Same definition applied to ReHo-glymphatic clearance match, mismatch1, and mismatch2 patterns. For each ROI, fALFF-glymphatic clearance mismatch index was defined as the negative absolute difference between $z\text{-fALFF}$ and $z\text{-39h PC}$ (i.e., $-|z\text{-fALFF} - z\text{-39hPC}|$). Higher score indicated greater mismatch between fALFF and 39h PC. ReHo-glymphatic clearance mismatch index was defined likewise, i.e., $-|z\text{-ReHo} - z\text{-39h PC}|$, with higher scores indicating greater mismatch between ReHo and 39h PC.

Statistical analyses

Statistical analyses were performed in SPSS packages (IBM, Chicago, version 22.0 for Windows) and MATLAB R2020a (MathWorks, Inc.). We checked the normality of all the characteristics by using the Kolmogorov-Smirnov test and histogram inspection. Statistical significance was accepted at the 0.05 level (two-tailed).

Comparison of PC

One-way repeated measure one-way analysis of variance (ANOVA) was used to compare regional PC across timepoints with post-hoc analyses. Independent two-sample t tests were used to compare regional PC between patients with neurodegenerative disease and peripheral neuropathy. Paired two-sample t test was used to compare the mean PC of patients with neurodegenerative disease and peripheral neuropathy across ROIs. Pearson correlations were used to investigate the association of PC between patients within different disease subgroups, and to assess accordance of gradient scores with PC in 4.5h or 39h across ROIs with spin test to adjust for spatial auto-correlation²⁵.

Association among fALFF, 39h PC, fALFF-39h PC mismatch index, and A β burden

To investigate the association between spontaneous neural activity and glymphatic outflow, Pearson correlation between fALFF and 39h PC was used with spin tests. Independent ANOVA was used to compare the A β burden among match, mismatch1, and mismatch2 regions with FDR-controlled multiple comparisons (i.e., two-stage linear step-up procedure of Benjamini, Krieger and Yekutieli). Furthermore, Spearman correlations were used to investigate the associations among z scores of fALFF and 39h PC, mismatch index, and A β burden in match, mismatch1, and mismatch2 regions. In association between mismatch index and A β burden, partial Spearman correlations were used when adjusting for z-scores of fALFF and 39h PC, and FDR-corrected.

Sensitivity analysis

To confirm the robustness of the fALFF-39h PC match and mismatch regions, a K-means clustering algorithm was programmed in Python. First, the fALFF and 39h PC were standardized using z-score normalization to mitigate scale-related biases. Then, the K-means clustering used $K = 4$ to determine if the clusters were concordant with those defined in fALFF-39h PC mismatch index. Since K-means clustering does not provide specific meaning, the clusters were manually assigned as distinct patterns in reference to clusters in fALFF-39h PC mismatch index. To replicate the results regarding neural activity based on fALFF, ReHo and ReHo-39h PC mismatch index were analyzed. The analysis procedure and statistics replicated those involving fALFF.

Results

Participant Characteristics

Following selection flowchart (Supplementary Fig. 1 [↗](#)), 96 participants were included to characterize the cortical glymphatic function (57 ± 14 [SD] years; 49 women). Disease diagnosis is listed in Supplementary Table 1 [↗](#). Among them, 15 participants who without intracranial diseases and with rs-fMRI were further included to investigate the association between spontaneous neural activity and cortical glymphatic function (53 ± 12 [SD] years; 8 women), of whom 11 were diagnosed as peripheral neuropathy and 4 as motor neuron disease. Demographic and clinical information are listed in Table 1 [↗](#).

Glymphatic dynamics across cortex

Regional glymphatic dynamics with their comparisons between different timepoints are illustrated in Fig. 2 [↗](#). For 4.5h, medial prefrontal cortex and insula were among regions with fast glymphatic influx, while temporal lobe was the slowest (Fig 2A [↗](#)). For 39h, periventricular regions like cingulate cortex, temporal lobe, and occipital lobe were with fast glymphatic clearance, while surface regions like dorsal prefrontal and parietal lobe were slower (Fig 2C [↗](#)). At individual level, PC significantly differed across timepoints ($P < 0.001$, Fig 2F [↗](#)). Overall, both at regional and individual levels, PC increased from 4.5h to 15h, then decreased to 39h. Thus, higher 4.5h PC could indicate better glymphatic influx, while higher 39h PC indicates worse glymphatic clearance.

While previous studies have widely posited that glymphatic transport is reduced in neurodegenerative diseases, it remains unclear whether this represents a uniform slowing with preserved spatial relationships between brain regions, or a more fundamental reorganization of the overall clearance pattern. Surprisingly, the general distribution patterns of CSF tracer were similar in each timepoints (Fig. 3A-F [↗](#)) and highly correlated (Fig. 3K, M [↗](#)) between neurodegenerative disease ($n = 17$) and peripheral neuropathy ($n = 49$, served as control) subgroups. On a group-level, pair-wise comparison of mean PC across 400 ROIs showed that neurodegenerative disease patients may have a generally delayed glymphatic influx and delayed clearance than those with peripheral neuropathy (Fig. 3J, L [↗](#)). In ROI-wise analysis, neurodegenerative disease patients had lower 4.5h PC in frontal cortex than peripheral neuropathy. Besides, patients with neurodegenerative diseases tended to exhibit higher PC in the cingulate cortex compared to those with peripheral neuropathy from 4.5h to 39h. By 39 hours, this trend had expanded to include the temporal lobe and frontal cortex; however, none of the observed differences survived FDR correction (Fig. 3G-I [↗](#)).

Cortical glymphatic gradient

To capture the intrinsic property of cortical glymphatic dynamics, gradients were calculated for 4.5h PC and 39h PC across cortex, respectively (Fig. 2G, I [↗](#)). In specific, gradient was positively correlated with PC value at 4.5h (Fig. 2H [↗](#)), while negatively correlated with PC value at 39h (Fig. 2J [↗](#)).

Transcriptomic correlates of glymphatic influx and clearance

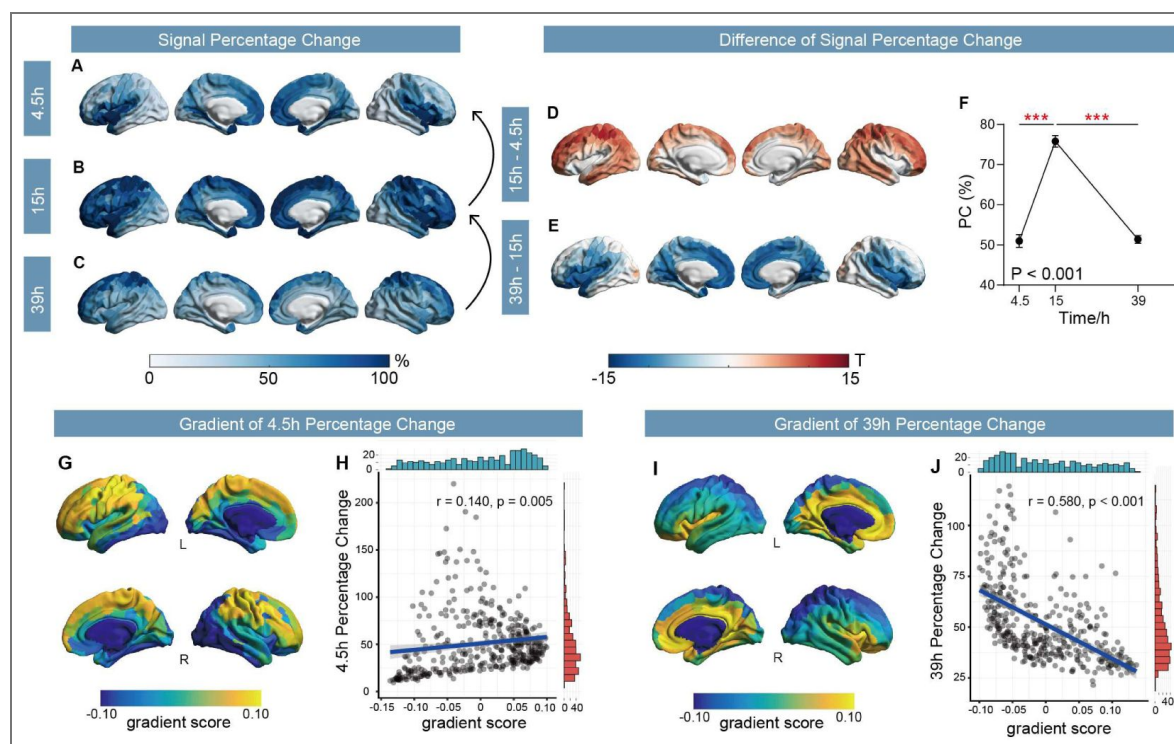
Using GO analysis with 4.5h PC gradient, we found several pathways enriched in regions with faster influx, broadly mapping to gene regulation and synapse function, such as “chromatin remodeling” (Count of genes = 113, $-\log_{10}(P) = 17.4$), “cell junction organization” (Count of genes = 99, $-\log_{10}(P) = 17.3$), “DNA repair” (Count of genes = 120, $-\log_{10}(P) = 14.0$), “regulation of synapse structure or activity” (Count of genes = 76, $-\log_{10}(P) = 12.8$) (Fig. 4A, B [↗](#)). Meanwhile, regions with slower influx enriched pathways related with metabolism, such as “aerobic respiration and respiratory electron transport” (Count of genes = 497, $-\log_{10}(P) = 70.7$), “mitochondrion organization” (Count of genes = 127, $-\log_{10}(P) = 45.8$), “mitochondrial protein degradation” (Count of genes = 47, $-\log_{10}(P) = 25.6$). (Fig. 4C, D [↗](#)).

Table 1. Population characteristics

	Whole cohort	fMRI subgroup
N	96	15
Age	57 ± 14	53 ± 12
Female	49 (51%)	8 (53.3%)
History of hypertension	44 (45.8%)	7 (46.7%)
History of diabetes mellitus	32 (33.3%)	3 (20.0%)
History of dyslipidemia	10 (10.4%)	4 (26.7%)
History of smoking	29 (30.2%)	4 (26.7%)
History of drinking	22 (22.9%)	5 (33.3%)

Fig. 2. Regional glymphatic dynamics across the cortex.

(A-C) Glymphatic signal percentage change at 4.5h (A), 15h (B), and 39h (C) across cortex based on Schaefer-400 atlas. (D-E) T statistics from paired t-tests comparing the signal percentage change between 15h and 4.5h (D), as well as 39h and 15h (E) across cortex. (F) Line graph of 4.5, 15, and 39h percentage change (PC) in 400 ROIs across cortex. Dot represents mean, and bar represents standard error of the mean. (G, I) The first gradient map across cortex based on Schaefer-400 atlas for 4.5h (G) and 39h (I) percentage change. The scatter plots show Pearson correlations between first gradient score and percentage change at 4.5h (H) and 39h (J). The translucent area around the regression lines represents the 95% confidential interval for the regression estimate, with fit line (blue) for significant association. The blue histogram represents the distribution of variables in x-axis, whereas the red histogram represents the distribution of variables in y-axis. L, left; R, right. ***, $p < 0.001$.



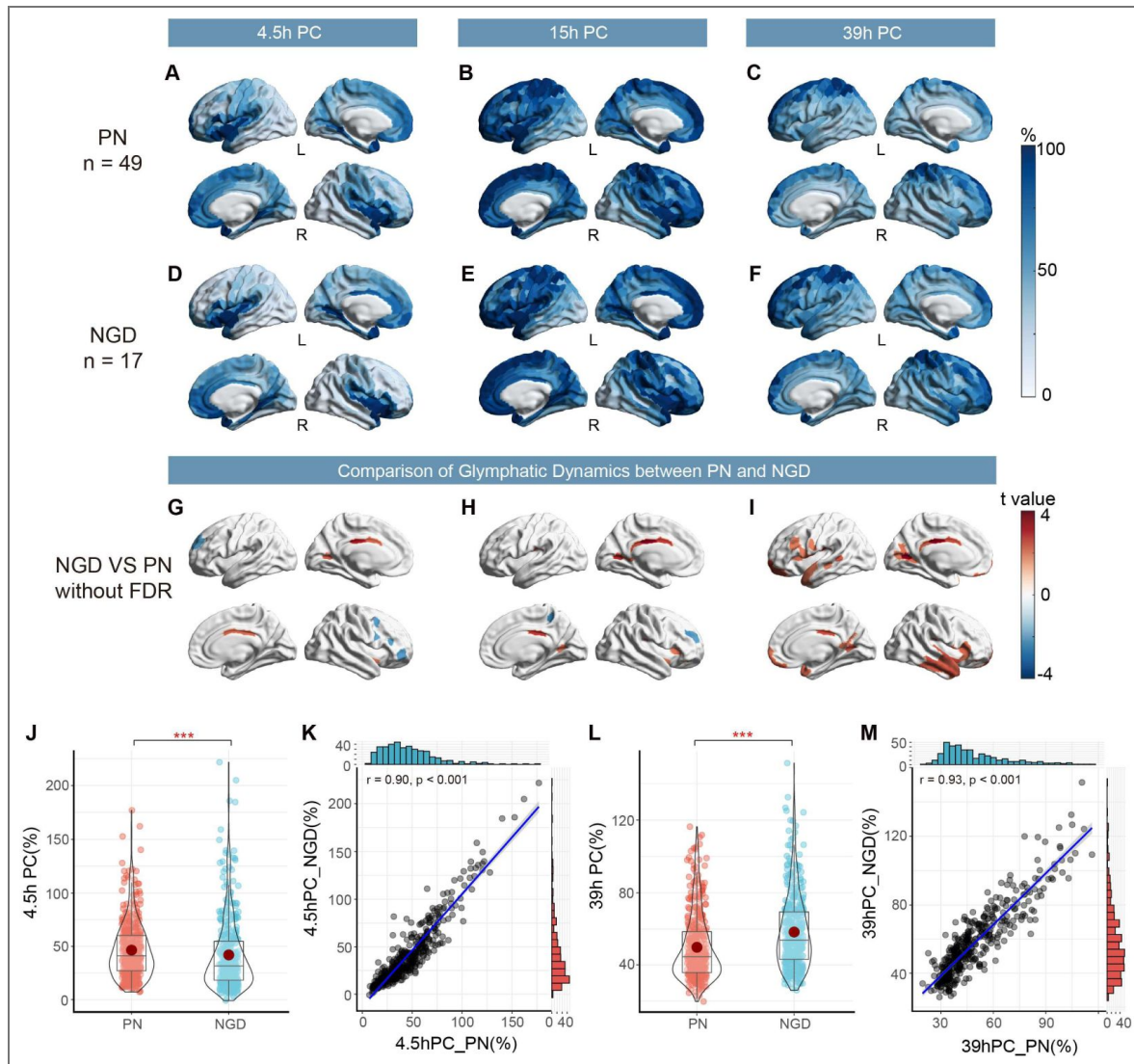


Fig. 3. Comparison of glymphatic dynamics between patients with peripheral neuropathy (PN) and neurodegenerative diseases (NGD).

(A-C) Glymphatic signal percentage change (PC) at 4.5h, 15h, and 39h in PN (n = 49). (D-F) Glymphatic signal PC at 4.5h, 15h, and 39h in NGD (n = 17). (G-I) T statistics from independent two sample t tests of PC at 4.5h, 15h, and 39h between NGD and PN without false discovery rate (FDR) correction. (J) Violin plot of paired two sample t test of 4.5h PC between PN and NGD. (K) Scatter plot of Pearson correlation of 4.5h PC between PN and NGD. (L) Violin plot of paired two sample t test of 39h PC between PN and NGD. (M) Scatter plot of Pearson correlation of 39h PC between PN and NGD. In K and M, the translucent area around the regression lines represents the 95% confidence interval for the regression estimate, with fit line (blue) for significant association. The blue histogram represents the distribution of variables in x-axis, whereas the red histogram represents the distribution of variables in y-axis. L, left; R, right. ***, $p < 0.001$.

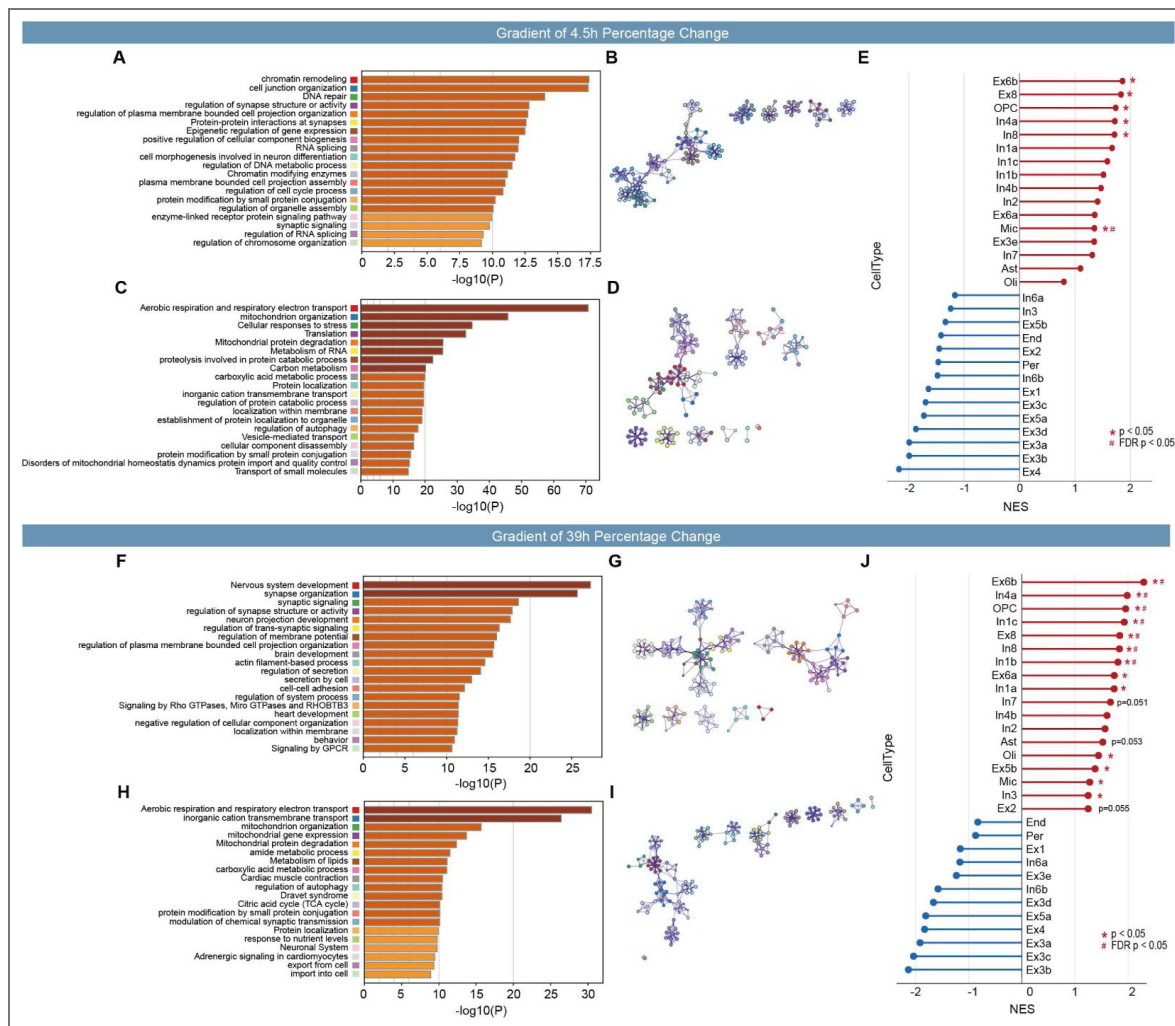


Fig. 4. Association between first gradient of glymphatic flow and gene expression profiles.

(A-E) shows the result for the first gradient of 4.5h percentage change. (A) The top 20 GO biological processes linked with gene sets positively correlated with the first gradient of 4.5h percentage change, and (B) shows the gene enrichment network. (C) The top 20 GO biological processes linked with gene sets negatively correlated with the first gradient of 4.5h percentage change, and (D) shows the gene enrichment network. (E) Cell type enrichment analysis of the first gradient map. (F-J) shows the result for the first gradient of 39h percentage change. (F) The top 20 GO biological processes linked with gene sets positively correlated with the first gradient of 39h percentage change, and (G) shows the gene enrichment network. (H) The top 20 GO biological processes linked with gene sets negatively correlated with the first gradient of 39h percentage change, and (I) shows the gene enrichment network. (J) Cell type enrichment analysis of the first gradient map. Colors indicate directions of association (red = positive, blue = negative). *, $p < 0.05$; #, false discovery rate (FDR) $p < 0.05$.

Similarly, in 39h PC gradient, regions with faster clearance enriched pathways related with synapse function, such as “synapse organization” (Count of genes = 107, $-\log_{10}(P) = 25.7$), “synaptic signaling” (Count of genes = 135, $-\log_{10}(P) = 18.6$), “regulation of synapse structure or activity” (Count of genes = 85, $-\log_{10}(P) = 17.9$) (Fig. 4F, G [↗](#)). Regions with slower outflow enriched pathways related with metabolism, such as “aerobic respiration and respiratory electron transport” (Count of genes = 385, $-\log_{10}(P) = 30.5$), “inorganic cation transmembrane transport” (Count of genes = 209, $-\log_{10}(P) = 26.4$), “amide metabolic process” (Count of genes = 92, $-\log_{10}(P) = 11.5$). (Fig. 4H, I [↗](#)).

Next, we assessed whether the genes associated with glymphatic dynamics, were strongly expressed in specific cell types. GSEA analysis found that, oligodendrocyte precursor cell (NES = 2.00, $p = 0.004$, FDR $p = 0.030$), astrocyte (NES = 1.77, $p = 0.012$, FDR $p = 0.06$), oligodendrocyte (NES = 1.62, $p = 0.002$, FDR $p = 0.030$), microglia (NES = 1.61, $p < 0.001$, FDR $p < 0.001$), and some types of inhibitory neurons (for example, In1c, NES = 1.65, $p = 0.004$, FDR $p = 0.030$) were enriched in regions with faster influx (Fig. 4E [↗](#)). As for clearance, both excitatory (for example, Ex6b, NES = 2.30, $p < 0.001$, FDR $p < 0.001$) and inhibitory (for example, In1c, NES = 1.93, $p < 0.001$, FDR $p < 0.001$) neurons, oligodendrocyte precursor cell (NES = 1.95, $p < 0.001$, FDR $p < 0.001$), oligodendrocyte (NES = 1.44, $p = 0.044$, FDR $p = 0.01$), microglia (NES = 1.28, $p = 0.006$, FDR $p = 0.022$) were enriched in regions with faster clearance (Fig. 4J [↗](#)). Astrocyte was enriched with a tendency (NES = 1.52, $p = 0.053$). Overall, above results showed a close relationship of synaptic structure and function with intact glymphatic function, as well as metabolism with impaired glymphatic function, triggering us to investigate the association between spontaneous neural activity and glymphatic function.

Association between spontaneous neural activity and glymphatic clearance across cortex

The cortical 39h PC of participants with fMRI ($n = 15$) was highly coherent with that of the whole cohort (Fig. 5A-B [↗](#)). Across 400 ROIs in cortex, z-score of fALFF was negatively correlated with 39h PC (Fig. 5C-D [↗](#)), suggesting that impaired glymphatic clearance often coupled with lower neural activity.

Cortical fALFF-glymphatic clearance mismatch pattern and its association with amyloid- β burden

Match regions mainly located in parietal and occipital lobes, while mismatch1 regions (high activity-slow clearance) in dorsal prefrontal cortex, and mismatch2 regions (slow activity-fast clearance) in anterior cingulate cortex and insula (Fig. 6A [↗](#)). Data-driven clustering revealed a similar pattern, further confirmed its robustness (Supplementary Fig. 2 [↗](#)). Utilizing open-source ^{11}C -PiB PET SUVR dataset¹⁵ (Fig. 6B [↗](#)), we found fALFF-39h PC mismatch regions were with higher A β burden than match regions (Fig. 6C [↗](#), one-way ANOVA $F = 4.391$, $p = 0.013$; Post-hoc: mismatch1 vs match, $p = 0.031$; mismatch2 vs match, $p = 0.010$).

In line with definition, with z-score of fALFF increasing, match regions showed decreased z-score of 39h PC, indicating better clearance; while mismatch regions showed weaker associations with z-score of 39h PC (Fig. 6D-F [↗](#)).

In match regions, z-score of fALFF was positively correlated with A β burden (Fig. 6E [↗](#)), but weaker in mismatch regions (Fig. 6G-I [↗](#)).

Across cortex, regional mismatch index was positively correlated with A β burden after adjustment for z-scores of fALFF and 39h PC, which was stronger in mismatch1 regions than in match regions or mismatch2 regions. Both match and mismatch1 regions survived adjustment for z-scores of fALFF and 39h PC, leaving mismatch2 regions with a tendency (Fig. 6J-L [↗](#)).

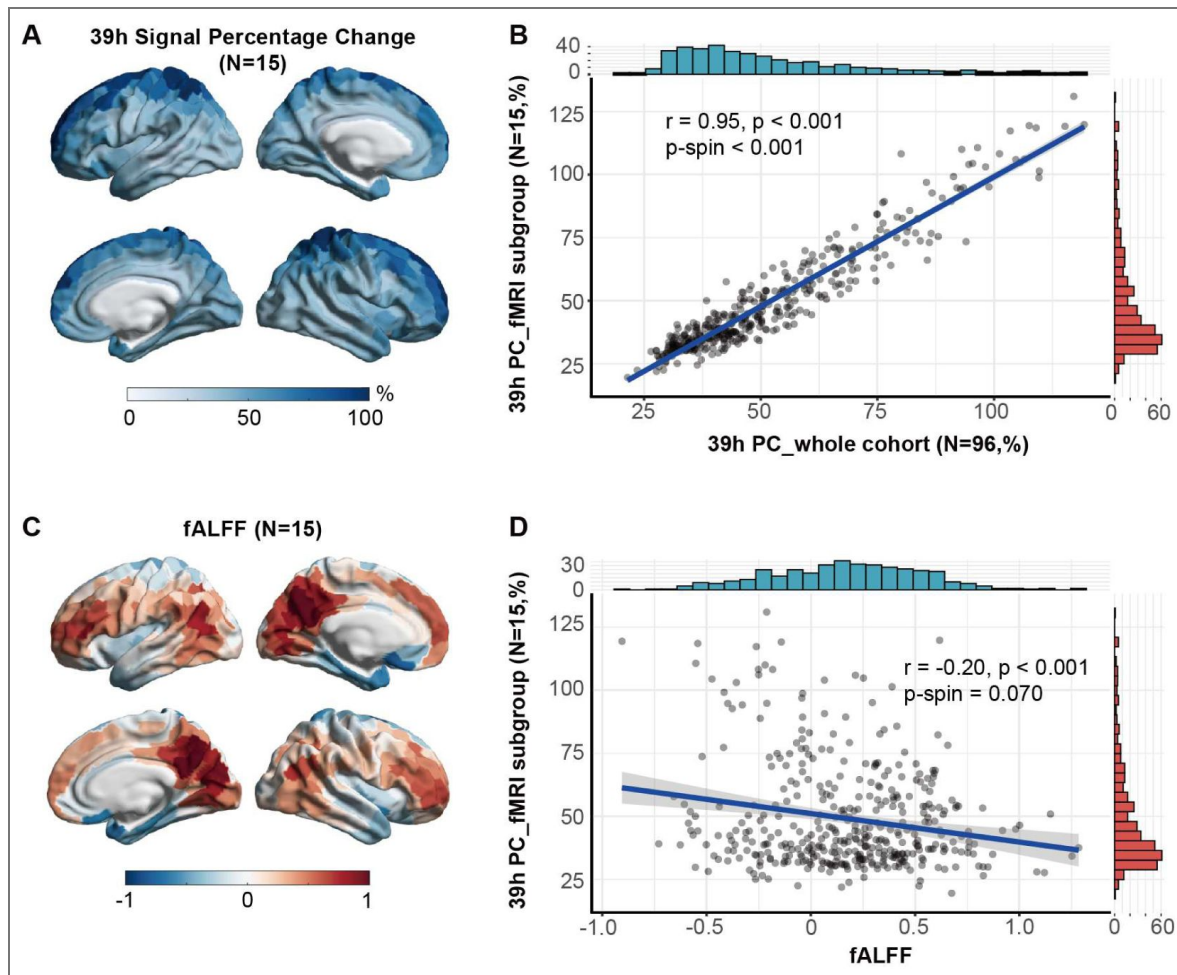


Fig. 5. Association between fractional ALFF (fALFF) and 39h percentage change (PC) across cortex.

(A) The 39h PC across cortex based on Schaefer-400 atlas in patients with fMRI ($n = 15$). (B) The scatter plot of Pearson correlation between 39h PC in whole cohort ($n = 96$) and subgroup with fMRI ($n = 15$). (C) The fALFF across cortex based on Schaefer-400 atlas in patients with fMRI ($n = 15$). (D) The scatter plot of Pearson correlation between fALFF and 39h PC and across cortex based on Schaefer-400 atlas in patients with fMRI ($n = 15$). In B and D, the translucent area around the regression lines represents the 95% confidential interval for the regression estimate, with fit line (blue) for significant association. The blue histogram represents the distribution of variables in x-axis, whereas the red histogram represents the distribution of variables in y-axis.

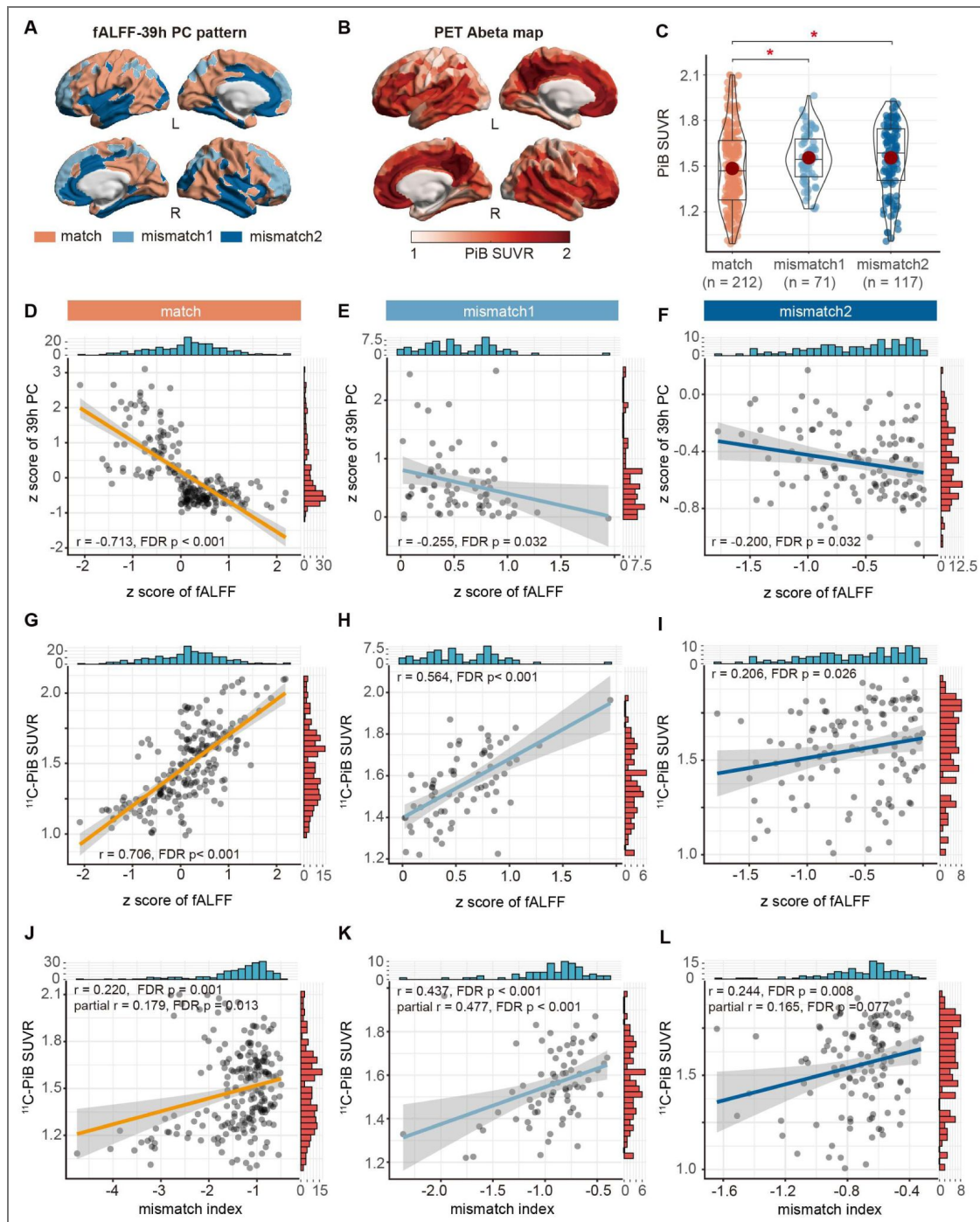


Fig. 6. Association between fALFF-39h percentage change (PC) mismatch and amyloid- β (A β) burden.

(A) fALFF-39h PC match (orange), mismatch1 (high activity with low clearance, light blue) and mismatch2 (low activity with high clearance, dark blue) regions. (B) Open-source ^{11}C -PiB SUVR for A β PET imaging based on Schaefer-400 atlas. (C) Independent one-way ANOVA test of PiB SUVR among regions in match (orange), mismatch1 (light blue) and mismatch2 (dark blue) pattern. p values are calculated using FDR-controlled multiple comparison: two-stage linear step-up procedure of Benjamini, Krieger and Yekutieli. (D-F) The scatter plot of Spearman correlation between z score of fALFF and 39h PC in match (D), mismatch1 (E) and mismatch2 (F) regions. (G-I) The scatter plot of Spearman correlation between z score of fALFF and PiB SUVR in match (G), mismatch1 (H) and mismatch2 (I) regions. (J-L) The scatter plot of Spearman correlation between fALFF-39h PC mismatch index and PiB SUVR in match (J), mismatch1 (K) and mismatch2 (L) regions. In D-L, the translucent area around the regression lines represents the 95% confidential interval for the regression estimate, with fit line for significant association; r indicates the Spearman correlation coefficient, and partial r indicates the Spearman partial correlation coefficient when adjusting for z scores of fALFF and 39h PC. FDR, false discovery rate. *, $p < 0.05$. L, left; R, right.

Sensitivity Analysis

Clustering analysis showed great similarities between the ReHo-39h PC pattern and fALFF-39h PC pattern (Supplementary Fig. 3A-B [↗](#)), with their mismatch index strongly correlated (Supplementary Fig. 3C [↗](#)).

Z-score of ReHo was negatively correlated with z-score of 39h PC. Notably, in mismatch2 regions, this association was insignificant (Supplementary Fig. 3D-F [↗](#)). Consistent with fALFF, z-score of ReHo was positively correlated with A β burden in match regions (Supplementary Fig. 3E [↗](#)), though weaker in mismatch regions (Supplementary Fig. 3G-I [↗](#)). Across cortex, regional ReHo-39h PC mismatch index was positively correlated with A β burden after adjustment for z-scores of ReHo and 39h PC, which was stronger in mismatch1 regions than in match regions or mismatch2 regions (Supplementary Fig. 3J-L [↗](#)).

Discussion

Capitalizing on Glymphatic MRI, this study deciphered the tripartite interplay among glymphatic dynamics, neural activity, and amyloidosis. At the molecular level, neuroimaging-transcriptional analyses on the glymphatic pattern demonstrated that genes associated with synapse organization and activity were significantly enriched in brain regions with better glymphatic influx and clearance. At the cellular level, the linked genes were predominantly expressed in specific cell types, including excitatory or inhibitory neurons, and several glial cells. At the regional level, based on a subgroup of participants with rs-fMRI, we demonstrated that glymphatic clearance was positively correlated with spontaneous neural activity. Mismatch index was defined to evaluate decoupling between spontaneous neural activity and glymphatic clearance function, which turned out to be positively associated with regional severity of amyloidosis using public A β map.

Our systematic mapping of cortical glymphatic dynamics revealed a conserved spatiotemporal architecture, demonstrating notable interindividual consistency. Overall, glymphatic fluid inflows at 4.5h and clears at 39h, aligning with previous studies²⁶. To decode the underlying cortical hierarchy^{27,28}, we derived distinct gradients for cortical glymphatic influx and clearance function. Generally, the influx gradient follows an anterior-posterior hierarchy, orchestrating with the distribution of gamma frequency band²⁹, a neurophysiological signature previously linked to glymphatic potentiation³⁰. Meanwhile, the clearance gradient follows a hierarchy from periventricular to surface area, leaving questions on how extracellular diffusion or advection contribute to this process. Notably, though coherent in pattern distribution, patients with neurodegenerative diseases exhibited regionally impaired glymphatic clearance capability compared with peripheral neuropathy controls. This deficit was particularly prominent in regions predisposed to A β aggregation^{1,31} like cingulate and frontal cortex, reinforcing the hypothesized role of glymphatic failure as a neurodegeneration accelerant³². Together, our results present an intrinsically stable glymphatic dynamic pattern. Elucidating its structural basis will advance understanding of fluid dynamics and clearance processes in the brain.

Evidence for coupling between neuronal activity and glymphatic function remains scarce in human. Utilizing AHBA dataset, we demonstrated that the transcriptomic profiles of 10 excitatory or inhibitory neuronal subtypes were positively correlated with glymphatic clearance function, with 4 subtypes enriched in regions with better glymphatic influx. Zooming in, we also found that spatial variation in glymphatic influx and clearance mirrored variation in neural activity-related molecular pathways, particularly genes governing synaptic architecture and plasticity. Strikingly, glymphatic clearance was associated with spontaneous neural activity including activity amplitude (fALFF) and synchronization (ReHo). Our results, together with other studies^{10,33,34}, identifying an important yet overlooked player in driving glymphatic flow beside vascular pulsations: the neurons. Neurons can drive parenchymal convection flow. Experimental models demonstrate that coordinated neuronal firing generates interstitial ionic waves capable of enhancing cerebrospinal fluid perfusion and clearance¹⁰, while neuromodulatory interventions, including transcranial optogenetics in rodents¹⁰ or visual stimulation in humans³³, markedly amplify clearance efficacy. Intriguingly, neurons can also regulate vasomotion through distant

neurovascular coupling³⁴, or by regulating vasoactive intestinal peptide interneurons through non-invasive 40Hz stimulation³⁰. Recently, Zhang et al demonstrated that glutamatergic axons dilate innervating arterioles via synaptic-like transmission at neural-arteriolar smooth muscle cell junctions³⁵. This synaptic-like structure not only provide novel insights in communication between neurons and hemodynamic fluctuation, but also offer a potential structural basis for loop between neurons, vasomotion and perivascular CSF dynamics. Future research is required to delineate the intricate interactions between neurons and glymphatic system.

Importantly, spatial heterogeneity was observed in the relationship between neuronal activity and glymphatic function. Using novel fALFF- or ReHo-39hPC mismatch index and data-driven k-means clustering, a relatively robust activity-clearance pattern was observed. Notably, match regions localized predominantly to parietal and occipital lobes, mismatch1 regions to dorsal prefrontal cortex, and mismatch2 regions to anterior cingulate cortex and insula. Previously, based on ADNI data with evaluation of CSF movement in the fourth ventricle, Han et al creatively depicted the coupling between regional BOLD signal and CSF³⁶. Regions with higher coupling in their findings align substantially with our match pattern located in motor/sensory, auditory and visual networks, which mostly belong to the lower-order regions. Meanwhile, both mismatch1 and mismatch2 localized to regions capable of higher-order cognition³⁷, yet how mismatch influence their functioning remains elusive. The detailed biological mechanisms driving or influencing mismatch still needs further investigations.

Activity-clearance mismatch could be a novel mechanism underlying regional proteopathy in brain. Intriguingly, we discovered that both mismatch1 and mismatch2 regions exhibited higher A β burden than match regions. Moreover, the mismatch index was generally positively associated with A β burden after adjustment for neural activity and glymphatic clearance, especially in mismatch1 regions. While both neural activity and glymphatic clearance dysfunction have been implicated in A β decomposition^{3,32,38}, their cooperative role in A β accumulation remains understudied. Notably, A β spreads preferentially through neural pathways^{39,40}, not fully compatible with the direction of glymphatic flow. This paradox may be partially reconciled considering the mismatch mechanism, suggesting the hypothesis that the imbalance between neural activity and glymphatic function may affect the final spreading pattern of toxic proteins. Previously, the coupling between resting-state global activity and CSF movement has been found associated with neocortical tau deposition⁴¹ and various AD-related pathology. While informative, these studies only evaluate CSF movement in the fourth ventricle but not glymphatic flow in parenchyma, with regional variability largely ignored. Our quantitative studies based on Glymphatic MRI shed light on this question by detailing in vivo assessment of the mismatch between regional glymphatic clearance and neuronal activity. At the preclinical stage of Alzheimer disease, A β mainly accumulates in default mode network and, to a lesser degree, the frontoparietal network³¹, overlapping partially with our mismatch1 pattern. It is also worth noting that brain regions showing age-invariant glucose metabolism are more susceptible to AD-related hypometabolism⁴ and overlap with our mismatch2 pattern, potentially involving activity-clearance mismatch. Whether augmenting glymphatic clearance function could mitigate mismatch and alleviate subsequent proteopathy in neurodegenerative diseases remains a question for future research.

Astrocytes are indispensable components in glymphatic system as CSF flushes along the perivascular spaces formed by their end-feet, with deletion of astrocyte AQP4 resulting in waste deposition⁵. In our cell type analysis, astrocyte enriched in regions with faster glymphatic influx and tended to enrich in regions with faster clearance, partially supporting its role in glymphatic maintenance. Except for the well-known astrocytes, better glymphatic clearance function was found correlated with genes expressed in other glia cells including oligodendrocyte, oligodendrocyte progenitor cell, and microglia. Typically, oligodendrocytes myelinate axons and provide metabolic support to neurons⁴²; microglia provide immune surveillance, synaptic pruning, and debris removal⁴³. Anatomically, using state-of-the-art technics, Allen et al confirmed that oligodendrocytes were enriched in the corpus callosum or near the CSF⁴⁴. In ageing, glial aging was particularly accelerated in white matter compared with cortical regions⁴⁵, while white

matter serves as a preferential, low-resistance glymphatic pathway under normal conditions^{6,46}. Meanwhile, aging-induced activation of microglia showed a strong dependence on their proximity to oligodendrocytes⁴⁴. Together, above evidence suggest oligodendrocyte and microglia as vulnerable foci when glymphatic system impairs. Consistently, in human, glymphatic dysfunction is closely related with demyelination diseases including white matter hyperintensity⁴⁷ and multiple sclerosis⁴⁸. It is plausible that glymphatic clearance impairment promotes accumulation of inflammatory and neurotoxic elements, activates microglia, and disrupts oligodendrocyte lineage determination, ultimately exacerbates demyelination. These results encourage future investigations on the mechanism between glymphatic function and other neuroglia.

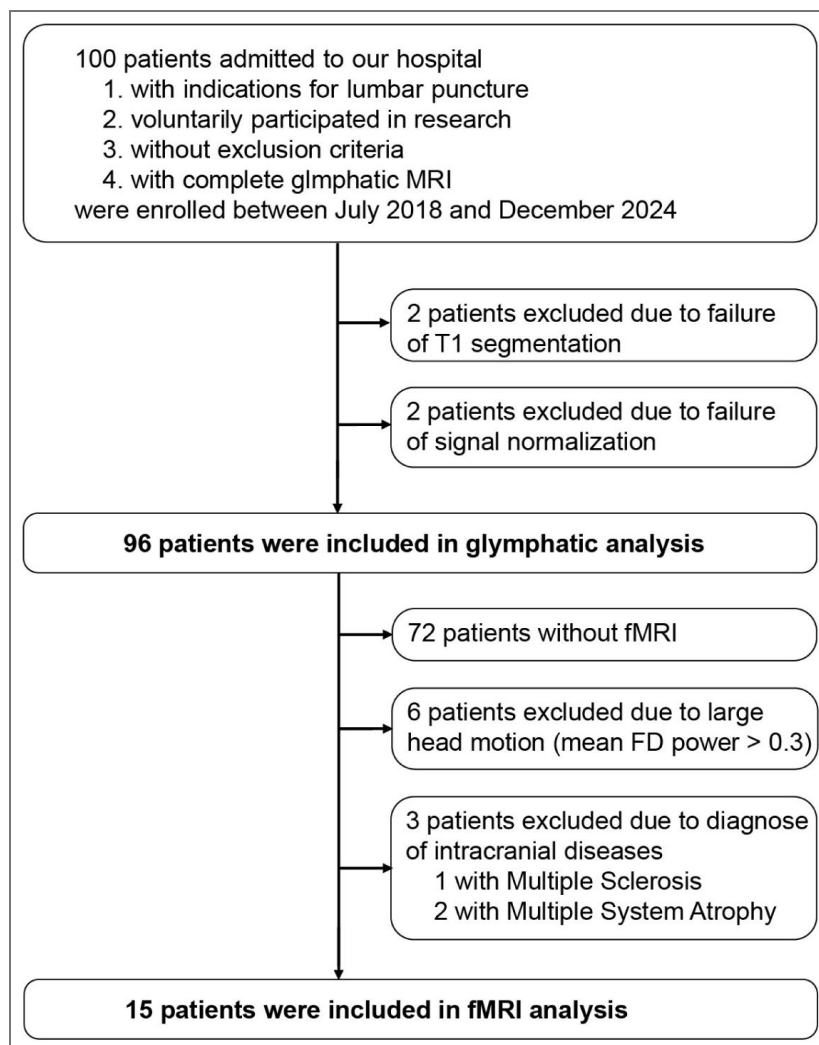
Several issues warrant consideration. First, our participants were with different diseases, though a stable glymphatic dynamic pattern was established. Second, only three time points after intrathecal injection were obtained, leaving relatively long period of blank. Third, since glymphatic dynamic is under circadian control^{32,38} and influenced by posture⁴⁹, scan time (though set relatively fixed), individual sleep-wake cycle, sleeping posture or day-time activity may constitute potential confounders. Forth, the cross-sectional design precludes causal inferences regarding glymphatic-neural activity mismatch and A β deposition. Fifth, transcriptomic analyses relied solely on the AHBA data in left hemisphere, precluding lateralization assessment. As the gene expression profiles derive from healthy participants, our conclusions reflect intrinsic variability rather than disease-associated changes in transcriptomics. Sixthly, the regional A β presented in this study is derived from population-average PET data rather than participant-specific measurements, potentially neglecting individual variability and involving deficits of spatial correspondence across datasets despite inter-study consistency in A β -susceptible regions^{1,31,39}. Future validation requires concurrent PET, fMRI, and glymphatic MRI to confirm this association. Moreover, this study mainly focused on general relationship between neural activity and glymphatic clearance across regions, neglecting the possibility that hub regions in network may have different properties. Lastly, the single-center design and modest sample size necessitate cautious interpretation. Larger multi-center cohorts with longitudinal designs are needed to validate and extend these findings.

In summary, our study provides preliminary evidence that the glymphatic dynamics especially the clearance is interwind with neuronal activity, and the mismatch between these two processes may explain, in part, regional vulnerability to proteinopathy like A β accumulation. Our glymphatic clearance map could be analyzed integrative with a series of cerebral molecular, physiological, and cognitive-related features^{29,50}, potentially advancing our understanding towards the intricate role of glymphatic system in multimodal and multiscale neuroscience. Further investigations are necessary to determine the cellular and molecular constituents in interaction between neurons and glymphatic system, and how they are affected by diseases.

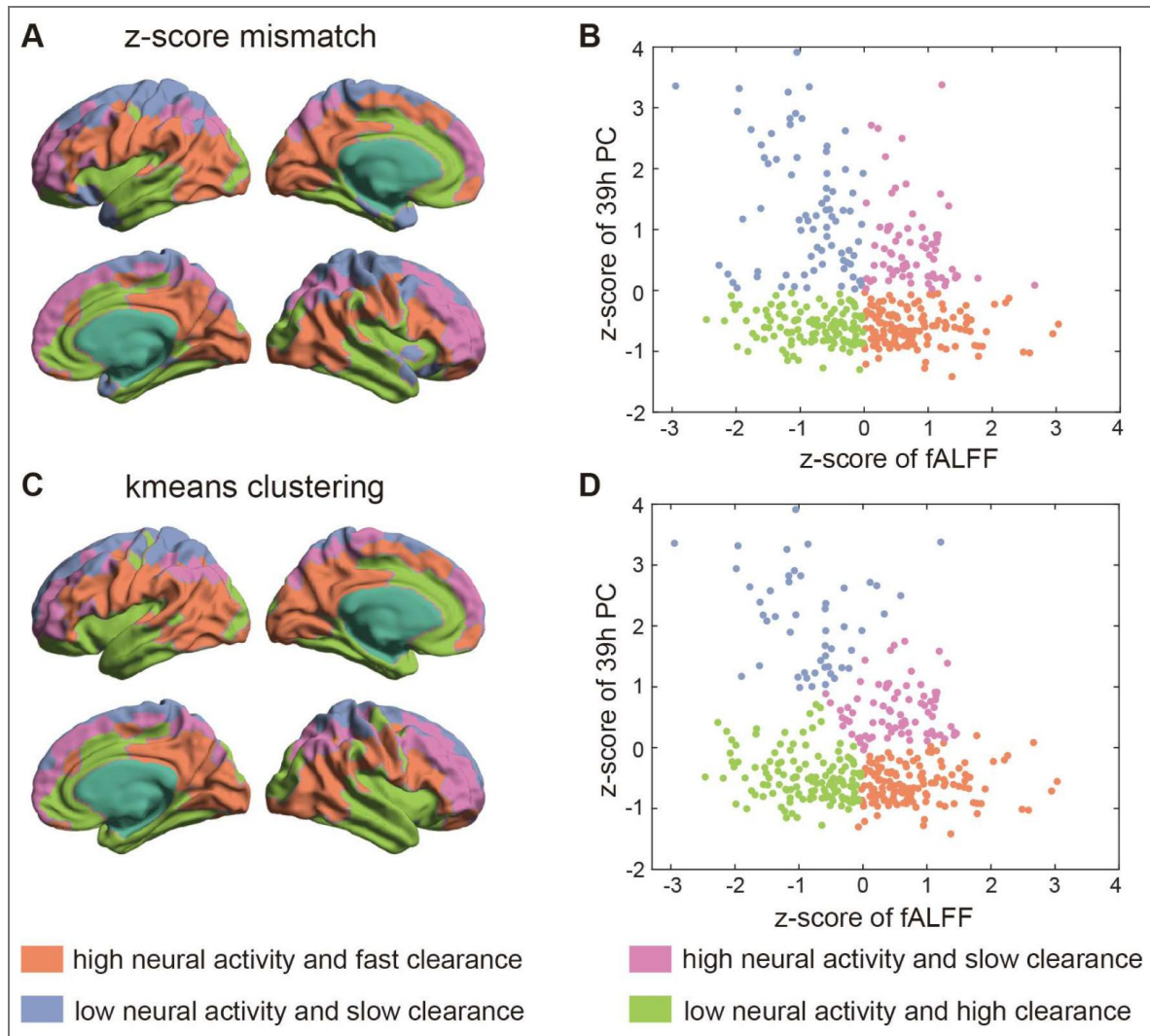
Supplementary Material

Diagnosis	N (%)
Peripheral neuropathy	49 (51.0%)
Motor neuron disease	8 (8.3%)
Encephalitis	16 (16.7%)
Suspected CSF leakage	3 (3.1%)
Hepatic encephalopathy	1 (1.0%)
Multiple sclerosis	2 (2.1%)
Neurodegenerative diseases	17 (17.7%)
Normal pressure hydrocephalus	5 (5.2%)
Parkinson's disease	4 (4.2%)
Possible cerebral amyloid angiopathy	3 (3.1%)
Multiple system atrophy	3 (3.1%)
Alzheimer's disease	1 (1.0%)
Lewy body dementia	1 (1.0%)

Supplementary Table 1.

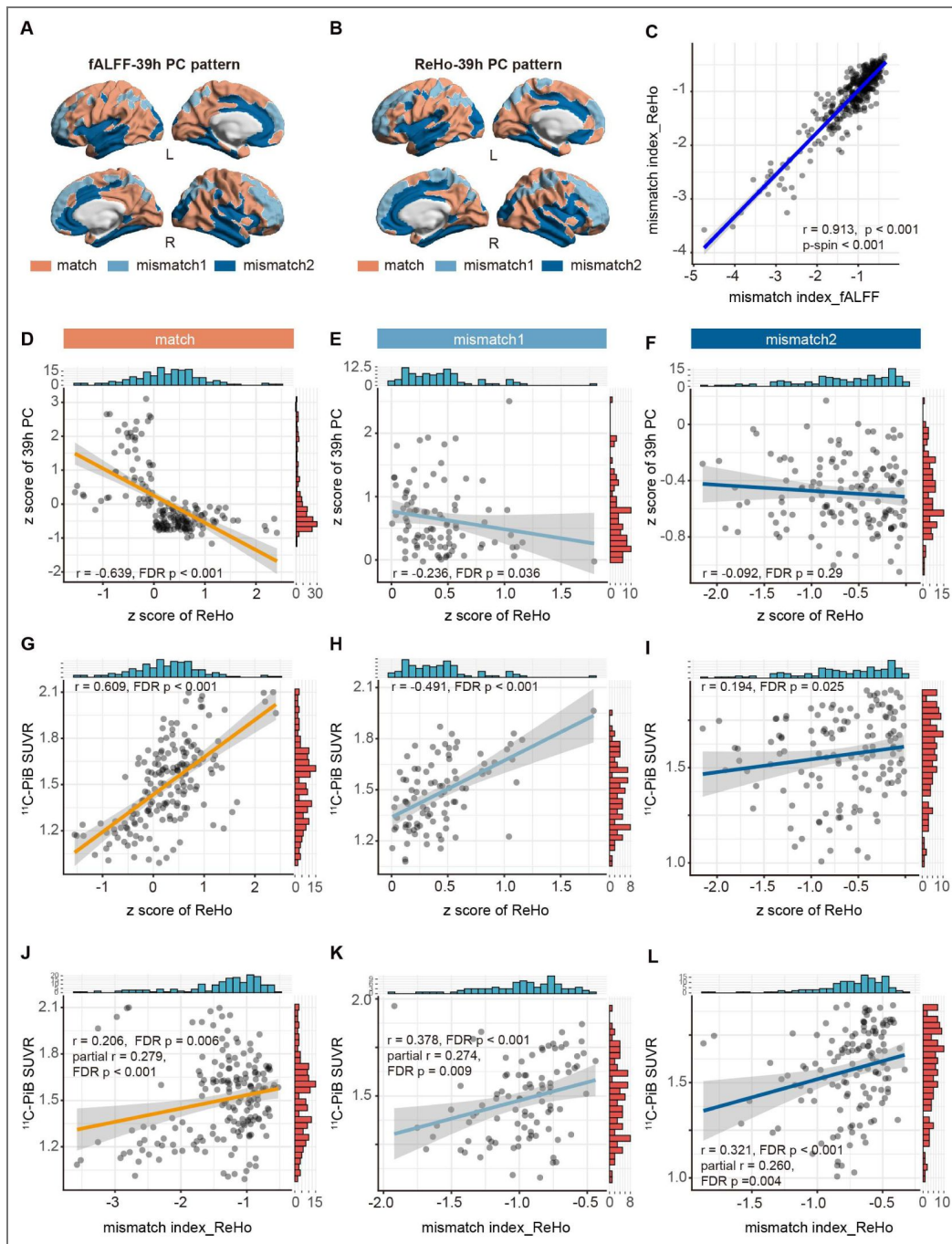


Supplementary Figure 1. Flowchart of patient selection.



Supplementary Figure 2. fALFF-39h percentage change (PC) pattern based on different clustering methodology.

(A-B) The spatial location (A) and scatter plot (B) of clusters based on fALFF-39h PC mismatch index. (C-D) The spatial location (C) and scatter plot (D) of data-driven k-means clustering based on z-scores of fALFF and 39h PC. Orange, high neural activity and fast clearance; pink, high neural activity but slow clearance; purple, low neural activity and slow clearance; green, low neural activity but fast clearance.



Supplementary Figure 3. Association between ReHo-39h percentage change (PC) mismatch and amyloid- β ($A\beta$) burden.

(A) fALFF-39h PC match (orange), mismatch1 (high activity with slow clearance, light blue) and mismatch2 (low activity with fast clearance, dark blue) regions. (B) ReHo-39h PC match (orange), mismatch1 (high activity with slow clearance, light blue) and mismatch2 (low activity with fast clearance, dark blue) regions. (C) The scatter plot of Pearson correlation between fALFF-39h PC mismatch index and ReHo-39h PC mismatch index. (D-F) The scatter plots of Spearman correlation between z score of ReHo and 39h PC in match (D), mismatch1 (E) and mismatch2 (F) regions. (G-I) The scatter plots of Spearman correlation between z score of ReHo and PiB SUVR in match (G), mismatch1 (H) and mismatch2 (I) regions. (J-L) The scatter plots of Spearman correlation between ReHo-39h PC mismatch index and PiB SUVR in match (J), mismatch1 (K) and mismatch2 (L) regions. In D-L, the translucent area around the regression lines represents the 95% confidential interval for the regression estimate, with fit line for significant association; r indicates the Spearman correlation coefficient, and partial r indicates the Spearman partial correlation coefficient when adjusting for z scores of ReHo and 39h PC. FDR, false discovery rate. L, left; R, right.

Data availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Acknowledgements

This study was supported by the National Natural Science Foundation of China (82171276, U23A20426) and Research Project on Prevention and Treatment Techniques for Stroke of Development Center for Medical Science & Technology, National Health Commission of the People's Republic of China (WKZX2023CZ0220).

Additional information

Author Contributions

Y.L., X.Z., and M.L. conceptualized and designed the study; Y.L., X.Z., Y.Z., X.Z., Z.Z., K.W., and J.S. collected the data; Y.L. and X.Z. analyzed the data and prepared the figures; Y.L. and X.Z. wrote the original manuscript draft; Y.Z. and M.L. reviewed and edited the manuscript; M.L. supervised the study. All authors have read and approved the final version of this manuscript.

Funding

Funder	Grant reference number	Author
MOST National Natural Science Foundation of China (NSFC)	82171276	Min Lou
MOST National Natural Science Foundation of China (NSFC)	U23A20426	Min Lou
Research Project on Prevention and Treatment Techniques for Stroke of Development Center for Medical Science & Technology, National Health Commission of the People's Republic of China	WKZX2023CZ0220	Min Lou

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Peer reviews

Reviewer #1 (Public review):

Summary:

Regional differences in the brain's waste-clearance system may interact with neural activity to influence where amyloid-B accumulates. Using intrathecal GBCA administration to produce "Glymphatic MRI" in 96 subjects, the authors mapped cortical glymphatic influx and clearance and found distinct spatial patterns, with transcriptomic analyses linking better glymphatic function to neuronal cell types (through genes). In a subgroup with resting-state fMRI, regions with stronger resting-state activation generally showed higher contrast clearance, indicating a positive coupling between these processes. Notably, cortical regions where neural activity and glymphatic clearance were mismatched showed greater amyloid- β burden in a separate, publicly available PiB-PET dataset, suggesting that activity-clearance decoupling may contribute to regional vulnerability and neurodegeneration.

Strengths:

This is a rare and valuable dataset. Intrathecal contrast injection in ~100 subjects is quite a remarkable accomplishment alone, but the addition of resting-state fMRI, a correlative PiB cohort, and gene-expression pattern data is impressive.

Weaknesses:

This is a cross-sectional study, and we can't determine whether neural activity drives glymphatic clearance, whether glymphatic dysfunction alters neural activity, or whether both are shaped by a third factor. Language describing "flow", "influx", and "clearance" could be made more specific so the reader can more easily follow the methodological approach.

<https://doi.org/10.7554/eLife.111724.1.sa2>

Reviewer #2 (Public review):

Summary:

In this study, Li et al. investigated the relationships among regional cortical tracer dynamics following intrathecal gadolinium administration, neural activity, and amyloid- β deposition in humans. Using serial MRI acquisitions after intrathecal gadodiamide administration in 96 participants, the authors characterized regional signal enhancement and clearance patterns across the human cortex. They integrated these imaging measures with transcriptomic data (Allen Human Brain Atlas), resting-state fMRI outcomes, and an external amyloid PET dataset. The authors report that regions with more efficient tracer clearance are enriched for genes related to synaptic organization and neuronal cell types, that tracer clearance patterns are in parts spatially coupled to spontaneous neural activity, and that regional mismatch between neural activity and tracer clearance is associated with increased amyloid burden according to the PET dataset.

Strengths:

The study addresses an important and very timely question about the interaction among neural activity, cerebrospinal fluid dynamics (waste clearance), and regional vulnerability to neurodegeneration. Integrating serial post-contrast MRI, transcriptomics, resting-state fMRI, and amyloid imaging is ambitious and conceptually very interesting. The spatial characterization of cortical tracer dynamics is potentially valuable for the field, particularly given the increasing interest in human glymphatic imaging approaches and intrathecal contrast MRI, which provides an opportunity to assess CSF tracer dynamics without confounding tracer signal from the blood. The imaging preprocessing pipeline includes normalization of regional cortical signal intensity to a reference region within each session before calculation of longitudinal percentage change, which helps reduce inter-session variability within individuals for conventional T1-weighted imaging. The transcriptomic analyses linking tracer dynamics to neuronal and synaptic gene expression patterns are also interesting. In addition, the manuscript addresses recent literature on neurovascular coupling, glymphatic function, and amyloid vulnerability.

Weaknesses:

Several issues limit the strength of the conclusions. One concern relates to the interpretation of repeated post-intrathecal contrast MRI measurements as direct indicators of glymphatic influx and clearance. The approach presented by the authors measures regional signal changes following intrathecal gadodiamide administration, but does not directly visualize paravascular flow or establish that the observed signal dynamics specifically reflect glymphatic transport mechanisms. Although it is widely accepted that CSF influx occurs primarily along periaxonal spaces as part of the glymphatic system, and the terminology "glymphatic MRI" is increasingly used in the literature, the physiological processes contributing to delayed parenchymal enhancement, including CSF-interstitial exchange mediated by convective bulk flow and/or extracellular diffusion, as well as transient and, in the case of linear gadolinium agents, even long-term tracer retention remain incompletely resolved. Importantly, tracer kinetics may not directly reflect interstitial fluid kinetics, as solute transport may also be influenced by compartmental and extracellular barriers, diffusion constraints, and tissue retention effects. As currently written, several sections of the manuscript appear to overstate what can be directly inferred from the imaging data. This issue may be particularly relevant given the intrathecal use of gadodiamide (Omniscan), a linear gadolinium-based contrast agent with known long-lasting tissue retention due to lower kinetic stability compared to macrocyclic agents. Sustained signal at later imaging time points may therefore not only reflect impaired glymphatic clearance dynamics but also be influenced by tissue retention of contrast material, particularly in the context of neurological disease. In addition, the participant cohort is heterogeneous and includes individuals with neuroinflammatory and neurodegenerative diseases, peripheral neuropathy, and motor neuron disease. Although the authors argue that the spatial tracer patterns are relatively preserved across neurodegenerative groups, this heterogeneity complicates interpretation of

imaging data and raises the possibility that disease-related factors and altered tracer-tissue interactions contribute to the observed effects. Thus, the rationale for interpreting a greater tracer signal at 39h as evidence of impaired glymphatic clearance should be explained more carefully, particularly given the highly heterogeneous patient population.

In addition, the analyses linking spontaneous neural activity and tracer clearance are based on a very small rs-fMRI subgroup ($n = 15$), limiting the generalizability. The interpretation of the "mismatch" analysis also requires caution. The mismatch index was computed from z-scored fALFF and tracer clearance and is subsequently associated with amyloid burden derived from the external PET dataset rather than from the studied participants themselves. Therefore, the observed spatial associations should be interpreted with greater caution rather than as evidence for a direct mechanistic relationship. The cross-sectional nature of the analyses also limits conclusions regarding the directionality and temporal sequence of the relationships between neural activity, tracer dynamics, and amyloid burden. Several statements in the Discussion currently imply stronger causal or biological conclusions than are directly supported by the data.

Despite these limitations, the study presents an interesting dataset and proposes a framework for understanding regional vulnerability to protein accumulation in neurodegeneration. This work hopefully motivates further investigation into the important relationships among neural activity, CSF dynamics, and neurodegeneration in humans.

<https://doi.org/10.7554/eLife.111724.1.sa1>

Reviewer #3 (Public review):

This manuscript addresses an interesting and timely question: whether regional glymphatic clearance in the human cortex is spatially coupled to neural activity and whether a mismatch between activity and clearance may help explain regional vulnerability to amyloid- β deposition. The authors use intrathecal gadolinium-based glymphatic MRI in 96 participants, derive cortical influx and clearance maps, integrate these with Allen Human Brain Atlas transcriptomic data, and then relate regional clearance to resting-state fMRI measures in a smaller subgroup. They further compare the resulting activity-clearance mismatch map with an open-source ^{11}C -PiB amyloid PET dataset. The overall concept is attractive because it attempts to connect glymphatic physiology, neuronal activity, and proteopathy at the regional level of the human brain, an important and understudied area.

The main strength of the study is the use of direct intrathecal contrast-enhanced MRI to generate cortical maps of glymphatic tracer dynamics. This is a technically demanding approach and provides a richer spatial readout than indirect MRI proxies of glymphatic function. The authors show that the cortical tracer signal increases from 4.5 h to 15 h and then decreases by 39 h, allowing them to interpret the early signal as reflecting influx and the persistent signal at 39 h as impaired clearance. They further identify regional patterns, with faster influx in medial prefrontal/insular areas and slower clearance in dorsal prefrontal and parietal surface regions. The analysis is visually clear, and the use of cortical gradients is a useful way to reduce complex regional data into interpretable spatial axes.

The multimodal integration is also interesting. The transcriptomic analysis suggests that regions with faster glymphatic clearance are enriched for synaptic organisation and neuronal activity-related pathways, while regions with slower clearance show enrichment for metabolic and mitochondrial pathways. The cell-type enrichment analysis further implicates excitatory and inhibitory neurons, oligodendrocyte lineage cells, microglia and, to a lesser extent, astrocytes. This provides a plausible biological bridge between regional neural activity and clearance function, and the sensitivity analysis using ReHo in addition to fALFF is a useful robustness check.

However, the manuscript should be more careful in its causal interpretation. The study is cross-sectional and largely correlative in space. The finding that regions with higher spontaneous neural activity tend to show better glymphatic clearance is intriguing, but it does not establish that neural activity drives clearance in these participants. Conversely, it remains possible that better tissue integrity, vascular function, CSF access, cortical geometry, vascular density, or disease composition jointly influence both fMRI measures and tracer clearance. The authors do acknowledge some of these limitations, but the abstract and discussion should more consistently frame the findings as associations rather than evidence of an activity-clearance mechanism in humans.

The most important limitation is the small size of the fMRI subgroup. Although the whole glymphatic MRI cohort includes 96 participants, the key activity-clearance analysis is based on only 15 individuals, including 11 with peripheral neuropathy and 4 with motor neuron disease. This is a very small and clinically heterogeneous sample on which to build a central conclusion about regional neural activity and glymphatic clearance. The authors show that the 39 h PC map in the fMRI subgroup resembles the whole-cohort map, which is helpful, but this does not address whether the fALFF-clearance relationship is robust at the individual level. The paper would be strengthened by reporting subject-level stability, leave-one-out analyses, and whether the association persists after excluding the four motor neuron disease cases.

A second major concern is the interpretation of the amyloid analysis. The ^{11}C -PiB map is derived from an external open-source Alzheimer's disease dataset, not from the same participants who underwent glymphatic MRI and fMRI. Therefore, the association between activity-clearance mismatch and amyloid burden is a spatial correspondence across group-average maps, not an individual-level relationship. This is valuable for hypothesis generation, but should not be presented as evidence that a mismatch in the present cohort predicts amyloid deposition. The authors should clearly state that this analysis tests whether mismatch regions overlap with known amyloid-prone cortical regions, rather than directly linking mismatch to amyloidosis in individual participants.

The definition of "mismatch" also needs clarification. The text defines the mismatch index as the negative absolute difference between z-fALFF and z-39h PC, and states that higher scores indicate greater mismatch. Because the index is negative, values closer to zero would normally indicate a smaller absolute difference rather than a greater mismatch. This should be checked carefully and corrected if necessary. More broadly, because a higher 39 h PC indicates worse clearance, the interpretation of match and mismatch categories is not intuitive. The authors should provide a clearer schematic and ensure that the mathematical definition, biological interpretation and figure labelling are fully aligned.

Several technical confounds require more attention. Intrathecal gadolinium MRI is influenced by CSF dynamics, posture, sleep, circadian timing, renal clearance, age, intracranial pathology, and potentially diagnosis-specific differences. The authors acquired scans at fixed time points and noted that patients slept as usual, but individual sleep duration, sleep quality, posture, and daytime activity were not objectively measured. Given that the central claim concerns glymphatic clearance, these are not minor confounders. The authors should consider adjusting for age, sex, diagnosis, vascular risk factors, and relevant clinical variables where possible, and be more explicit about how heterogeneous disease indications may influence cortical tracer kinetics.

The statistics are generally good. However, many correlations are performed across 400 cortical parcels, which are not independent biological samples. The paper would benefit from clearer separation between participant-level inference and region-level spatial inference. For example, the fALFF-clearance and mismatch-amyloid analyses are regional map correlations, not correlations across individuals. This should be clearly stated throughout. The authors should also report effect sizes and confidence intervals more consistently, and explain how

multiple comparisons were controlled across transcriptomic, cell-type, fMRI, ReHo and amyloid analyses.

The transcriptomic analysis is useful but should be presented as indirect. AHBA data come from six post-mortem brains; only the left hemisphere was used, and the donors were healthy and younger than the clinical cohort. Therefore, these data capture intrinsic regional gene-expression patterns rather than disease-state expression in the same individuals. The authors should avoid implying that the transcriptomic findings directly explain glymphatic function in their participants. The current discussion partly acknowledges this, but the framing in the abstract and results could be more cautious.

There are also several points of presentation that should be improved. The manuscript should consistently distinguish glymphatic influx, glymphatic clearance, CSF tracer retention, and waste clearance. A 39 h residual gadolinium signal is a useful proxy for delayed clearance, but it is not the same as direct measurement of amyloid or tau clearance. The language around "waste clearance" and "amyloidosis" should therefore be precise. The authors should also clarify whether "higher clearance" corresponds to lower 39 h PC across all analyses, as this inversion is easy for readers to misinterpret.

<https://doi.org/10.7554/eLife.111724.1.sa0>

Author response:

We would like to express our deepest gratitude to the Editors and Reviewers for their highly rigorous and constructive evaluation of our manuscript. We are greatly encouraged by the recognition of our study's ambition, the unique value of the in vivo intrathecal contrast MRI dataset, and the conceptual novelty of linking macroscopic glymphatic physiology with neural activity and regional proteopathy.

We fully agree with the thoughtful limitations and methodological concerns raised in the eLife Assessment and the Public Reviews. In our upcoming revised manuscript, we are implementing a comprehensive set of revisions to address these points. Specifically, our planned revisions focus on the following key areas:

- **Tempering Causal Interpretations:** We agree that our cross-sectional design precludes definitive causal inferences. We are systematically revising the manuscript to soften causal language (e.g., replacing "drives" with "is spatially associated with"). We will explicitly frame our findings as macroscopic spatial associations and discuss the potential influence of joint physiological confounders.
- **Tightening Terminology and Imaging Physics:** We are refining our terminology to more accurately reflect our MRI measurements. We will replace assertive terms like "direct glymphatic flow" with precise descriptors such as "imaging proxies for tracer enhancement and retention." Furthermore, we are expanding the Limitations section to explicitly acknowledge the confounding effects of Partial Volume Averaging (PVE), systemic tracer redistribution, and renal clearance kinetics.
- **Conducting Supplementary Imaging & Robustness Analyses:** To address concerns regarding cohort heterogeneity and the sample size of the rs-fMRI subgroup (n=15), we are performing a series of rigorous supplementary analyses. This includes conducting sensitivity analyses (e.g., excluding the motor neuron disease subgroup) and applying leave-one-out cross-validation to rigorously assess the subject-level stability and robustness of the spatial coupling between neural activity and tracer clearance.
- **Clarifying the Conceptual Model and "Mismatch" Index:** To improve readability, we are moving the anatomical definitions of the cortical gradients directly into the Results section.

Additionally, we are introducing schematic diagram to intuitively explain the mathematical formulation and biological interpretation of the "activity-clearance mismatch" index.

- Re-framing External Dataset Analyses: We are carefully re-framing the interpretations of the Allen Human Brain Atlas (AHBA) transcriptomic data and the external PiB-PET amyloid dataset, emphasizing that these reflect spatial correspondences of intrinsic regional vulnerability across groups, rather than individual-level direct interactions.

We believe these revisions will significantly enhance the scientific rigor, clarity, and precision of our study.

<https://doi.org/10.7554/eLife.111724.1.sa4>