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**Human CSF Movement Influenced by Vascular Low Frequency Oscillations and
Respiration**

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Abstract

Background: Cerebrospinal fluid movement (CSF) through the pathways within the central nervous system is of high significance for maintaining normal brain health and function. Low frequency hemodynamics and respiration have both been shown to independently drive CSF in humans. Here, we hypothesize that CSF movement may be driven simultaneously (and in synchrony) by both mechanisms and we study their independent and interactive effects on CSF movement using novel neck fMRI scans.

Methods: Caudal CSF movement at the fourth ventricle and hemodynamics of the major neck blood vessels (internal carotid arteries and internal jugular veins) were captured from 11 young healthy volunteers using novel neck fMRI scans with simultaneous measurement of respiration. Two distinct models of CSF movement (1. Low-frequency hemodynamics and 2. Respiration) were independently investigated in corresponding frequency ranges. Possible interactions between these mechanisms were also studied using cross-frequency coupling.

Results: The results from this study validated that the caudal CSF movement may be driven by both low frequency hemodynamics (0.01 Hz – 0.1 Hz) and respiration (0.2 Hz - 0.4 Hz), through different mechanisms. We show that the dynamics of brain fluids can be assessed from the neck, by studying the interrelationships between major neck blood vessels and the CSF movement at the fourth ventricle. We also demonstrate that there exists a cross-frequency interaction between two separable mechanisms.

Conclusions: The human CSF system is capable of responding to multiple interacting physiological forces at the same time. This information may help inform the pathological mechanisms behind CSF movement-related disorders and facilitate new approaches to therapeutic interventions.

Keywords

Cerebrospinal fluid, Low-frequency oscillations, Respiration, Neck fMRI scans

1. Introduction

Cerebrospinal Fluid (CSF), an ultrafiltrate of blood plasma, flows through the ventricles of the human brain and the subarachnoid spaces of the cranium and spine. Predominantly secreted by the choroid plexuses, CSF is known to perform several critical functions essential for brain health and function [1]. In addition to offering mechanical protection and support to the brain and spinal cord, it also maintains the brain homeostasis by transporting nutrients, hormones and other immunologic factors through the central nervous system (CNS) [2–4].

The flow of CSF in the CNS garnered increased interest after the relatively recent proposal of the glymphatic system. According to this model, the flow of CSF through the brain's perivascular pathways and further exchange with the interstitial fluid (ISF) is instrumental in removing the metabolic waste products of the brain [5,6]. Furthermore, emerging evidence has also linked reduction in CSF flow to increased amyloid-beta concentrations in the context of neurodegenerative disorders such as Alzheimer's disease [7,8] and elevated tau concentrations with chronic traumatic encephalopathy [9].

Despite the crucial physiological roles and clinical significance of CSF dynamics, the exact driving forces behind CSF flow in humans are still poorly understood. To date, animal model studies have explained a brain-wide perivascular pathway that aids the exchange between CSF and ISF for interstitial waste clearance, primarily driven by cerebral arterial pulsations [5,10,11], although some studies have argued that these pulsations alone are too weak to drive the clearance [12,13]. A recent study on awake mice has reported spontaneous contractions and relaxations of

the vascular smooth muscle cells in the low frequency range (<0.1 Hz), referred to as vasomotion, to be the principal driver for this perivascular clearance pathway [14].

Different mechanisms are currently debated in the literature as the motive force propelling CSF flow in humans. Studies using phase contrast magnetic resonance imaging (MRI) have identified respiration as a major regulator of CSF flow [15–18]. Specifically, Dreha-Kulaczewski et al., quantified a net upward flow of CSF into the cranium from the spinal canal and its relation to the venous outflow, during a deep breathing regimen. More recently, a functional MRI (fMRI) study by Fultz et al. reported coupling between cranially directed CSF flow in the fourth ventricle and cerebral hemodynamic signals in the low frequency range (<0.1 Hz) during non-rapid eye movement (NREM) sleep [19]. Expanding on this line of work, we recently proposed a biomechanical model of CSF dynamics, suggesting low frequency brain hemodynamic signals (0.01 Hz - 0.1 Hz) as the fundamental driving force for CSF flow in both directions (i.e., cranial and caudal), in the awake state [20].

In this study, we use fMRI to explore the different mechanisms driving the CSF flow. We hypothesize that more than one mechanism simultaneously (and in synchrony) drives the CSF movement: 1) A hemodynamic mechanism that drives CSF movement in the low-frequency range (0.01 Hz - 0.1 Hz), and 2) respiration, which explains CSF movement in the respiratory frequency range (0.2 Hz - 0.4 Hz). For this study, we conducted neck fMRI scans (instead of typical brain scans) for the following reasons: 1) All the brain fluids (blood, CSF) are transported into/out of the brain through the neck so that the flow dynamics in the neck offers a simple, direct way to assess the input/output fluid dynamics of the brain, as well as their relationships; 2) Respiration has been shown to directly and mechanically affect the large draining veins in the neck (i.e., inspiration leading to collapse of the internal jugular veins (IJV)), in turn leading to internal

pressure changes in the brain. Therefore, measuring these signals in the neck and their relationships to respiration are crucial. Furthermore, we explore the cross-frequency interactions between respiration and low-frequency oscillations (LFOs), particularly in the context of the observed CSF dynamics.

2. Methods

2.1. Participants

This study included a total of 11 healthy participants (7 females and 4 males) aged 20 – 25 (21.4 ± 1.4) years. The study was approved by Purdue University's Human Research Protection Plan (IRB-2020-1329) and was conducted in accordance with application of Belmont Report principles (Respect for Persons, Beneficence, and Justice) and federal regulations 45 CFR 46, 21 CFR 50, 56. Written informed consent was obtained from all participants.

2.2. Mechanisms of CSF Dynamics

The unique feature of the neck fMRI scan [20] is used in this study to explore different mechanisms that contribute to CSF dynamics. Here we investigate two distinct models based on the classic Monro-Kellie doctrine which stipulates that the total volume of brain parenchyma, CSF, and intracranial blood remains constant: 1) A hemodynamic model in the low frequency range: 0.01 Hz – 0.1 Hz and 2) A respiration model in the respiratory frequency range: 0.2 Hz – 0.4 Hz. The Monroe-Kellie doctrine implies that a change in volume of any one brain component would lead to a compensatory change in volume of at least one of the other components [21].

2.2.1. Hemodynamic Model

For the hemodynamic model, we hypothesize that the net cerebral blood volume (CBV) changes in both the arteries (main contribution) and veins are the dominant forces moving the CSF. As found previously, a CBV change initially detected in ICAs can be detected later in the IJVs

[22]. Since ICAs are the main blood supply to the brain, the arterial volume changes in ICAs precede the CBV changes in the brain tissue, before eventually producing a net BOLD signal change in the IJVs. (Note that the ICAs produce a BOLD signal change, but for normal oxygen saturation >98% the arterial blood is more diamagnetic than water and this produces an inverted BOLD signal compared to the later positive BOLD signal in the IJVs.) The cumulative effects of CBV changes within the brain parenchyma (i.e., net CBV) exert forces on the lateral ventricle walls. As a result, the CSF will be forced in/out via the fourth ventricle [20]. In parallel, the CBV change in arteries and arterioles leads to passive blood volume and CBF changes in the draining veins. The latter evokes detectable BOLD signal in the IJVs a few seconds later.

Based on the model (see figure 1), we predict the following: 1) Dilation of the ICAs leads to 2) a CBV increase in the brain which causes 3) caudally directed CSF movement at the fourth ventricle and leads to 4) a BOLD signal change in IJVs. Since the low frequency CBV changes progress at the speed of the blood flow [23], the delays between ICA signal and CSF movement in the LFO range (0.01 Hz – 0.1 Hz) should be around 5 - 6 seconds in a healthy subject.

Figure 1: The hemodynamic model of CSF dynamics. (A) Model illustrating changes in vessel volume dynamics in the LFO range sequentially starting with 1) Dilation of ICA which leads to 2) Expansion of cerebral blood vessels which finally leads to 3) Caudally directed CSF movement out of the brain and (4) with the same LFOs in the brain travelling to the IJVs with the blood flow (B) Illustrative time series representation of changes in vessel volume dynamics that

leads to caudally directed CSF movement and corresponding fMRI signals. The crests in the representation indicates positive change/increase. ICA – Internal Carotid Artery; IJV – Internal Jugular Vein; CSF - Cerebrospinal Fluid; fMRI – functional Magnetic Resonance Imaging.

2.2.2. Respiration Model

Respiration has recently been identified as a major driver of CSF flow, in the context of Monro-Kellie hypothesis. Dreha-Kulaczewski et al. reported CSF flows upward from the spinal canal in the cranial direction almost instantaneously to compensate for the deep inspiration-induced venous outflow of blood from the brain through the draining veins in the neck [17] (see figure S1 in the supplementary material).

It is well known that the expiration phase causes the intrathoracic volume to decrease and the intrathoracic pressure to increase, leading to reduced venous return to the heart. During inspiration, decreasing intrathoracic pressure causes increased venous return. As illustrated in figure 2, the increase in intrathoracic pressure leads to the following: the venous drainage from the cerebral blood vessels into the Internal Jugular Veins (IJV) decreases, causing cerebral blood vessels to expand [24] and thereby leading to an increase in the CBV. In addition, the undisturbed arterial input from ICAs might also contribute to this increase in CBV. This then, as per the Monro-Kellie doctrine, immediately leads to a compensatory movement of CSF from the brain into the spinal canal through the fourth ventricle. It is to be noted here that the CSF flow, according to this model, happens only with a change in intrathoracic volume/pressure.

Based on the model (see figure 2), we predict the following: 1) Intrathoracic pressure increase (volume decrease) during expiration causes 2) an IJV drainage decrease which leads to 3) CBV increase and consequent parenchyma pressure increase that invokes 4) caudally directed CSF movement at fourth ventricle. We expect that the delays between the detectable signals in the

respiratory frequency range (0.2 Hz – 0.4 Hz) will be no more than 1 – 2 seconds, since the venous drainage slows down almost immediately with expiration-related pressure change [24].

Figure 2: The Respiration model of CSF dynamics. (A) Model illustrating how a negative change in intrathoracic volume/positive change in intrathoracic pressure affect CSF dynamics sequentially starting with 1) Pressure coupling to IJV which leads to 2) Reduction in venous drainage in the IJV which in turn leads to 3) Increase in the volume of intracranial blood and finally results in 4) Caudally directed CSF movement out of the brain. (B) Illustrative time series representation of intrathoracic pressure-change derived mechanism of CSF flow and corresponding fMRI signals. The crests and troughs in the representation indicates positive change/increase and negative change/decrease respectively. ICA – Internal Carotid Artery; IJV – Internal Jugular Vein; CSF - Cerebrospinal Fluid; fMRI – functional Magnetic Resonance Imaging.

2.3. Experimental Design

A pictorial description of the experimental design and data analysis stream is given in figure 3.

Figure 3: Experimental Design and Data Analysis Stream. (A) Typical fMRI scan design illustrating the capture of CSF movement into the neck scan volume utilizing inflow effect and the (D) corresponding raw CSF movement signal. (B) Large blood vessels of the neck (Left and Right ICA (red) and IJV (blue)) identified and overlaid on structural T1-weighted image and the (E) corresponding ICA (red) and IJV (blue) fMRI signals. (C) Placement of MRI chest belt and the corresponding (F) Raw chest-belt respiratory signal. LFOs and ROs were extracted from CSF and blood vessels and used in the (G) data analysis techniques to study the effects on CSF dynamics. ICA – Internal Carotid Artery; IJV – Internal Jugular Vein; CSF - Cerebrospinal Fluid; LFO – Low frequency Oscillations (0.01 Hz – 0.1 Hz); RO – Respiratory Oscillations (0.2 Hz – 0.4 Hz).

2.3.1 MRI Data Acquisition

All participants were scanned using a 3T SIEMENS MRI scanner (Magnetom Prisma, Siemens Medical Solutions, Erlangen, Germany) with a 64-channel head coil. Structural T1-weighted MPRAGE (Magnetization Prepared Rapid Acquisition Gradient Echo) images were

acquired with the following parameters: TR/TE: 2300/2.26 ms, 192 slices per slab, flip angle: 8° , resolution: $1.0\text{mm} \times 1.0\text{mm} \times 1.0\text{mm}$. Structural 3D SPACE (Sampling Perfection with Application optimized Contrasts using different flip angle Evolution) T2-weighted images were acquired with the following parameters: TR/TE: 2800/409 ms, 208 slices per slab, resolution: $0.8\text{mm} \times 0.8\text{mm} \times 0.7\text{mm}$. Resting state functional MRI (fMRI) scans of the neck were acquired using: FOV = 230 mm, acquisition matrix = 92×92 , 48 slices, voxel size = $2.5 \times 2.5 \times 2.5\text{ mm}$, TR/TE = 960/30.6 ms, echo-spacing = 0.51 ms, flip angle = 35° , multiband acceleration factor = 8, multi-slice mode: interleaved. TR was changed to 440 ms in three participants and to 480 ms in two of them. All participants wore a chest belt (figure 3C) to measure chest motion.

The fMRI scans of the neck region were designed to capture the caudally directed CSF movement utilizing the inflow effect [19]. As illustrated in figure 3A, by placing the top edge of the scan volume appropriately at the fourth ventricle, an intense CSF flow signal directed into the scan volume can be obtained. Consequently, the slice of interest at the fourth ventricle, was always acquired first in the scan. We chose to evaluate the CSF movement at the level of the fourth ventricle, since it is the channel through which the CSF exits the brain into the spinal canal. Moreover, the narrow-tapered structure of the fourth ventricle limits the flow of CSF in other directions, thereby strengthening the inflow effect. It is worth noting that the flow-weighted signal in the opposite direction (i.e., CSF flowing towards the brain) is very weak in the top slice of the scan (figure 3D) and we do not attempt to interpret these small negative signal changes.

2.3.2. Data Processing

Pre-processing: MR data were processed using FSL (FMRIB Expert Analysis Tool, v6.01; Oxford University, UK [25]) and MATLAB (MATLAB 2020b, The MathWorks Inc., Natick, MA, 2000). Firstly, the CSF signal was extracted from a voxel in the fourth ventricle identified by

overlaying the fMRI data over the structural T1-weighted image registered to the fMRI data (see figure 3A and 3D). Care was taken to identify the signal from the center of the fourth ventricle, in such a way that the CSF signal comes from a voxel with negligible partial-volume effects from surrounding tissues. The fMRI data were only corrected for slice-timing (FSL SLICETIMER) before extracting the single voxel CSF signal. No motion-correction was applied [19,20]. To confirm that the CSF signals were not corrupted by motion, we assessed and document no significant correlations between motion parameters (FSL MCFLIRT) and the CSF signals (see table S1 in the supplementary material). Having obtained a CSF-only signal from the fourth ventricle without motion correction, motion correction (FSL MCFLIRT) and slice-timing correction (FSL SLICETIMER) were applied to fMRI data used in subsequent analyses.

Blood Vessel Extraction: The left and right ICAs and IJVs were identified using T1-weighted and T2-weighted structural images and these vessel masks (figure 3B), registered on to the functional space, were used to extract the corresponding fMRI time series (figure 3E). These methods have been used and validated in previous studies [22,23,26].

Hemodynamic Model Verification Analysis: The ICA, IJV and CSF signals were linearly detrended and demeaned. For analysis of the hemodynamic model based on LFOs, the signals were bandpass filtered to the range of 0.01 Hz – 0.1 Hz, using a zero delay fourth-order Butterworth filter to extract the corresponding LFOs. The LFOs of left and right ICAs and IJVs exhibited high correlations (Right-Left ICAs: 0.57 ± 0.10 and Right-Left IJVs: 0.64 ± 0.13). Hence, the vessel signals of the same kind were averaged for each participant to increase the signal-to-noise ratio (SNR).

As we demonstrated in our previous work [20], the changes of CBV rather than CBV itself, are the driving force of CSF movement. Without the CBV changes, no force will be exerted on the

ventricles. Therefore, instead of the fMRI signal itself, we used the derivative of the fMRI signal to reflect CBV changes in the ICAs and the corresponding downstream CBF changes in the IJVs. Further, maximum cross-correlation coefficients (MCCCs) and corresponding time delays for each participant were calculated (MATLAB xcorr, maximum lag range: ± 15 seconds) between (1) $\frac{d}{dt}(\text{LFO}_{\text{ICA}})$ and LFO_{CSF} and (2) $\frac{d}{dt}(\text{LFO}_{\text{IJV}})$ and LFO_{CSF} . The absolute maximum value from the calculated CCCs was identified as the MCCC with its original arithmetic sign. Based on previous research, only the MCCCs above the statistically established threshold of 0.3 (p-value<0.01 for positive MCCCs) or below -0.3 (p-value<0.01 for negative MCCCs) are considered significant in the LFO range [23,27]. Accordingly, non-parametric one sample Wilcoxon signed rank test against 0.3/-0.3 was applied on MCCCs in the LFO range.

Respiration Model Verification Analysis: For analysis of the respiratory model of CSF dynamics, the fMRI signals and the chest belt respiratory signals were bandpass filtered to the range of 0.2 Hz – 0.4 Hz, using a zero delay fourth order Butterworth filter to extract the corresponding respiratory oscillations (RO). The frequency range of respiration affecting the CSF signals for each participant was identified from the results of cross-spectral analysis between them (see figure S2 in the supplementary material). As in the hemodynamic model verification analysis, the ROs (high correlations between right-left ICAs: 0.37 ± 0.12 and right-left IJVs: 0.48 ± 0.12) from the same kind of vessels were averaged for each participant to increase the SNR.

The difference in pressure between the right atrium of the heart and the brain is the key modulator of venous return from the brain [24]. The intrathoracic pressure changes during respiration are coupled to the right atrial pressure and thereby modulate the venous drainage from the brain. (A detailed explanation of how the intrathoracic pressure changes modulate the venous return from the brain is provided in figure S3 in the supplementary material.) To estimate the

instantaneous changes in this pressure differential, we use the derivative of chest respiratory oscillations ($\frac{d}{dt}(\text{RO}_{\text{Chest}})$) to represent the instantaneous changes in intrathoracic volume, hence the intrathoracic pressure. Maximum cross-correlation coefficients (MCCCs) and corresponding time delays for each participant were calculated (MATLAB xcorr, maximum lag range: ± 15 seconds) between: (1) $\frac{d}{dt}(\text{RO}_{\text{Chest}})$ and RO_{CSF} ; (2) $\frac{d}{dt}(\text{RO}_{\text{Chest}})$ and RO_{IJV} ; and (3) $\frac{d}{dt}(\text{RO}_{\text{Chest}})$ and ICA respiratory oscillations (RO_{ICA}). In this model, MCCCs and time delays were calculated by forcing the $\frac{d}{dt}(\text{RO}_{\text{Chest}})$ to lead the other signals, under the assumption that respiration is the driver.

Since the signals in the respiratory range can be highly periodic, there is a risk of spurious cross correlation errors. To reduce false positives on our cross-correlation results in the respiration model, we first limited the cross-correlation window to reflect the fact that $\frac{d}{dt}(\text{RO}_{\text{Chest}})$ signal always leads the MRI signals (based on the model that respiration initiates the whole process). Second, we sought to establish a statistically significant threshold for the MCCCs to be qualified as physiologically meaningful using existing data. To establish this threshold, we calculated the MCCCs between (1) $\frac{d}{dt}(\text{RO}_{\text{Chest}})$ and RO_{CSF} ; (2) $\frac{d}{dt}(\text{RO}_{\text{Chest}})$ and RO_{IJV} ; and (3) $\frac{d}{dt}(\text{RO}_{\text{Chest}})$ and RO_{ICA} , after mismatching the signals across participants in random orders. The whole procedure was then repeated 5000 times for each mismatched time-series combination and a distribution of about 55000 MCCCs were generated in each of the three cases to determine a statistically significant threshold for MCCCs in the respiratory range.

Cross – Frequency Coupling (CFC): CFC quantifies the interaction between signals across different frequency bands. In this study, CFC was employed to assess if there is any interaction between RO and LFO bands. Phase-Amplitude Coupling (PAC), a type of CFC, was used to quantify the intensity of CFC between amplitude of RO_{Chest} and phase of LFO_{CSF} , LFO_{IJV} and

LFO_{ICA}, using Mean Vector Length based Modulation Index [28]. Statistically significant values of modulation indices between amplitude of RO_{Chest} and phase of LFO_{CSF}, LFO_{IIV} and LFO_{ICA} were calculated at 5 percent level of significance as outlined previously [29].

3. Results

3.1. Hemodynamic Model

The results of the cross-correlation analysis for the hemodynamic model of CSF dynamics in LFOs are illustrated in figure 4. MCCCs and corresponding delay between $\frac{d}{dt}(\text{LFO}_{\text{ICA}})$ and LFO_{CSF} as well as between the $\frac{d}{dt}(\text{LFO}_{\text{IIV}})$ and LFO_{CSF} from a representative participant can be found in figure 4A. The data shows that the $\frac{d}{dt}(\text{LFO}_{\text{ICA}})$ is negatively correlated with LFO_{CSF}, with a time delay of -6.7 seconds. The negative delay indicates that the $\frac{d}{dt}(\text{LFO}_{\text{ICA}})$ signal leads the LFO_{CSF}. An earlier work on resting state fMRI has shown that LFO_{ICA} signal leads the global brain LFO signal by ~3 seconds [22]. It can therefore be inferred that global brain LFO precedes the LFO_{CSF} in the spinal canal by 3-4 seconds. Across all participants (figure 4B) there is a significant mean negative correlation of -0.43 ± 0.12 (p-value < 0.05) between $\frac{d}{dt}(\text{LFO}_{\text{ICA}})$ and LFO_{CSF}, with a time delay of -6.3 ± 1.8 seconds.

The MCCCs and corresponding delay times between $\frac{d}{dt}(\text{LFO}_{\text{IIV}})$ and LFO_{CSF}, for the same representative participant (figure 4A) yields a positive correlation with a delay of 1.92 seconds. The positive time delay indicates that the $\frac{d}{dt}(\text{LFO}_{\text{IIV}})$ signal lags the LFO_{CSF}. Across all participants (figure 4B) there is a significant mean positive correlation of 0.59 ± 0.11 (p-value < 0.05) between $\frac{d}{dt}(\text{LFO}_{\text{IIV}})$ and LFO_{CSF}, with a time delay of 1.5 ± 0.79 seconds. A detailed explanation of how the

298 LFOs move from the ICA to the IJV through the brain influencing the CSF movement to the spinal
 299 canal is provided in discussion section 4.1.

301 *Figure 4: Caudally directed CSF movement in the LFO range. Results of MCCCs and corresponding delays between*
 302 *$\frac{d}{dt}(LFO_{ICA})$ and $\frac{d}{dt}(LFO_{IJV})$ to LFO_{CSF} for (A)a representative participant and (B)for all participants enrolled in the*
 303 *study. ICA – Internal Carotid Artery; IJV – Internal Jugular Vein; CSF - Cerebrospinal Fluid; LFO – Low frequency*
 304 *Oscillation (0.01 Hz – 0.1 Hz); A.U – Arbitrary Units; MCCC – Maximum Cross-Correlation Coefficient. In group*
 305 *results (B), the green solid line represents the mean, the green dotted line represents the median, the brown whiskers*
 306 *represent one standard deviation of the raw data points jittered over a 95 percent confidence interval in cream and*
 307 *the gray dashed lines represent the thresholds of statistical significance for MCCCs in the LFO range.*

309 3.2. Respiration Model

310 3.2.1. MCCC distribution in the RO range of frequencies

311 The distribution of all the spurious MCCCs (and corresponding delays) calculated from
 312 mismatched RO range time series, i.e., between mismatched $\frac{d}{dt}(RO_{Chest})$ and RO_{CSF} ; $\frac{d}{dt}(RO_{Chest})$

313 and RO_{IJV} and $\frac{d}{dt}(RO_{Chest})$ and RO_{ICA} are displayed respectively in figure 5A, 5B and 5C. The
 314 MCCC's are mostly clustered around -0.18 and 0.18 (Fig 5, top row). In addition, the p-values from
 315 these distributions show that MCCC's less than -0.26 (for negative MCCC's) or greater than 0.26
 316 (for positive MCCC's) are statistically significant (p-value<0.05) (Fig 5, middle row). Hence, the
 317 MCCC's in the RO range were tested against 0.26/-0.26 using the Wilcoxon signed rank test. The
 318 delays (figure 5, bottom row) are evenly distributed across the cross-correlation window, which is
 319 consistent with the randomization in data pairing. A comparatively higher concentration at the
 320 boundary (12-15 seconds in figure 5A, bottom row), arises from delays which are outside the cross-
 321 correlation search window.

322
 323 Figure 5: Distribution of MCCC's and corresponding delays calculated from randomly mismatched (A) $\frac{d}{dt}(RO_{Chest})$
 324 and RO_{CSF} ; (B) $\frac{d}{dt}(RO_{Chest})$ and RO_{IJV} and (C) $\frac{d}{dt}(RO_{Chest})$ and RO_{ICA} . ICA – Internal Carotid Artery; IJV – Internal
 325 Jugular Vein; CSF - Cerebrospinal Fluid; RO – Respiratory Oscillation (0.2 Hz – 0.4 Hz); A.U – Arbitrary Units;
 326 MCCC – Maximum Cross-Correlation Coefficient.

As a final step to ensure that our cross-correlation results in the RO range were not spurious errors arising from the periodicity of the signals, we also calculated the MCCCs and delays between mismatched halves of RO range time series data for each participant, i.e., between the first half of $\frac{d}{dt}(\text{RO}_{\text{Chest}})$ and second half of each of the RO_{CSF} , RO_{IJV} and RO_{ICA} time series in turn (see figure S4 in the supplementary material). The results fall mostly outside the significant range of MCCCs, thus validating our findings in the respiration model.

3.2.2. Caudally directed CSF movement in the RO Range

Figure 6: Caudally directed CSF movement in the RO range. Results of MCCCs and corresponding delays between $\frac{d}{dt}(\text{RO}_{\text{Chest}})$ to RO_{CSF} , RO_{IJV} and RO_{ICA} for (A) a representative participant (entire signal in top row and first 50 seconds in bottom row to illustrate the correlations) and (B) for all participants enrolled in the study. ICA – Internal Carotid Artery; IJV – Internal Jugular Vein; CSF – Cerebrospinal Fluid; RO – Respiratory Oscillation (0.2 Hz – 0.4 Hz); A.U – Arbitrary Units; CCC – Cross-Correlation Coefficients; MCCC – Maximum Cross-Correlation Coefficient. In group results (B), the green solid line represents the mean, the green dotted line represents the median, the brown whiskers

represent one standard deviation of the raw data points jittered over a 95 percent confidence interval in cream and the gray dashed lines represent the thresholds of statistical significance for MCCCs in the RO range.

Figure 6A shows the MCCCs and the corresponding time delays between $\frac{d}{dt}(\text{RO}_{\text{Chest}})$ and RO_{CSF} , $\frac{d}{dt}(\text{RO}_{\text{Chest}})$ and RO_{IJV} and $\frac{d}{dt}(\text{RO}_{\text{Chest}})$ and RO_{ICA} from a representative participant. As postulated in the respiratory model, the $\frac{d}{dt}(\text{RO}_{\text{Chest}})$ is negatively correlated to RO_{CSF} , with a time delay of -1.92 seconds. Here, the negative value of time delay indicates that the $\frac{d}{dt}(\text{RO}_{\text{Chest}})$ leads the RO_{CSF} . For the same participant (illustrated in figure 6A), $\frac{d}{dt}(\text{RO}_{\text{Chest}})$ is positively correlated with the RO_{IJV} , leading it by 0.96 seconds. From these two results, we can infer that the RO_{IJV} leads the RO_{CSF} by around a second. For the group (figure 6B), $\frac{d}{dt}(\text{RO}_{\text{Chest}})$ is significantly negatively correlated to RO_{CSF} (MCCC: -0.41 ± 0.17 , p-value < 0.05), leading it by 1.73 ± 1.28 seconds, and is also significantly positively correlated to RO_{IJV} (MCCC: 0.35 ± 0.11 , p-value < 0.05), leading it by 0.89 ± 0.78 seconds (Mean and standard deviation values here were calculated after excluding the results from one participant that exhibited unrealistic delays greater than 10 seconds, that does not fit into the proposed model, due to lower SNR of the chest-belt signal). For the group, we can again infer that RO_{IJV} leads RO_{CSF} by around a second. Taken together with the significant difference between these average delay values (p-value < 0.05), these results confirm that RO_{Chest} changes affect IJVs first, leading to a change in the cerebral blood volume through the mechanism of reduced venous drainage, thereby leading to the caudally directed CSF movement into the spinal canal.

To determine if respiration might also affect the arterial supply within a time frame short enough to induce an effect measurable under the proposed venous return model, we also calculated the correlations between $\frac{d}{dt}(\text{RO}_{\text{Chest}})$ and RO_{ICA} . However, the results were not consistent, with low

correlation mean values of 0.24 ± 0.07 (p-value > 0.05) and delays of around -4 seconds (p-value < 0.05) that are outside of the time-frame of communication predicted by the respiration model.

3.3. Coupling between Respiration and LFOs

The results of cross-frequency interactions between amplitude of RO_{Chest} signal and the phase of LFO_{CSF} , LFO_{IJV} and LFO_{ICA} signals for a representative participant are delineated in figure 7A, 7B and 7C, respectively. The plots only show the values of CFC at 5% level of significance [29].

Figure 7: Cross-frequency interactions between respiration and LFOs. Results of coupling quantified by modulation index, between amplitude of RO_{Chest} signal and the phase of (A) LFO_{CSF} , (B) LFO_{IJV} and (C) LFO_{ICA} signals for a representative participant and the corresponding instantaneous time-series plots (D, E, F) of RO_{Chest} amplitude and phases of LFO_{CSF} , LFO_{IJV} and LFO_{ICA} respectively for the same participant. ICA – Internal Carotid Artery; IJV – Internal Jugular Vein; CSF - Cerebrospinal Fluid; LFO- Low frequency Oscillations (0.01 Hz – 0.1 Hz); RO – Respiratory Oscillations (0.2 Hz – 0.4 Hz).

The highest coupling is between amplitude of RO_{Chest} signal in the frequency range 0.25 – 0.3 Hz with the phases of LFO signals in the frequency of range 0.016 - 0.23 Hz. It is to be noted that the range 0.25 – 0.3 Hz is the peak frequency range of respiration for the given participant. All participants exhibited a similar pattern, having the maximum coupling with LFO signals at the peak frequency range of the respiration for the individual. However, the LFO range of maximal coupling varies across subjects (see figure S4 in the Supplementary material). This implies that respiration mostly affects a narrow range of frequencies in the LFO range. In addition, the instantaneous time-series plots (figure 7D, 7E, 7F) show that the RO_{Chest} amplitude and phases of LFO_{CSF} , LFO_{IJV} and LFO_{ICA} , appear to fluctuate in a similar manner.

4. Discussion

In this study, we have demonstrated several significant findings. First, we show that the dynamics of brain fluids can be assessed from the neck, by studying the interrelationships between major neck blood vessels and the CSF movement at the fourth ventricle. Second, we validate via fMRI neck scan that both low frequency hemodynamics (0.01 Hz – 0.1 Hz) and respiration (0.2 Hz - 0.4 Hz) influence CSF movement in the respective frequency ranges, albeit through different mechanisms. Finally, we show that there exists a cross-frequency interaction between the amplitude of respiration and phase of LFOs found in CSF, IJV and ICA. Below, we provide a physiological interpretation of these findings and their implications for CSF dynamics. In addition, we discuss the potential clinical impact of our results.

4.1. Effects of low frequency hemodynamics

In this study, we investigated the vascular LFOs (0.01 Hz – 0.1 Hz), which originated outside the brain, as a possible driving force responsible for CSF movement in humans. Our results demonstrate that the derivative of these low frequency hemodynamic oscillations extracted from

ICA and IJV in the neck, correlate with LFO_{CSF} directed toward the spinal canal within a physiologically relevant time frame. This is the first study to establish the fMRI temporal relationship between ICA and caudally directed CSF movement in the LFO range.

Evidence from our prior resting state fMRI study in awake humans illustrate that LFOs within the brain (i.e., global mean fMRI signal) represent the global cerebral blood volume fluctuations, whose collective force on the lateral ventricles results in CSF movement [20]. Results from the current hemodynamic model support and extend this phenomenon by tracing these LFOs at the ICA. We show that these LFOs enter the brain through the ICAs and take an average of 6 seconds to have the accumulative CBV effects (in the brain), finally culminating in caudally directed CSF movement observed at the fourth ventricle (Figure 4). Lastly, these LFOs appear at the IJV seconds later.

Various physiological mechanisms have been suggested as the possible origin of vascular LFOs. Variations in blood CO_2 (a potent vasodilator) is a possible major global source of vascular LFOs, as it is also known to dilate vessels as it travels along with blood [30,31]. Vasomotion is another potential source of LFOs [32,33]. Results from two recent studies using mathematical modeling [34] and in-vivo two photon microscopy in rodents [14] support the idea that local vasomotion generated from vascular smooth muscle cells might be driving CSF dynamics. Although our study establishes the influence of low frequency hemodynamics in driving CSF, the exact origin of these LFOs is not clear.

Lastly, it is worth noting that there are various fMRI endogenous contrasts used in this study, which include the inflow effect at the fourth ventricle, the BOLD effect at the draining veins, and an obscure BOLD effect at ICAs. The ICAs in healthy subjects carry blood with ~100% oxygenation. However, a weak positive BOLD effect might arise through variations in arterial

saturation [35]. Alternatively, a weak negative BOLD effect might arise via changes in arterial diameter since fully saturated arterial blood has a lower magnetic susceptibility than tissues. Given the negative correlations between the derivative of LFO_{ICA} (i.e., representing dilation) and LFO_{CSF} , the most likely contrast in ICA is a partial volume effect arising from changes in arterial diameter, producing an fMRI signal inversely correlated with arterial blood volume [22,23,26].

4.2. Effects of Respiration

Respiration is another important mechanism that has been suggested as a potential engine for CSF movement [15,17,18,36,37]. Our results point to the possibility of a highly interdependent and tightly communicating CSF-venous system (i.e., in the respiratory frequency range). In detail, our results document a negative change in intrathoracic volume (i.e., a positive change in intrathoracic pressure) leads to a reduction in venous drainage from the brain observed within a second at the jugulars, further leading to an increase in blood volume in the brain (due to reduced drainage) and finally resulting in a compensatory outward movement of CSF from the brain within an average time of 2 seconds from the start of the cycle. The observed time delays, although small, show that respiration indeed influences CSF dynamics through the venous system in a sequential and continual fashion.

We further explored the possibility that the intrathoracic volume/pressure changes also affect the arterial system. The results showed that these signals correlate weakly at time delays much longer (~ 4.4 s) than that taken by respiration to affect the venous system (< 1 s). This is likely to be due to different mechanisms by which respiration couples with venous and arterial systems and to the relatively lower SNR of fMRI signal in the arteries. Our respiratory model (Figure 2) did not include a specific route by which changes of intrathoracic volume/pressure might couple with ICA but there are a few candidate mechanisms, including variations in minute

ventilation (the volume of gas inhaled/exhaled per minute) leading to changes in arterial oxygen saturation and arterial CO₂ concentration. The weak positive correlation of chest motion and RO_{ICA}, with chest motion leading by around 4.4 seconds, would fit a standard BOLD signal model in which increased minute ventilation (larger breaths) leads to greater arterial oxygen saturation and dilution of the small concentration of arterial deoxyhemoglobin. Variation of arterial deoxyhemoglobin has been proposed by Aso et al. [35] as one possible source of LFOs. Future work might expand the respiratory model to include minute ventilation as an independent measure. Together with measurements of expired CO₂ it might then be possible to determine a precise mechanism for the weak coupling between chest motion and RO_{ICA}.

4.3. Interactions and Relative Effects

The results from our study have shown that both low frequency hemodynamics and respiration generate fluctuations of CSF flow in the fourth ventricle in humans. We found that the amplitude of respiration measured from the chest is tightly coupled with the phases of LFOs found in CSF movement. We also found a similar coupling between respiration amplitude and the phases of vascular LFOs extracted from ICAs and IJVs. These results point to the possibility that the depth of respiration (i.e. minute ventilation) contributes to the changes in blood vessel volume dynamics that occurs in LFO range. This concept is physically plausible, since depth of respiration is capable of inducing changes in blood vessel volume dynamics through intrathoracic pressure changes as well as CO₂ concentration changes [38]. As suggested by the results from cross-frequency coupling, it is possible that respiration exerts its effects on CSF dynamics not only through the pressure-related venous return changes in the RO range, but also through the blood gas related vessel volume changes, within a narrow range of low frequency oscillations (0.0175 Hz – 0.028 Hz) in CSF signals (see figure S6 in the supplementary material). On average, respiration

explained $73.46 \pm 15.83\%$ of variability in the LFO range of 0.0175 Hz – 0.028 Hz. Natural variations in blood CO₂ during respiration could be thought of as primarily contributing to this narrow range of LFOs, consistent with a previous report that the end-tidal CO₂ fluctuations during rate-controlled resting state breathing were significantly correlated to BOLD fMRI signal fluctuations at ~ 0.03 Hz [39].

Finally, it is imperative to figure out the relative contributions of each of these mechanisms in regulating CSF dynamics. A power spectrum analysis of the caudally directed CSF signals observed at the fourth ventricle showed that LFOs represent the major component of the signal in all of the participants in this study. In fact, the percent of power in the LFO range is significantly higher than the percent of power in the normal respiration range (see figure S7 in the Supplementary material). On average, about 67 percent of power in CSF signal comes from the low frequency oscillations, whereas only about 15 percent of power is present in the respiration range. However, this does not entirely mean that respiration has a negligible effect on CSF movement. Cardiac pulsations have also been suggested as a potential driver of CSF dynamics [40,41]. However, this mechanism has been identified through mathematical modelling to be too weak to become the principal force driving CSF [12,13]. In our study, cardiac pulsations (>0.6 Hz) were sampled in only 5 participants explained only an average of 11 percent of the power in the caudally directed CSF signals observed at the fourth ventricle.

4.4. Clinical Impact

This study demonstrated that close coupling exists between fluid dynamics in the brain and that this valuable information can be extracted from a neck scan. This opens the possibility of developing a simple neck scan sequence or applying other imaging modalities (e.g., Doppler ultrasound or Near Infrared Spectroscopy) to get indirect, or inferred, CSF measures from the

blood vessels in the neck. We caution, however, that the first step towards such a clinical tool would be to repeat the current study in patient groups, in case abnormal vascular or CSF dynamics, as found in stroke and hydrocephalus patients, respectively, reduces or eliminates the relationships we observe in healthy subjects. In addition, the study also validated the mechanism of respiration in the CSF movement and its relationship with that of LFOs. These findings can inform the development of clinically relevant breathing tasks that might further couple with LFOs and enhance CSF flow in patients where low natural flow may be a concern, e.g., due to insomnia. In addition, these results will also be beneficial in further delineating the pathophysiology of impaired CSF clearance of proteins associated with neurodegenerative disorders such as Alzheimer's disease [7] and chronic traumatic encephalopathy [9].

5. Limitations and Conclusions

A limitation of this study is the lack of a simultaneous end-tidal CO₂ measurement. This would have helped to separate the contributions of respiration to CSF dynamics from both pressure changes and CO₂ changes. Future studies will incorporate simultaneous measurement of end-tidal CO₂ values. The method used in our study was also unable to simultaneously detect the CSF movement into the brain. Another minor limitation is the lower fMRI temporal resolution that made it impossible to sample the cardiac pulsations in 6 participants.

In conclusion, the results of our study illustrate that valuable brain fluid dynamic information can be obtained from a neck scan, from which it was found that multiple physiological forces contribute to driving CSF movement in humans. Two simple yet distinct mechanisms simultaneously regulating caudally directed CSF movement in humans, based on 1) low frequency hemodynamics from the neck blood vessels (ICA and IJV) and 2) respiration were validated. Cross-frequency interactions between these motive forces were also identified. The capability of

the CSF system to respond to multiple physiological forces at the same time will help unveil the pathological mechanisms behind CSF flow related disorders and thereby develop new approaches to therapeutic interventions.

List of Abbreviations

BOLD: Blood Oxygen Level Dependent

CNS: Central Nervous System

CSF: Cerebrospinal Fluid

fMRI: functional Magnetic Resonance Imaging

ICA: Internal Carotid Artery

IJV: Internal Jugular Veins

LFO: Low-frequency Oscillations

MCCC: Maximum Cross-Correlation Coefficients

RO: Respiratory Oscillations

SNR: Signal-to-Noise Ratio

Declarations

Ethics approval and consent to participate

The study was approved by Purdue University's Human Research Protection Plan (IRB-2020-1329) and was conducted in accordance with application of Belmont Report principles (Respect for Persons, Beneficence, and Justice) and federal regulations 45 CFR 46, 21 CFR 50, 56. Written informed consent was obtained from all participants.

Consent for publication

539 Not applicable

540 **Availability of data and materials**

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

543 **Competing interests**

544 The authors declare that they have no known competing financial interests or personal
545 relationships that could have appeared to influence the work reported in this paper.

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550 **Authors' contributions**

551 YT, VVN and BI conceived of the presented idea. VVN, BRK and HY performed data collection.
552 VVN performed data analysis. YT, VVN and BI interpreted the results and developed the theory.
553 VVN took the lead in writing the manuscript. AJS, YW, XZ and others provided critical feedback
554 and helped shape the research, analysis and manuscript.

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557

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678 **Supplementary Material**

679

Parameter Participant #	r_x	r_y	r_z	t_x	t_y	t_z
1	0.0123	-0.0644	-0.0106	-0.0816	-0.1122	-0.1282
2	0.2019	0.0982	0.1975	0.1494	-0.1948	-0.0532
3	-0.0285	0.1861	-0.1109	0.2218	-0.1447	-0.0119
4	0.1439	-0.0907	0.0744	0.1289	-0.1117	0.1289
5	0.0133	0.1135	-0.1124	-0.1193	0.0957	-0.0181
6	-0.0006	0.0761	-0.0516	0.0634	0.0021	0.0901
7	0.1611	0.1996	-0.0121	0.1664	-0.1408	0.2266
8	-0.0158	0.0436	0.0095	0.0079	-0.0184	0.0553
9	0.1788	-0.0315	-0.0026	0.0851	-0.1323	0.0432
10	0.3231	0.0057	0.4047	0.1533	-0.4397	-0.0281
11	0.2244	0.0667	0.0823	0.0178	-0.1607	0.0525
Mean± SD	0.1104 ± 0.12	0.0549± 0.09	0.0426± 0.15	0.0721± 0.11	-0.1235± 0.14	0.0325± 0.09

680

681 *Table S1: Correlations between motion parameters (FSL MCFLIRT) and caudally directed CSF signals for each*

682 *participant. From the results, it can be seen that there exist no noteworthy correlations between motion parameters and the*

683 *CSF signals. This therefore confirms that the CSF signals extracted from the fMRI scans were not affected by motion.*

684 *r_(x-z): MCFLIRT estimated rotations; t_(x-z): MCFLIRT estimated translations.*

685

Figure S1: CSF movement to the brain and Respiration. Cranially directed CSF movement to the brain was captured in 3 participants by performing a brain scan with the fourth ventricle still maintained at the first slice of the scan volume. (A) Model illustrating how a positive change in intrathoracic volume/negative change in intrathoracic pressure affect CSF dynamics sequentially starting with 1) Pressure coupling to IJV which leads to 2) Increase in venous drainage in the IJV which in turn leads to 3) Decrease in the volume of intracranial blood and finally results in 4) Cranially directed CSF movement into the brain. Based on this relationship, the $d/dt(RO_{Chest})$ should be positively correlated with cranially directed RO_{CSF} and negatively correlated with global brain-RO (RO_{GM}). Results of MCCCs and corresponding delays between $d/dt(RO_{Chest})$ to RO_{CSF} , and RO_{GM} for (B) a representative participant and (C) for all three participants with the brain scans. It can be seen that as predicted by the model, the $d/dt(RO_{Chest})$ leads the RO_{CSF} by an average of 1.03 ± 0.25 seconds with an average MCCC of 0.53 ± 0.06 and leads the RO_{GM} by an average time delay of 0.44 ± 0.44 seconds and an average MCCC of -0.57 ± 0.16 . The negative delays indicate that the $d/dt(RO_{Chest})$ signal leads the others. Cross-correlation calculations were performed by forcing the $d/dt(RO_{Chest})$ signal to lead, under the assumption that respiration is the driver to the observed GM and CSF responses in the respiratory frequency range. IJV – Internal Jugular Vein; CSF - Cerebrospinal Fluid; RO – Respiratory Oscillations (0.2 Hz – 0.4 Hz); A.U – Arbitrary Units; CCC – Cross-Correlation Coefficients; MCCC – Maximum Cross-Correlation

Coefficient. In group results (B), the brown solid line represents the mean, the brown dotted line represents the median, the brown whiskers represent one standard deviation of the raw data points jittered over a 95 percent confidence interval in cream.

Figure S2: Cross-Spectral analysis of caudally directed CSF signals and Chest-belt respiration signals. The figure illustrates the results of cross-spectrogram between raw CSF signals and chest-belt respiratory oscillations for all the 11 participants enrolled in this study. It can be seen that the maximum coherence between the signals occur at the normal respiratory range of 0.2 Hz – 0.4 Hz throughout the entire scan duration of 5 minutes, in all participants. The cross-spectrograms were calculated using the MATLAB function ‘xspectrogram’ with window lengths of 50 seconds and a 90 percent overlap. Plots are numbered according to participant number. Colorbars represent power/frequency (dB/Hz).

Figure S3: Coupling of intrathoracic pressure changes during respiration to venous return. (A) During inspiration, entry of air into the lungs, leads to simultaneous expansion of lungs, chest wall and descent of the diaphragm. As a result, intrathoracic pressure (i.e., pressure in the thoracic cavity between the organs – lungs, heart and chest wall) decreases (i.e., becomes negative). This leads to further expansion of the lungs and the chambers of the right heart (right atrium and right ventricle). This in turn leads to a decrease in the right atrial pressure and thereby in an increase of the pressure gradient between right atrium and the brain blood vessels, aiding the venous return from the brain to the right atrium. On the other hand (B) during expiration, the reverse occurs. That is, passage of air out of the lungs, leads to simultaneous collapse of lungs, chest wall and ascent of the diaphragm. As a result, intrathoracic pressure increases (i.e., becomes positive). This leads to further collapse of the lungs and the chambers of the right heart (right atrium and right ventricle). This in turn leads to an increase in the right atrial pressure and thereby in a decrease of the pressure gradient between right atrium and the brain blood vessels, reducing the venous drainage from the brain to the right atrium.

Figure S4: MCCCs and delays between mismatched halves of RO range time series data for each participant. In order to ensure that our cross-correlation results in the RO range were not spurious errors arising from the periodicity of the signals, we also calculated the MCCCs and delays between mismatched halves of RO range time series data. For example, the MCCC and the corresponding time delay was calculated between the first half of $d/dt(\text{RO}_{\text{Chest}})$ and second half of $\text{RO}_{\text{CSF}}/\text{RO}_{\text{IJV}}/\text{RO}_{\text{ICA}}$ for each participant. The results illustrate that the spurious MCCCs cluster outside the range of statistical significance ($-0.26 < \text{MCCC} < 0.26$) in the RO range, thereby validating that our results are not due to spurious errors. ICA – Internal Carotid Artery; IJV – Internal Jugular Vein; CSF - Cerebrospinal Fluid; RO – Respiratory Oscillations (0.2 Hz – 0.4 Hz).

Figure S5: Cross-frequency interactions between respiration and LFOs. Results of coupling quantified by modulation index, between amplitude of RO_{Chest} signal and the phase of (A) LFO_{CSF} , (B) LFO_{IJV} and (C) LFO_{ICA} signals for all 11 participants enrolled in the study. Colorbars represent modulation index. ICA – Internal Carotid Artery; IJV – Internal Jugular Vein; CSF - Cerebrospinal Fluid; LFO – Low frequency Oscillations (0.01 Hz – 0.1 Hz); RO – Respiratory Oscillations (0.2 Hz – 0.4 Hz).

(A)

Parti pant #	Peak frequency range of respiration (Hz)	LFO _{CSF} band with maximum correlation (Hz)	Variation of LFO _{CSF} band explained by peak respiration amplitude (%)
1	0.3 - 0.35	0.015 - 0.025	50.12
2	0.2 - 0.25	0.015 - 0.025	55.22
3	0.25 - 0.3	0.016 - 0.023	89.01
4	0.3 - 0.38	0.02 - 0.03	91.93
5	0.25 - 0.3	0.025 - 0.036	72.24
6	0.25 - 0.3	0.01 - 0.02	47.22
7	0.25 - 0.3	0.02 - 0.03	72.95
8	0.3 - 0.35	0.02 - 0.04	77.05
9	0.3 - 0.35	0.011 - 0.028	83.54
10	0.2 - 0.25	0.01 - 0.02	83.37
11	0.23 - 0.28	0.01 - 0.02	85.34

Figure S6: Variation in caudally directed LFO_{CSF} at the fourth ventricle explained by respiration. Specific narrow frequency ranges in LFO_{CSF} with maximum coupling with corresponding peak frequency ranges of respiration was identified for each participant and the percent variability of these narrow LFO_{CSF} bands explained by corresponding peak respiratory frequencies were calculated. (A) Table shows the peak respiratory frequencies and LFO_{CSF} frequencies with maximum correlation for each participant and the corresponding percent variation of peak respiration frequencies in the specific LFO_{CSF} frequency bands. (B) Bar plots of the amplitudes of the average power in the specific LFO_{CSF} frequency bands with maximum correlation with peak respiration show that these LFO_{CSF} bands of all participants overlap in the narrow range of 0.0175 Hz – 0.028 Hz. On average, respiration was found to explain 73.46±15.83% of variability in this narrow LFO range of 0.0175 Hz – 0.028 Hz. The amplitude of average power in each specific LFO_{CSF} frequency band was calculated using the MATLAB function ‘bandpower’. The purple-colored plot shows the average power spectral density curve for all participants. The cyan colored bar plots illustrate the amplitude of average power in the specific LFO_{CSF} bands identified to maximum correlation with their corresponding peak respiratory frequencies. The red highlighted part shows the narrow LFO range with maximum overlap across the participants. CSF - Cerebrospinal Fluid; LFO – Low frequency Oscillations.

Figure S7: Power analysis of caudally directed CSF signals. (A) Power spectrum of CSF signals of all participants enrolled in the study, clearly illustrates the significant contribution of LFOs, compared to respiration. (B) Average power (amplitude in linear units) of LFO band (0.01 Hz – 0.1 Hz) and respiration band (0.2 Hz – 0.4 Hz) in CSF signals for all participants illustrate that the LFO band power is significantly higher than the respiration band power ($p < 0.001$). The amplitude of average power in each frequency band was calculated using the MATLAB function 'bandpower'. In the box plot, the red solid line represents the median, box represents the interquartile range, whiskers represent the extreme data points and outliers are represented by the '+' symbol. CSF - Cerebrospinal Fluid; LFO – Low frequency Oscillations (0.01 Hz – 0.1 Hz).