Sex-specific age-related changes in glymphatic function assessed by resting-state functional magnetic resonance imaging

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Abstract

The glymphatic system that clears out brain wastes, such as amyloid-β (Aβ) and tau, through cerebrospinal fluid (CSF) flow may play an important role in aging and dementias. However, a lack of non-invasive tools to assess the glymphatic function in humans hindered the understanding of the glymphatic changes in healthy aging. The global infra-slow (<0.1 Hz) brain activity measured by the global mean resting-state fMRI signal (gBOLD) was recently found to be coupled by large CSF movements. This coupling has been used to measure the glymphatic process and found to correlate with various pathologies of Alzheimer’s disease (AD), including Aβ pathology. Using resting-state fMRI data from a large group of 719 healthy aging participants, we examined the sex-specific changes of the gBOLD-CSF coupling, as a measure of glymphatic function, over a wide age range between 36-100 years old. We found that this coupling index remains stable before around age 55 and then starts to decline afterward, particularly in females. Menopause may contribute to the accelerated decline in females.
Introduction

Aging is the leading risk factor for cognitive decline and neurodegenerative disorders that are often associated with excessive accumulation of misfolded proteins, including the amyloid-β and tau, in the brain (Hou et al., 2019; Mawuenyega et al., 2010; Peng et al., 2016). Recent studies suggested the aggregation of toxic proteins could be partly attributed to impaired glymphatic clearance at advancing ages (Benveniste et al., 2019; Boland et al., 2018; Jessen et al., 2015). The glymphatic system, as “glia-lymphatic system”, constitutes a pathway for brain waste clearance in the central nervous system (Iliff et al., 2012; Jessen et al., 2015). In this clearance pathway, cerebrospinal fluid (CSF) moves from the periarterial space, facilitated by aquaporin-4 (AQP4) channels in astroglial endfeet, into the interstitial space to wash out interstitial solutes, including Aβ and tau, into the perivenous space surrounding deep-draining veins (Jessen et al., 2015; Tarasoff-Conway et al., 2015). The paravascular CSF recirculation and interstitial solute efflux have been found to decrease in aged mice due to widespread loss of perivascular AQP4 polarization and reduced pulsatility of intracortical arterioles (Kress et al., 2014). In humans, the clearance along the glymphatic pathway and downstream meningeal lymphatic vessels, measured by contrast-agent MRI, decreased and delayed in older patients as compared with younger ones (Zhou et al., 2020). However, the invasive nature of these imaging tools has hindered a large-scale study of glymphatic function in healthy aging subjects. As a result, it remains unclear how the glymphatic function changes in aging, which is vital to understanding the mechanisms of age-related neurodegenerative disorders and cognitive decline.
Global infra-slow (< 0.1 Hz) brain activity measured by resting-state fMRI (rsfMRI) was recently linked to the glymphatic function (Kiviniemi et al., 2016) and used to quantify its changes in Alzheimer’s disease (AD) (Han et al., 2021b) and Parkinson’s disease (PD) patients (Han et al., 2021a). Increasing evidence suggested the brain exhibits highly structured, brain-wide infra-slow (< 0.1 Hz) activity during the resting state (Gu et al., 2021; Liu et al., 2021; Raut et al., 2021; Thompson et al., 2014). This global brain activity is evident in neural signals of distinct scales, ranging from single neuron recordings to whole-brain fMRI, and closely related to transient arousal modulations (Gu et al., 2021; Liu et al., 2021). The fMRI measure of this activity, i.e., the global mean rsfMRI blood-oxygenation-level-dependent (gBOLD) signal, is coupled by large CSF movements (Fultz et al., 2019) and astroglial calcium waves (Wang et al., 2018), suggesting its potential link to the glymphatic function. The gBOLD is greatly enhanced during sleep (Fukunaga et al., 2006; Larson-Prior et al., 2009; Olbrich et al., 2009), in accordance with the sleep-enhanced nature of the glymphatic function (Xie et al., 2013). In contrast, arterial and respiratory pulsations, which had been traditionally regarded as the main glymphatic drivers (Iliff et al., 2013; Yamada et al., 2013), actually lack of this attribute with the decreased amplitude during sleep (Boudreau et al., 2013; Douglas et al., 1982; Snyder et al., 1964). For all these reasons, the strength of gBOLD-CSF coupling has been proposed as a surrogate measure of the glymphatic function and found to correlate with various AD pathologies and cognitive decline in PD (Han et al., 2021b, 2021a). Recently, the disengagement of gBOLD from the default mode network (DMN) regions were found to account for early, preferential Aβ accumulation in these higher-order brain areas in the early stage of Aβ pathology (Han et al., 2022). In these early studies, the gBOLD-CSF coupling also was found to correlate significantly with age and sex (Han et al., 2021b, 2021a), but the related findings were limited by narrow age ranges and complicated by the inclusion of patient data.
The fMRI-based glymphatic measurement, together with the widely available rsfMRI data, provides us a unique opportunity to study the change of the glymphatic function over a wider age range and in a larger population of healthy aging subjects. In this study, we used rsfMRI data of 719 healthy aging subjects in the Human Connectome Project Aging (HCP-A) (Harms et al., 2018) to study the age-related changes in glymphatic function in a sex-specific way. We found that the fMRI-based glymphatic measure remained relatively stable within the range of age 36-54 and then started to decrease around age mid-50s. Compared with males, females showed a larger and more abrupt decline of the glymphatic function at this transitioning point.

Results

Nonlinear age trajectory of the gBOLD-CSF coupling

We used rsfMRI data of 719 healthy subjects (403 females) from the HCP-A project with age ranging from 36 to 100. Following previous procedures (Fultz et al., 2019; Han et al., 2021b, 2021a), we obtained the whole-brain gBOLD signal from the gray matter regions and the bottom-slice CSF signal around the bottom of the cerebellum to measure CSF movements via MR inflow effects (Fultz et al., 2019; Gao et al., 1996; Gao and Liu, 2012) (Fig. S1A and S1B). Consistent with the previous studies, the averaged cross-correlation function of the two signals displayed a biphasic pattern with a negative peak ($r = -0.33$) at the +3.2 sec lag (Fig. S1C). The gBOLD-CSF correlation at this +3.2 sec time lag was then computed for individual subjects to quantify their coupling and thus the glymphatic function. The gBOLD-CSF coupling was then averaged within
10 equal-size groups of subjects at different ages, and the resulting age-related trend displayed a clear non-linear trajectory: it remains relatively stable between ages 36 to 54 and then begins to decline at around 55 years old (i.e., yrs) (Fig. 1A). We then divided the entire cohort into younger and older groups according to the age boundary (53.9 yrs) between the fourth and fifth groups, which had the largest drop among pairs of consecutive groups. The gBOLD-CSF coupling is not correlated ($r = -3.7 \times 10^{-3}, p = 0.95$) with age in the younger group whereas this correlation is significant ($r = 0.14, p = 3.9 \times 10^{-3}$) in the older group (Fig. 1B).

We examined potential factors mediating this age-coupling association. Neither the coupling index nor age was significantly correlated with the total Pittsburgh Sleep Quality Index (PSQI) score, but they were similarly correlated with a few PSQI items, including sleep medication, trouble sleeping, and sleep hours. Nevertheless, the age-related changes in the gBOLD-CSF coupling remain largely unchanged with adjusting for these sleep-related measurements (Fig. 2). Likewise, the age trajectory of the gBOLD-CSF remained similar with controlling for other non-sleep factors, including the head motion assessed by mean framewise displacement (FD) (Fig. S2), the brain volume (Fig. S3), and the CSF volume (mainly from the ventricles) (Fig. S4), even though these factors showed a significant dependence on age (all $p < 3.9 \times 10^{-7}$ for linear regression).

**Sex-specific differences in age-related changes**

The gBOLD–CSF coupling is different between females and males. The mean gBOLD-CSF strength is significantly ($p = 7.9 \times 10^{-6}$, two-sample t-test) weaker in females than males (Fig. 3A), consistent with the previous finding in an AD cohort (Han et al., 2021b).
The coupling index are different for the two groups. The gBOLD–CSF coupling decreases more evidently and abruptly with aging in the females, particularly around 55 yrs, whereas the males showed a more gradual and steady age-related changes (Fig. 3B).

We further tested whether and how the gBOLD-CSF coupling is affected by menopause, which often occurs before age 55. This analysis was focused on the age range of 36-55, which includes both post-menopause and other females. The menopause status had no significant effects on the gBOLD-CSF coupling strength for two subgroups of subjects of similar ages (both $p > 0.13$; two sample t-test). However, it showed a marginally significant ($p = 0.061$) interaction with age on the coupling strength. Consistent with this result, the gBOLD-CSF coupling in the postmenopausal females appeared to decline earlier in the age mid-40s (Fig. 3C), making the drop around age 55 less abrupt and significant ($p = 0.058$). The result suggests the potential effect of the menopause to accelerate the decline of the fMRI-based glymphatic measure.

Discussion

Here we study the sex-specific age-related change in the glymphatic function in a large healthy aging population based on the gBOLD-CSF coupling measured by rsfMRI. We showed that the gBOLD-CSF coupling changes with age in a non-linear way: it remains relatively stable from 36 yrs to 54 yrs and then begins to decrease afterwards. Importantly, the decrease at age mid-50s is larger and more abrupt in females than in males.
Converging evidence has suggested a link between the glymphatic function and the resting-state global brain activity, often measured by the gBOLD signal of rsfMRI. The gBOLD, once regarded as noise, measured a brain-wide, low-frequency (< 0.1 Hz) activity linked to transient arousal modulations, which also has been observed in monkey electrocorticography (ECoG) (Liu et al., 2015, 2018) and mice spiking data as highly structured patterns (Liu et al., 2021). The low-frequency rsfMRI signals were first linked to the glymphatic function due to their potential link to CSF dynamics and vasomotor waves (Kiviniemi et al., 2016). It was found later that it is the global component of rsfMRI, i.e., gBOLD, that is coupled to CSF movements in a sleep-dependent way (Fukunaga et al., 2006; Fultz et al., 2019; Helakari et al., 2022; Larson-Prior et al., 2009; Olbrich et al., 2009). The sleep dependency makes it more suitable for driving the sleep-dependent glymphatic clearance (Holth et al., 2019; Xie et al., 2013), as compared with the cardiac and respiratory pulsations that are actually suppressed during sleep (Baust and Bohnert, 1969; Boudreau et al., 2013; Douglas et al., 1982; Guazzi and Zanchetti, 1965; Snyder et al., 1964).

Nevertheless, the gBOLD is not independent from these physiological drivers but shows strong correlations with the low-frequency modulation of cardiac and respiratory functions (Birn et al., 2006; Chang et al., 2009; Gu et al., 2020; Özbay et al., 2019, 2018; Power et al., 2018). Consistent with these human findings of strong low-frequency physiological modulation, a recent mice study demonstrated very strong arterial constrictions/dilations during sleep in the same frequency range (<0.1 Hz) (Turner et al., 2020). Such low-frequency vessel modulations were coupled by pupil size changes suggestive of transient arousal modulations (Turner et al., 2022), similar to the global brain activity measured by gBOLD (Liu et al., 2021, 2018; Pais-Roldán et al., 2020; Turchi et al., 2018). Animal research also suggested that the gBOLD is coupled by large calcium signals of astrocytes (Pais-Roldán et al., 2020), and the AQP4 channels on the endfeet of these cells are a
key player of the glymphatic system (Iliff et al., 2012; Jessen et al., 2015). To date, the key evidence linking the gBOLD to the glymphatic function came from a human study showing that the gBOLD is coupled to large CSF movements (Fultz et al., 2019). Based on this, the gBOLD-CSF coupling was used to quantify glymphatic function and found correlated with various pathologies of AD and cognitive decline in PD (Han et al., 2021b, 2021a). The preference reduction of gBOLD signal in the higher-order default mode network was found to account for early, preferential β-amyloid accumulations in the same regions at the early stage of AD (Han et al., 2022). All these findings established the foundation for using the gBOLD-CSF coupling to measure the glymphatic function. But a direct proof of their relationship would need future experiments capable of recording brain signals across distinct spatial and temporal scales. It is also worth noting that the debate is ongoing regarding specific components of glymphatic theory, e.g., the convective flow in the interstitial space and the involvement of AQP4 channels in the process (Abbott et al., 2018; Hladky and Barrand, 2022). Nevertheless, a consensus view is reached regarding the existence of periarterial CSF flow (Mestre et al., 2018) and its role in waste clearance (van Veluw et al., 2020), which is more related to the gBOLD-CSF coupling in the present study.

Brain aging affects the glymphatic function. The CSF inflow of larger tracers or macromolecules decreased up to 85% in old wild-type mice as compared to young counterpart (Da Mesquita et al., 2018; Nedergaard and Goldman, 2020). The decreased glymphatic flow in aged mice has been partly attributed to dysregulation of astroglial water transport due to the widespread loss of AQP4 polarization (Kress et al., 2014), the decline of CSF pressure (Fleischman et al., 2012), and the changes of CSF secretion and protein content (Chen et al., 2009). Arterial wall stiffening and associated reduction of arterial pulsatility (Zieman et al., 2005) may also account for age-related
glymphatic reduction (Iliff et al., 2013). Human studies of glymphatic function remain sparse (Eide et al., 2018; Zhou et al., 2020). Previous studies using the gBOLD-CSF coupling found a consistent association between the age and glymphatic function, but only in patients of relatively old ages (Han et al., 2021a, 2021b). A retrospective study combined contrast-agent MRI data from various patient groups to study the glymphatic function and its change with age (Zhou et al., 2020). Despite different methodologies and patient cohorts from the present study, a similar age trajectory was observed for the glymphatic function: it remains stable before 50 yrs and then begins to decline since then (Zhou et al., 2020). The age-related glymphatic changes could be critical for the aging-related risk for neurodegenerative diseases (Hou et al., 2019). The glymphatic dysfunction may result in inadequate clearance and thus accumulation of toxic proteins, such as Aβ and tau, and thereby increase the vulnerability to developing cognitive impairments and neurodegenerative diseases (Jessen et al., 2015; Tarasoff-Conway et al., 2015). Epidemiologic research suggested the late-onset AD, the most common AD variant, starts around the mid-60s with the prevalence doubled every 5 years afterwards (Qiu et al., 2009). But the pathophysiological process, including the accumulation of aggregated Aβ, could begin more than a decade before the dementia (Jack et al., 2013; Sperling et al., 2014). Together, these findings suggested a timeline consistent with our finding that the glymphatic function begins to decrease at age mid-50s.

The female sex is another leading risk factor for developing AD (Mielke et al., 2014). It has been found a significantly weaker glymphatic function, as measured by gBOLD-CSF coupling strength, in females than in males (Han et al., 2021b). Here we confirmed the finding with a much larger dataset from healthy populations. Importantly, we further showed that the glymphatic function had different age trajectories in the two groups with the females displaying a larger and more abrupt...
Decline at around 55 yrs. In fact, women indeed show larger and faster cognitive declines than men with aging (Levine et al., 2021; Nooyens et al., 2022). Together with increasing evidence that links the glymphatic dysfunction and cognitive impairments (Da Mesquita et al., 2018; Iliff et al., 2014; Zou et al., 2019), the sex-specific glymphatic change with aging may provide a possible explanation for the sex differences in age-related cognitive decline. The menopause and associated hormone loss have been suggested to contribute to cognitive decline in females (Brown and Gervais, 2020; Hachul et al., 2015). Our result is not inconsistent with this notion by showing a marginally significant ($p = 0.061$) interaction between age and menopause on the coupling metrics. Among the females of age 36-55, the post-menopausal group appeared to show a decline of gBOLD-CSF coupling with age, which is absent in the non-menopause group. A limited sample size and information related to menopause could partially account for statistical non-significance. But the finding should warrant future studies looking into the menopause effects on the glymphatic function with a refined design and/or augmented dataset.

Sleep quality appeared not to be a major contributor to the age-related glymphatic changes. The gBOLD-CSF coupling and age are similarly correlated with a few sleep measures, including the frequency of using sleep medication and the sleep troubles in the month prior to the experiments. However, the age-related changes in the gBOLD-CSF coupling remained largely unchanged after adjusting for these sleep measures. Nevertheless, the change in sleep architecture might be related to the sex difference seen in the age-related glymphatic changes. The age and sex are known to have a strong interaction effect on the composition of sleep stages. Aging in males is associated with a significant increase of light sleep (stages 1&2) but decrease in slow wave sleep (SWS: sleep stages 3&4), whereas this age-related change is absent in women (Mander et al., 2017; Redline et
al., 2004). It is known that the glymphatic function increases during sleep and anesthesia featuring strong slow wave activity (SWA) (Hablitz et al., 2019; Ju et al., 2017; Xie et al., 2013), and one would thus expect an improved glymphatic clearance with a higher percentage of SWS. The empirical evidence, however, suggested an opposite by showing higher SWS is associated with lower CSF Aβ42 level (Varga et al., 2016), which is an early indicator of preclinical AD and often accompanied by cortical Aβ accumulation (Jack et al., 2013; Palmqvist et al., 2017). The paradox might be explained by an observation about the gBOLD and its coupling with CSF flow. They were more specifically related to ultra-slow (0.6-1 Hz) component of SWA (often related to K-complexes) (Özbay et al., 2019) and phasic changes of SWA power (Fultz et al., 2019; Gu et al., 2022), which could be stronger during the light sleep than SWS. Indeed, the SWA was found to decrease in subjects with more cortical Aβ and poorer memory consolidation (Mander et al., 2015; Winer et al., 2020), as well as AD patients (De Gennaro et al., 2017), but the reduction was specific to its ultra-slow component (0.6-1 Hz) with the delta-band (1-4 Hz) power showing opposite changes. Based on all these findings, it is possible that the age-related increase in the percentage of light sleep in males may help to offset some age-related decline in glymphatic function and thus lead to its slow deterioration as compared with females. However, the test of this hypothesis would have to be left for future studies, particularly those with assessment of subjects’ sleep architecture.

Methods

Participants and study data

This study included 719 healthy human subjects (36–100 yrs; 403 females) who have participated in all 4 sessions of rsfMRI scanning in the HCP-A project.
For these subjects, we also used their T1-weighted structural MRI imaging and demographic data, such as the age, sex, and the menstrual cycle of females. These “typical aging” subjects were healthy for their age without identified pathological causes of cognitive declines, such as stroke or clinical dementia (Bookheimer et al., 2019). All participants provided written informed consent, and investigators at each HCP-A participating site obtained ethical approval from the corresponding institutional review board (IRB).

The use of de-identified data from the HCP-A and the sharing of analysis results have been reviewed and approved by the Pennsylvania State University IRB (IRB#: STUDY00008766) and also strictly followed the National Institute of Mental Health (NIMH) Data Archive-data use certification (DUC).

**Image acquisition and preprocessing**

The rsfMRI data were acquired at 3T MR scanners (Siemens Medical Solutions; Siemens, Erlangen, Germany) with a matched protocol (Harms et al., 2018) across four acquisition sites including Washington University St. Louis, University of Minnesota, Massachusetts General Hospital, and University of California, Los Angeles (researchers in Oxford University dedicating to the data analysis). For each subject, 4 sessions of rsfMRI (including the anterior to posterior phase encoding (PE) from Day1, i.e., AP1, as well as PA1, AP2, and PA2) were followed by one T1-weighted structural MRI session (MPRAGE sequence, echo time (TE)= 1.8/3.6/5.4/7.2 ms [multi-echo], repetition time (TR) = 2,500 ms, field of view (FOV) = 256 × 256 mm², 320 × 300
matrix, number of slices = 208, voxel size = 0.8 × 0.8 × 0.8 mm³, flip angle = 8°) (Harms et al., 2018). The T1-weighted MRI served to provide the whole brain and CSF volume information and was used for the anatomical segmentation and registration. For rsfMRI acquisition, 488 fMRI volumes were collected with a multiband gradient-recalled (GRE) echo-planar image (EPI) sequence (TR/TE=800/37 ms, flip angle=52°, FOV = 208 mm, 104 × 90 matrices, 72 oblique axial slices, 2 mm isotropic voxels, multiband acceleration factor of 8).

We referred to the previous study (Han et al., 2021b) in preprocessing the rsfMRI data using the FSL (version 5.0.9; https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FSL) (Smith et al., 2004) and AFNI (version 16.3.05; https://afni.nimh.nih.gov/) (Cox, 1996). The general fMRI preprocessing procedures included motion correction, skull stripping, spatial smoothing (full width at half maximum (FWHM) = 4mm), temporal filtering (bandpass, approximately 0.01 to 0.1 Hz), and the co-registration of each fMRI volume to corresponding T1-weighted structural MRI and then to the 152-brain Montreal Neurological Institute (MNI-152) space. The motion parameters were not regressed out to avoid attenuating the gBOLD signal (Gu et al., 2020; Han et al., 2021b). The preprocessing of structural images was performed using FSL. Processing steps included spatial normalization and skull stripping.

**Extract gBOLD and the CSF inflow signals and compute their coupling**

We followed the previous studies (Han et al., 2021b, 2021a) to extract the gBOLD signal and CSF inflow signal. We defined the mask of the gray matter regions based on the Harvard-Oxford cortical and subcortical structural atlases (https://neurovault.org/collections/262/). We then
transformed the gray-matter mask from the MNI-152 space back to the original space of each session to avoid spatial blurring from the registration process (Fultz et al., 2019), and spatially averaged the Z-normalized gray-matter rsfMRI signals to obtain the gBOLD signal. Following the previous study (Han et al., 2021b), the CSF inflow signal was extracted from the CSF region at the bottom edge of fMRI, with a similar voxel number of CSF ROI for all subjects/sessions (see an example in Fig. S1A). We extracted the CSF ROI from the preprocessed fMRI signal at the original individual space referring to the corresponding CSF region below the bottom of the cerebellum from the high-resolution T1-weighted MRI (see an exemplary time-series of gBOLD and bottom CSF fMRI in Fig. S1B).

The cross-correlation function was calculated on the extracted gBOLD signal and the CSF inflow signal for each fMRI session from each individual subject. The cross-correlation function quantified the Pearson’s correlation at different time lags. We first averaged all the cross-correlation functions from the 4 individual fMRI sessions (AP1, PA1, AP2, and PA2) for each subject, and further averaged the functions across all subjects (Fig. S1C). Referring to the previous studies (Han et al., 2021b, 2021a), we quantified the gBOLD–CSF coupling with the session-mean cross-correlation at the lag of +3.2 seconds, where the negative peak of the subject-mean cross-correlation located, for each subject.

The relationship between the gBOLD–CSF coupling and age

To access the association between the gBOLD–CSF coupling and age, we first divided all the subjects into 10 sub-groups with different ages (based on the deciles), and further calculated and
compared the mean gBOLD–CSF coupling for each sub-group. Moreover, all the subjects were separated into the younger (age < 53.9 yrs) and older (age ≥ 53.9 yrs) groups. The linear regression was used to evaluate the association between the ages and the coupling measures for the subjects in each sub-group.

Several sensitivity analyses were then performed to test whether the age-coupling association would be driven by the various sleep quality measures (i.e., PSQI measures), the brain volume, as well as the CSF volume. We included the total PSQI score, as well as 4 different PSQI items, which were selected due to their strong dependence ($p < 0.05$ for linear regression or ordinal regression) with the coupling measures or age, covering the components/aspects of sleep medication, trouble sleeping, and sleep hours in the sensitivity test. In the test, each of these PSQI measures was first linked to the coupling measures and age, and then regressed out from the coupling measures to further examine the age-coupling associations in Fig. 1. Similar age-coupling association tests were applied on the whole brain volume and CSF volume, respectively. The whole brain volume was accessed by the volume number of all brain regions excluding the CSF area from the T1-weight MRI, where the CSF regions mainly from all the ventricles were extracted to quantify the CSF volume. To test whether the head motion would drive the age-coupling association, we adjusted the gBOLD-CSF coupling for the head motion from each rsfMRI acquisition, which was quantified by the session-mean framewise displacement (FD), following the previous study (Han et al., 2021b) and replicated the analysis in Fig. 1. The FD was calculated as the sum of the absolute value of all 6 translational and rotational realignment parameters derived from the preprocessing (Power et al., 2012). We did not use motion-censoring methods (Power et
al., 2014, 2012) to avoid the influence from the cross-correlation of the concatenated timeseries of gBOLD and CSF fMRI signals.

Sex-specific coupling changes with aging

We further compared the gBOLD–CSF coupling strength between the male and female subjects, as well as the respective trajectory of coupling changing with aging. First, we compared the coupling measures between sexes with a two-sample t-test. Second, we divided the males or females into 7 sub-groups (one group per 10 years; starting from 36–45 yrs, i.e., 36 ≤ age < 46), respectively, and compared the coupling measures across these sub-groups.

To examine whether the menopause would affect the tendency of the coupling measure changing with aging, we separated all the females into the “post-menopause” and “other females” groups (based on the measure of “whether having no period for 12 months” in the “menstrual cycle” data), divided the “other females” into two stages of the “36–45 yrs” and “46–55 yrs”, and compared the coupling measures between the two stages. Similarly, we also selected the same age range/sub-groups for the post-menopausal subjects and contrasted the corresponding coupling measures between the two sub-groups, as well as compared the coupling across the “post-menopause” and “other females” subjects for each age-stage. Furthermore, we applied the same grouping metric on the “post-menopause” subjects as the entire group of females above, i.e., 7 groups with an age duration of 10 years (from 36–45 yrs), and then replicated the analyses for whole females to observe the trajectory of the coupling changing with aging.
Statistical analysis

The present study used the two-sample t-test for the group comparison of continuous variables, including the coupling difference of neighboring groups and that between males and females. The linear regression was applied to estimate the association between age and gBOLD–CSF coupling for the younger group and older group, respectively. The ordinal regression was used for responses with natural ordering among categories (i.e., the association between the coupling measure or age and these PSQI measures). The cross-correlation function was used to evaluate the relationship between the gBOLD signal and CSF inflow signal at different time lags. We also tested the interaction effects of age and menopause on the coupling measure. In the study, Pearson’s correlation was employed to access the inter-subject associations between different variables. A p-value less than 0.05 was considered statistical significance.

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Competing Interests:

The authors report no financial interests or potential conflicts of interest.

Data and Materials Availability:

The multimodal data, including subject characteristics, structural MRI, and rsfMRI are all publicly available at the NDA website upon the approval of the data use application (https://nda.nih.gov/get/access-data.html). All the code used in the present study are available from the corresponding author upon request.
Fig. 1 The change of gBOLD–CSF coupling with age. (A) The gBOLD–CSF coupling remains relatively stable before age mid-50s and then begins to decrease since then. The entire cohort was grouped into 10 subgroups of equal size. Each error bar represents one standard error of the mean. (B) The gBOLD-CSF coupling was significantly correlated with age for the group of subjects over 53.9 yrs \( (r = 0.14, p = 3.9 \times 10^{-3}; \text{gray dots at the right}) \), but not so \( (r = -3.7 \times 10^{-3}, p = 0.95; \text{black dots at the left}) \) for the younger group. Each dot represents one subject.
Fig. 2 The age-related changes in gBOLD-CSF coupling remain similar with controlling for sleep measures. The gBOLD-CSF coupling (left) and age (middle) are similarly correlated with a few sleep-related measurements in PSQI, including the sleep medication frequency (2nd row), the frequency of bathroom-use during sleep-time (3rd row), the frequency of having pain during sleep-time (4th row), and sleep hours (5th row), but not the total PSQI score (1st row). However, the
age-related changes in gBOLD-CSF coupling remain similar with adjusting for these PSQI measures (right). Each dot represents one subject, and error bars represent the standard error of the mean.
Fig. 3 The age-coupling association showed distinct patterns between males and females. (A) The gBOLD-CSF coupling is significantly weaker in females (red) than in males (blue) ($p = 7.9 \times 10^{-6}$, two-sample t-test). (B) The age-related changes of the gBOLD-CSF coupling were summarized separately for the females and males and showed different patterns of trajectory: gBOLD–CSF coupling in the females showed a steep decline around 55 yrs ($p = 1.1 \times 10^{-3}$ for two consecutive subgroups around that age), in contrast to the slow and gradual decreases in the males. (C) The gBOLD-CSF coupling trends in the “Post-menopause” and “Other females” groups. All females above age 55 are post-menopause. Within the age range of 36-55, the menopause status and age showed a marginally significant ($p = 0.061$) interaction effect on the gBOLD-CSF coupling. The coupling index started to decrease early at age mid-40s and thus its reduction around age mid-50s is less significant ($p = 0.058$) as compared with the entire female group.
Fig. S1 gBOLD is coupled with CSF changes in HCP-Aging data. (A) Top: CSF region at bottom fMRI slice shown in an example subject; Bottom: location of bottom rsfMRI slice marked in corresponding structural MRI (dashed line). (B) gBOLD (blue) and CSF (red) rsfMRI signals showed a coupled change (black arrows) from a representative example. (C) Averaged cross-correlation function between gBOLD and CSF across 719 subjects. The red dashed line marks the time lag (+3.2-sec) where the negative peak of the mean cross-correlation occurs. The shaded regions represent the area within one standard error of the mean.
Fig. S2 The age-coupling association was not affected by head motion during fMRI acquisition. Similar results as Fig.1 were found when the mean FD was regressed out from the gBOLD-CSF coupling for each rsfMRI session.
Fig. S3 The age-coupling association was not dominant by the whole brain volume. Consistent results as Fig.1 were found when we regressed out the whole brain volume (ventricles excluded) from the gBOLD-CSF coupling for each rsfMRI session.
Fig. S4 The age-coupling association was not affected by the whole CSF volume. Similar results as Fig.1 were found when the volume of the whole CSF area was regressed out from the gBOLD-CSF coupling for each rsfMRI session.


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