Aging affects the phase coherence between spontaneous oscillations in brain oxygenation and neural activity*

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Abstract

The risk of neurodegenerative disorders increases with age, due to reduced vascular nutrition and impaired neural function. However, the interactions between cardiovascular dynamics and neural activity, and how these interactions evolve in healthy aging, are not well understood. Here, the interactions are studied by assessment of the phase coherence between spontaneous oscillations in cerebral oxygenation measured by fNIRS, the electrical activity of the brain measured by EEG, and cardiovascular functions extracted from ECG and respiration effort, all simultaneously recorded. Signals measured at rest in 21 younger participants (31.1±6.9 years) and 24 older participants (64.9±6.9 years) were analysed by wavelet transform, wavelet phase coherence and ridge extraction for frequencies between 0.007 and 4 Hz. Coherence between the neural and oxygenation oscillations at ∼0.1 Hz is significantly reduced in the older adults in 46/176 fNIRS-EEG probe combinations. This reduction in coherence cannot be accounted for in terms of reduced power, thus indicating that neurovascular interactions change with age. The approach presented promises a noninvasive means of evaluating the efficiency of the neurovascular unit in aging and disease.

Keywords: Neurovascular unit, aging, neurovascular dynamics, EEG, fNIRS, wavelet analysis

1. Introduction

A healthy brain requires sufficient supplies of glucose and oxygen to function properly, and any impairment of the vasculature will affect their delivery to the target cells. The brain and cardiovascular system work closely together in a common endeavour to match energy supply to demand. Their intimate relationship is reflected in the concept of the neurovascular unit (NVU) (35), corresponding to consideration of the neurons, astrocytes, microglia, pericytes, endothelial cells and basement membrane as a single functioning entity. In the process of aging, the brain undergoes structural (24) and functional changes, and so also does the cardiovascular system. Knowledge of healthy aging can aid understanding of the mechanisms of pathological aging, as age is the biggest risk factor in the etiology of neurodegenerative diseases, such as Alzheimer’s disease which appears to include accelerated aging of the brain (28).

The neurophysiological changes in the aging brain have been well documented through measures of its electrical and magnetic activities using electroencephalogram (EEG) and magnetoencephalogram (MEG) recordings, respectively (31; 2; 34; 4; 22; 109; 88). Both the power of brain waves, and the functional connectivity patterns in the brain, have been shown to change with age.

The cardiovascular system is a closed system of vessels, where blood circulates, cyclically pumped by the heart and oxygenated by the lungs. It is well known that heart rate variability (1) decreases with aging, whereas the blood pressure (78; 76) increases. This has been linked to altered cognition in healthy people below 70 years old (107), thereby indicating the importance of a well-functioning cardiovascular system for brain health. More local to the brain, changes in cerebral blood oxygenation can be measured non-invasively using functional Near-Infrared Spectroscopy (fNIRS). Several investigations have found differences in oxygenation dynamics between younger and older subjects, both in the resting state and during task activation (114). In elderly subjects, the power and connectivity in the 0.052–0.145 Hz range are reduced compared to younger ones (57; 110). This frequency range is associated with vasomotion, the mechanism through which smooth muscle cells modulate the blood flow, by altering the diameter of the blood vessels (10; 35; 97). However, despite general awareness that all components of the NVU are individually affected by aging (58), no quantitative method is available for non-invasive assessment of the function of the NVU as a whole. Nor has any study to date inves-

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Table 1: Participants’ data. Age, body mass index (BMI), systolic blood pressure (sBP) and diastolic blood pressure (dBP) are given as means ± standard deviations. $p$ is obtained from the Wilcoxon rank-sum test between the two groups.

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Age (yrs)</th>
<th>Sex</th>
<th>BMI (kg m$^{-2}$)</th>
<th>sBP (mmHg)</th>
<th>dBP (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Younger</td>
<td>21</td>
<td>31.1 ± 6.9</td>
<td>11F/10M</td>
<td>23.6 ± 3.6</td>
<td>122 ± 18</td>
<td>79 ± 9.8</td>
</tr>
<tr>
<td>Older</td>
<td>24</td>
<td>64.9 ± 6.9</td>
<td>15F/9M</td>
<td>26.9 ± 3.0</td>
<td>136 ± 17</td>
<td>83 ± 11</td>
</tr>
<tr>
<td>$p$</td>
<td>-</td>
<td>1.02 × 10$^{-8}$</td>
<td>-</td>
<td>0.002</td>
<td>0.004</td>
<td>0.067</td>
</tr>
</tbody>
</table>

2. Methods

2.1. Participants

All participants provided written informed consent, and the study was conducted in accordance with the Declaration of Helsinki. The study protocols were approved by the Commission of the Republic of Slovenia for Medical Ethics and/or by the Faculty of Science and Technology Research Ethics Committee (FSTREC) at Lancaster University. The study involved the recording and analysis of data from 45 participants. The younger group consisted of 21 participants between 20 and 39 years. The older group consisted of 24 participants between 56 and 77 years. Participant details are provided in Table 1. The exclusion criteria were neurodegenerative disorders, clinically diagnosed neurological disorders, psychiatric disease and/or diabetes. Three participants were excluded because they fell asleep during the measurements, and one participant was excluded on account of poor probe contact resulting in noisy data.

Based on two groups with 21 and 24 participants, a statistical power of 0.8 and a significance level of 0.05 we expected, at minimum, to reliably detect effects of size 0.92, which were considered large effects (23). Effect size was calculated using Cohen’s $d$ (15). Further details are reported in the Supplementary Material (SM) Sec. 2.

2.2. Data acquisition

Data were recorded in quiet rooms at the Neurological Clinic, Ljubljana, Slovenia or in the Nonlinear and Biomedical Physics Lab, Physics Department, Lancaster University, Lancaster, UK (see SM, Sec. 5). The same system was used in both locations. Each participant was seated in a comfortable chair and had their eyes open during the approximately 30 minutes of measurement. No fixation points were used. An electroencephalogram (EEG) was recorded at 1 kHz using a 16-channel system (V-Amp, Brain Products, Germany). Simultaneously, functional Near-Infrared Spectroscopy (fNIRS) measurements detected changes in oxygenated hemoglobin. Note that we refer to these measurements as “brain oxygenation” although, strictly speaking, we investigate brain oxygenation dynamics, because fNIRS does not measure absolute hemoglobin concentrations. An 8-source/8-detector LED system (NIRScout, NIRx, Germany) was used and the...
recordings were made at 31.25 Hz. The probe layout is shown in Fig. 1B.

The heart rate was evaluated from an electrocardiogram (ECG), obtained with a bipolar precordial lead similar to the standard D2 lead. To maximize R-peak sharpness, electrodes were positioned on the right and left shoulders and over the lower left rib. The respiration rate was evaluated from the respiratory effort recorded using a belt wrapped around the participant’s chest, fitted with a Biopac TSD201 Respiratory Effort Transducer (Biopac Systems Inc., CA, USA). Both were sampled at 1.2 kHz using a signal conditioning system (Cardiosignals, Institute Jožef Stefan, Slovenia). Fig. 1A depicts signals from a participant in the younger group.

2.3. Data preparation and preprocessing

Signal processing was done in MATLAB, and the analysis was completed using the toolbox MODA (69) to implement the methods illustrated by Clemson et al. (13). A continuous 25-minute signal, mostly free of movement artifacts, was extracted for each participant. The data were detrended by subtracting a best-fit third-order polynomial, and bandpass filtered in the range 0.007–4 Hz. The preprocessing procedures were as described by Iatsenko et al. (37). To reduce computational load, the EEG, ECG and respiration signals were each downsampled using a moving average. The resultant frequencies are listed in Table 2. The artefact in the EEG signals due to cross-talk between brain electrical activity and the electrical activity of the heart was extracted using nonlinear mode decomposition (38).

As we do not have individual 3D head geometry data, such as MRI scans, and as we use a relatively low-density EEG set-up, we chose to do the analysis on the sensor level rather than the source level. This is because a lack of geometrical data coupled with a low-density of EEG sensors is known to result in a low accuracy of source localisation (9, 63). Increasing the number of electrodes would have improved spatial localisation to some extent, but would also have increased the set-up time for the experiment, constituting a limiting factor in clinical applications.

2.4. Time-frequency analysis

Time-frequency analysis provides information on how the frequency of an oscillation changes through time. We used the continuous wavelet transform (WT) and, at each discrete time $t_n$ and frequency $\omega_k$, obtained a complex number $X_{k,n} = a_{k,n} + ib_{k,n}$. From this a phase $\Phi$ and amplitude $A$ were found:

$$\Phi_{k,n} = \arctan\left(\frac{b_{k,n}}{a_{k,n}}\right),$$

$$A_{k,n} = |X_{k,n}|.$$ Power was found by squaring the amplitude. The WT has a logarithmic frequency scale. When analysing low frequency oscillations, the WT therefore provides better frequency resolution than, for example, the windowed Fourier transform. After taking the transforms, the time-averaged WT power spectra were calculated for each of the 11 fNIRS signals, and for the instantaneous heart/respiration rates. The Morlet wavelet was used for the WT. An overview of the parameters used, including the frequency resolution and sampling frequencies, is provided in Table 2.
## Table 2: Summary of the methods and parameters used in the analyses.

<table>
<thead>
<tr>
<th>Analysis</th>
<th>Method</th>
<th>Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate</td>
<td>Peak detection and ridge extraction</td>
<td>WT: f&lt;sub&gt;0&lt;/sub&gt; = 2 ( f \in [0.6, 1.7] ) ( f_s = 100 \text{ Hz} )</td>
</tr>
<tr>
<td>Respiration rate</td>
<td>Peak detection and ridge extraction</td>
<td>WT: f&lt;sub&gt;0&lt;/sub&gt; = 1 ( f \in [0.1, 0.6] ) ( f_s = 100 \text{ Hz} )</td>
</tr>
<tr>
<td>( \gamma ) instantaneous frequency</td>
<td>Ridge extraction</td>
<td>WT: f&lt;sub&gt;0&lt;/sub&gt; = 5 ( f \in [20, 30] ) ( f_s = 142 \text{ Hz} )</td>
</tr>
<tr>
<td>( \gamma ) instantaneous power</td>
<td>WT and frequency average</td>
<td>WT: f&lt;sub&gt;0&lt;/sub&gt; = 5 ( f \in [20, 30] ) ( f_s = 142 \text{ Hz} )</td>
</tr>
<tr>
<td>IHR/IRR power</td>
<td>Time-averaged WT</td>
<td>WT: f&lt;sub&gt;0&lt;/sub&gt; = 1 ( f \in [0.007, 2] ) ( f_s = 20 \text{ Hz} )</td>
</tr>
<tr>
<td>EEG wavelet power</td>
<td>Time-averaged WT</td>
<td>WT: f&lt;sub&gt;0&lt;/sub&gt; = 1 ( f \in [0.007, 4] ) ( f_s = 31.25 \text{ Hz} )</td>
</tr>
<tr>
<td>fNIRS wavelet power</td>
<td>Time-averaged WT</td>
<td>WT: f&lt;sub&gt;0&lt;/sub&gt; = 1 ( f \in [0.007, 4] ) ( f_s = 31.25 \text{ Hz} )</td>
</tr>
<tr>
<td>Power of ( \gamma ) instantaneous frequency/power</td>
<td>Time-averaged WT</td>
<td>WT: f&lt;sub&gt;0&lt;/sub&gt; = 1 ( f \in [0.007, 4] ) ( f_s = 31.25 \text{ Hz} )</td>
</tr>
<tr>
<td>fNIRS-EEG coherence</td>
<td>Wavelet phase coherence</td>
<td>WT: f&lt;sub&gt;0&lt;/sub&gt; = 1 ( f \in [0.007, 4] ) ( f_s = 31.25 \text{ Hz} )</td>
</tr>
<tr>
<td>fNIRS-fNIRS coherence</td>
<td>Wavelet phase coherence</td>
<td>WT: f&lt;sub&gt;0&lt;/sub&gt; = 1 ( f \in [0.007, 4] ) ( f_s = 31.25 \text{ Hz} )</td>
</tr>
<tr>
<td>EEG-EEG coherence</td>
<td>Wavelet phase coherence</td>
<td>WT: f&lt;sub&gt;0&lt;/sub&gt; = 1 ( f \in [0.007, 4] ) ( f_s = 31.25 \text{ Hz} )</td>
</tr>
<tr>
<td>IHR/IRR/Respiration fNIRS/EEG coherence</td>
<td>Wavelet phase coherence</td>
<td>WT: f&lt;sub&gt;0&lt;/sub&gt; = 1 ( f \in [0.007, 2] ) ( f_s = 20 \text{ Hz} )</td>
</tr>
<tr>
<td>( \gamma )F-fNIRS/( \gamma )P-fNIRS coherence</td>
<td>Wavelet phase coherence</td>
<td>WT: f&lt;sub&gt;0&lt;/sub&gt; = 1 ( f \in [0.007, 4] ) ( f_s = 31.25 \text{ Hz} )</td>
</tr>
</tbody>
</table>

The wavelet phase coherence is then defined as

\[
C_{\Phi}(\omega_k) = \sqrt{(\cos \Delta \Phi_{k,n})^2 + (\sin \Delta \Phi_{k,n})^2},
\]

where \( (\cos \Delta \Phi_{k,n}) \) and \( (\sin \Delta \Phi_{k,n}) \) are averaged in time.

We assessed the fNIRS–fNIRS pairwise coherence (for all permutations of the 11 fNIRS probes), as well as the EEG–fNIRS, instantaneous heart rate (IHR)–respiration, IHR–EEG, IHR–fNIRS, respiration–fNIRS, respiration–EEG, instantaneous respiration rate (IRR)–fNIRS, and IRR–EEG coherences.

### 2.6. Frequency bands

The sampling frequency of the fNIRS is 31.25 Hz, and so the Nyquist frequency would be \(~15\text{ Hz}\). If the oscillations had constant frequencies, and there were no harmonics, then 15 Hz would have been the upper limit for investigation of oscillatory modes and their interactions in the fNIRS signal. Furthermore, fNIRS is known not to contain oscillations faster than the cardiac oscillation (~1 Hz). Consistent with this, we did not see any significant power above the cardiac frequency. So, we selected the upper frequency limit to be 4 Hz for the fNIRS and fNIRS–EEG interactions. The EEG signal was sampled at 1000 Hz, but we analysed it only up to 48 Hz, which allowed for investigation of the slow \( \gamma \) oscillatory modes. The other reason for our 48 Hz limit was to avoid the effect of the 50 Hz notch filter used by the monitoring system. For both the EEG and fNIRS, the lower frequency limit was set to 0.007 Hz.

The power and coherence values were divided into the conventional frequency bands (Table 3) [3], within each

Stationarity comes from a time-variation of the characteristic frequencies. The logarithmic frequency resolution of WPC is particularly suitable for signals with a large span of characteristic frequencies. It provides a model-free approach that does not assume the existence of an underlying stochastic process. Taken together with wavelet analysis, it provides information about potential oscillatory modes contributing to the measured signal, and their degree of coordination and interaction. However, it does not provide information about direction of interaction, nor about couplings between oscillatory modes. For the evaluation of directional couplings one may use dynamical Bayesian inference, Granger causality, or similar information- or permutation-based methods [13, 23, 90].

The phase coherence is evaluated at each frequency and takes a value between 0 and 1. If the phase difference remained constant throughout the whole length of the signals at a certain frequency, the phase coherence value would be 1 at that frequency. As the measure only depends on the phase difference, it is independent of the amplitudes of the oscillations. The phase difference between signals 1 and 2 at time \( t_n \) and frequency \( \omega_k \) is

\[
\Delta \Phi_{k,n} = \Phi^{(2)}_{k,n} - \Phi^{(1)}_{k,n}.
\]

The wavelet phase coherence is then defined as

\[
C_{\Phi}(\omega_k) = \sqrt{(\cos \Delta \Phi_{k,n})^2 + (\sin \Delta \Phi_{k,n})^2},
\]

where \( (\cos \Delta \Phi_{k,n}) \) and \( (\sin \Delta \Phi_{k,n}) \) are averaged in time.

We assessed the fNIRS–fNIRS pairwise coherence (for all permutations of the 11 fNIRS probes), as well as the EEG–fNIRS, instantaneous heart rate (IHR)–respiration, IHR–EEG, IHR–fNIRS, respiration–fNIRS, respiration–EEG, instantaneous respiration rate (IRR)–fNIRS, and IRR–EEG coherences.
of which an average value was calculated. The first five bands, representing the characteristic frequency intervals of the cardiovascular system [97], strongly overlap the slow oscillations in EEG [11]. The last five bands are the traditional EEG frequency bands. After obtaining single power/coherence values in each band for each subject, the two groups were compared.

<table>
<thead>
<tr>
<th>Name</th>
<th>Frequency range (Hz)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endothelial (V)</td>
<td>0.007 – 0.021</td>
</tr>
<tr>
<td>Neurogenic (IV)</td>
<td>0.021 – 0.052</td>
</tr>
<tr>
<td>Myogenic (III)</td>
<td>0.052 – 0.145</td>
</tr>
<tr>
<td>Respiratory (II)</td>
<td>0.145 – 0.6</td>
</tr>
<tr>
<td>Cardiac (I)</td>
<td>0.6 – 1.7</td>
</tr>
<tr>
<td>Delta (δ)</td>
<td>1.7 – 4</td>
</tr>
<tr>
<td>Theta (θ)</td>
<td>4 – 7.5</td>
</tr>
<tr>
<td>Alpha (α)</td>
<td>7.5 – 14</td>
</tr>
<tr>
<td>Beta (β)</td>
<td>14 – 22</td>
</tr>
<tr>
<td>Gamma (γ)</td>
<td>22 – 48</td>
</tr>
</tbody>
</table>

Table 3: Frequency ranges used in the analysis [97]. The cardiac and δ ranges are slightly changed from past studies (see text).

In previous studies of cardiovascular dynamics, the cardiac band was defined as 0.6–2 Hz [97]. In the present case, however, we also need to take account of EEG dynamics which potentially overlap the cardiac band. To separate the cardiac and δ bands, we therefore defined the cardiac band as 0.6–1.7 Hz and the δ band as 1.7–4 Hz. With the upper limit set to 1.7 Hz, the variation in heart rate is still accommodated.

The respiratory oscillations are manifested in the frequency interval 0.145–0.6 Hz. They can be detected even in the smaller vessels such as capillaries, as they generate pressure waves that propagate throughout the entire cardiovascular system [99].

The 0.052–0.145 Hz frequency interval is referred to as myogenic, and the neurogenic band is defined as 0.021–0.052 Hz. The origins of these two bands are still debated, with perceptions depending on whether interest is being focused on the vascular or cardiac regulation mechanisms (see discussion section). The neurogenic response is similar to the myogenic response in that it also depends on pressure changes, but additionally involves neuronal pathways.

The frequency intervals 0.005–0.021 Hz is called the NO-dependent endothelial frequency band, in view of evidence that NO-dependent endothelial activity manifests itself within this range [55, 97, 91].

2.7. Heart and respiration rates

Time-series of instantaneous heart and respiration rates were obtained in two ways: by peak detection and by the ridge extraction method. Peak detection was performed in the time domain with a customised program in MATLAB that searched for R-peaks in the ECG signals or maxima in the respiration signal. The instantaneous frequencies were extracted in the time-frequency domain by the ridge extraction method [39] using the toolbox MODA [69]. Note that “instantaneous heart rate” (IHR) is a time-series of heart frequency values. It is traditionally referred to as heart rate variability when derived in the time domain from the intervals between heart beats. Similarly, “instantaneous respiration rate” (IRR) is a time-series of respiration frequency values, and is usually called respiration rate variability when derived from the time intervals between maxima. The instantaneous heart and respiration rate time series were in close agreement whether obtained either by the peak detection method or by the ridge extraction method, as shown in Fig. 2 for the IHR. The average heart and respiration rates were obtained from their respective time-series.

Because the time-series obtained with the ridge extraction method are smooth functions, ready to use in time-series analysis, they were used in the wavelet and phase coherence analyses. Furthermore, the ridge extraction method is more appropriate for extracting IHR than the peak-detection method, as ridge extraction takes into account the whole ECG signal and not just the R-peaks, thus also capturing the effect of T-waves.

For the IHR, ridge extraction was applied to the WTs of ECG signals in the 0.6–1.7 Hz frequency range. The lognormal wavelet and a frequency resolution of 2 Hz were used for the WT. The sampling frequency of the IHR was the same as that of the ECG, and no interpolation was needed [36]. For the IRR, ridge extraction was applied to the WTs of respiration signals in the 0.1–0.6 Hz frequency range and with a frequency resolution of 1 Hz.

The standard deviation of the instantaneous rates (sd IHR and sd IRR), resulting in a single number in each case, was used to obtain a measure of their variability.

2.8. Frequency and amplitude modulation of the γ-band by low-frequency oscillations

From the EEG signals, the instantaneous frequency and power in the 20–30 Hz interval were obtained by ridge extraction [39], and are referred to as a γ–instantaneous frequency and γ–instantaneous power time-series. Fig. 3 illustrates the procedure. The frequency resolution parameter was 5 Hz.
Figure 3: \(\gamma\)-instantaneous frequency (projected onto the Frequency-Time plane) and \(\gamma\)-instantaneous power time-series (projected onto the Wavelet power-Time plane) as obtained by ridge extraction.

For the 8 locations where fNIRS and EEG sensors are co-located, the WPC was calculated between the \(\gamma\)-instantaneous frequency time-series and the fNIRS signal, to evaluate the effect of low frequency modulation on the oscillations in the \(\gamma\)-band. The WPC was also calculated between the \(\gamma\)-instantaneous power time-series and the fNIRS signal to evaluate the effect of low frequency modulation on the \(\gamma\)-band amplitude and the corresponding power.

2.9. Intersubject surrogates

To ensure that apparent coherence is statistically significant, we used intersubject surrogates \(52\). In addition to calculating coherence between the signals from one person, we calculated the apparent coherence between signals from different participants. This measure of coherence could not signify any underlying link between the signals, and was thus random. Inter-subject surrogates have previously been found suitable in the context of cardiorespiratory interactions \(50\). They are model-free and do not require stationary data.

Based on 154 intersubject surrogates a surrogate threshold was set as the 95th percentile of all these coherences at each frequency. In the plots throughout the paper, only the effective coherence (i.e. coherence after subtracting the surrogate threshold) is shown, and it was the effective coherence that we used in testing for differences between the groups. Each subject and signal pair had an individual significance threshold to account for different spectral biases in the signals. Due to the lower number of complete oscillations at low frequencies, the likelihood of apparent coherence is increased. Hence, the surrogate threshold is high for low frequencies and, correspondingly, the measurement time is not long enough for a reliable study of oscillations in the endothelial band in the case of fNIRS-EEG coherence.

2.10. Group statistics

To assess population differences, the non-parametric two-sided Wilcoxon rank-sum test was applied, and differences are considered significant for \(p < 0.05\). The data are presented as median values and violin plots \(33\). Additionally, for the fNIRS, EEG and fNIRS-EEG analyses, a Monte-Carlo permutation test \(15\) was applied to check the reliability of the significance. From the total of 45 participants, 21 were randomly placed in one group and 24 in the other. The Wilcoxon rank-sum test was applied to test for differences between the permuted groups. After 16587 permutations the original \(p\)-value was compared with the values obtained with permutation. If the initial \(p\)-value was smaller than 95% of the \(p\)-values obtained by permutations its significance was considered confirmed. Additional details are provided in Sec. 7 of the SM.

In time-frequency analysis, cluster-based permutation is a common method to correct for multiple comparisons \(58\). As we averaged in both time and frequency before applying statistical tests, we would only see differences in power/coherence that were present over many time-points and frequencies. For the spatial aspect of multiple comparisons, the expected false discovery rate, quantifying how many null-hypotheses would be incorrectly rejected with \(\alpha = 0.05\) assuming all null-hypotheses were true, was 0.8 for the EEG power analysis, 0.55 for the fNIRS power analysis, 6 for the EEG coherence analysis, 2.75 for the fNIRS coherence analysis and 8.8 for the fNIRS-EEG coherence analysis. From \(N\) trials, and assuming that there were no true differences, the probability of obtaining \(X\) or more positive findings was calculated from the binomial probability. This was used to assess the reliability of the results, keeping the multiple comparison problem in mind, as done in the literature \(64\ 70\).

2.11. Correlations

The correlations were found from the Spearman’s rank-order correlation, which is a non-parametric alternative to the Pearson linear correlation. It tests for a monotonic relationship between two variables. The \(p\)-value was found from permutation distributions.

3. Results

Here we present the results of the analyses summarised in Table \(2\). These include the central oscillations of the cardiovascular system (evaluated from the instantaneous heart and respiration frequencies), and the local vascular and neural oscillations in the brain (from fNIRS and EEG). The analyses relate to the transport of nutrients to the NVU, quantifying its efficiency and the impact of ageing.

3.1. Central oscillations: heart and respiration rates

We first present the cardio-respiratory characteristics. This enables a consistency check with earlier results, and
provides insight into systemic changes relevant to neurovascular interactions,

Heart rates (older: $1.04 \pm 0.16$ Hz; younger: $1.17 \pm 0.15$ Hz) and sd IHR (older: $0.052 \pm 0.011$ Hz; younger: $0.070 \pm 0.022$ Hz) are significantly different between the groups ($p = 0.014$, $p = 0.005$), as shown in Fig. 4A,B. No significant difference is seen in the respiration rate (older: $0.23 \pm 0.08$ Hz; younger: $0.24 \pm 0.05$ Hz, $p = 0.300$), or sd IRR (older: $0.039 \pm 0.009$ Hz; younger: $0.045 \pm 0.019$ Hz, $p = 0.26$). The corresponding plots are shown in the SM Sec. 3.

IHR power is reduced in the older group in the $0.01$–$0.11$ Hz range (see Fig. 4C). The IRR power is not significantly different between the groups (Fig. 4D).

Each group has significant IHR–respiration coherence in the respiratory band (see Fig. 4E; for the frequency band ranges, see Table 3). The younger group has significantly higher coherence around $0.3$ Hz, compared to the older group. For both groups the IHR power and IRR–respiration coherence were shown not to differ significantly between males and females (see SM Sec. 6), consistent with earlier results (36).

3.2. Interactions between instantaneous heart/respiration rates and brain oxygenation

The results presented here illustrate how the modulation of the heart and respiration rates is linked to the oxygenation of the brain. Fig. 5 shows the wavelet phase coherence between IHR and oxygenation, between IRR and oxygenation, and between the respiration signal and oxygenation, all at N5. For data from the other fNIRS probes see SM Sec. 3. The SM also includes the IHR–EEG, respiration–EEG and IRR–EEG coherence.

There are systematic differences in coherence, with the older group tending to have lower coherence. This difference is statistically significant for coherence between IHR and oxygenation (Fig. 5A), and is particularly pronounced in the myogenic and respiratory bands. The same significant reduction of coherence with age is observed in coherence between the IHR and all other oxygenation signals apart from the two temporal ones, where the coherence is reduced only in the respiratory band. Interestingly, the phase difference between oxygenation and IRR/respiration/IHR is found to be negative in the respiratory band, meaning that brain oxygenation is the leading signal. This result is consistent for both age groups. In contrast, the phase difference in the myogenic region is slightly positive, indicating that the brain oxygenation lags.

3.3. Brain oxygenation oscillations

Here we present the power calculated for all 11 fNIRS signals, and coherence between all possible signal combinations. The positions of the probes are shown in Fig. 3B.

The myogenic power (0.052–0.145 Hz frequency interval) in 8 of the 11 channels is significantly lower in the older group (Figs. 5A,B). A lower power is also found in the endothelial, neurogenic and respiratory bands (Fig. 5B), but the differences are statistically significant for fewer probes. In the endothelial band there are 3 fNIRS probes with a significant difference between the groups, while this number is 4 in the respiratory band, and 1 in the neurogenic and cardiac bands. The chance of obtaining 3 positive outcomes out of 11 is 1.5% when there were no true differences, while the chance of obtaining 1 positive outcome out of 11 is 43% when there were no true differences.

Significantly lower myogenic coherence in the older participants is found in 12 fNIRS signal combinations: across the frontal-parietal signals, the frontal signals and the occipital signals (Fig. 5C,D). In the neurogenic band significantly higher coherence in 12 fNIRS combinations (mainly from the temporal probes) is observed in the older group.
Further information is provided in the SM. It consists of

In the cardiac band in 50 of 55 combinations coherence is also significantly higher in the older group. The differences are found between the frontal-parietal, frontal-occipital and temporal signals. In the endothelial band coherence in 3 combinations is significantly higher in the older group, while in the respiratory band coherence in only one combination is significantly higher in the younger group. The chance of obtaining 12 positive outcomes out of 55 is 0.0014\% when there were no true differences, while the chance of obtaining 3 positive outcomes out of 55 is 52\%.

Brain oxygenation for males and females is summarised in Sec. 6 of the SM. The older male group has higher myogenic power at probes 1 and 9 compared to the older female group, while the older female group has higher myogenic coherence than the older male group in 7 signal combinations.

3.4. Brain neuronal activity evaluated by EEG

The EEG power and coherence are consistent with previous results (\textit{e.g.} (\textit{e}109\textit{~}88\textit{~}65\textit{~}83), and are summarised in the SM Sec. 4.

3.5. Coherence between neuronal activity and brain oxygenation

The coherence between neuronal activity, as evaluated by EEG, and brain oxygenation, as evaluated by fNIRS, differs significantly between the groups, in both the myogenic and cardiac bands (Fig. 7B,C). In the myogenic band, the coherence is lower in the older group in 46/176 probe combinations and the decrease does not seem confined to any specific areas. However, both groups have low myogenic coherence in the two temporal fNIRS probes (N8 and N9). In contrast, the coherence in the cardiac band is higher in the older group in 50/176 probe combinations. The chance of having 46 or more positive findings out of 176 is $1.2 \times 10^{-18}\%$ assuming there were no true differences. Further information is provided in the SM. It consists of neurogenic and respiratory coherence (Fig. 23), the coherence plots of all 176 fNIRS-EEG combinations (Sec. 10), and the results divided by sex (Sec. 6).

3.6. Frequency and amplitude modulation of the $\gamma$-band by low-frequency oscillations

Here we show analysis of possible amplitude and phase modulation of $\gamma$-band oscillations by low-frequency oscillations. There is non-zero power for both the $\gamma$-instantaneous frequency and $\gamma$-instantaneous power time-series between 0.007 and 4 Hz (Figs. 3A, B) for both groups indicating the existence of modulation. The coherence between oxygenation and these time-series, and the phase shifts for both instances, are shown in Fig. 3C–F for the signals measured at location O1. For the remaining locations, see the SM Sec. 11. For the $\gamma$-instantaneous frequency time-series the coherence is zero for all frequencies in the interval 0.007–4 Hz. For the $\gamma$-instantaneous power time-series the median coherence is zero, but there is evidence of some significant effective coherence (Fig. 3D). For the oxygenation–power there is a negative phase shift for the older group around 0.06–0.08 Hz (Fig. 3F), which is significantly different between the groups in 5/8 probe combinations. A negative phase difference indicates that the oxygenation is lagging.

3.7. Correlations

BMI is negatively correlated with neurovascular coherence in the myogenic band, IHR–respiration coherence in the respiratory band and IHR–respiration coherence in the myogenic band (Fig. 9A, B, C). The systolic blood pressure is also negatively correlated with neurovascular coherence in the myogenic band ($\rho = -0.435$, $p = 0.004$) and IHR–respiration coherence in the respiratory band ($\rho = -0.356$, $p = 0.022$) (SM Sec. 8).

As shown in Fig. 10 the neurovascular coherence in the myogenic band is correlated with the IHR–respiration coherence in the myogenic band ($\rho = 0.397$, $p = 0.008$),
Figure 6: fNIRS power and coherence. A) Time-averaged power spectra for N3. B) p-values indicating significant group differences between the powers in the frequency bands. Blue (yellow) indicates that the power is higher in the younger (older) group. C) Coherence between N11 and N7 (see Fig. 1 for locations). The blue and black lines are the median group coherences, while the shaded areas show the 25–75th percentiles. Significant differences between the groups at particular frequencies are indicated by blue stars on the x-axis. D) p-values indicating a significant group difference between the coherence in the frequency bands. Blue (yellow) indicates that the coherence is higher in the younger (older) group. For the frequency intervals see Table 3, and for the probe layout see Fig. 1.

4. Discussion

Based on 25-minutes signals recorded in participants in resting state and novel time-frequency analysis methods, our investigation of cardiovascular and neurovascular interactions reveals clear changes with aging. These are manifested through:

- Weakened 0.052–0.145 Hz coherence between the neural activity and brain oxygenation, reflecting reduced neurovascular interactions;
- Reduced coherence between instantaneous heart rate and brain oxygenation oscillations in the myogenic and respiratory frequency bands;
- Changes in the heart and respiration rates, and their coordination through respiratory sinus arrhythmia; and
- Altered brain oxygenation resting state networks in the brain.

We now discuss these changes in more detail.
Figure 7: A) Group median fNIRS–EEG coherence averaged over the frequency band 0.021–1.7 Hz. The results for the younger group (left) are compared with those for older group (middle) and p-values indicating a significant difference between the groups are shown on the right. Blue (yellow) coding indicate that coherence is higher in the younger (older) group. B) Same as for A but for the myogenic band. C) Same as for A but for the cardiac band. For the frequency intervals see Table 3 and for the probe lay-out see Fig. 1.
4.1. Central oscillations: heart and respiration activity

Consistent with previous studies (36), we found a decrease in the variability of the cardiac frequency with age, as quantified by the sd IHR. Additionally, the average resting cardiac frequency (heart rate) is higher in the younger group. We did not find a significant reduction with age in the respiratory frequency band of the IHR (in studies with linear frequency resolution and shorter recordings often referred to as the high frequency band, 0.15–0.4 Hz, linked to parasympathetic nervous activity (1)). The IHR power decreases with age in the myogenic frequency band, 0.052–0.145 Hz. We note here that when evaluated with linear frequency resolution, and based on shorter, usually 5-min recordings, this frequency interval is also referred to as the low frequency band, 0.04–0.15 Hz, and is linked to sympathetic nervous activity (1).

Note that the low/high frequency bands strongly overlap the myogenic/respiratory frequency bands. Low heart rate and insignificantly different respiratory band power in elderly participants could reflect relatively preserved parasympathetic tone. However, the changed parasympathetic/sympathetic activity is not sufficient to account for the variability in heart rate, which is generated by a complex interplay of nervous activity, respiration, smooth muscle cells and other factors (7; 14). Reduced variability with aging has previously been demonstrated (1; 27; 91).
also with wavelet-based methods (36).

A tendency for the IHR–respiration coherence to be lower in the older group reaches significance at around 0.3Hz. We did not, however, find a significant change in the respiration rate or its variability, as evaluated by the sd IHR, so this is an unlikely explanation for the reduced coherence. The significant IHR–respiration coherence reflects respiratory sinus arrhythmia (RSA), which is modulation of the heart frequency by the amplitude of respiration (113, 98). Wavelet based methods have previously been applied to investigate RSA (46, 36), and Iatsenko et al. (36) found the peak coherence in the respiratory band to decrease with age, suggesting that RSA is more time-variable and weaker in elderly subjects.

Consistent with the previous studies the present results show that the two central pumps of the cardiovascular system, heart and lungs, and their coordination, mainly through RSA, are affected by aging.

4.2. Propagation of the central oscillations: instantaneous heart/respiration rates and oxygenation

Next we investigated the effect of aging on the propagation of cardiovascular oscillations to the brain. Systemic cardiovascular oscillations naturally impact brain oxygenation (44), and their propagation may be affected by age-related structural changes in blood vessels. We investigated this latter possibility by evaluating the phase coherence between the cerebral blood oxygenation and the time-series of instantaneous heart or respiration rates.

The IHR–oxygenation coherence is significantly reduced in the older group in the myogenic and the respiratory frequency bands, across all non-temporal sites (Fig. 5A). These changes in coherence are consistent across combinations, indicating that the changes are systemic. The elastic properties of the vessels are known to change with aging (20), which could affect the propagation of pressure waves and therefore impact the myogenic response, causing reduced IHR–oxygenation coherence. This reduced coherence is attributable to the way in which smooth muscle cells respond to pressure changes. In mice, the myogenic response to pulsatile pressure in the middle cerebral arteries has been shown to decrease with age (94).

Systemic cardiovascular oscillations have been shown to affect the ~0.1 Hz oscillations in cerebral oxygenation: Katura et al. (44) estimated that such effects could only account for less than half of the observed changes. Note, however, that the study investigated heart rate and arterial blood pressure, but did not consider respiration. Furthermore, it has been shown that the Granger causality from heart rate to oxyHb during head-up tilt (83) at 45° decreased with age, which is in line with our findings of reduced coherence in the older group.

In the myogenic frequency band the phase difference between the oscillations in the time-series of IHR and fNIRS is positive, implying that in this frequency interval the oscillations in the IHR are preceding the oscillations recorded by the fNIRS signal. This furthermore confirms that the myogenic oscillations are propagating to the brain. The shift is significantly reduced with ageing, suggesting that the pulse propagates with less resistance to the small vasculature of the brain, as discussed in more detail below in Sec. 4.3.

The phase difference between the same signals in the respiratory band is negative (see Fig. 5B), suggesting that oxygenation is the leading signal. The reduction in phase coherence might, therefore, reflect decreasing efficacy of brain oxygenation with age. However, the phase difference between the two signals in the respiratory band is not altered by ageing.

There is a tendency for the respiration–oxygenation coherence to decrease with age in the respiratory band (at location N5 ~0.3 Hz $p < 0.1$, in several locations $p < 0.05$): see Fig. 5B and Fig. 6 in the SM). The phase difference is negative and similar for both groups, suggesting that oxygenation is the leading signal. The high coherence between respiration and each of the oxygenation signals implies a systemic orchestration of cortical oxygenation in rhythm with breathing, an effect that is reduced in the older group. The phase difference, indicating which signal leads or lags the other, can be explained as follows:

Figure 10: Spearman correlations between A) IHR–respiration coherence in the myogenic band and fNIRS–EEG coherence in the myogenic band, B) IHR–respiration coherence in the respiratory band and fNIRS–EEG coherence in the respiratory band. The black circles show the coherence values between fNIRS–EEG combinations (176 combinations per participant), while the red crosses show the median coherence for each participant. The correlation is found between the median coherence values and IHR–respiration coherence.
1. The oxygenation signal is leading. Respiration is controlled by the brain stem, and voluntary respiration can also be controlled by the motor cortex. The brain then controls the respiration signal.

2. The respiration signal is leading. The period of an oscillation at 0.2 Hz is 5 s, and the period of an oscillation at 0.3 Hz is 3.3 s. This means that if the lag is longer than these times the phase difference might appear to be negative when, in reality, it is not. Zhang et al. (115) found in mice that breathing rate is a key modulator of cerebral oxygenation, and that oxygenation was correlated with both the respiration rate and the phase of the respiration cycle, which was true across the brain. They found a time lag of around 1-3 seconds between respiration and PtO2 consistent with the transit time of blood from the lungs to the brain, which was similar for blood oxygenation too. What a similar lag would be in humans is not known, and the corresponding phase difference is therefore also not known. However, it might be the case that, although the respiration is actually leading the oxygenation, the latter is delayed by more than the time for one complete respiration cycle.

4.4. Neurovascular coherence

Our key findings are: that there is significant neurovascular phase coherence in the 0.052–0.145 Hz (myogenic) frequency range; that this coherence is greatly reduced in older participants, as compared to the younger group; and that there is higher neurovascular coherence in the cardiac band in the older group (Fig. 7). As can be seen by comparing Figs. 6B,C and 7B, the coherence is also reduced in some locations without a decrease in power, so that the reduction in coherence cannot be accounted for by reduced power. To our knowledge, this is the first report of such effects.

In both the myogenic and cardiac bands there was widely distributed coherence across the cortex, as seen in Fig. 7B,C. In comparison, the neurogenic and respiratory bands showed little or no significant coherence in either age group, so that little change in coherence with age could be detected (see SM Fig. 23). The altered neurovascular coherence in the older group reflects less effective neurovascular interaction. Magnitude squared coherence (which has linear frequency resolution) between fNIRS and EEG signals near 0.1 Hz was found in a previous study of healthy participants aged around 30 years (70). This is in agreement with the coherence found in the younger group of the present study.

Grooms et al. (29) studied slow oscillations in EEG and blood oxygen level dependent (BOLD) signals in the default mode network. The authors concluded that there was evidence of a relationship between infra-slow (< 0.1 Hz) EEG and BOLD oscillations at the same frequencies, which was also found by Hiltunen et al. (52) and Keinänen et al. (45). These correlations were shown to span several brain regions and to be time-varying. Both fNIRS and BOLD signals reflect changes in oxygenation, and the BOLD signal has been shown to correlate with both oxyHb and deoxyHb (100 90). These studies investigated linear correlation between BOLD signals and infraslow EEG time-series, whereas the wavelet phase coherence used in our present study has logarithmic frequency resolution and evaluates coherence at each frequency step. The earlier studies did not consider frequencies above 0.1 Hz, while our present results show coherence centred around approximately 0.1 Hz. Although the studies are not directly comparable, they all provide evidence of a significant relationship between electrical neural activity and oxygenation oscillations in the brain at low frequencies. Mitra et al. (61) found a similar relationship in mice, using laminar electrophysiology and hemoglobin imaging. Such invasive recordings have the advantage of measuring activity that is more local but, given that our goal was in-vivo, non-
invasive measurements in humans, we chose to use EEG and fNIRS.

In fMRI studies it is found that typically, only 10% of the variability in the hemodynamic signal can be explained by neural activity \(^{(21)}\). Similarly, we show low, but significant, coherence between the EEG and fNIRS signals. BOLD signals are often thought of as a convolution of the neural activity with what is known as the hemodynamic response function (HRF) \(^{(79)}\). The HRF contains vascular factors, such as vasomotion, which is also present in the fNIRS signals. The difference in coherence between the younger and older groups illustrates that care should be taken in studies estimating the HRF, as the response is age-dependent.

4.5. Neurovascular coupling

In the awake resting state the brain consumes around 11% of the cardiac output and 20% of the body’s total metabolic energy, despite only making up about 2% of the body’s weight \(^{(30)}\). Resting state functional networks are consistently observed both with fMRI \(^{(8; 32)}\) and fNIRS \(^{(87)}\), in addition to EEG \(^{(4)}\), indicating that the resting state activity is not random. Neurovascular coupling, mediating the adjustment of local cerebral blood flow to match the energy demand of neurons, is maintained continuously by the diverse cells constituting the NVU \(^{(45)}\).

Studies of neurovascular coupling usually consider information flow from neurons to the vasculature. However, Kim et al. \(^{(48)}\) introduced the term vascular-neuronal coupling to describe information flow from vessel to astrocyte to neuron. From experiments on mice, both in vivo and in vitro, the authors concluded that neurons adjust their resting state activity based on brain perfusion changes in flow and pressure \(^{(47; 48)}\), probably to match the energy supply and demand. Changes in the blood flow and perfusion are characterised by oscillatory processes, and so is energy metabolism \(^{(11)}\). Hence, the energy exchange to the brain is also likely to occur in an oscillatory manner. To be efficient, this is coordinated between the cardiovascular system and the brain, leading to coherent oscillations. It therefore seems likely that the degree of myogenic phase coherence is a proxy for neurovascular efficiency, and that the neurovascular interaction can be considered as arising through the cardiovascular system and brain behaving as interacting oscillators.

Myogenic coherence is reduced in the older group of participants, indicating that the interaction between the oscillators has decreased. From the current results we cannot be certain of the direction of the interaction, but it could be bi-directional. The neurovascular coherence in the myogenic frequency band is negatively correlated with BMI (Fig. 9), an observation that could be further investigated in future studies.

In the present work we focused on quantifying the functioning of the neurovascular unit. Our reasoning is that the efficiency of coordination between neuronal and vascular activities can be evaluated by their phase coherence. It provides a measure of neurovascular coupling. Establishment of the directionality and strength of the coupling between the vascular and neuronal oscillatory modes, as identified in this work, will be the next step in the investigation. The efficiency of the neurovascular unit, and the neurovascular coupling, are of particular interest in relation to the older population, as decreased neurovascular coupling has been linked to cognitive decline and dementia \(^{(103; 17)}\). Especially promising is the recent report of a treatment that can improve neurovascular coupling in mice \(^{(102)}\). Evaluation of neurovascular phase coherence therefore has potential as a biomarker for the efficiency of the NVU, and could be used to evaluate the effects of treatment and lifestyle changes in humans.

4.6. Origins of 0.1 Hz oscillations

Having established that oxygenation and neural activity are coherent around 0.1 Hz, reflecting neurovascular interactions, the next question is: what are the mechanisms underlying the coherence? There are several possible origins of 0.1 Hz oscillations in the brain and cardiovascular system, which we now consider.

Systemic cardiovascular fluctuations. IHR is coherent with oxygenation at \(\sim0.1\) Hz (see Sec. 3.2), and, to a much lesser degree respiration is also coherent with oxygenation at \(\sim0.1\) Hz. However, the systemic cardiovascular fluctuations cannot fully explain the oscillations in oxygenation \(^{(44)}\), indicating that the 0.1 Hz oscillations could have additional origins. Most EEG probes have low but non-zero coherence with the \(\sim0.1\) Hz IHR signal, but the IHR–EEG coherence is generally lower than the neurovascular coherence evaluated from the EEG and fNIRS time-series: see SM Fig. 5 and SM Sec. 10.

Vascular origin. In 1902 Bayliss \(^{(6)}\) considered how smooth muscle cells respond to changes in intravascular pressure. This myogenic hypothesis was later studied by Folkow \(^{(25)}\) who found it was important for blood autoregulation. Myogenic oscillations tend to manifest between 0.052-0.145Hz \(^{(60; 97; 101; 53)}\). Local 0.1 Hz oscillations consistent with myogenic activity have been observed in vivo in the human cortex \(^{(51; 72)}\). These oscillations are believed to contribute to the clearance of substances like amyloid-beta proteins from the brain \(^{(3)}\).

Vascular neural origin. The hemodynamic bases of Meyer waves are oscillations of the sympathetic vasomotor tone of arterial blood vessels \(^{(42)}\). Note that this would contribute to systemic cardiovascular fluctuations by impacting the heart rate and arterial blood pressure. In studies on blood flow with neural blockers, however, it was shown that 0.1 Hz activity continues, suggesting at least a contribution from the myogenic activity \(^{(48; 101)}\). Rayshubskiy et al. \(^{(51)}\) found that 0.1 Hz oscillations in the human cortex were spatially localised, and correlated with the diameter of local vessels, suggesting that the 0.1 Hz hemodynamic oscillation in the human cortex are primarily myogenic in nature.
Electrophysiological origin in the brain. Oscillations around or below 0.1 Hz detected with EEG in the brain are not traditionally referred to as myogenic, but rather as infra-slow (<0.1 Hz) or slow oscillations (11). Such studies usually do not include measurements of cardiovascular activity, and rather focus on metabolic processes. The origin of these oscillations is still debated (71; 108; 70; [111] 49). Mitra et al. (54) have shown that, in mice, the infra-slow oscillations have unique dynamics when compared to higher frequencies, and should be considered as a separate physiological process. There is evidence for both a neuronal and a non-neuronal generator of these oscillations, and possibly both of them contribute.

One feature of the infra-slow oscillations is that their phases were found to be correlated with the amplitude of faster oscillations and with performance (67; 19). It has been suggested that infra-slow oscillations are related to gross cortical excitability (73) and to arousal (80; 92). Changes in arousal level would be reflected in the heart rate, which could explain why we observe IHR–EEG coherence. Non-neuronal infra-slow oscillations in EEG could stem from a potential difference across the blood-brain barrier (BBB) (74; 105; 52; 104; 106). This difference is sensitive to pH (104), and can be manipulated by hyperventilation, hypocapnia (105) or postural changes that affect intracranial hemodynamics (106). The BBB, consisting of endothelial cells, is known to be affected by aging (91). Further, electrical coupling through the endothelium is a mechanism for neurons to modulate smooth muscle cell activity and therefore arteriole diameter (21). At the molecular level, another component that could affect the slow EEG oscillations might be neural mitochondrial calcium signalling, which is known to be altered in aging (56). Neuron-glia interactions are also thought to contribute to the slow oscillations (55; 10), as are extracellular ion fluxes which have been shown to contribute to the coupling of brain activity and blood flow (59).

Other origins. Another potential origin of infra-slow fluctuations is movement artifacts from fidgeting, which has been observed in both animal and human studies. It has been shown in mice that both flow in arterioles and also brain electrical activity can be impacted by these artifacts (21), however in humans it is hardly likely that such movement artefacts would be oscillatory.

We find widely-distributed ~0.1 Hz coherence across the cortex, which does not in itself represent evidence of a single generator. Neurovascular coherence in the myogenic band is correlated with the IHR–respiration coherence in the myogenic band, while the neurovascular coherence in the respiratory band is not correlated with the IHR–respiration coherence in the respiratory band. This result suggests that the myogenic frequency band and the 0.1 Hz oscillation are key to understanding aging from both the neural and vascular perspectives.

4.7. Frequency and amplitude modulation of the γ-band by low-frequency oscillations

An interesting question to explore is whether the amplitude and/or frequency of γ oscillations in the EEG is modulated by the slower oxygenation/vascular oscillations. Murta et al. (68) have reported evidence for amplitude modulation from combined fMRI and EEG studies. There is also some evidence from previous fNIRS studies that β oscillations are modulated by brain oxygenation (77). The ~0.1 Hz variations in the oxygenation level of brain blood are generally used as an fMRI-based surrogate of “resting-state” neuronal activity, implying that it is the gamma band which is most closely correlated with BOLD signals (21).

To investigate possible amplitude and frequency modulation of neuronal activity by low-frequency oxygenation oscillations, we focused on the higher β / lower γ band (20–30 Hz). Our results revealed that the spatial coherence between EEG signals has a peak in this frequency range. They also showed non-zero power for γ–instantaneous frequency and γ–instantaneous power time-series between 0.007 and 4 Hz, as shown in Figs. 5A, B).

We therefore calculated the WPC of the γ instantaneous frequency-time-series with fNIRS (frequency modulation), and of the γ–instantaneous power-time-series with fNIRS (amplitude modulation) for the 8 locations where the fNIRS and EEG are co-located. However, we found little to no coherence in the frequency band considered here (Fig. 5C) indicating that there was no significant frequency modulation. We comment however, that a single γ instantaneous frequency provides only a rough measure of the collective neuronal activity in the γ band.

On the other hand, a non-zero coherence was observed for amplitude modulation, as shown in Fig. 5D), though not for all participants. What is more interesting is that we observed a negative phase shift for the older group around 0.06–0.08 Hz. This frequency range is often linked to periodic breathing, which appears in hypoxia (51). This may indicate that some effects of hypoxia appear with aging, even in the resting state. These results suggest an exciting direction for future research through more detailed investigations of how fast neural activity measured by EEG is modulated by slow hemodynamic oscillations measured by fNIRS. Further investigation of the coherence between the band power and oxygenation should also include a broader γ frequency band, and could explore other frequency bands too. This may elucidate additional information about neurovascular interactions.

In addition, neuro–respiratory interactions with the γ band may be investigated using the IRR and respiration signals. Our results show that both the instantaneous γ–frequency and instantaneous γ–power are modulated by respiration (Figure 5A and B). Earlier studies in both humans and animals (12; 105; 26) have provided evidence of respiration-related oscillations in several brain regions. Distinct from respiration-related artefacts in fMRI,
respiration-related networks have been shown to be linked with the $\gamma$-band power $[105]$. Respiration-related oscillations might aid coordination between different brain regions $[26]$. In humans, the phase of respiration has an impact on memory encoding and perception, further indicating the importance of respiration for cognitive function.

The close relationship of neural activity to both hemodynamics and respiration illustrates the importance of simultaneous measurements to investigate interactions between the underlying systems, e.g. as done in systemic physiology augmented fNIRS $[89]$.  

4.8. Effect of increased BMI and BP

The two age groups differ in BMI and sBP (Table 3).

From Fig. 9A it is clear that BMI is correlated with neurovascular coherence in the myogenic band. From Fig. 9B it is clear that BMI is correlated with neurovascular coherence in the myogenic band. To separate these effects, we created a smaller data-set, matching the BMI and BP values between the younger and older groups. This modified data-sets consisted of 13 younger and 13 older participants with comparable BMI ($p = 0.80$) and $\text{sBP}$ ($p = 0.86$). We then compared the subgroups’ power/coherence values. The results and subgroup details are shown in the SM Sec. 9. We conclude that, while it is difficult to disentangle the influence of aging from that of the increased BMI/BP, there is evidence for an effect of aging on the parameters considered, independent of the BMI/BP differences.

It is likely that BMI/BP differences also contribute, but some of the loss of significance can be attributed to loss of statistical power due to having smaller groups.

Further investigation of the impact of increased BP and BMI could be useful given that raised BMI is associated with increased risk of cardiovascular diseases such as coronary heart disease $[54]$, and increased mid-life BMI is associated with the development of dementia in later life $[74]$.

5. Conclusions

We have investigated the function of the neurovascular unit at macroscopic level, evaluating the coherence between the oscillations in the cardiovascular system (simultaneously monitored centrally via ECG and respiration effort, and locally by whole-brain fNIRS) and oscillations in neuronal activity (monitored locally by EEG), thereby gaining insight into the mechanisms of ageing in the NVU.

Most notably, the neurovascular coherence near $0.1\, \text{Hz}$ is significantly reduced by ageing. This presumably reflects progressively impaired control of cerebral blood flow. The changes in cardio-respiratory coherence with blood oxygenation confirm that age affects significantly brain vascular function and oxygenation. It seems that this then impacts neuronal activity.

The methods described here, combined with state-of-the-art time-frequency analysis focusing on phase dynamics, have yielded new insights into the neurovascular dynamics of the aging brain. In particular, they have provided a quantitative measure of the neurovascular efficiency and health of the NVU, information that cannot be obtained in other ways. The approach could thus be used for non-invasive evaluation of the decline of neurovascular function in normal aging, as well as for monitoring the efficacy of treatment or lifestyle changes in a wide range of neurodegenerative disorders.

Code availability

MODA is a numerical toolbox developed by the Lancaster University Nonlinear Dynamics group (available at https://doi.org/10.5281/zenodo.3470856).


In addition, these MATLAB functions were used for plotting: Rob Campbell (2021), https://github.com/raacampbell/sigstar Bastian Bechtold (2016), Violin Plots for MATLAB, Github Project, https://github.com/bastibe/Violinplot-Matlab

Data availability

The data analysed are available in Lancaster University’s Pure database:
https://doi.org/10.17635/lancaster/researchdata/427

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Author contributions

GL did the measurements and preliminary analysis of the data. JK and BM organised all clinical aspects of the study. JB analysed the data completely, prepared the figures and a draft of the text. PVEMcC contributed to writing the funding proposal. TJC supervised JB and advised on writing the manuscript. AS conceived the study, wrote the funding proposal, provided the theoretical framework for the time-series analysis methods, selected and discussed the analysis methods, supervised GL and JB and closely discussed the results. She was also involved in structuring the manuscript. All authors contributed to editing the manuscript, and accepted the final version.

Conflict of interest

The authors have no conflict of interest.