

**INTEGRATION OF SIMULTANEOUS FMRI, EEG AND  
PHYSIOLOGICAL MEASURES TO DETERMINE AND  
VERIFY VIGILANCE STATE**

by

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PHYSIOLOGICAL MEASURES TO DETERMINE AND  
VERIFY VIGILANCE STATE**

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## ACADEMIC ETHICS AND INTEGRITY STATEMENT

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## ABSTRACT

### INTEGRATION OF SIMULTANEOUS FMRI, EEG AND PHYSIOLOGICAL MEASURES TO DETERMINE AND VERIFY VIGILANCE STATE

A major challenge in resting-state fMRI (rsfMRI) analysis lies in its sensitivity to fluctuations in vigilance—the state of wakefulness and attention. Vigilance-driven variations can alter fMRI signal amplitudes and correlations between brain regions. These effects, often combined with systemic physiology effects, can be misinterpreted if not properly accounted for. This study aimed to demonstrate how vigilance states modulate rsfMRI metrics and physiological signals in a healthy young population. Using an EEG-based vigilance index, we delineated the effects of vigilance on fMRI signals and their links to physiological dynamics. Heart rate, respiratory volume, photoplethysmography amplitude (PPG-AMP) were analyzed to understand their contribution to these dynamics. Our work advances the field through several key innovations: developing vigilance classification methodologies, integrating cerebrospinal fluid (CSF) flow oscillations from the fourth ventricle as a signal of interest, utilizing autonomic physiology biomarkers which are often overlooked in rsfMRI studies, and leveraging time-lagged correlation matrices to uncover spatiotemporal brain dynamics. Data from 17 participants across multiple short, eyes-open resting-state scans were analyzed. We observed co-variations between CSF oscillations and respiratory volume, correlations between sensory regions and heart rate, and between the default mode network (DMN) and PPG-AMP. Additionally, we demonstrated reduced connectivity in drowsy states compared to wakefulness. Our findings demonstrate the systemic physiology and neural activity relationship across vigilance states and enhances the interpretative accuracy of fMRI, broadening its potential applications in both research and clinical domains.

**Keywords:** EEG, FMRI, Physiology, Vigilance

## ÖZET

### EŞZAMANLI FMRI, EEG VE FİZYOLOJİK ÖLÇÜMLERİN ENTEGRASYONU İLE UYANIKLIK DURUMUNUN BELİRLENMESİ VE DOĞRULANMASI

Dinlenim durumu fonksiyonel manyetik rezonans görüntüleme (rsfMRI) analizinde karşılaşılan en büyük zorluklardan biri, uyanıklık ve dikkat düzeyindeki dalgalanmalara olan duyarlılığıdır. Uyanıklığa bağlı değişimler, fMRI sinyal genliklerini ve beyin bölgeleri arasındaki korelasyonları etkileyebilir. Bu etkiler doğru bir şekilde ele alınmadığında, sistemik fizyoloji kaynaklı değişimlerle de birleşerek yanlış yorumlanabilir. Bu çalışma, sağlıklı genç bireylerde uyanıklık durumlarının rsfMRI metriklerini ve fizyolojik sinyalleri nasıl modüle ettiğini ortaya koymayı amaçlamaktadır. EEG tabanlı bir uyanıklık indeksi kullanarak, fMRI sinyalleri üzerindeki uyanıklık etkilerini ve bunların fizyolojik dinamiklerle ilişkisini inceledik. Kalp atış hızı, solunum hacmi ve fotopletismografi genliği (PPG-AMP) gibi sistemik belirteçler analiz edilerek katkıları değerlendirildi. Çalışmamız, alanı ileriye taşıyan birkaç önemli yenilik içermektedir: uyanıklık sınıflandırma yöntemlerinin geliştirilmesi, dördüncü ventriküldeki beyin omurilik sıvısı salınımlarının sistemik bir sinyal olarak entegre edilmesi, rsfMRI çalışmalarında sıklıkla göz ardı edilen fizyoloji biyobelirteçlerinin kullanımı ve zaman gecikmeli korelasyon matrislerinin uygulanması. 17 katılımcının kısa süreli, gözler açık dinlenim hali tarama verileri analiz edilmiştir. BOS salınımları ile solunum hacmi arasında eş değişimler, duyuşal beyin bölgeleri ile kalp atış hızı arasında korelasyonlar ve varsayılan mod ağı (DMN) ile PPG-AMP arasında ilişkiler gözlemlenmiştir. Ayrıca, kısa süreli taramalarda bile uyku durumlarında bağlantısallığın uyanıklığa kıyasla azaldığını gösterdik. Bulgularımız, sistemik fizyoloji ile beyin aktivitesi arasındaki uyanıklığa bağlı ilişkiyi ortaya koyarak fMRI ölçümlerinin tutarlılığını artırmakta, araştırma ve klinikte daha geniş uygulama alanları açmaktadır.

**Anahtar Sözcükler:** EEG, FMRI, Fizyoloji, Uyanıklık

## TABLE OF CONTENTS

|                                                                                           |     |
|-------------------------------------------------------------------------------------------|-----|
| ACKNOWLEDGMENTS . . . . .                                                                 | iii |
| ACADEMIC ETHICS AND INTEGRITY STATEMENT . . . . .                                         | v   |
| ABSTRACT . . . . .                                                                        | vi  |
| ÖZET . . . . .                                                                            | vii |
| LIST OF FIGURES . . . . .                                                                 | x   |
| LIST OF TABLES . . . . .                                                                  | xv  |
| LIST OF SYMBOLS . . . . .                                                                 | xvi |
| LIST OF ABBREVIATIONS . . . . .                                                           | 1   |
| 1. INTRODUCTION . . . . .                                                                 | 2   |
| 2. BACKGROUND . . . . .                                                                   | 7   |
| 2.1 Vigilance . . . . .                                                                   | 7   |
| 2.1.1 Neural Activity and Physiological Signal Patterns Across Vigilance Levels . . . . . | 8   |
| 2.1.2 EEG Vigilance Metrics . . . . .                                                     | 10  |
| 2.2 Magnetic Resonance Imaging (MRI) . . . . .                                            | 12  |
| 2.2.1 Functional Magnetic Resonance Imaging . . . . .                                     | 12  |
| 2.2.2 Resting-state fMRI . . . . .                                                        | 14  |
| 2.3 Electroencephalography (EEG) . . . . .                                                | 15  |
| 2.4 Simultaneous EEG-fMRI . . . . .                                                       | 19  |
| 2.4.1 Gradient Artifacts . . . . .                                                        | 20  |
| 2.4.2 Cardioballistic Artifacts . . . . .                                                 | 21  |
| 2.5 Measurement of Physiological Signals . . . . .                                        | 23  |
| 2.5.1 Pulse Oximeter . . . . .                                                            | 23  |
| 2.5.2 Respiratory Bellow . . . . .                                                        | 25  |
| 3. MATERIALS AND METHODS . . . . .                                                        | 28  |
| 3.1 Experimental Design and Subjects . . . . .                                            | 28  |
| 3.2 Setup and Data Acquisition . . . . .                                                  | 29  |
| 3.3 Data Preprocessing . . . . .                                                          | 33  |
| 3.3.1 MRI Data Preprocessing Pipeline . . . . .                                           | 33  |

|       |                                                                  |    |
|-------|------------------------------------------------------------------|----|
| 3.3.2 | EEG Artifact Removal . . . . .                                   | 36 |
| 3.3.3 | Preprocessing of Physiological signals . . . . .                 | 38 |
| 3.4   | Data Analysis . . . . .                                          | 42 |
| 3.4.1 | Regions of Interest . . . . .                                    | 42 |
| 3.4.2 | EEG Power Spectrum Analysis and Vigilance Metric . . . . .       | 43 |
| 3.4.3 | Time-lagged Cross Correlations . . . . .                         | 45 |
| 3.4.4 | Time-Lagged Correlation Matrix . . . . .                         | 46 |
| 3.4.5 | Functional Connectivity . . . . .                                | 46 |
| 4.    | RESULTS . . . . .                                                | 48 |
| 4.1   | Classification of Vigilance Levels: Awake vs Drowsy . . . . .    | 48 |
| 4.2   | Impact of Drowsiness on Brain Activity Synchronization . . . . . | 51 |
| 4.3   | Brain and Systemic Physiology Relationship . . . . .             | 52 |
| 4.3.1 | fMRI and Heart Rate Interactions . . . . .                       | 52 |
| 4.3.2 | fMRI and PPG Amplitude Interactions . . . . .                    | 57 |
| 4.3.3 | fMRI and Respiratory Variation Interactions . . . . .            | 60 |
| 4.4   | fMRI and EEG Vigilance Metrics . . . . .                         | 63 |
| 5.    | DISCUSSION . . . . .                                             | 68 |
| 6.    | CONCLUSION . . . . .                                             | 73 |
|       | REFERENCES . . . . .                                             | 75 |

## LIST OF FIGURES

- Figure 2.1 Visualization of the BOLD response, showing increased blood flow and volume in the arteries upon neural activation, accompanied by an increased fMRI signal. Bottom shows the signal profile of the electrical versus hemodynamic responses for neural activity. 13
- Figure 2.2 Color coded brain regions that are masked and analyzed within this study, shown on an exemplary data from this study. Visual (light green), auditory (red), motor (purple); default mode network (DMN) regions: precuneus (brown), posterior cingulate (dark blue), angular gyrus (dark green); anterior cingulate (pink), thalamus (lilac), middle cingulate (light blue), insula (yellow). 15
- Figure 2.3 The EEG electrode placement employed within this study. It is the international 10-20 system of EEG electrode placement. 17
- Figure 2.4 An exemplary data showing activity of different EEG bands for 8 seconds of data from O1 channel of 1 subject. The vertical axis is in  $10^{-5}\mu V$ . 17
- Figure 2.5 A) Example of a gradient artifact observed in the raw EEG signal recorded during simultaneous EEG-fMRI acquisition. The artifact appears as regular, high-frequency oscillations synchronized with the MR gradient pulses. B) Example of a cardioballistic artifact in the raw EEG signal. These artifacts are caused by pulsatile blood flow within the MR environment and manifest as periodic deflections in the signal. The red box highlights one such artifact, emphasizing its distinct shape and periodicity. C) Gradient- and cardioballistic artifact-cleaned EEG data. 22
- Figure 2.6 Raw PPG and respiration signals from an exemplary subject. 24



- Figure 2.7 (A) Example of raw photoplethysmogram (PPG) data from an exemplary subject, illustrating key morphological features, including the systolic peak, diastolic peak, anacrotic phase, catacrotic phase, systolic upslope, diastolic decay, and the overall waveform width. (B) A pulse oximeter positioned on a subject's finger, the typical setup used for acquiring PPG data. 25
- Figure 2.8 (A) Example of raw respiratory bellow data from an exemplary subject, showing the cyclic pattern of respiratory activity over time. The signal amplitude reflects changes in abdominal expansion during breathing. (B) A respiratory bellow device fitted on a subject, demonstrating its placement around the torso to measure respiratory movements during simultaneous recordings. 26
- Figure 3.1 An exemplary multimodal resting state scan. Image shows *left* MRI computer, *middle-left* eye camera computer recording the eye, *middle-right* stimuli computer where a cross is presented for the subject to focus on, and finally *right* the EEG computer. 29
- Figure 3.2 A) Modalities used in our study. B) The schematic of multimodal integrations, both for data acquisition and for TTL trigger. 30
- Figure 3.3 MR Compatible 32 Channel EEG Cap (Brain Products) used in our study. 32
- Figure 3.4 Preprocessing steps for anatomical and functional MRI datasets. On top of these major steps, there are additional preprocessing such as removal of first 4 TRs in fMRI, Gaussian blur etc. as described in chapter 3.3.1. 34
- Figure 3.5 An exemplary data showing at (top) three different times during the scan, subject had moved their finger, resulting in movement artifacts, and (bottom) PPG-AMP calculated from noisy PPG signal (background with huge changes) where movement reflects as huge amplitude changes vs PPG-AMP calculated from cleaned PPG (on top of the noise PPG-AMP). 40

- Figure 3.6 Example of pulse oximetry data and its derived metrics. The top plot shows the preprocessed photoplethysmogram (PPG) signal, highlighting the cleaned waveform. The middle shows the PPG amplitude (PPG-AMP), extracted from the peak-to-peak analysis of the PPG signal, reflecting blood volume changes. On the bottom heart rate (HR) is presented, calculated as beats per minute from the PPG signal. 40
- Figure 3.7 Example of respiratory bellow data and its derived metric. The top Figure shows the preprocessed respiratory bellow signal, with the cyclic changes in thoracic or abdominal expansion during breathing. The bottom panel displays the respiratory volume (RV), calculated from the bellow signal to quantify respiratory volume changes over time. 42
- Figure 3.8 A masking being done in AFNI software. Figure shows precuneus region of an example subject being selected for a mask to be created. Functional data are then masked and averaged using these masks. Masks for other regions were shown in Figure 2.2. 43
- Figure 3.9 (Left) Typical connectivity matrix for 300 regions of interest for different resting state networks. (Right) Surface representations of the regions of interest with color coding. Figure from [1]. 47
- Figure 4.1 The drowsy stage counts for each subject in number of TRs. 49
- Figure 4.2 Connectivity analysis between awake and drowsy subjects of the *comprehensive* group (B threshold = 58). 51
- Figure 4.3 Time-lagged correlation plots of *comprehensive* group's heart rate signal with various regions of interest. 53
- Figure 4.4 Time-lagged correlation plots of the heart rate signal with various regions of interest for *boundary* group. 54
- Figure 4.5 Time-lagged correlation matrix of the heart rate signal with various ROI in comprehensive group. First row auto-correlation, x-axis represents time-lags. 55

|             |                                                                                                                                                                                      |    |
|-------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----|
| Figure 4.6  | Voxel-wise correlation group averages of the heart rate signal with whole brain for <i>comprehensive</i> group, left for awake, right for drowsy subjects.                           | 56 |
| Figure 4.7  | Time-lagged correlation plots of the 4th ventricle signal (as a representative of CSF signal) with HR and RV signals for A. <i>comprehensive</i> group and B. <i>boundary</i> group. | 57 |
| Figure 4.8  | Time-lagged correlation plots of <i>comprehensive</i> group's PPG-AMP and RV signals with precuneus and angular gyrus - active regions of the default mode network.                  | 58 |
| Figure 4.9  | Time-lagged correlation plots of <i>boundary</i> group's PPG-AMP and RV signals with precuneus and angular gyrus - active regions of the default mode network.                       | 58 |
| Figure 4.10 | Time-lagged correlation plots of the PPG-AMP signal with insula and primary visual regions for A. <i>comprehensive</i> group and B. <i>boundary</i> group.                           | 60 |
| Figure 4.11 | Time-lagged correlation matrix of the PPG amplitude signal with various ROI in <i>comprehensive</i> group. First row auto-correlation, x-axis represents time-lags.                  | 61 |
| Figure 4.12 | Voxel-wise correlation group averages of the PPG amplitude signal with whole brain for <i>comprehensive</i> group, left for awake, right for drowsy subjects.                        | 62 |
| Figure 4.13 | Time-lagged correlation matrix of the respiratory variation signal with various ROI in <i>comprehensive</i> group. First row auto-correlation, x-axis represents time-lags.          | 64 |
| Figure 4.14 | Voxel-wise correlation group averages of the respiratory variation signal with whole brain for <i>comprehensive</i> group, left for awake, right for drowsy subjects.                | 65 |
| Figure 4.15 | Time-lagged correlation matrix of the Olbrich's frontal metric with various ROI in <i>comprehensive</i> group. First row auto-correlation, x-axis represents time-lags.              | 66 |

Figure 4.16 Time-lagged correlation matrix of the Olbrich's occipital metric with various ROI in *comprehensive* group. First row auto-correlation, x-axis represents time-lags. 67



LIST OF TABLES

|           |                                        |    |
|-----------|----------------------------------------|----|
| Table 3.1 | Anatomical MRI acquisition parameters. | 31 |
| Table 3.2 | Functional MRI acquisition parameters. | 31 |



## LIST OF SYMBOLS

|          |                      |
|----------|----------------------|
| $\alpha$ | Alpha Frequency Band |
| $\beta$  | Beta Frequency Band  |
| $\gamma$ | Gamma Frequency Band |
| $\delta$ | Delta Frequency Band |
| $\theta$ | Theta Frequency Band |



## LIST OF ABBREVIATIONS

|         |                                                     |
|---------|-----------------------------------------------------|
| AP      | Action Potential                                    |
| AUD     | Auditory Network                                    |
| BC      | Ballistocardiogram Artifact                         |
| BOLD    | Blood Oxygen Level-Dependent                        |
| CBA     | Cardioballistic Artifact                            |
| CBF     | Cerebral Blood Flow                                 |
| CBV     | Cerebral Blood Volume                               |
| CSF     | Cerebrospinal Fluid                                 |
| DMN     | Default Mode Network                                |
| EEG     | Electroencephalography                              |
| EPSP    | Excitatory Postsynaptic Potential                   |
| FC      | Functional Connectivity                             |
| fMRI    | Functional Magnetic Resonance Imaging               |
| GA      | Gradient Artifact                                   |
| HR      | Heart Rate                                          |
| iEEG    | Intracranial Electroencephalography                 |
| MRI     | Magnetic Resonance Imaging                          |
| PA      | Pulse Artifact                                      |
| PPG     | Photoplethysmography                                |
| PPG-AMP | Photoplethysmography Amplitude                      |
| PSP     | Postsynaptic Potential                              |
| RF      | Radiofrequency                                      |
| RSN     | Resting State Networks                              |
| rs-fMRI | Resting State Functional Magnetic Resonance Imaging |
| RV      | Respiratory Variation                               |
| SMN     | Somatomotor Network                                 |
| VIS     | Visual Network                                      |

## 1. INTRODUCTION

Functional magnetic resonance imaging (fMRI) is a powerful tool for its ability to image the dynamically changing activity of the brain, allowing for the investigation of both the functional and the structural organization that gives rise to brain function in health and disease. During both resting-state and active task involvement, fMRI can be used to measure the fluctuations in the neural activity, capturing the signal patterns when a subject is not engaged in any specific task or during particular task performance. Resting-state fMRI (rs-fMRI) has been increasingly used in research settings to explore the intrinsic functional connectivity of the brain. There is mounting evidence that the level of vigilance during rs-fMRI scans contributes to the global fluctuations in brain activity as measured by fMRI, thus the functional connectivity inferred from it [2],[3]. Vigilance is a state within the range from wakefulness to drowsiness, and is influenced by various internal and external factors such as sleep deprivation, fatigue, stress levels, time of day and medication use [4]. Also, vigilance level is subject to oscillation with certain diseases, or with aging [5]. As shown by Tagliazucchi et al. (2014) using both eyes-open and eyes-closed rs-fMRI data of 1147 subjects from 1000 Functional Connectomes Project datasets, 1/3 of subjects exhibit drifts between wakefulness and sleep within the first 3 minutes of scan [6]. The varying vigilance level of subjects during the scans is a confounder, impacting the reliability and consistency of fMRI data. Moreover, vigilance fluctuations co-vary with autonomic nervous system (ANS) responses, meaning that external measurements of systemic physiology such as heart rate and respiration can be used to track changes in vigilance. These systemic physiology changes can also lead to variations in the BOLD signal, further complicating the interpretation of neuroimaging data [7]. Thus, the level of vigilance should be correctly accounted for when analyzing rs-fMRI, in order to avoid misinterpretations. This is particularly important in clinical settings, where accurate interpretation of rs-fMRI data is crucial for diagnosis and anticipating the course of various neurological and psychiatric conditions. There is a need for defining objective biomarkers to characterize vigilance level in fMRI studies.



A key contributor to fMRI signal is the hemodynamic response to neural activity that causes changes in the cerebral blood volume. Cerebral blood volume (CBV) changes in turn cause cerebrospinal fluid (CSF) flow in or out of the brain, so that the fluid volume within the head remains unchanged [8]. Fultz et al. (2019) showed that a sequence of changes in electrocortical activity, CBV, and CSF signals follow each other in sleep. During N1 and N2 sleep, changes in CSF flow detected by fMRI from the fourth ventricle covary with EEG slow waves. Low-frequency neural activity oscillations during non-rapid eye movement (NREM) sleep, which are important for memory consolidation and neuronal processing, are shown to be strongly associated with fluid inflow [9]. The occurrence of fluid pulsations during sleep is tied to both the neurovascular response (hence the CBV changes), and the changes in autonomic activity (sympathetic arousal). Picchioni et al. (2022) later showed that during both sleep and wake, vasoconstriction events result in CSF pulsations. CSF fluctuations are highly coupled with variations in vascular tone as detected by the PPG amplitude (PPG-AMP) and respiratory volumes [10]. The vasoconstrictions occur either via active, due to electrocortical activity, or via passive vascular responses, resulting from blood pressure changes. Pulsations that are of vasoactive origin are likely larger in amplitude than that of cardiac and respiratory origin, which was previously thought as the single origin of CSF inflow. These large amplitude CSF pulsations may have originated either from widespread neural activity changes with arousal events, or changes in autoregulatory mechanisms [8]. Signal fluctuations observed in the fourth ventricle, thought to be CSF flow, were previously observed during times when subjects were prone to drifting into sleep. Study conducted by Gonzalez-Castillo et al. (2021) showed that an inflow-like behavior observed in the fourth ventricle can be used to predict drowsiness/alertness based on eye closure as a vigilance metric [11]. When combined, these findings suggest that changes in neural activity during altered vigilance states are closely linked to CSF pulsations.

Previous studies determined vigilance levels during fMRI with the use of metrics obtained from concurrent modalities, such as EEG [12],[13],[14] and eye camera [15],[16]. Furthermore, as the link between fMRI signal in gray matter and changes in sympathetic activity [10],[17] have been established before, there are some studies

investigating the use of physiological measures as markers of drowsiness and alertness. Drowsiness and reduced wakefulness are associated with changes in autonomic regulation, often manifesting as changes in PPG-AMP [10] and altered cardiovascular variability [18]. Changes in HR have been associated with modulations in brain regions responsible for attention and emotional regulation [19]. Furthermore, during transitions from wakefulness to drowsiness, HR often decreases and becomes more variable, reflecting the down-regulation of sympathetic activity [20]. RV can be used as another indirect physiological measure of vigilance since changes in RV can reflect alterations in autonomic activity, reflecting transitions between different states of arousal. For instance, during drowsiness, respiration may become shallower and more irregular, leading to detectable changes in the RV signal [21]. Incorporating physiological measurements into assessment of vigilance can help link the physiological state of the vascular system with brain activity in order to understand how systemic factors like blood flow and autonomic regulation interact with neurocognitive processes.

The main focus of the previous literature was on relating the electrophysiological or autonomic nervous system responses to different wakefulness levels, by investigating the temporal characteristics of these signals in relation to fMRI BOLD signal. Liu & Falahpour (2020) reviewed studies regarding the vigilance fluctuations in relation to both the independent vigilance metrics that can be applied to rsfMRI and the BOLD amplitude changes in relation to vigilance. Although most of the recent studies used EEG metrics to determine vigilance, fMRI derived measures, such as functional connectivity-based vigilance measures have also been explored [4]. However, these studies were either limited to determining different sleep stages [22],[23], or heavily relied on the metrics based on only a single modality [13],[15],[24],[25].

Previous studies using EEG or simultaneous EEG-fMRI along with behavioral measures have reported that with increased vigilance, fMRI signal increases in thalamus, with widespread decreases in occipital, parietal and cingulate cortices [13],[26],[27]. Another study reported negative correlations between fMRI and behavioral arousal in widespread areas of cortex, such as insula, extrastriate visual, and limbic cortex, along with positive correlations in thalamus and cerebellum [25]. These

approaches, along with techniques using connectivity estimates, provide insight into the variability of vigilance across participants. We expect that as vigilance profoundly shapes rsfMRI signals, accounting for its effects will be essential for accurate interpretation of fMRI and advancing both research and clinical applications.

Simultaneous measurements of physiology or electrical activity of the brain are not always available for use with fMRI in clinical settings, and require expertise to set up or analyze. Thus, the inability to identify the vigilance-related signal changes in the fMRI data limits the clinical use of fMRI, since the information obtained from it becomes compromised.

The primary goal of this thesis is to investigate how vigilance states influence resting-state fMRI (rs-fMRI) and physiological signals in a healthy young population. Our central hypothesis is that vigilance states have a significant impact on rs-fMRI inferences and can be reliably detected using concurrent EEG, supported by physiological markers and CSF signals derived from fMRI. We utilized a multimodal approach, integrating simultaneous fMRI and EEG measurements with physiological markers, such as heart rate, respiratory volume, and PPG amplitude (PPG-AMP), during short, eyes-open resting-state scans. One relatively overlooked area of vigilance research is the integration of CSF signals and PPG measures, which this study seeks to address, offering a unique contribution to the field. Unlike some previous studies that focus on long-duration (around half an hour [13],[28],[29],[30]), eyes-closed resting-state scans [13],[30], we tailored our methodology to shorter, eyes-open resting-state scans, aligning more closely with conventional scanning protocols. Beyond characterizing the distinct patterns in highly alert and drowsy states, we investigated subtle transitions between wakefulness and drowsiness. This was achieved through innovative classification strategies: one employing a strict criterion and the other a more inclusive, comprehensive approach. Our findings aim to bridge the gaps in vigilance research, offering deeper insights into its influence on rs-fMRI and the underlying systemic physiology changes. In order to achieve our goal, we investigated cross-modal correlations between fMRI, EEG, systemic signals (HR, RV), and sympathetic biomarkers (PPG-AMP) to reveal the spatio-temporal relations between these measures, and their relation to vigilance

during resting-state sessions. We gave a specific focus on the CSF signal for its relation to certain diseases [31],[32],[33] and drowsiness [11]. We expected that by tracking these CSF fluctuations, we would be able to better detect transitions between wakefulness and drowsiness. Overall, we exploited neural, physiological and CSF signal oscillations that co-occur with vigilance fluctuations in fMRI, to provide a more comprehensive overview of the neurophysiology of vigilance. We ultimately aimed to advance our understanding of the neural activity behind the vigilance mechanisms, with a focus on the brain region signals from fMRI data. In the long run, this work has the potential to inform applications in monitoring and managing vigilance-related states, such as those influenced by sleep deprivation, fatigue, stress levels, medication use or disease, aiding in the development of targeted clinical tools.

## 2. BACKGROUND

### 2.1 Vigilance

The purpose of this section is to define and give background on what vigilance is, and why it matters in the context of fMRI to understand the dynamics that govern vigilance. Vigilance has varying definitions in the literature, with mostly encountered ones being sustained attention or arousal level within the sleep-wake spectrum [12]. Vigilance encompasses the level of alertness, attention, and cognitive engagement an individual maintains during a specific task or, in this context, during a resting-state fMRI scan. Here, we refer to vigilance, same as arousal, as the level of wakefulness within sleep-wake axis, or readiness to stimuli. Lower levels of vigilance have been related to occurrences of microsleep. Poudel et al. (2014) showed the association between microsleeps and the activity decrease in brain regions governing arousal, such as thalamus, posterior cingulate, and midbrain, with an increased activity in frontal, posterior parietal, and parahippocampal cortices. As wakefulness decreases and drowsiness emerges, slowing of responses and increases in attentional lapses occur [34]. With increasing levels of drowsiness, microsleep events start to take place. These microsleep events manifest themselves as behavioral cues as well, such as unresponsiveness, increased eye close, or lower eyelids. The occurrence of such attentional lapses, or microsleeps becomes more critical in professions regarding public or personal safety, such as drivers, pilots, traffic control, or factory process control [26].

During resting-state scans, due to the fact that subjects may experience a variety of cognitive states, the nature of the neural activity obtained from rs-fMRI has been a topic of debate. Resting-state is simply wakefulness where the attention is not directed specifically, extensively including various wakefulness states, including increased arousal to mind-wandering, daydreaming and even light sleep. Thus, it is natural to observe dramatic changes in functional integration between brain regions throughout the scan. Different views have been put forward on the meaning of the measures in-

ferred from rs-fMRI signal, converging on a shared view that fluctuations in vigilance is a major contributor [11]. Vast evidence has pointed out that changes in BOLD signal coincide with marked changes in vigilance over time. Previous research has linked different vigilance levels to different functional connectivity states [2] and established that accounting for the different vigilance levels during the scans is necessary to avoid misinterpretations [4]. The transient behavior of fMRI signal during resting-state imply the existence of a potential contributor causing moment-to-moment changes in the hemodynamic response. The changes in vigilance level is thought to have caused these widespread fluctuations in the fMRI signal, and the changes in functional integration of the brain networks [5]. The vigilance changes naturally co-occur with large amplitude alterations in autonomic physiology responses, leaving a question mark on whether these BOLD signal fluctuations occur partially due to the changes in autonomic nervous system activity and the changes they implicate on cerebral hemodynamics [30]. A multitude of studies investigated low-frequency oscillations in the fMRI signal. Research has investigated the relation of the low-frequency oscillations in the fMRI signal to heart rate and respiratory volumes [35],[36],[20],[37]. Chang et al, 2009 proposed that the underlying mechanism for this variation is neuronal activity associated with changes in arousal levels. Given the deficiency of constraints on the cognitive processes of a subject during resting-state scans, the time-varying changes in fMRI based brain network metrics might be reflecting alterations in the arousal level [38]. Thus, the ability to detect and track changes in vigilance holds the potential to provide fMRI studies with a more profound and accurate understanding of the functional organization of the human brain.

### **2.1.1 Neural Activity and Physiological Signal Patterns Across Vigilance Levels**

The challenges associated with vigilance levels become apparent in recent studies which uncover a significant occurrence of individuals falling asleep during typical resting-state experiments [6]. There may arise pronounced differences in the mean level of vigilance between subjects, or within subjects, in the deviation from mean

vigilance level, and in the magnitude of changes in the vigilance. This occurs mainly due to internal and external differences, such as sleep deprivation, time of the day, medication use, disease, age, or stress levels. Variations in vigilance levels present challenges, because they can introduce confounding factors and impact the interpretation of resting-state fMRI findings, particularly when comparing resting-state fMRI findings between healthy subjects and psychiatric patients. The challenges arise from the dynamic nature of vigilance, as individuals may transition from wakefulness to drowsiness or sleep during a resting-state scan. Fluctuations in vigilance can lead to alterations in functional connectivity (FC) patterns [39] and global signal variance [24] affecting the reliability and validity of the obtained neuroimaging data. Numerous studies have documented the effects of vigilance on BOLD signal amplitude and FC, highlighting its importance in interpreting resting-state data [21],[39],[40]. Studies, such as those by Tagliazucchi and Laufs (2014), have shown that vigilance states directly influence BOLD signal amplitude and functional connectivity patterns [6]. Transitions from wakefulness to drowsiness induce changes in global signal variance and network connectivity. Reduced wakefulness is associated with increased global BOLD variance, often driven by enhanced low-frequency fluctuations in sensory areas. Altered intra and between network connectivity in the default mode network (DMN) or sensorimotor network occurs during vigilance transitions [7]. Drowsy subjects exhibited higher and more positive cortico-cortical functional connectivities compared to the alert group [21]. These changes can obscure true connectivity patterns, leading to misinterpretations of network activity.

As previously demonstrated, brain activity measured via fMRI and EEG is affected by the state of vigilance, or arousal, over time periods as brief as seconds [41],[42]. The vigilance changes also alter the physiological measurements, when combined leading to further alterations in the fMRI signal. Physiological signals such as heart rate (HR), photoplethysmography amplitude (PPG-AMP), and respiratory variation (RV) are also closely tied to changes in vigilance levels. As vigilance increases, PPG-AMP tends to decrease, reflecting reduced peripheral vasodilation and heightened sympathetic nervous system activity [10]. This drop in PPG-AMP aligns with the autonomic shift that accompanies heightened states of arousal or attention. Conversely, during

transitions to lower vigilance states, such as drowsiness, RV exhibits a characteristic oscillatory behavior, indicating more irregular and shallow breathing patterns [21]. These changes in RV signal can be attributed to altered respiratory control mechanisms mediated by the autonomic nervous system, which become more pronounced as arousal levels decrease. Additionally, HR has been shown to be lower in drowsy subjects compared to alert individuals [21]. This reduction in HR during drowsiness suggests a dominance of parasympathetic activity, reflecting the body's shift toward a less aroused state. These physiological fluctuations, when combined, not only modulate cerebral blood flow and blood oxygenation but also affect the fMRI signal indirectly by influencing the vascular and metabolic dynamics underlying the BOLD response. Furthermore during vigilance transitions, cerebral blood volume changes facilitate CSF flow. The changes in the fMRI signal with altered vigilance states result partly from changes in cerebral blood flow and cerebral blood volume, physiological bases of BOLD mechanism, in turn altering the flow of CSF to the brain. This implies a link between CSF movements and the arousal state, with further associations to wakefulness and glymphatic clearance mechanisms mediated by CSF pulsations [8].

### 2.1.2 EEG Vigilance Metrics

EEG has been used as a standard for separating wakefulness from sleep, and for determining different sleep stages [43]. Although how to differentiate between vigilance levels has not been fully established in the literature, various studies investigated the link between EEG and vigilance states. There are various studies that have developed and applied EEG based metrics [12],[13],[14].

The metrics are proposed generally in the form of either the ratio of the power in the alpha band to the power in the delta and theta bands, or the square root of this ratio, often referred to as alpha slow-wave index [5]. An example follows in Eq. 2.1 from Olbrich et. al. (2009), where they have employed two vigilance metrics using channels F3, 4 and O1, 2 (channel locations on the scalp can be viewed from Figure 2.3) in segments of 3 second periods, then classified subjects into 5 categories of wakefulness



[13]. They also have assessed the relation of the metrics with BOLD signal and reported negative correlations across various brain regions of interest. One example reported is the anterior cingulate cortex where they have reported an increase in BOLD signal amplitude with increasing drowsiness.

$$\frac{P_{\alpha} : [8 - 12Hz]}{P_{\delta, \theta, \alpha} : [2 - 12Hz]} \quad (2.1)$$

In another study, Knaut et. al. recently demonstrated a different index for measuring wakefulness that takes advantage of both EEG frequency bands and topography [14]. Eq. 2.2 describes their proposed metric, where they take the powers from F3, 4, O1, 2 and C3, 4 channels in different frequency bands in segments of 2 second periods. In their study, they report the opposite of what was previously reported for BOLD signal from the anterior cingulate cortex, where they see an increase in BOLD signal contrast with increasing wakefulness.

$$\frac{P_{\theta_f, \alpha_O, \beta_f} : [4 - 30Hz]}{P_{\delta_f, \theta_O, \alpha_f, \beta_f} : [0.5 - 30Hz]} \quad (2.2)$$

The relation between EEG metrics and fMRI BOLD signal from different brain regions can be further investigated. As reported differently in these studies and various others, there is a gap of knowledge about which brain regions or networks can be indicative of drowsiness/wakefulness and how. One motivation of this study is to deepen the understanding in this regard to be able to more decisively comment on how the BOLD signal is effected from drowsiness.

## 2.2 Magnetic Resonance Imaging (MRI)

Magnetic resonance imaging (MRI) is an imaging tool widely used in research and clinical settings to monitor the structural and functional architecture of the human body. An MRI scanner is a giant magnet that creates a strong static magnetic field that aligns the magnetization of spin-active nuclei. Due to the abundance of hydrogen in tissues, especially in water and fat molecules, MRI is particularly efficient at producing high-resolution images of soft tissues and organs throughout the body, including the brain. MRI utilizes radiofrequency (RF) waves to excite nuclei by generating an RF pulse that matches the precessional frequency (i.e., Larmor Frequency under a given magnetic field strength) of the nucleus spins in the molecules within the tissue of interest [44]. As the excited spin systems relax back to their original magnetization state by precessing around the main magnetic field, the changes in the magnetic field become detectable by a coil placed around the body part (essentially the same coil that emits the RF pulses in the first place). In order to create spatially distinguishable signals, i.e., understanding where in the body the signal comes from, magnetic field gradients are created within the scanner bore. This allows for spatial encoding of the signal, thus giving rise to spatial resolution in the resulting image. Finally, the signal detected by the coils are processed by the scanner's computer system, and image reconstruction is performed to yield the final detailed images of the internal structures of interest [45]. Within the field of neuroimaging, MRI is adopted by researchers for a vast variety of applications ranging from diagnosis, treatment, and prevention of neurological and psychiatric conditions, to infer the structural and functional neuroanatomy of the human brain.

### 2.2.1 Functional Magnetic Resonance Imaging

Functional Magnetic Resonance Imaging (fMRI) is a noninvasive neuroimaging technique that offers a way of studying human brain function. It was introduced in rat brain studies as an indirect measure of regional neuronal activity, by measuring the changes in blood oxygenation and blood flow [46],[47]. It is a popular technique

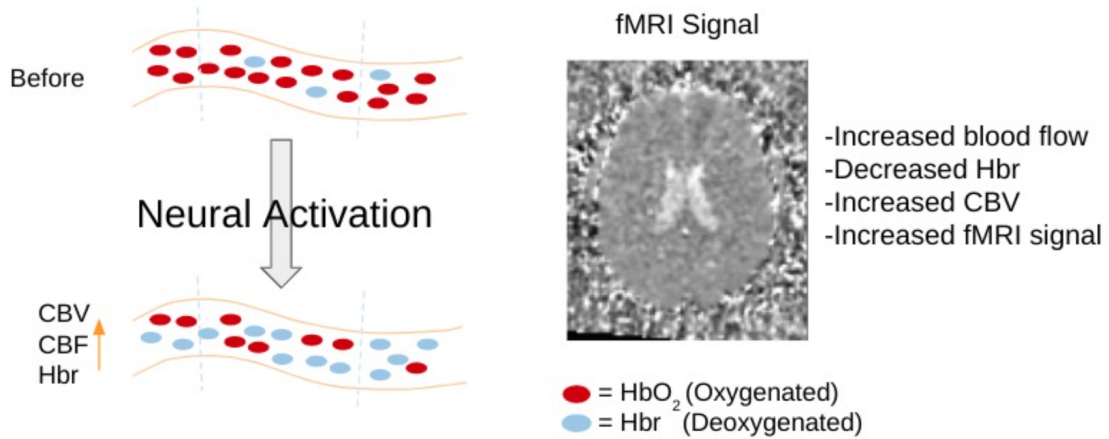


Figure 2.1: Visualization of the BOLD response, showing increased blood flow and volume in the arteries upon neural activation, accompanied by an increased fMRI signal. Bottom shows the signal profile of the electrical versus hemodynamic responses for neural activity.

among researchers and clinicians to investigate brain functions under task or resting state conditions.

The underlying physical principle of fMRI is to exploit a phenomenon called blood oxygen level dependent (BOLD) contrast, which can be expressed as changes in the inhomogeneity of the magnetic field resulting from changes in the blood oxygenation within the brain vessels. Neural activity in the brain is accompanied by metabolic activity, supplying oxygen and glucose to the activated region. However, since the brain does not store oxygen, the activity of the brain is dependent on a continuous supply of fresh blood by cerebral blood flow (CBF). Upon neural activation, oxygen and glucose mechanisms, along with CBF and cerebral blood volume (CBV) increase locally. Hemoglobin, the oxygen carrier molecule in the blood, becomes paramagnetic when it is deoxygenated, meaning an oxygen molecule is removed from it to the tissue. The change in the magnetic properties causes a slight decrease in the fMRI signal. This constitutes the physical basis for the BOLD contrast combined with a second biophysical event, that is, the CBF increases more than the increase of oxygen consumption in the activated area, creating an overcompensation mechanism, leading to a reduction in the oxygen extraction fraction (OEF). fMRI BOLD contrast arises from these biophysical changes in local blood oxygenation within the brain. Upon oxygen

consumption, deoxyhemoglobin is increased, and the BOLD signal is decreased [45].

### 2.2.2 Resting-state fMRI

Resting-state is characterized by the absence of a controlled stimulus. Even in the absence of any stimuli, there are organized activations within the brain shown to be consistent across many subjects. These organized activations between brain networks are referred to as resting state networks (RSNs). There are many different networks in resting state scans identified consistently across studies and being used to assess subject cognition under no stimuli [1],[48],[4],[15]. Even under no stimuli, RSNs are shown to be related to sensory processing and sensorimotor networks [49],[1]. Figure 3.9 shows some of these networks as connectivity matrices and on the surface of the brain, such as somatomotor, auditory and visual networks (also referred to as SMN, AUD and VIS respectively), as well as attention networks and default mode network (DMN), which has been increasingly studied. DMN, shown in Figure 2.2, is noted to be activated when the subjects do not engage in any particular goal-directed task in both humans and apes [50],[51]. Until its discovery by fMRI, DMN was not suspected to be present, even though it is the largest RSN in terms of brain areas involved within the network.

Commonly used resting-state experimental designs include instructions such as: keep your eyes open, fixate to a cross in the screen, or to keep your eyes closed, try not to fall asleep and try not to focus on a specific thought. In the field of neuroimaging, resting-state functional magnetic resonance imaging (rs-fMRI) has emerged as a powerful tool to study the intrinsic brain activity and functional connectivity patterns during periods of wakefulness and relaxation, without the influence of external stimuli. It is even argued that resting state networks (RSNs) can offer help to neurosurgeons in pre-surgical planning by enabling them to consider not only motor or language areas, but additional brain regions within resting state networks [48]. These passive yet essential states can be useful to investigate the brain's default network operations, providing insights into both the organized synchronization of different brain regions and

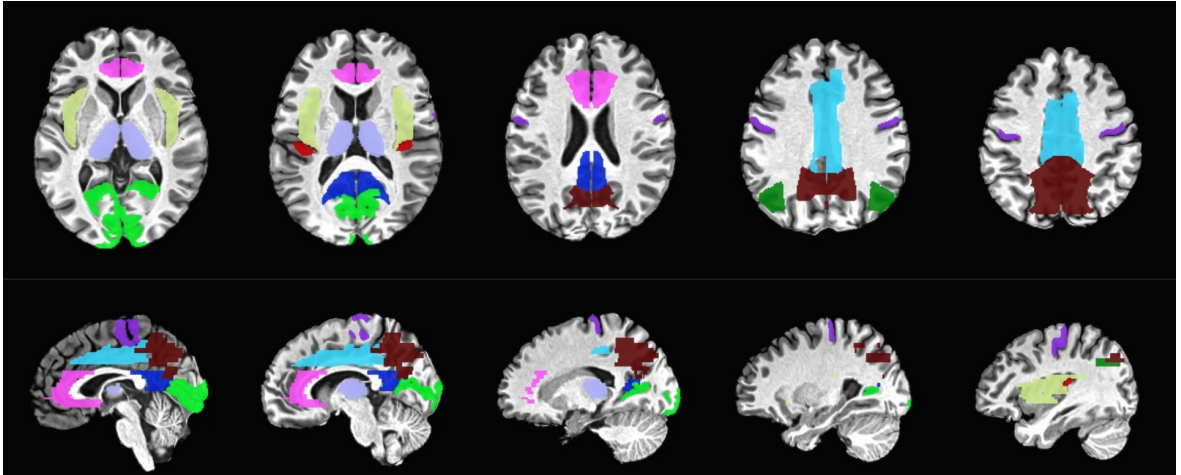


Figure 2.2: Color coded brain regions that are masked and analyzed within this study, shown on an exemplary data from this study. Visual (light green), auditory (red), motor (purple); default mode network (DMN) regions: precuneus (brown), posterior cingulate (dark blue), angular gyrus (dark green); anterior cingulate (pink), thalamus (lilac), middle cingulate (light blue), insula (yellow).

the underlying neurobiological processes that drive cognitive and emotional functions of the human brain.

### 2.3 Electroencephalography (EEG)

Electroencephalography (EEG) is a technique used to record the electrical activity generated within the brain. There are two main types of EEG; invasive and non-invasive. Intracranial EEG (iEEG) is an invasive method that involves placing an array of electrodes directly on the cortical surface or within the brain tissue to record local field potentials of the surrounding neurons. On the other hand, scalp EEG is a non-invasive alternative that involves placing electrodes on the scalp to measure the surface potentials that are the result of the collective activity in the brain. While it provides less spatial resolution compared to iEEG, scalp EEG is widely used due to its safety and ease of application. EEG provides a direct, millisecond-resolution measure of neurotransmission-mediated neural activity. It has a superior temporal resolution compared to fMRI, which takes advantage of BOLD signal, a secondary consequence

of neuronal activity; though it lacks in spatial resolution.

Underlying cause of the EEG signal is directly related to postsynaptic potentials (PSPs). When a neuron fires, there happens a rapid sequence of changes in the voltage across the neuron's membrane potential (i.e., a temporary shift from negative to positive) caused by ions suddenly flowing in and out of the neuron. This phenomenon is called the action potential (AP). APs are really short events that last around 1 to 4 milliseconds. An AP is transmitted through the neuron's axon, towards the synapse, which is the neural junction, connecting one neuron to the other. The AP results in the release of neurotransmitter molecules (in the pre-synaptic axon) which open ion channels in the post-synaptic neuron. The local voltage changes caused by the release of these chemicals are called PSPs. PSPs can be excitatory (EPSPs) or inhibitory (IPSP). In the excitatory case, the binding of a neurotransmitter increases the voltage (depolarizing the membrane), and in the inhibitory case, the binding decreases the voltage (hyperpolarizes the membrane). Any change in voltage, whether it's an increase or decrease, spreads passively to the surrounding areas of the neuronal membrane. The summation of PSPs across numerous neurons creates the electrical field fluctuations that are detectable at the scalp by EEG electrodes.

A fundamental aspect of EEG analysis is the classification of brainwaves based on the frequencies, measured in Hertz (Hz). These frequency bands, each with distinct characteristics and associated brain states, provide information about different stages of the brain. The frequency bands of EEG signals are categorized into 5 intervals: delta ( $\delta$ , 0.5-4 Hz), theta ( $\theta$ , 4-8 Hz), alpha ( $\alpha$ , 8-13 Hz), beta ( $\beta$ , 13-30 Hz), and gamma ( $\gamma$ , 30-50 Hz). The waveforms for these bands are illustrated in Figure 2.4 for an exemplary subject from this study.

### *Delta Waves*

Delta waves are the slowest EEG waveforms, typically associated with deep sleep. They are characterized by their high amplitude and slow, regular pattern. Delta waves are predominantly observed during the deep, restorative stages of sleep, particu-

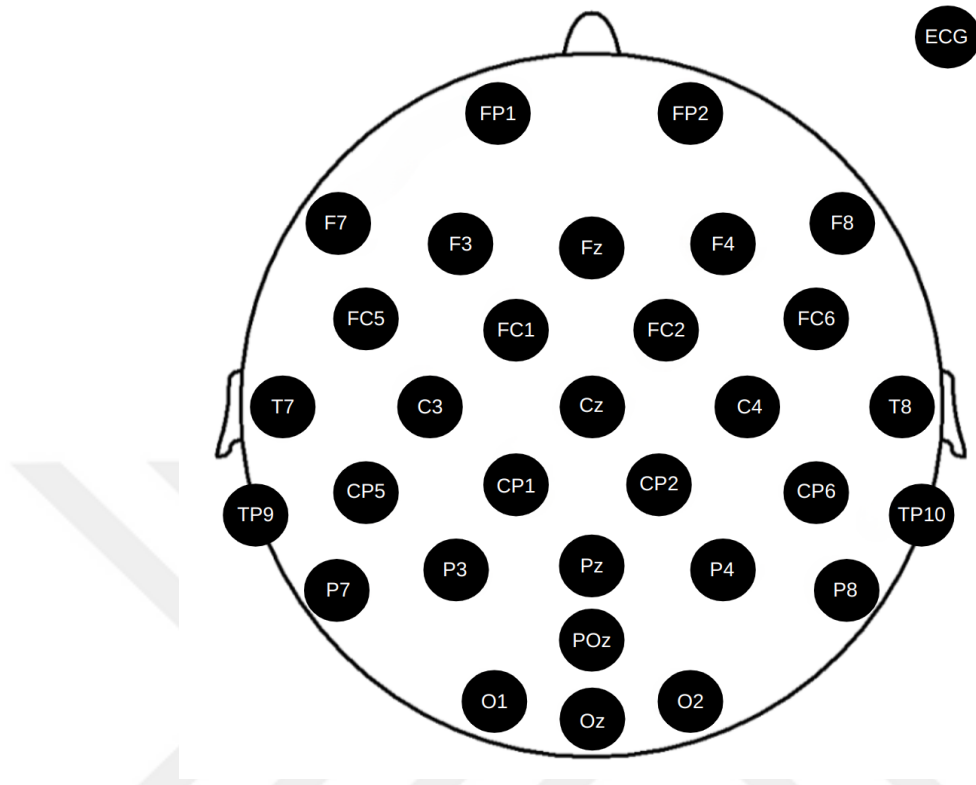


Figure 2.3: The EEG electrode placement employed within this study. It is the international 10-20 system of EEG electrode placement.

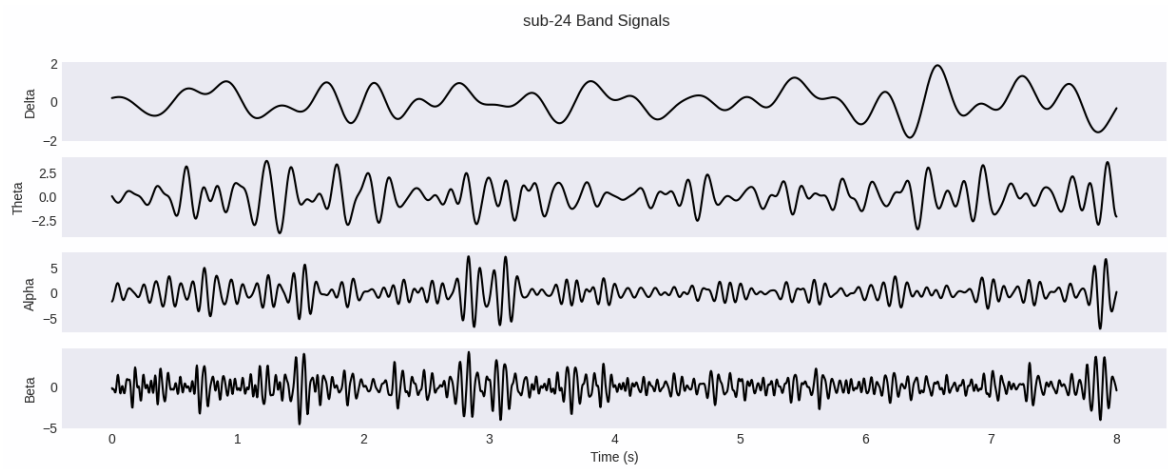


Figure 2.4: An exemplary data showing activity of different EEG bands for 8 seconds of data from O1 channel of 1 subject. The vertical axis is in  $10^{-5}\mu V$ .

larly during non-REM stage 3 (also known as slow-wave sleep). During this stage, the body undergoes important processes such as tissue repair, immune system strengthening, and energy replenishment. In addition, the role of delta band activity in cognitive processing is being researched and delta activity is proposed to facilitate internal concentration [52].

### *Theta Waves*

Theta waves occupy the frequency range between delta and alpha waves. They are commonly associated with light sleep, dreaming, and deep meditation. However, theta activity can also be observed during wakefulness, particularly in states of drowsiness, relaxation, or focused attention [13],[25], as well as memory processes [53].

### *Alpha Waves*

Alpha waves are characterized by their relatively high amplitude and regular sinusoidal shape. While there is no universally accepted definition of alpha activity, research has demonstrated that it is linked to arousal, attention, perceptual processing, and the semantic memory of the cortical system necessary for performing critical tasks, with evidence supporting both inhibitory and active processing functions [54]. The presence of alpha waves is often seen as an indicator of an awake but restful state, where the brain is not actively engaged in processing specific tasks. This is also interpreted as alpha band activity playing a role in attentional selectivity, or attentional suppression, where alpha waves may act to filter out irrelevant information and allow the brain to focus on pertinent stimuli [55]. This filtering mechanism can facilitate attentional selectivity by moderating the processing of relevant sensory inputs while suppressing potential distractions.

### *Beta Waves*

Beta waves are typically associated with active, conscious mental activity, such as problem-solving, decision-making, and focused attention. They are characterized by



their low amplitude and rapid, irregular pattern. The presence of beta waves is often linked to a state of heightened alertness and cognitive engagement. During tasks that require concentration, logical reasoning, or analytical thinking, beta activity increases, moderating the brain's ability to process information quickly and efficiently [56]. In everyday life, beta waves are dominant during activities that involve active conversation, intellectual work, and other forms of mental stimulation. However, excessive beta activity can indicate a state of heightened arousal or stress, which may manifest as anxiety, restlessness, or even insomnia.

### *Gamma Waves*

Gamma waves are the highest frequency EEG waveforms, traditionally considered beyond the standard clinical EEG bandwidth. However, advancements in EEG technology have made it possible to study gamma activity in more detail. Gamma waves are associated with high-level cognitive functions, including perception and learning. Abnormal gamma activity has been implicated in various neurological disorders, such as epilepsy and schizophrenia.

## **2.4 Simultaneous EEG-fMRI**

Combining the high temporal resolution of EEG with the detailed spatial resolution of fMRI provides a powerful way of studying the human brain. Simultaneous EEG-fMRI studies are getting increasingly popular, however, the integration of electroencephalography (EEG) within magnetic resonance imaging (MRI) environments presents many challenges due to the inherent incompatibility of these two diagnostic modalities. While certain modalities, such as transcranial ultrasound and near-infrared optical spectroscopy, can be relatively easily integrated; integrating fMRI and EEG is much more complex and poses major technical difficulties [57] due to the magnetic environment of MRI. In MR imaging, there are intense static and time-varying magnetic fields, leading to electromagnetic interference that introduces problems to the quality of EEG recordings, where the radiofrequency (RF) pulses used in MRI for image

acquisition introduce immense noise in the EEG signals [58].

EEG measures changing surface potentials on the head, happening due to voltage fluctuations generated by neuronal activity. The measurement is done via electrodes, which is a combination of electrode head and connecting lead. The use of non-invasive MR-compatible EEG is utilized within our study, which is placed on top of the scalp. The electrodes of the EEG measure the activity from the induced electromotive forces in a conductive loop, which is defined as

$$V_{induced} = A \times \frac{dB}{dt} \quad (2.3)$$

where  $V_{induced}$  is the emf induced in the loop,  $A$  is the loop area perpendicular to the field and  $dB/dt$  is the rate of change of magnetic flux cutting the loop.

#### 2.4.1 Gradient Artifacts

The main challenge of integrating EEG within MRI environments is the issue of MRI gradient artifacts. The RF fields applied during scanning induce eddy currents in the electrodes according to Faraday's law of induction or by the relative motion of a conductor in a magnetic field. MRI utilises gradient magnetic fields during imaging sequences, leading to rapid changes in magnetic field strength. These gradients induce voltages in EEG electrodes due to Faraday's law, resulting in what is commonly referred to as gradient artifacts. As the MRI gradient fields switch rapidly, they generate electric fields that cause current flow in the conductive electrodes of EEG, described as above, contributing to the obtained signals, contaminating the EEG recordings. These artifacts manifest as sharp spikes or oscillations in the EEG data, easily visible as they create really dark areas within the EEG graph.

### 2.4.2 Cardioballistic Artifacts

Since the static magnetic field in MR environment ( $B_0$ ) is not changing over time, it does not introduce any noise itself into the EEG signal. However, there is the subject motion which create movement within the MR bore, in which case, there occur smaller (compared to the gradient artifact) noises within the signal [59]. The subject motion can be head movement due to uncomfortable environment of MRI, or autonomic movement such as heart [60], which create cardioballistic artifacts.

The cardioballistic artifact (CBA, also referred to as ballistocardiogram artifact, BC, or lastly, as pulse artifact, PA, we will continue with cardioballistic artifact terminology here) occurs as a result of heart pulsations creating movement, which results in moving electrodes and thus the magnetic field -even though it is static- inducing currents.

The unconscious bodily movements occur as the heart performs its pumping function by forcing the blood through the vessels. The formation of a CBA is a time-locked cardiac cycle waveform with several polarities and a power spectrum that partly covers the essential EEG frequencies of interest, thus posing a challenge in analysis if the artifact is left within the signal.

The CBA's changing behavior in the cardiac cycle is one of the primary difficulties. The CBA, unlike other stable types of artifacts, shows variability in both amplitude and morphology making it impossible to apply the traditional method of averaging multiple occurrences. Also, the detailed mechanisms of the formation of the CBA are not fully understood. CBA is believed to stem from three primary sources: bulk head motion caused by blood entering the head, pulsatile scalp dilation due to arterial pulsations, and potential Hall effects within the moving blood [61]. These physical movements, coupled with the electromagnetic properties of the MRI environment, contribute to the generation of the PA, which is detected as a waveform in EEG signals.

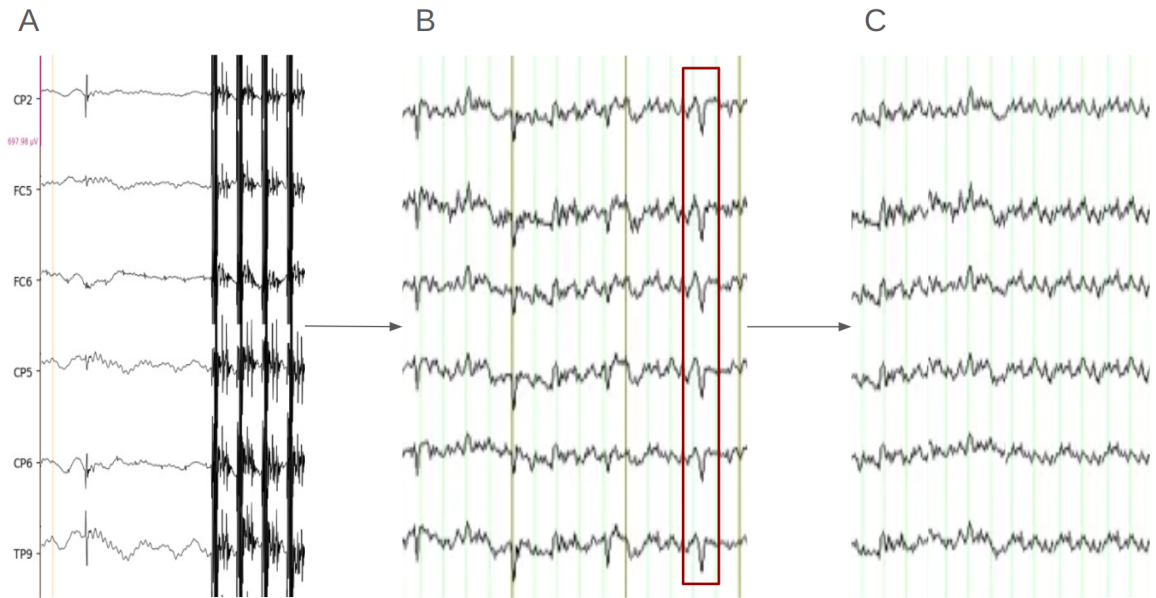


Figure 2.5: A) Example of a gradient artifact observed in the raw EEG signal recorded during simultaneous EEG-fMRI acquisition. The artifact appears as regular, high-frequency oscillations synchronized with the MR gradient pulses. B) Example of a cardioballistic artifact in the raw EEG signal. These artifacts are caused by pulsatile blood flow within the MR environment and manifest as periodic deflections in the signal. The red box highlights one such artifact, emphasizing its distinct shape and periodicity. C) Gradient- and cardioballistic artifact-cleaned EEG data.

## 2.5 Measurement of Physiological Signals

### 2.5.1 Pulse Oximeter

A plethysmograph is an instrument that measures changes in volume within different body parts. A photoplethysmogram measures blood volume changes using an optically obtained plethysmogram, often using pulse oximetry, a non-invasive optical technique. In order to obtain the PPG signal, a pair of LEDs of 660 nm and 940 nm wavelength (red and infrared respectively) are placed in one side, of typically a translucent body part such as an earlobe or fingertip. On the other side of the body part, a photodetector is placed for detecting the transmitted wavelength. Absorption of light at these wavelengths differs significantly between oxygenated and deoxygenated blood. When hemoglobin, the oxygen-carrying molecule within the blood, is oxygenated it absorbs more of the infrared light and allows more red light to pass through. Deoxygenated hemoglobin allows more infrared light to pass through while absorbing more red light. The amount of light that is transmitted (in other words, that is not absorbed) is measured with the detectors of the pulse oximeter.

PPG signal has two components; namely a direct current (DC) component and an alternating current (AC) component. The DC component refers to the baseline level of light absorption mainly due to the constant factors like tissues, bones while the AC component represents the pulsating changes in light absorption caused by heart beat. With each cardiac cycle, there is a variation in blood volume that causes the light reaching the detector to fluctuate, creating a wave in the signal. That fluctuation is present even though it is dampened when reaching the fingertip or earlobe. Breathing is also noted to influence the PPG signal at subcardiac frequencies [62].

The typical shape of the PPG waveform is shown in Figure 2.7. The components of the PPG waveform are: systolic peak, which represents the blood inflow following the ejection from the heart to arteries, a small dip called dicrotic notch, corresponding to the brief period of reduced blood flow immediately after the aortic valve closes, followed by diastolic peak thought to result from the wave reflections from the stiffness

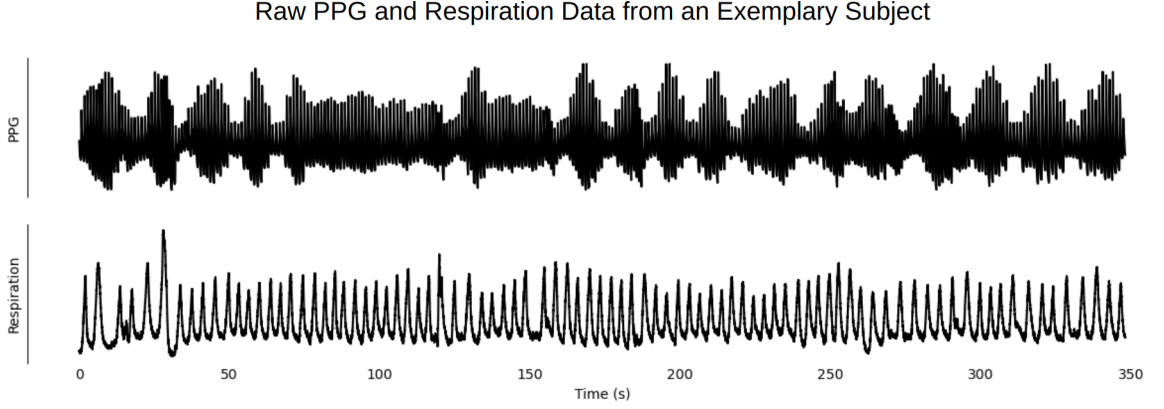


Figure 2.6: Raw PPG and respiration signals from an exemplary subject.

of the arteries. Lastly, diastolic decay is the gradual return of blood to the baseline level during diastole. These waveform components can be analyzed to provide metrics for autonomic nervous system function; such as heart rate variability (HRV), which is calculated from the distance between systolic peaks. Moreover, the systolic and diastolic peaks can be used to assess arterial stiffness and blood pressure. The dicrotic notch gives information about the aortic valve and arterial elasticity.

The physiological phenomena measured by pulse oximetry is called peripheral oxygen saturation and referred to as  $SpO_2$ . It is calculated from the formula

$$SpO_2 = \frac{HbO_2}{HbO_2 + Hb} \quad (2.4)$$

where  $HbO_2$  is oxygenated hemoglobin (oxyhemoglobin) and  $Hb$  is deoxygenated hemoglobin (deoxyhemoglobin).

The signal obtained from a PPG carries beneficial information on autonomic nervous system activity and cardiovascular function. In order to make use of this information, it is crucial to develop proper denoising and signal processing techniques.

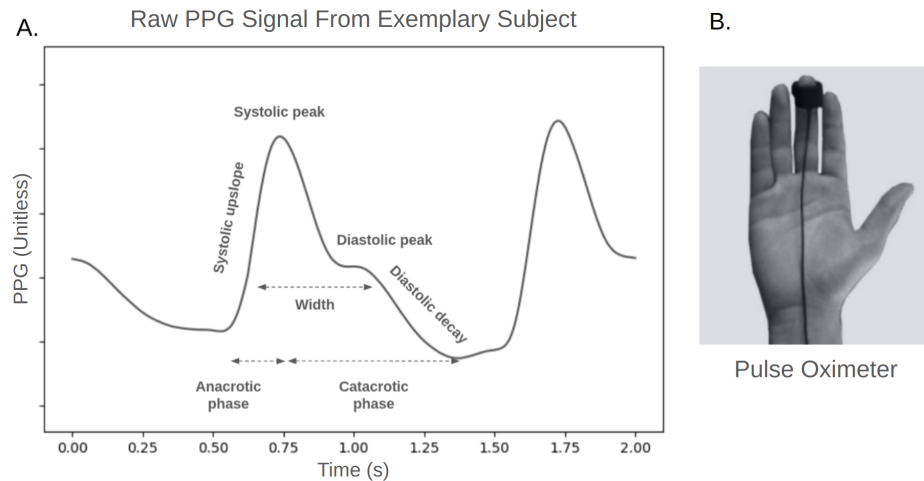


Figure 2.7: (A) Example of raw photoplethysmogram (PPG) data from an exemplary subject, illustrating key morphological features, including the systolic peak, diastolic peak, anacrotic phase, catacrotic phase, systolic upslope, diastolic decay, and the overall waveform width. (B) A pulse oximeter positioned on a subject's finger, the typical setup used for acquiring PPG data.

### 2.5.2 Respiratory Bellow

Respiratory bellows are non-invasive devices used to monitor respiratory patterns by measuring changes in thoracic or abdominal circumference during breathing. These devices operate based on the principle of mechanical displacement, wherein the expansion and contraction of the chest or abdomen during the respiratory cycle cause changes in the bellows' structure. The bellows, typically a flexible elastic band or inflatable mechanism, are placed around the subject's thoracic or abdominal region and secured snugly to ensure accurate measurement of respiratory effort.

As a person inhales, their lungs expand, causing the chest or abdominal cavity to increase in circumference. This expansion stretches the bellows, producing a measurable displacement or pressure change. Conversely, exhalation causes the chest or abdomen to contract, allowing the bellows to return to their original state. This cyclical movement generates data that can be captured by sensors integrated into the bellows, such as strain gauges, pressure transducers, or optical encoders, depending on the design.

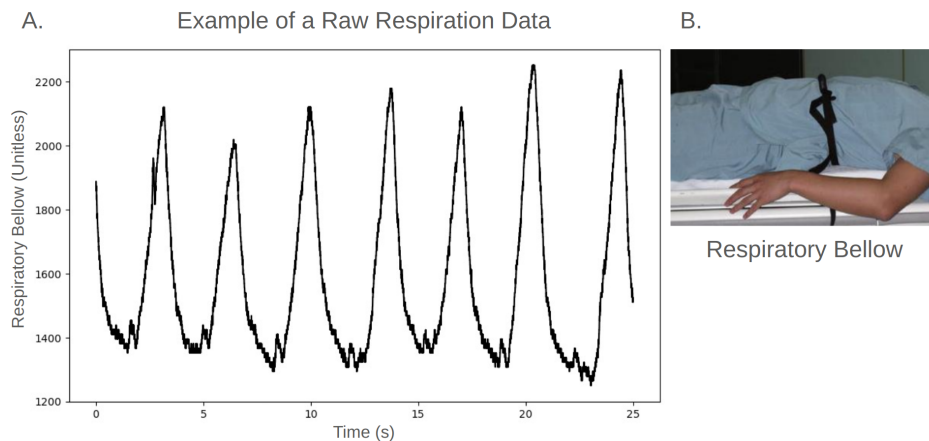


Figure 2.8: (A) Example of raw respiratory bellow data from an exemplary subject, showing the cyclic pattern of respiratory activity over time. The signal amplitude reflects changes in abdominal expansion during breathing. (B) A respiratory bellow device fitted on a subject, demonstrating its placement around the torso to measure respiratory movements during simultaneous recordings.

The data captured by respiratory bellows (an exemplary data is shown in Figure 2.8) are typically analog signals corresponding to the degree of displacement or pressure change during breathing. These signals are then digitized for further analysis. Modern systems often incorporate advanced algorithms to convert raw signals into clinically relevant parameters, such as respiratory rate, tidal volume, and respiratory effort.

The resolution and accuracy of these measurements depend on the sensitivity of the sensors and the fit of the bellows on the subject. To ensure the quality of data obtained from respiratory bellows, several steps are implemented during setup and data acquisition. Proper positioning of the bellows is important; they must be securely placed around the thoracic or abdominal region to capture accurate displacement without being too tight, which could restrict breathing or alter natural movement. Calibration of the system is another essential step, typically involving zeroing the signal before data collection to eliminate baseline drift or noise. Additionally, artifacts caused by body movements or environmental factors, such as vibrations, can be mitigated by using appropriate filters or algorithms during signal processing.

Respiratory bellows are widely used in clinical, research, and experimental set-



tings due to their simplicity, ease of use, and non-invasiveness. They are particularly valuable in situations requiring real-time respiratory monitoring, such as during polysomnography, anesthesia, or simultaneous multimodal imaging studies. While they provide a reliable approximation of respiratory effort, it is important to note that they measure external changes rather than direct airflow, which can introduce certain limitations, such as reduced sensitivity to shallow breathing or interference from body movement.



### 3. MATERIALS AND METHODS

#### 3.1 Experimental Design and Subjects

This study utilized a multimodal neuroimaging approach, integrating EEG-fMRI, cardiac and respiratory monitoring, and pupillometry data from an eye camera. Such a combination of techniques allows for a comprehensive exploration of neural and physiological dynamics, bridging the gap between brain activity and systemic physiology. Each experiment included anatomical scans, resting-state and task-based experiments.

Resting-state fMRI sessions consisted of 6-minutes long scans where subjects were instructed to keep their eyes open, and focus on the fixation cross on the screen at the back of the MRI scanner, which subjects see through a mirror fixed on the head coil. The fixation cross is selected as a black colored cross on a grey background in order to avoid any color inferences within the data. Two-three runs of resting-state and a T1-weighted anatomical scan in between (all scan parameters can be seen in Table 3.1 and 3.2). Total scan time within the MR has been limited to 1 hour. The number of runs varied between subjects, ranging from 2 to 3, and included both resting-state and task-based experiments. While the experiments also involved controlled caffeine interventions, the scope of the current work has been limited to blind resting-state analysis. Hence, all resting-state data were pooled together regardless of the experimental conditions.

Data were collected from 17 healthy subjects with a total of 37 rs-fMRI scans, ensuring robust within-subject comparisons (15 males, 2 females, 20-35 years of age). All subjects gave informed consent and experiments took place with ethical considerations.

The focus of this work is on the resting-state experiments, capturing individual

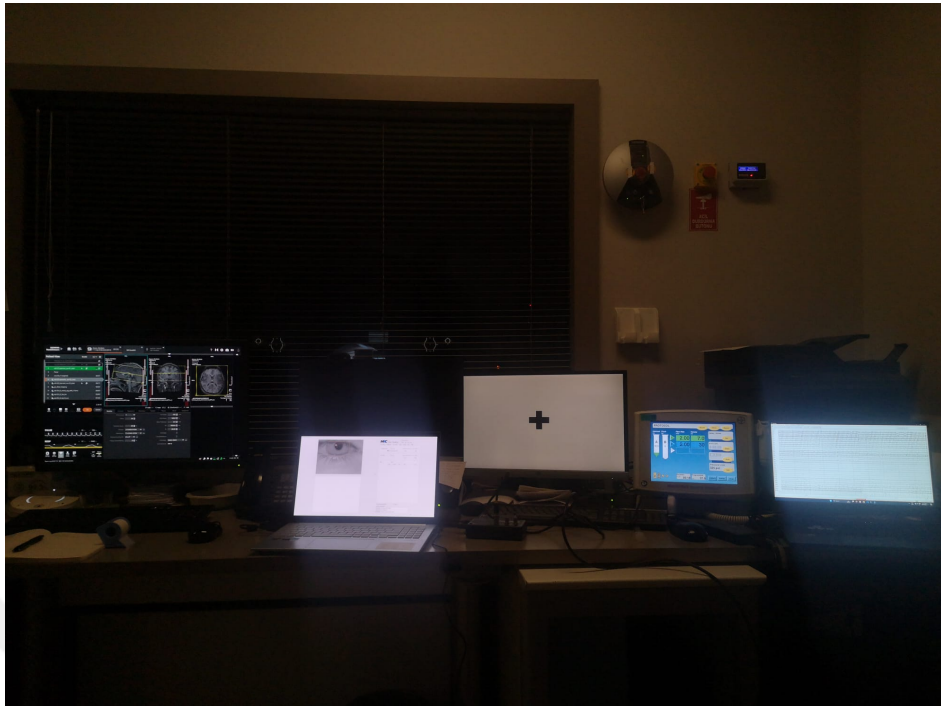


Figure 3.1: An exemplary multimodal resting state scan. Image shows *left* MRI computer, *middle-left* eye camera computer recording the eye, *middle-right* stimuli computer where a cross is presented for the subject to focus on, and finally *right* the EEG computer.

variability. EEG-fMRI data were synchronized with physiological recordings, providing a rich dataset to investigate the interplay between cortical activity and autonomic functions.

## 3.2 Setup and Data Acquisition

### *MRI*

Magnetic resonance imaging data is obtained using a Siemens Magnetom Prisma Fit 3 Tesla scanner at a local hospital. Figure 3.1 shows an example setup from one session where the subject is inside the scanner. The MRI sequences and parameters are adjusted in the computer on the left, from Siemens' interface. For the anatomical data, a spoiled gradient echo is used to obtain the image, Table 3.1 shows the parameters employed in this study.

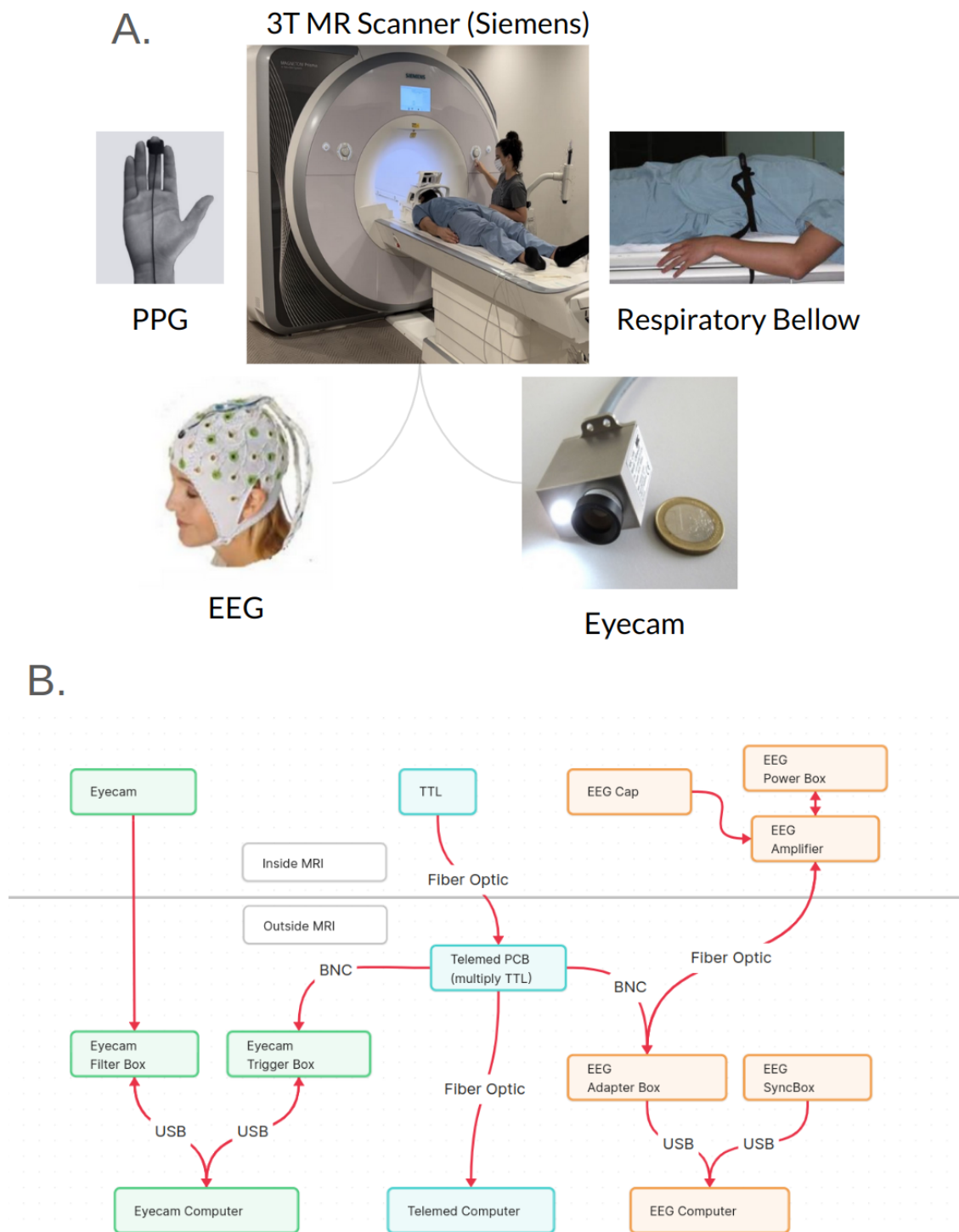


Figure 3.2: A) Modalities used in our study. B) The schematic of multimodal integrations, both for data acquisition and for TTL trigger.

Table 3.1: Anatomical MRI acquisition parameters.

|                      |        |
|----------------------|--------|
| Flip angle (FA)      | 12     |
| Repetition time (TR) | 2.16 s |
| Echo time (TE)       | 3.3 ms |

For the functional MRI, a gradient-echo echo-planar-imaging (GE EPI) is used with the parameters described in Table 3.2.

Table 3.2: Functional MRI acquisition parameters.

|                      |                                 |
|----------------------|---------------------------------|
| Flip angle (FA)      | 90                              |
| Repetition time (TR) | 3 s                             |
| Echo time (TE)       | 36 ms                           |
| Voxel size           | 2.5 x 2.5 x 2.5 mm <sup>3</sup> |
| Number of TRs        | 135                             |

### *EEG*

Electroencephalography data is collected simultaneously using BrainAmp MR series 32 channel head coil (31 EEG channels, 1 ECG channel).

The EEG setup has a preparation phase in each experiment outside the MR room. The subjects are fitted with the EEG cap before being taken into the MR room. First, the skin is wiped with alcohol in order to get rid of the excess oil on the skin. Then, electrolyte gel is poured into each electrode. The impedance for each electrode are checked and dropped to below 5 kOhm to increase signal-to-noise ratio. In order to be able to check, the EEG amplifier with the power box are setup outside the MR room, in a similar fashion it is setup within the MR. Using the BrainVision Recorder application, the quality checks are done, which are the aforementioned impedance check, and ECG signal check to be sure that the R-peaks are clearly visible in the signal. The R-peaks play an important role in the preprocessing of the EEG signal, as is described in the following section.

When the subject is brought into the MR, the amplifier and power pack is put



Figure 3.3: MR Compatible 32 Channel EEG Cap (Brain Products) used in our study.

right behind the head and the cap is connected with the amplifier via a short cable. In order to reduce the vibrations of the MR being reflected on the EEG setup, a soft platform using hard foam is placed beneath the amplifier and the power box. We also put small sandbags that can be commonly found in MR rooms on top of the amplifier in order to further stabilize the EEG setup. The data is transmitted outside the MR room via a fiber optic cable. Full EEG connections within the MR room are represented in Figure 3.2, which also shows TTL trigger as an input to the adapter box.

### *Physiological signals*

PPG signal is collected with a pulse oximeter attached to the fingertip, and respiratory signal is obtained using a pneumatic belt positioned at the level of the abdomen to measure respiratory related variations. In Figure 3.1, MR computer shows the real-time recording of both of these signals where they are monitored during the experiments to make sure of subjects' health conditions. In addition to monitoring, using Siemens' IDEA command-line tool in the MR computer, we also record both signals for all the functional scans.

### 3.3 Data Preprocessing

#### 3.3.1 MRI Data Preprocessing Pipeline

##### Anatomical MRI

All MRI data are obtained in DICOM format from the scans, and they are converted to NIFTI format using *dcm2nii* commandline tool.

The T1-weighted MRI scans saved in NIFTI format are subjected to warping and skull stripping using AFNI's @SSwarper command. The @SSwarper script performs skull stripping and non-linear warping (SSwarper is used as an abbreviation for skull stripping (SS) and warping). Skull stripping is the process that removes non-brain tissues from the anatomical images, removing irrelevant signals for the analysis. Non-linear warping aligns the anatomical images with the MNI152 2009 template, ensuring that individual anatomical differences are minimized and that the data from different subjects are in a standardized MNI space. The processed anatomical images, after skull stripping and warping, are saved for each subject. These images are used in further preprocessing, such as functional alignment and masking for region of interest (ROI). Figure 3.4 shows from a representative subject, how the raw data is transformed into a preprocessed anatomical data.

##### Functional MRI

Preprocessing of functional data followed the suggested '*afni proc*' pipeline (AFNI [63]). Here, the pipeline in Figure 3.4 is described step-by-step in more detail.

The first four time points of the fMRI data were removed to allow for magnetization equilibrium. During the initial moments of the scan, the MRI signal can be unstable as the tissue magnetization builds up to a steady state. By discarding these time points, we ensure that only stable and reliable signal data are used in the anal-

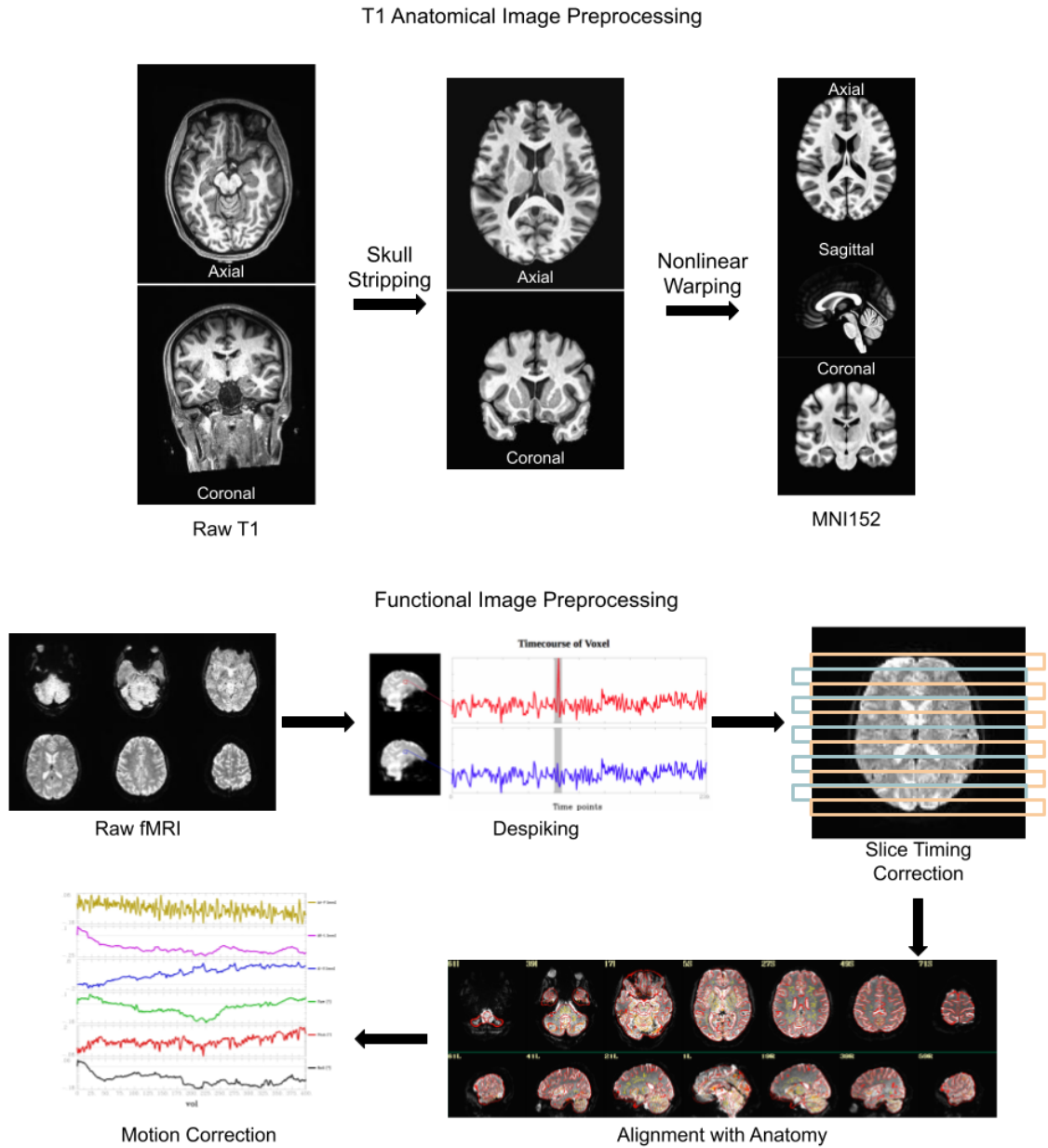


Figure 3.4: Preprocessing steps for anatomical and functional MRI datasets. On top of these major steps, there are additional preprocessing such as removal of first 4 TRs in fMRI, Gaussian blur etc. as described in chapter 3.3.1.



ysis. This step helps prevent artificial activations or noise from affecting subsequent analyses.

Despiking was performed to reduce transient noise spikes in the fMRI time series. These spikes are often caused by sudden head movements, scanner artifacts, or physiological noise, and can distort the analysis if left uncorrected. By applying a despiking algorithm, these brief, high-amplitude fluctuations in the signal are attenuated, improving the quality of the time series and reducing the impact of outlier points on subsequent steps.

To correct for slice timing differences, a time shift (tshift) was applied. In fMRI, images are acquired one slice at a time, so different slices within the same volume are captured at slightly different times. Slice timing correction ensures that all slices are aligned in time, as if they were acquired simultaneously. This step is important for capturing the precise timing of neural activity across the brain.

The functional images were aligned to the anatomical images, which had been previously skull-stripped and warped. Skull stripping involves removing non-brain tissues from the anatomical image, allowing for better accuracy in alignment. Warping refers to the process of transforming the images into a standardized space, such as Talairach or MNI space, to enable comparisons across participants. By aligning the functional and anatomical images, we can localize neural activity within specific brain regions, and use masks in order to obtain signals from specific regions of interest.

The time series volumes were aligned to the first volume of each run to correct for head motion during the scan. Even small head movements can introduce noise and distort the data. By realigning each volume to a consistent reference point, this step makes sure that the data reflect neural activity rather than artifacts caused by motion. Consistent alignment of volumes helps maintain the integrity of the time series and prevents false correlations due to misalignment.

A Gaussian blur with a full-width half-maximum (FWHM) of 3.0 mm was ap-

plied to the data to increase the signal-to-noise ratio. Spatial smoothing helps reduce the impact of noise in the data by averaging the signal from neighboring voxels. Blurring is useful for increasing the detection of distributed neural activity, as it enhances signals that are spread across multiple voxels, while reducing the effect of random noise.

Finally, motion parameters were regressed out of the data using a threshold of 0.2 mm to account for remaining motion artifacts. This involves modeling and removing the effects of head motion on the fMRI signal.

With use of *proc.py*, we created anatomical masks, functional image mask with whole brain coverage, and region-specific masks for areas of interest, such as the motor and primary visual regions.

### 3.3.2 EEG Artifact Removal

2 main artifacts of simultaneous EEG-fMRI were described in the background section, that are gradient artifacts due to the gradient coil switching on and off, and cardioballistic artifacts that happen because of the movement caused by pulsations of the heart. To remove these artifacts, general approaches that are employed in hardware and software are as follows:

Several methodologies are combined in MR compatible EEGs. First, a shielded amplifier is used which can be taken directly inside the MRI chamber and be placed in the bore right behind the subject's head. Short cables are used to connect the EEG cap with the amplifier, which also avoids creating loops within this cable, thus avoiding any induction currents. From the amplifier, the digitized signal is transferred via fiber optic cable to the USB interface within the control room, which ensures no artifacts are being added along the way to the outside of the MRI chamber. In other systems that use copper cables, the gradient noise is reportedly transferred via the cable outside the MR room, creating noise within the acquired images. Using fiber optic cables makes the most sense given how easily metallic cables can be influenced by the magnetic fields

present within the MR room.

The EEG caps that contain the electrodes have current-limiting resistors, which limit additional currents that would be induced by the changing gradient fields. The electrodes contain small gaps within their rings to avoid induction in closed loops. The cables are tied together in a way that avoids any loops on the cables which would create induction currents. The electrodes are shielded to reduce the electromagnetic interference induced by the MRI environment.

In our setup, in order to reduce any artifacts that may be caused by vibrations of the MR, we first create a stable platform using MR compatible materials right to the back of the head-coil when it is fully within the MR. Furthermore we place sandbags on top of the amplifier. By this, we aim to prevent any sudden movement that may occur in the amplifier and cables connecting amplifier and EEG cap, given that additional movement directly influences the data as artifacts as described in the 2.4.

#### *Postprocessing for Artifact Correction*

Since the gradient artifacts are predictable noise sources, caused by the sequences that MRI physicists write, they can be cleared rather easily from the signal. Template-based subtraction methods, as implemented in software packages like Brain-Vision Analyzer, involve creating a template of the artifact waveform based on MRI sequence parameters and subtracting this template from the EEG signal to eliminate the artifact. Independent Component Analysis (ICA) provides another approach, separating the mixed EEG and artifact signals into independent components. By identifying components corresponding to the artifacts, ICA can effectively remove them, preserving the underlying brain signal. Lastly, Adaptive filtering offers a dynamic solution, where filters continuously adjust to the changing characteristics of gradient artifacts, enabling effective artifact removal while maintaining EEG signal integrity.

For the cardioballistic artifact, high-pass filtering, ICA, and artifact rejection are general methods that are used. There are many methods being studied on how

to remove CB effectively. The AAS algorithm is the most commonly used method for reducing pulse artifacts (PA) in EEG data [64]. It operates by creating a template of the PA waveform based on multiple occurrences of the artifact. This template is then subtracted from each detected PA instance in the EEG signal. To identify PA occurrences, the algorithm typically relies on an electrocardiogram (ECG) signal, using the R-peaks as reference points. A fixed delay is applied to account for the time difference between the R-peak and the onset of the PA in the EEG. The effectiveness of AAS depends on several assumptions: the PA and EEG signal are uncorrelated, the PA waveform remains consistent across occurrences, and the delay between the R-peak and PA onset is stable. However, the number of PA occurrences used to create the template is crucial; a large number improves template accuracy but reduces sensitivity to PA variations, while a small number increases sensitivity but may also remove parts of the EEG signal.

In this study, we have used a BrainVision 32-channel EEG cap, and BrainVision Analyzer 2.0 software to reduce the artifacts. Brain Products Analyzer's template approach is used to eliminate the MRI gradient artifacts in the contaminated EEG data. This method requires using average artifact subtraction (AAS) [65]. For the cardiobalistic artifact correction, the first step involves writing R markers on the ECG channel. After this, template subtraction is performed using the CB Correction transformation, setting appropriate values for peak detection and template intervals. Subsequently, an ICA subtraction is conducted if needed, involving component analysis and careful selection of components for removal to ensure cardiobalistic artifacts are minimized. The EEG data is subjected to a band-pass filter with a frequency range of 0.5-35 Hz after applying ICA, and Fz is used as a reference channel.

### **3.3.3 Preprocessing of Physiological signals**

#### **Pulse Oximetry**

Photoplethysmographic amplitude (PPG-AMP) is a valuable measure for assess-

ing vascular dynamics and their potential interactions with neurophysiological states. As discussed in the previous section 2.5.1, PPG-AMP reflects changes in blood volume in the microvasculature, which are influenced by both central autonomic outputs and peripheral vascular tone. As such, it serves as an indirect marker of sympathetic and parasympathetic nervous system activity, which are tied to arousal states such as wakefulness and drowsiness. In the context of neuro-dynamics, fluctuations in vascular tone and cardiac function have been linked to variations in the brain’s functional organization and signal patterns, including those captured through BOLD fMRI [10],[66].

PPG amplitude (PPG-AMP), as an index of finger skin vascular tone [67] is calculated. Figure 3.5 shows an example PPG recording from this study that contains motion artifacts. The finger motion creates immense noise in the PPG recording where the signal saturates at the top. This artifact can best be avoided by asking the subject not to move their finger that the PPG is being recorded on. However, there are still datasets that contained movement.

Each physiological data underwent a manual inspection as a first approach, in which especially the motion artifacts are inspected. Even though there are sophisticated artifact removal approaches when a study can include accelerometer to record the motion, in the absence of one, best approach to deal with the motion artifacts is to filter out and equalize the noisy part to the mean [68]. In the bottom of Figure 3.5 we see how a motion artifact can reflect as big amplitude change in the PPG-AMP calculation. Amplitude calculated from the cleaned PPG however, represent the trend of the PPG-AMP realistically. This figure implies the importance of manual inspection and correction of motion artifact in PPG data, in order to avoid wrong inferences in the derivatives such as PPG-AMP and HRV.

Another derived metric from the physiological data in our study is the heart rate (HR), which is a fundamental physiological measure. Variations in HR are modulated by the sympathetic and parasympathetic activities, which regulate cardiac function in response to arousal, stress, and relaxation [66].

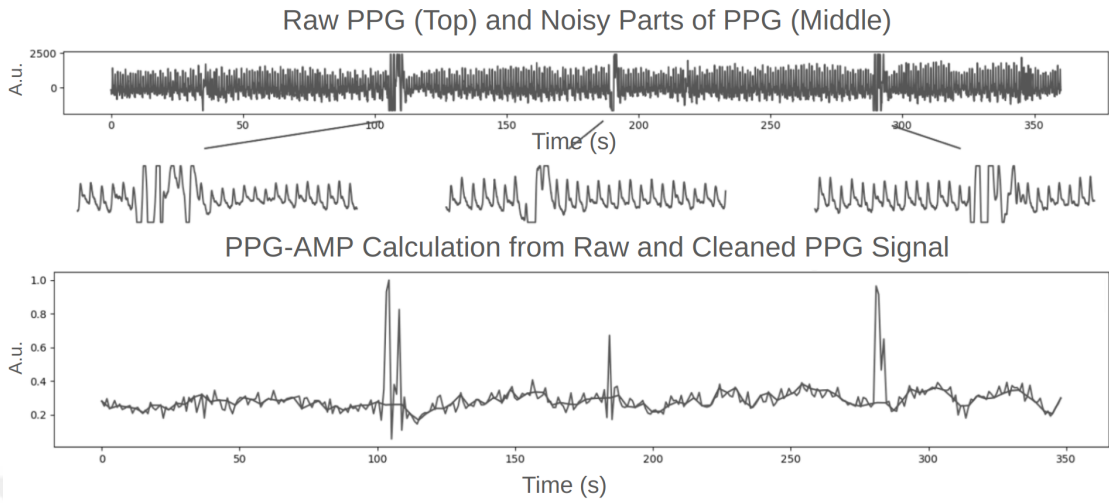


Figure 3.5: An exemplary data showing at (top) three different times during the scan, subject had moved their finger, resulting in movement artifacts, and (bottom) PPG-AMP calculated from noisy PPG signal (background with huge changes) where movement reflects as huge amplitude changes vs PPG-AMP calculated from cleaned PPG (on top of the noise PPG-AMP).

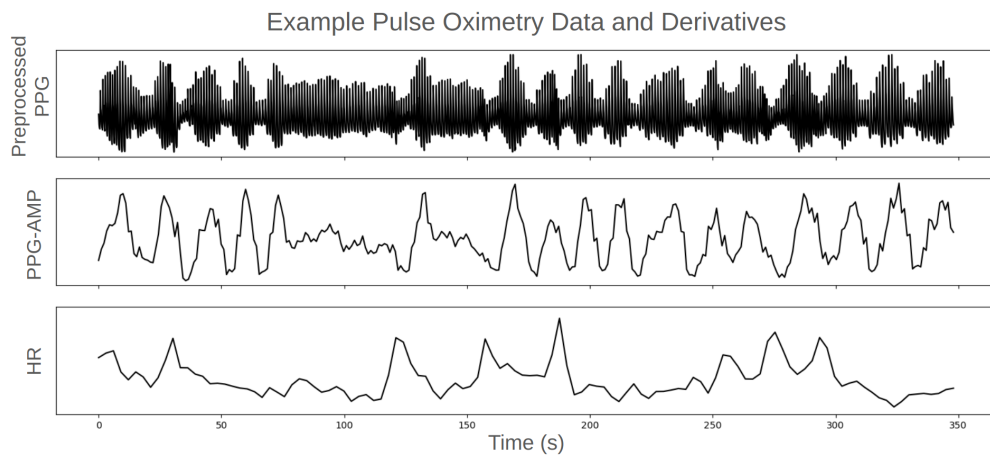


Figure 3.6: Example of pulse oximetry data and its derived metrics. The top plot shows the preprocessed photoplethysmogram (PPG) signal, highlighting the cleaned waveform. The middle shows the PPG amplitude (PPG-AMP), extracted from the peak-to-peak analysis of the PPG signal, reflecting blood volume changes. On the bottom heart rate (HR) is presented, calculated as beats per minute from the PPG signal.

In our study, the variation in HR is computed by averaging the differences between pairs of adjacent heartbeats contained in the 6s window around each trigger and dividing the result by 60 (beats per minute) [20].

## Respiratory Bellows

Respiration rate and depth can vary due to physiological demands or psychological states, making it an important marker of autonomic nervous system activity. The act of breathing introduces changes in thoracic pressure, which in turn affect cardiac function, cerebral blood flow, and other physiological processes. These interactions are of particular interest in studies examining states of vigilance, as respiration is closely tied to arousal levels, emotional states, and overall autonomic balance.

The respiratory bellow signal, often derived from thoracic/abdominal strain gauges, provides a continuous measure of the mechanical motion associated with breathing. This signal captures the cyclic expansion and contraction of the chest/abdomen during respiration. It is a proxy for the respiratory cycle's timing and amplitude, allowing researchers to track the rhythm and depth of breathing over time. The respiratory bellow is useful in combination with neuroimaging and physiological datasets, as it can be used as an indication of respiratory patterns' influence or co-variance with brain activity and autonomic states.

The respiratory variation (RV) signal represents the relative variability in the respiratory cycle and is often derived from the respiratory bellow [35]. An example of a raw respiration signal and its derived RV signal is shown in Figure 3.7. RV quantifies the fluctuations in respiration amplitude and timing.

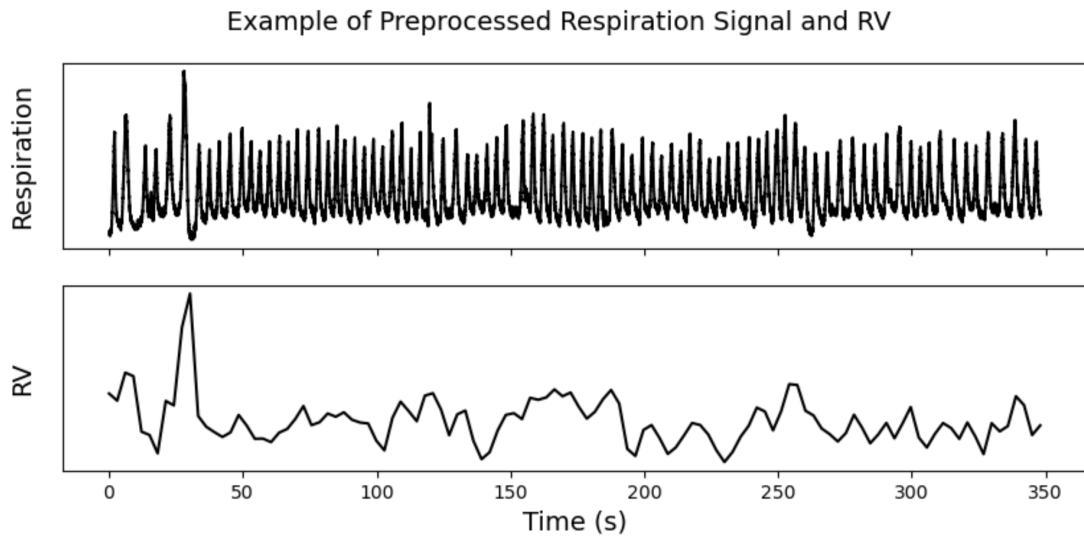


Figure 3.7: Example of respiratory bellow data and its derived metric. The top Figure shows the preprocessed respiratory bellow signal, with the cyclic changes in thoracic or abdominal expansion during breathing. The bottom panel displays the respiratory volume (RV), calculated from the bellow signal to quantify respiratory volume changes over time.

## 3.4 Data Analysis

### 3.4.1 Regions of Interest

The preprocessed functional data has been masked in order to get the average signals of various brain regions, that include but not limited to DMN regions, sensory regions, anterior cingulate cortex and insula. The selection of the regions are based on the previous literature. For example anterior cingulate is reported to have increased activity as well as the occipital cortex, in higher stages of vigilance [13],[14]. Furthermore DMN regions (namely precuneus, posterior cingulate cortex and angular gyrus) are reported to be activated in the resting state scans of previous studies [1],[69],[70].

Figure 3.8 shows an exemplary subject's data being masked for the precuneus region using AFNI's draw dataset functionality. This step is done after preprocessing, using the functional masks created by AFNI's *proc\_py*, for each region of interest separately. The manual selection of regions of interest allows for visual inspection of the brain region. The masks are saved, then *3dmaskave* command (of AFNI's) is



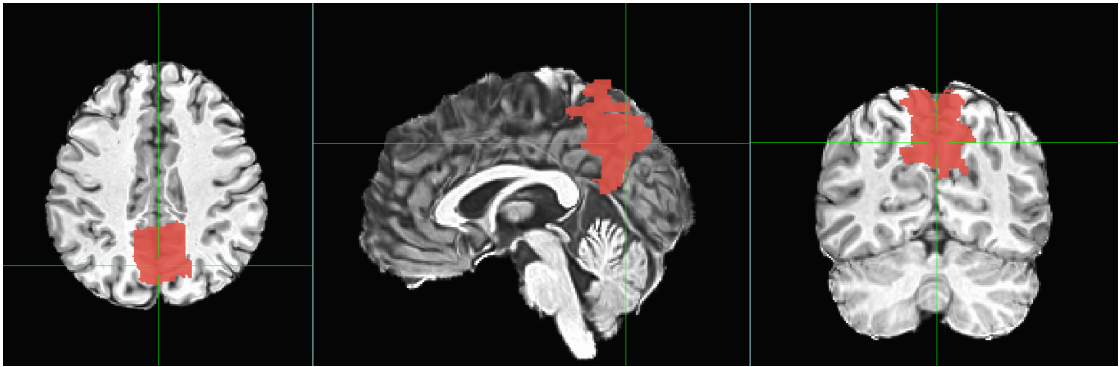


Figure 3.8: A masking being done in AFNI software. Figure shows precuneus region of an example subject being selected for a mask to be created. Functional data are then masked and averaged using these masks. Masks for other regions were shown in Figure 2.2.

used with the mask on the functional data in order to average signals from all voxels corresponding to that mask.

### 3.4.2 EEG Power Spectrum Analysis and Vigilance Metric

EEG-based vigilance metrics are taken into analysis in this study as they have been proven to be successful indicators of vigilance, and is noted to be the most commonly studied physiologic measure of vigilance [12]. While various methodologies exist for measuring vigilance using EEG signals, a common approach for EEG-based vigilance metric is related to the ratio of the power in middle frequency bands, such as alpha and beta bands, to the power in lower frequency bands, such as gamma or theta [71],[12],[13],[14]. These metrics were described in the background are calculated using preprocessed EEG data. Calculations are based on the Eq. 2.1; which, as also described in earlier chapters, captures the relative balance between alpha and theta-alpha band power for vigilance assessment. Our study takes into account the previously applied metric [13] in which its features include alpha, delta and theta frequency powers, and the ratio between these powers. In order to get the whole-brain EEG vigilance, the time-course of EEG vigilance of all channels will be averaged [72].

All calculations are done using python's *mne* library. First, channels are picked

in the dataset; for the occipital region, channels O1 and O2 are chosen, while for the frontal region, channels F3 and F4 are used (channels were decided based on the previous literature [13] as discussed in chapter 2.1.2). Then the data is segmented in 3 second intervals to equalize the length of events with the number of TRs of the functional scan. The epochs are created without overlap to maintain discrete, non-redundant segments of data. Welch's method is used to obtain the powers for the appropriate frequencies for each segment of 3 seconds. Specifically, two PSD calculations are performed; in 8-12 Hz frequency band, which represents alpha power, which is commonly associated with alertness and relaxation, and in 2-12 Hz frequency band, which includes both theta and alpha frequencies and serves as the denominator to normalize the alpha power. The PSD values for each band are summed across all frequencies within the specified range. The summed values are then averaged across the two channels within each region (occipital or frontal) to obtain a representative power value for the epoch.

$$P_f = \frac{1}{N} \sum_{f_1 \leq f \leq f_2} |X(f)|^2 \quad (3.1)$$

In this study, we chose Welch's method for power calculation as described above. Welch's method and regular power density calculation differ in their approach to estimating power. Regular power density calculation, as represented in Eq. 3.1, directly computes the power within a specific frequency range by summing the squared magnitudes of the Fourier-transformed signal and normalizing by the number of frequency components. While this approach is straightforward, it can be sensitive to noise and variations in the signal, leading to less reliable power estimates. In contrast, Welch's method is more robust to power estimation by dividing the signal into overlapping segments, applying a windowing function (e.g., Hamming or Hann) to each segment to minimize spectral leakage, and averaging the power spectral densities of these segments. This process reduces the variance of the power estimate and provides a smoother, more stable representation of the signal's frequency content. The use of Welch's method in this analysis ensures reliable PSD values for calculating vigilance metrics based on EEG

data.

Higher values of this metric indicate a stronger presence of alpha activity relative to lower-frequency activity, which corresponds to a relaxed but alert state, while lower values suggest increased theta activity, potentially reflecting reduced vigilance or drowsiness. This rationale is grounded in the association of alpha and beta bands with heightened wakefulness, reflecting increased cognitive engagement and alertness, while gamma and theta bands are indicative of reduced wakefulness, suggesting a state of decreased cognitive arousal and attentiveness [73]. Therefore, this metric captures the relation between distinct EEG frequency bands, providing insight into the dynamic nature of vigilance states.

### 3.4.3 Time-lagged Cross Correlations

All time-lagged correlations (cross correlations) of EEG, fMRI and physiological signals in this study are calculated according to Equation 3.2 where one signal is shifted in samples (for all our cases, shifted in time) in order to calculate correlation at each shift, or within this study, time lag.

$$R_{xy}(k) = \sum_{n=-\infty}^{\infty} x(n)y(n+k) \quad (3.2)$$

In our fMRI signal, we first identify the RSN regions, particularly the regions for DMN, and check time-lagged correlations within the brain, both within the RSN and with RSN and other regions. This process gives us insight on identifying RSN regions and enables us to see that our datasets indeed agree with previous research. Then, our methodology will involve using the previously obtained vigilance index as a regressor for our blood oxygen level-dependent (BOLD) signal analysis. By this approach, we aim to discern the relationship between arousal states and BOLD signals, and reveal the spatial and temporal dynamics of vigilance levels during resting-state sessions.

To highlight the spatial and temporal properties of the brain’s vigilance response, we utilized the cross-correlations between fMRI, EEG (alpha, beta, TBR), systemic signals (HR, RVT), and sympathetic biomarkers (PPG-AMP). We calculated lag-dependent correlations based on Pearson’s correlations, and also performed voxel-wise correlation maps. Moreover, we included PPG-AMP in our analysis as a marker of vascular tone, to extend our findings by establishing connections between our observations and variations in the PPG-AMP.

#### 3.4.4 Time-Lagged Correlation Matrix

As a visualization approach for exploring the relationship between brain activity and physiological signals, we created a time-lagged correlation matrix. Unlike traditional connectivity matrices as described below, this approach represents the x-axis as time lags and the y-axis as the correlation values between a single physiological signal and different regions of interest (ROIs). This visualization allows us to capture dynamic spatio-temporal interactions between systemic physiology and neural activity.

#### 3.4.5 Functional Connectivity

To analyze the connectivity patterns, we included various brain regions of interest in the functional connectivity analysis. These regions are anterior cingulate, insula, DMN regions (namely precuneus, posterior cingulate and angular gyrus), and sensory regions (primary auditory, primary motor and primary visual). These regions are masked as described in section 3.4.1, and global signal average was obtained by taking average of all voxels in the functional datasets. By functional connectivity analysis within fMRI data, we aimed to reveal the dynamic relationships and communication patterns associated with vigilance states during resting-state sessions.

To assess connectivity, we extracted time series data from these regions and computed correlation matrices. The correlation matrix was derived by evaluating the

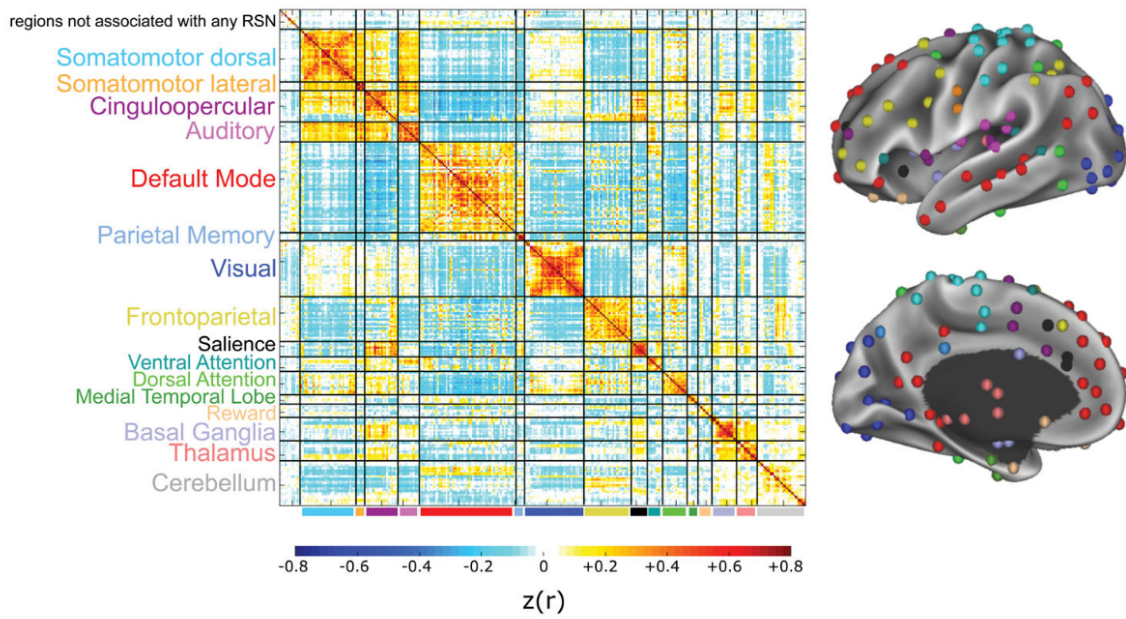


Figure 3.9: (Left) Typical connectivity matrix for 300 regions of interest for different resting state networks. (Right) Surface representations of the regions of interest with color coding. Figure from [1].

pairwise correlations between the time series from each selected region. Additionally, a global signal was included as a reference to account for any widespread signal fluctuations that might affect regional connectivity measurements. This methodological approach allowed us to explore the connectivity dynamics between different brain regions and to assess how these interactions vary between drowsy and awake states.

## 4. RESULTS

### 4.1 Classification of Vigilance Levels: Awake vs Drowsy

To classify subjects into EEG stage A or B in this study, we analyzed the EEG signals based on the distribution of alpha power (8-12 Hz) across specific channels. The channels selected for this analysis were F3 and F4 from frontal regions, and O1 and O2 from occipital regions. These regions are chosen in accordance with Olbrich et. al. study [13] for monitoring the brain's transition between alertness and drowsiness, as they are reported to show distinct changes in alpha activity during these states.

#### TR-Based Classification of Vigilance Levels

For each subject, we calculated the alpha power in the 8-12 Hz frequency band for each channel for every 3-second interval of EEG data. This power was compared to the total power in the broader frequency range of 2-12 Hz within the same time frame. The comparison between alpha power and total power allowed for a determination of the subject's EEG stage. Specifically, if the alpha power in any of the monitored channels (F3, F4, O1, or O2) exceeded 50% of the total power in the 2-12 Hz range, the 3-second interval was classified as EEG stage A. This stage is associated with higher levels of alertness.

Conversely, if the alpha power in all channels remained below or equal to 50% of the total power in the 2-12 Hz range, the 3-second interval was classified as EEG stage B. This stage indicates reduced alertness, or a drowsier state.

The use of 3-second intervals for the classification was chosen to match the TR (repetition time) of 3 seconds in our resting-state functional MRI data acquisition. This synchronization allowed for a direct comparison between the EEG-based classification of alertness and the fMRI-based connectivity data. By classifying each subject's EEG

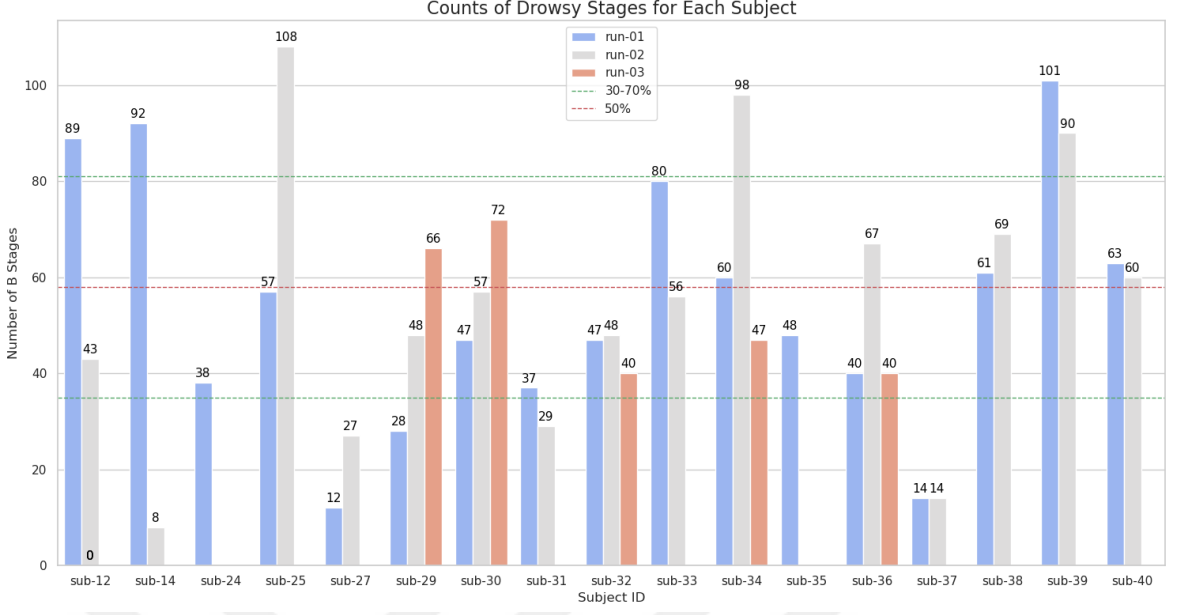


Figure 4.1: The drowsy stage counts for each subject in number of TRs.

data into alternating periods of EEG stage A and B, we were able to classify the fluctuations in drowsiness/alertness stages.

### Subject-Based Classification of Vigilance Levels

For the subject-level classification, the number of EEG stage B intervals (representing drowsy states) was used as the key metric to categorize subjects into different alertness groups. Our datasets contained a total of 116 TRs, corresponding to 116 three-second intervals of EEG data. To classify subjects as either awake or drowsy, we established a threshold of 58 intervals, which is half of the total TR count. If the count of stage B intervals for a subject was less than 58, the subject was labeled as awake, indicating that they spent more time in alert (stage A) periods. Conversely, if the count of stage B intervals was greater than 58, the subject was labeled as drowsy, reflecting a dominance of drowsy intervals. Dashed red line in Figure 4.1 visualizes this separation; the data that show below the red line would fall into the awake category, while the ones above the red line would fall into the drowsy category. In the analysis below, this group will be referred to as *comprehensive group*.

In addition to this basic classification, we also conducted a more focused anal-

ysis to investigate edge cases. For this, we introduced stricter thresholds based on 30% and 70% of the total TR count, with the aim of focusing on more extreme cases of alertness and drowsiness. Specifically, if a subject exhibited fewer than 35 stage B intervals (30% of 116 TRs), they were classified as awake for the edge case analysis, representing a state of sustained alertness. On the other hand, if a subject had more than 81 stage B intervals (70% of 116 TRs), they were classified as highly drowsy, indicating a pronounced shift towards drowsiness. The green lines in Figure 4.1 visualizes these thresholds, similarly as described above. This stricter thresholding allowed us to exclude subjects who fell in the middle range of drowsiness (between 35 and 81 stage B intervals) and focus on more clear-cut cases of alert or drowsy states. By separating these edge cases, we aimed to investigate whether more extreme states of alertness or drowsiness produced stronger or more distinct effects in the brain connectivity data and its relations with physiology, offering a more focused perspective on how drowsiness influences neural activity patterns and physiology. In the following sections, this group will be referred to as *boundary group*, given the fact that all of the subjects that fall into this "edge-case" analysis were included within the *comprehensive group* group and are merely the subset of all the data.

As visible in the Figure 4.1; 15 datasets were classified as drowsy in the whole set group, while 6 datasets were classified as drowsy in the edge cases, having above 70% B stages. Similarly, 22 datasets were classified as awake for the whole set, 7 of which are also below the 30% threshold, which will be included in the subset.



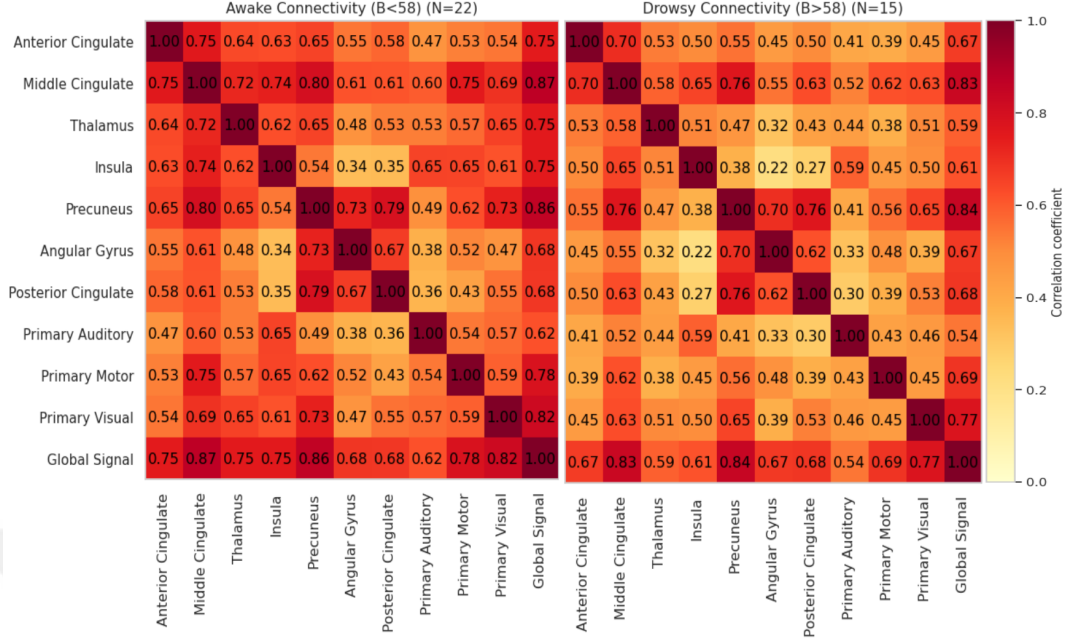


Figure 4.2: Connectivity analysis between awake and drowsy subjects of the *comprehensive* group (B threshold = 58).

## 4.2 Impact of Drowsiness on Brain Activity Synchronization

The connectivity analysis revealed prominent differences between the drowsy and awake groups across the examined brain regions, which included the anterior cingulate, insula, DMN regions (namely precuneus, angular gyrus and posterior cingulate), and sensory regions (primary auditory, primary motor, and primary visual cortices). We also include the global signal, which is obtained by averaging all voxels in the functional MRI datasets. Figure 4.2 show the average connectivity matrices for fMRI BOLD signal for 2 groups (awake and drowsy) with threshold 58, as described in section 4.1.

Connectivity values across all regions were consistently lower in the drowsy group compared to the awake group, consistent with the general reduction in neural synchronization typically associated with drowsiness. Notably, the Default Mode Network (DMN) regions-specifically the precuneus, angular gyrus, and posterior cingulate demonstrated relatively higher connectivity in both groups compared to the other regions assessed.

### 4.3 Brain and Systemic Physiology Relationship

Since we did not regress out the physiological-related variation from the fMRI signal, we expect to see the effect of physiology in the fMRI BOLD signal.

#### 4.3.1 fMRI and Heart Rate Interactions

##### Gray Matter Dynamics and Heart Rate

Gray matter and heart rate correlations are shown for *comprehensive* group in Figure 4.3 and for *boundary* group in Figure 4.4. The *comprehensive* group shows significantly different correlation in anterior cingulate region with the HR signal at 0 seconds lag ( $p = 0.023$ ) with the drowsy group being much more positively correlated than the awake group. Even though the other regions of interest show no significant difference in this group, for the *boundary* group, the correlations between these brain regions (insula, primary auditory cortex, primary visual cortex and again, anterior cingulate) and HR is positive at lag 0 in the drowsy group (with anterior cingulate  $r = 0.33$ , with insula  $r = 0.19$ , with primary auditory cortex  $r = 0.20$ , and with primary visual cortex  $r = 0.26$ ). All values are for 0 lag drowsy group, and represented more visually in Figure 4.5 and on brain plots in Figure 4.6. At 0 second lag, the signals are simultaneously changing, but note that our TR value used in our study is 3 seconds, which limits our temporal resolution to 3 second intervals.

Time-lagged correlation matrix shows colorfully the broader correlation increase in drowsy group at 0 lag, while also showing the broad negative correlations at positive lags in awake group. The strong negative correlation occurring in positive lags in awake group, starting after 9 seconds lag in all these regions. A continuum of negative correlations are observed for all ROIs. Strongest (negative) correlations for each ROI occur at, anterior cingulate  $r = -0.16$  at 18 seconds lag (note that  $r = -0.15$  for 9 seconds lag), insula  $r = -0.23$  at 9 seconds lag, primary auditory cortex  $r = -0.24$  at 9 seconds lag, and finally primary visual cortex shows  $r = -0.27$  at 9 seconds lag,

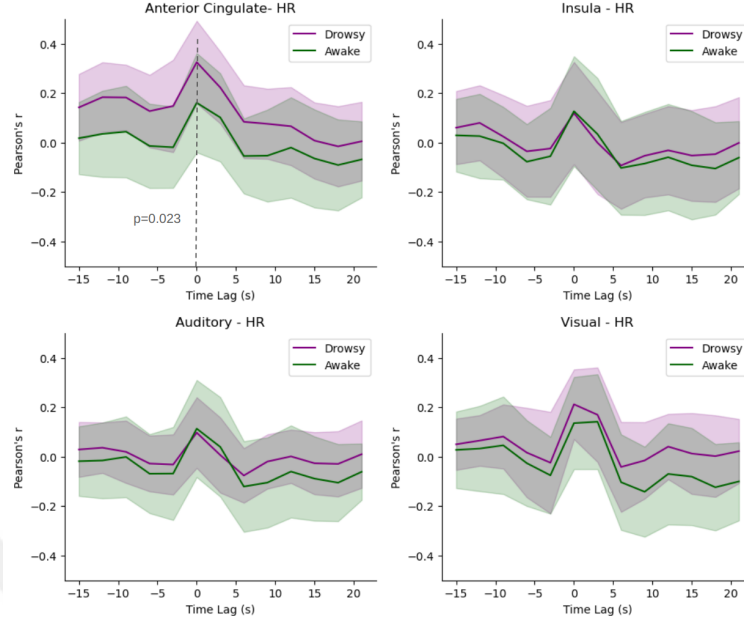


Figure 4.3: Time-lagged correlation plots of *comprehensive* group's heart rate signal with various regions of interest.

as can be seen in Figure 4.4. In other words, most regions show strongest negative correlation at 9 seconds lag with HR signal in awake group. Positive lag means that HR signal leads the fMRI signal, meaning that a change occurring in HR might be reflected onto the fMRI signal. In Figure 4.5 we visualize the aforementioned results in a more compact way; top matrix shows the awake group correlation values with HR for each ROI with x-axis showing time-lags, while bottom matrix shows results for drowsy group in a similar fashion (first row is the auto-correlation of HR for sanity check reasons). In almost all ROI, it is visible that around 0 second lag, drowsy group has higher correlations while the prolonged negative correlation of awake group in positive lags are visualized in blue color in the top matrix (in positive lags).

## CSF Oscillations and Heart Rate

Figure 4.7 shows in top cross-correlation results for *comprehensive* group, and in bottom results for *boundary* group. In drowsy state, subjects in both group did not show a correlation in lags of interest, although the awake group had shown significant difference in correlation ( $r = 0.13$  for *comprehensive* group, top-left, and  $r = 0.15$  for

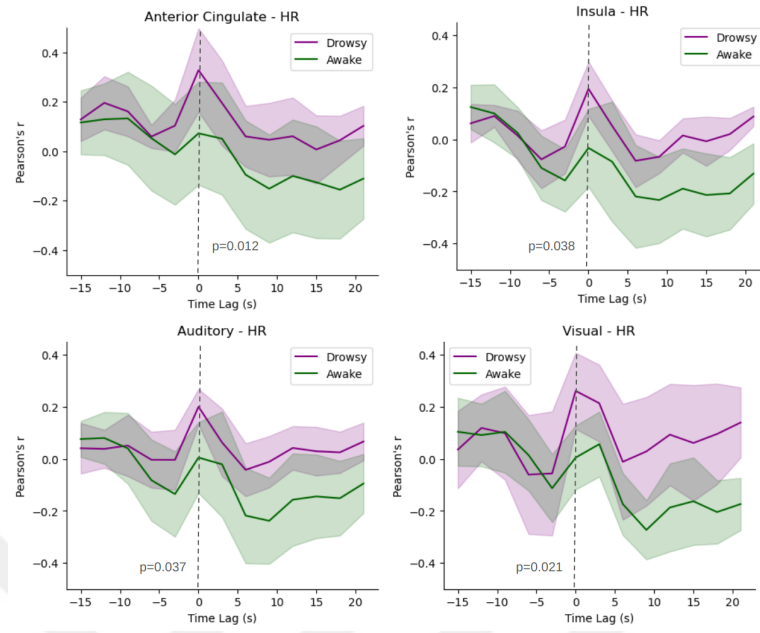


Figure 4.4: Time-lagged correlation plots of the heart rate signal with various regions of interest for *boundary* group.

*boundary* group, bottom-left) at 6 seconds lag. Since the peaks occur at positive lag value, HR signal leads the CSF signal.

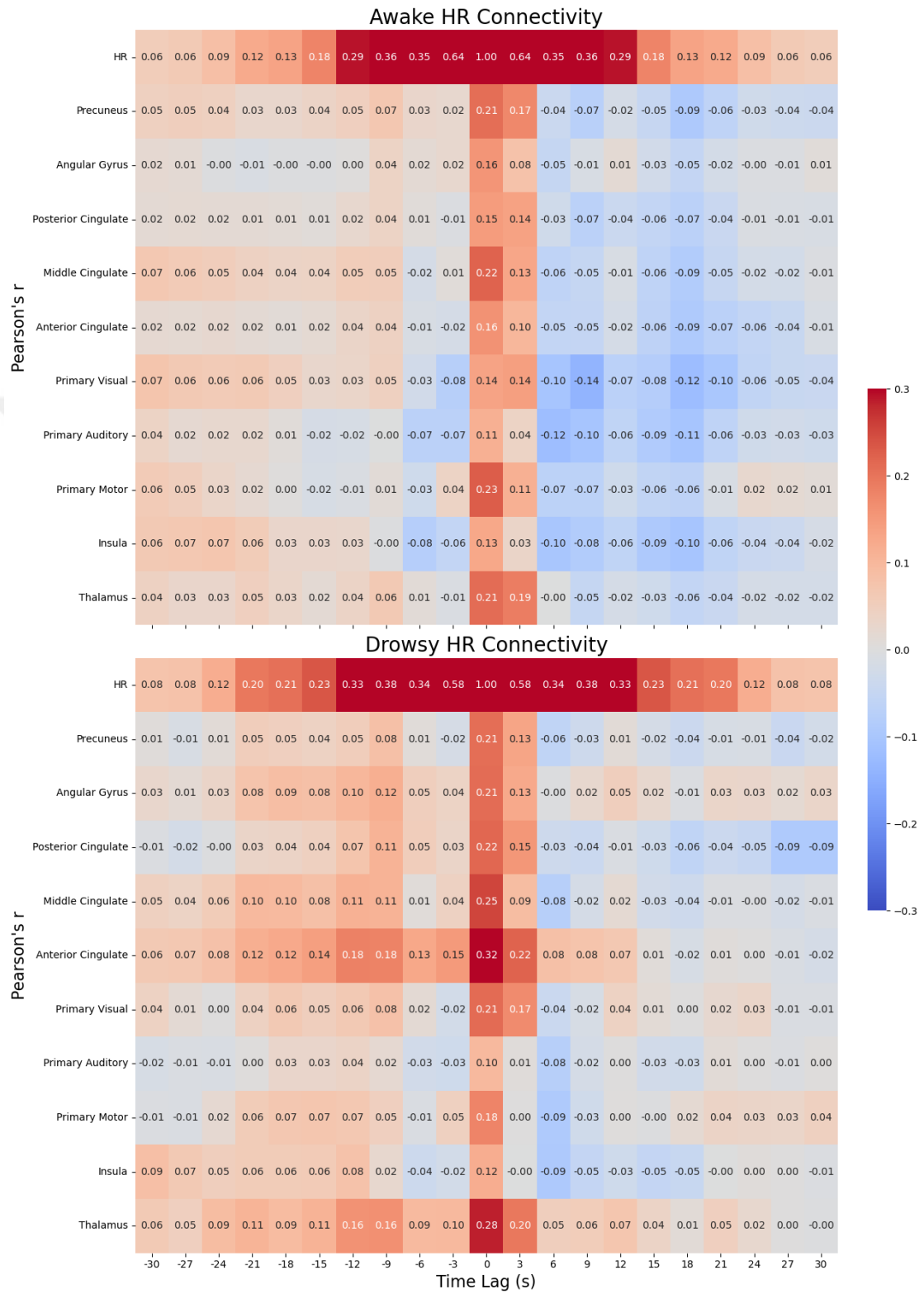


Figure 4.5: Time-lagged correlation matrix of the heart rate signal with various ROI in comprehensive group. First row auto-correlation, x-axis represents time-lags.

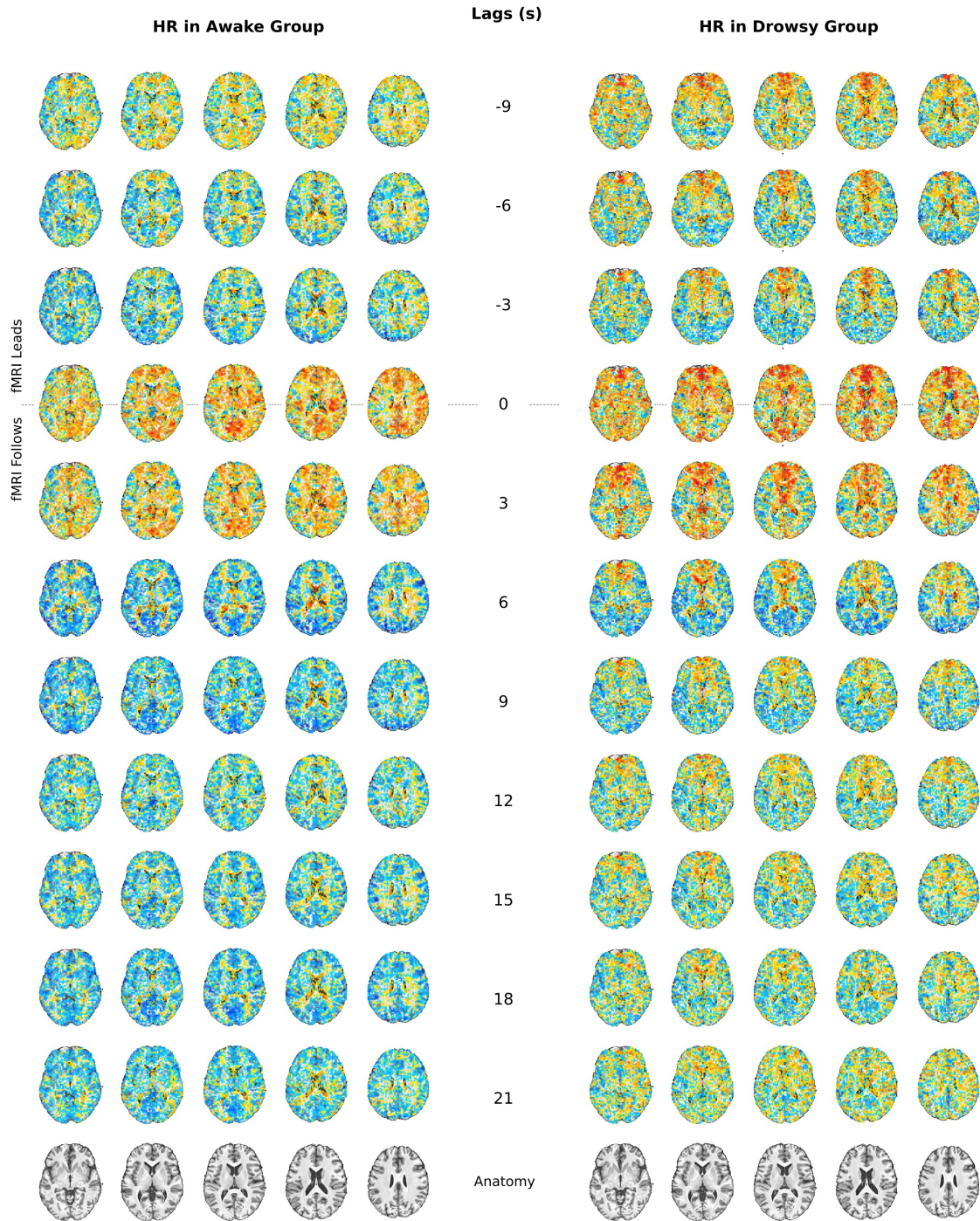


Figure 4.6: Voxel-wise correlation group averages of the heart rate signal with whole brain for *comprehensive* group, left for awake, right for drowsy subjects.



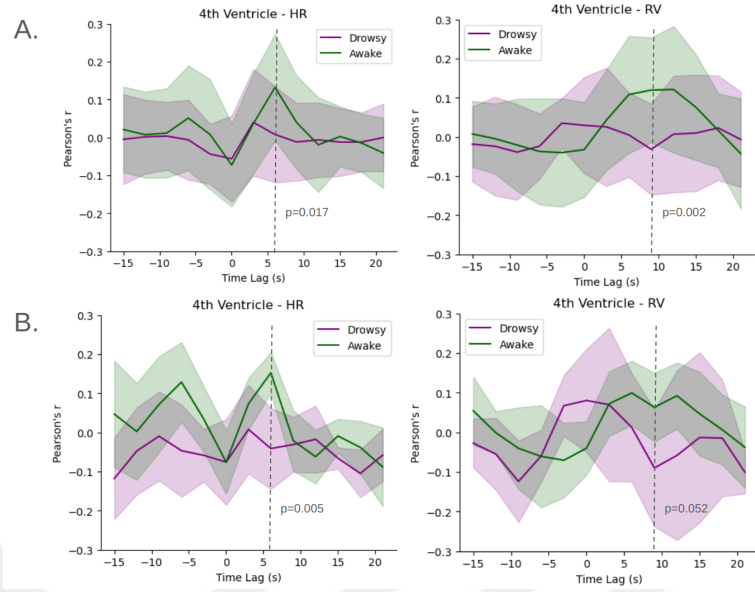


Figure 4.7: Time-lagged correlation plots of the 4th ventricle signal (as a representative of CSF signal) with HR and RV signals for A. *comprehensive* group and B. *boundary* group.

### 4.3.2 fMRI and PPG Amplitude Interactions

#### Default Mode Network and PPG Amplitude

Regions of the default mode network revealed contrasts in dynamics of awake and drowsy groups. Figure 4.9 shows for *boundary* group, strong positive correlations between angular gyrus and PPG-AMP in the drowsy group at lag 0 seconds ( $p < 0.05$ ). Correlations between precuneus and PPG-AMP follow a similar trend, as the drowsy group exhibits a strong positive correlation at lag 0 seconds. In both precuneus and angular gyrus, correlations at lag 0 with the awake group are either negative (precuneus) or around 0 (angular gyrus). We also noted a negative peak (strong negative correlation) in drowsy group occurring around -10 seconds lag for precuneus and angular gyrus (DMN signal changes leading an opposite change in PPG-AMP signal). Significant differences in correlations between precuneus & PPG-AMP and angular gyrus & PPG-AMP are at 0 second lag as noted down on Figure 4.9, and also +3 seconds lag in all regions with  $p < 0.05$ , with drowsy group being positively correlated.

#### Gray Matter Dynamics and PPG Amplitude

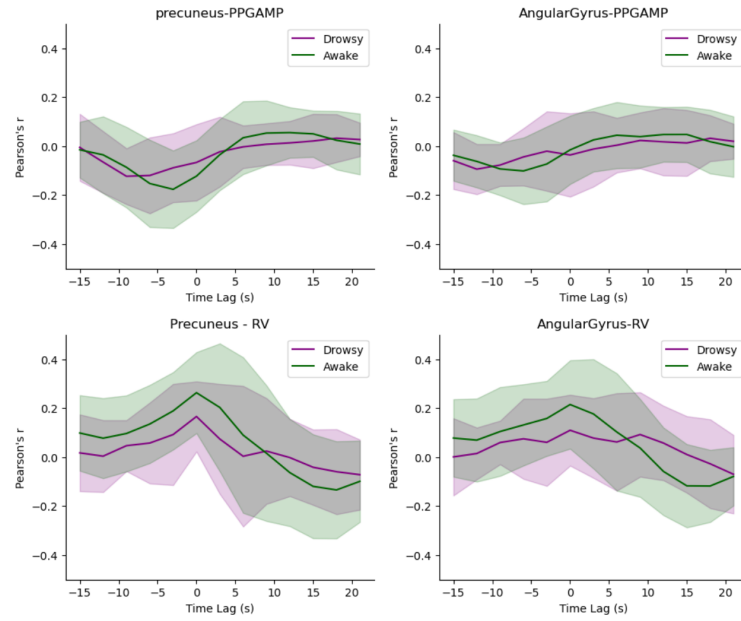


Figure 4.8: Time-lagged correlation plots of *comprehensive* group's PPG-AMP and RV signals with precuneus and angular gyrus - active regions of the default mode network.

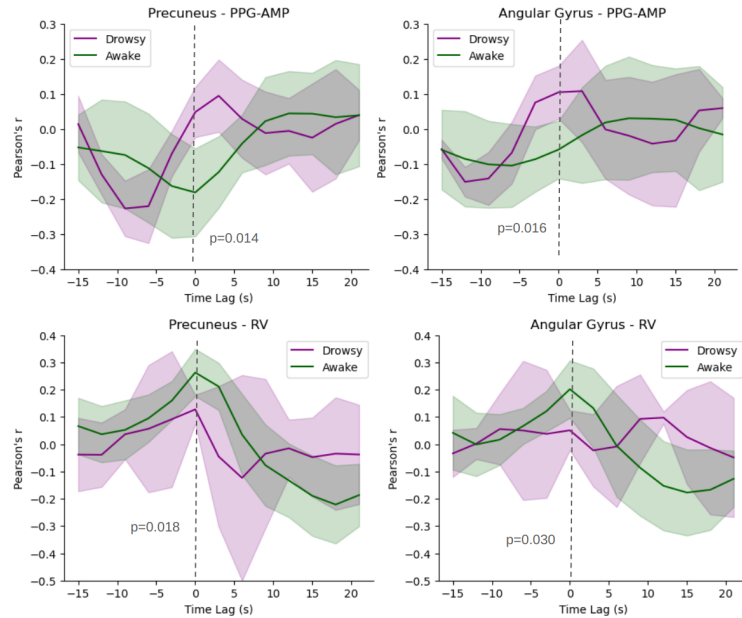


Figure 4.9: Time-lagged correlation plots of *boundary* group's PPG-AMP and RV signals with precuneus and angular gyrus - active regions of the default mode network.



Figures 4.10 and 4.11 show PPG-AMP signal having stronger negative correlations in awake group at -3 seconds lag (where fMRI leads) for various ROI. While the same observation may be argued for many ROI in this Figure, we note the correlations of primary visual cortex and insula regions especially. Top-right plot in Figure 4.10 shows for the correlations between primary visual cortex and PPG-AMP, awake group is significantly more correlated than the drowsy group at -3 seconds lag, with negative correlation values for awake and drowsy groups ( $p < 0.05$ ,  $r = -0.23$  for awake group and  $r = -0.12$  for drowsy group) in *comprehensive* group. This effect strengthens with lower p-value for the *boundary* group as seen in the bottom-right plot of the same figure, with  $p < 0.05$ ,  $r = -0.22$  for awake group and  $r = -0.08$  for drowsy group.

A similar result is also shown for insula region. Again in Figure 4.10, top-left and bottom-left plots show for both *comprehensive* and *boundary* groups awake group having negative correlation peak at -3 seconds lag, though in *boundary* group (bottom-left plot) the negative peak of drowsy group is shifted towards -6 seconds negative lag. Numerically, in *comprehensive* group,  $r = -0.18$  for awake group and  $r = -0.09$  for drowsy group for -3 seconds lag; and in *boundary* group  $p < 0.05$ ,  $r = -0.17$  at -3 seconds lag for awake group, and  $r = -0.17$  at -6 seconds lag for drowsy group (-3 seconds lag for drowsy group shows  $r = -0.05$ ).

In Figure 4.12 we visualize the correlations in a voxel-wise fashion for PPG-AMP signal with the brain. The awake group at -3 seconds lag show broader negative correlations across the brain, especially towards posterior regions (including visual areas), compared to the drowsy group.

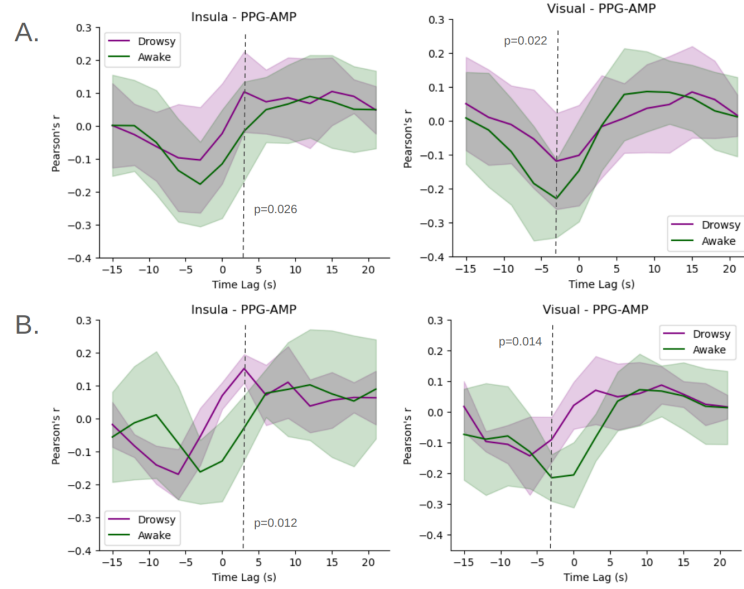


Figure 4.10: Time-lagged correlation plots of the PPG-AMP signal with insula and primary visual regions for A. *comprehensive* group and B. *boundary* group.

### 4.3.3 fMRI and Respiratory Variation Interactions

#### Default Mode Network and Respiratory Variation

The relationship between DMN and respiration is also altered in wakefulness and drowsiness. Significant differences are observed in correlations of both angular gyrus and precuneus with RV. Figure 4.9 shows awake group having higher positive correlations than the drowsy group at 0 seconds lag ( $p < 0.05$ , with  $r = 0.26$  for awake and  $r = 0.13$  for drowsy in boundary group). Also note that a negative, prolonged correlation is seen after 12 seconds lag in awake *boundary* group with significantly different correlation ( $p < 0.05$ ,  $r = 0.13$  for awake and  $r = -0.001$  for drowsy at exactly 12 seconds lag). Generally, drowsy group exhibits weaker correlations with the DMN region & RV signal.

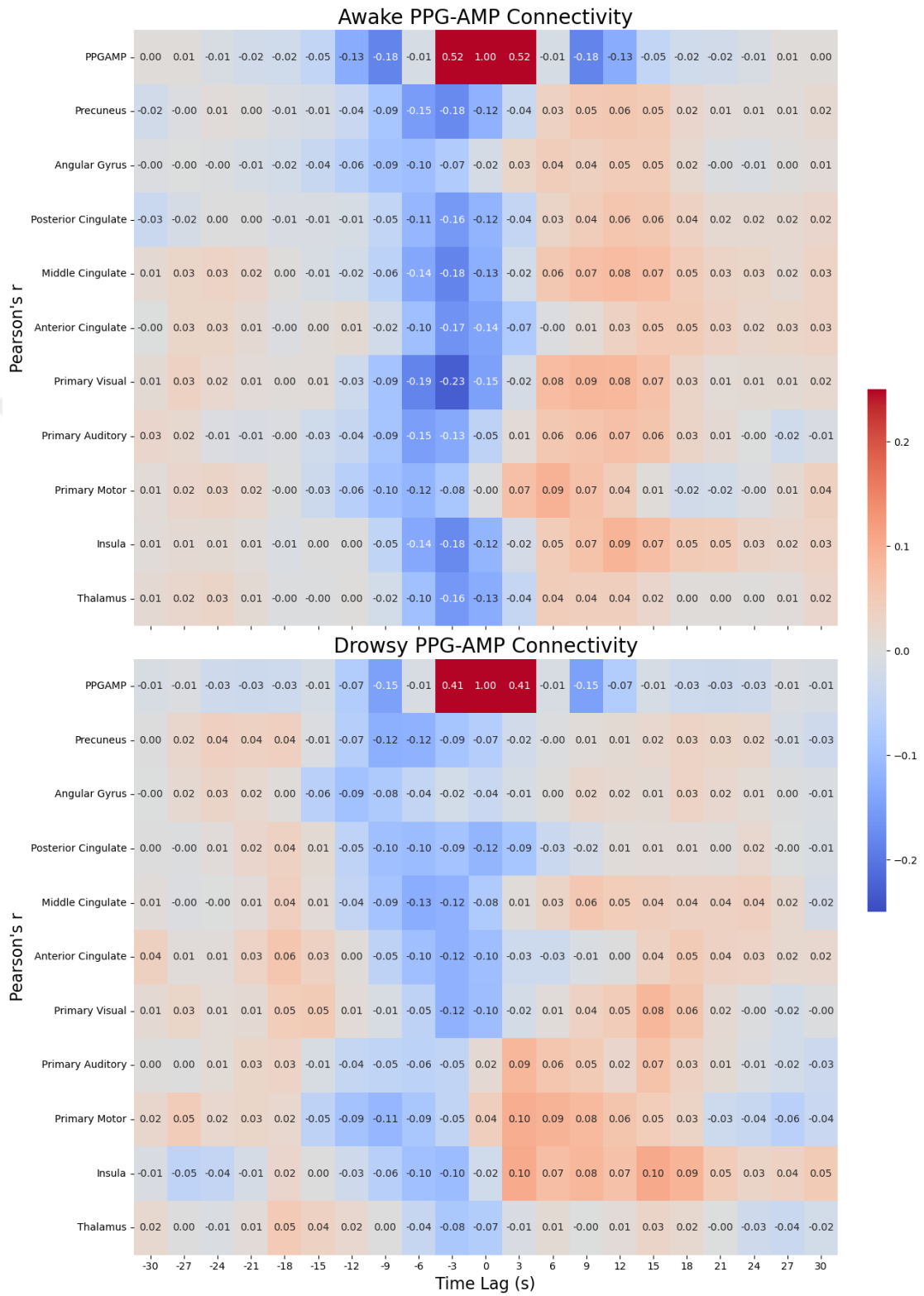


Figure 4.11: Time-lagged correlation matrix of the PPG amplitude signal with various ROI in *comprehensive* group. First row auto-correlation, x-axis represents time-lags.

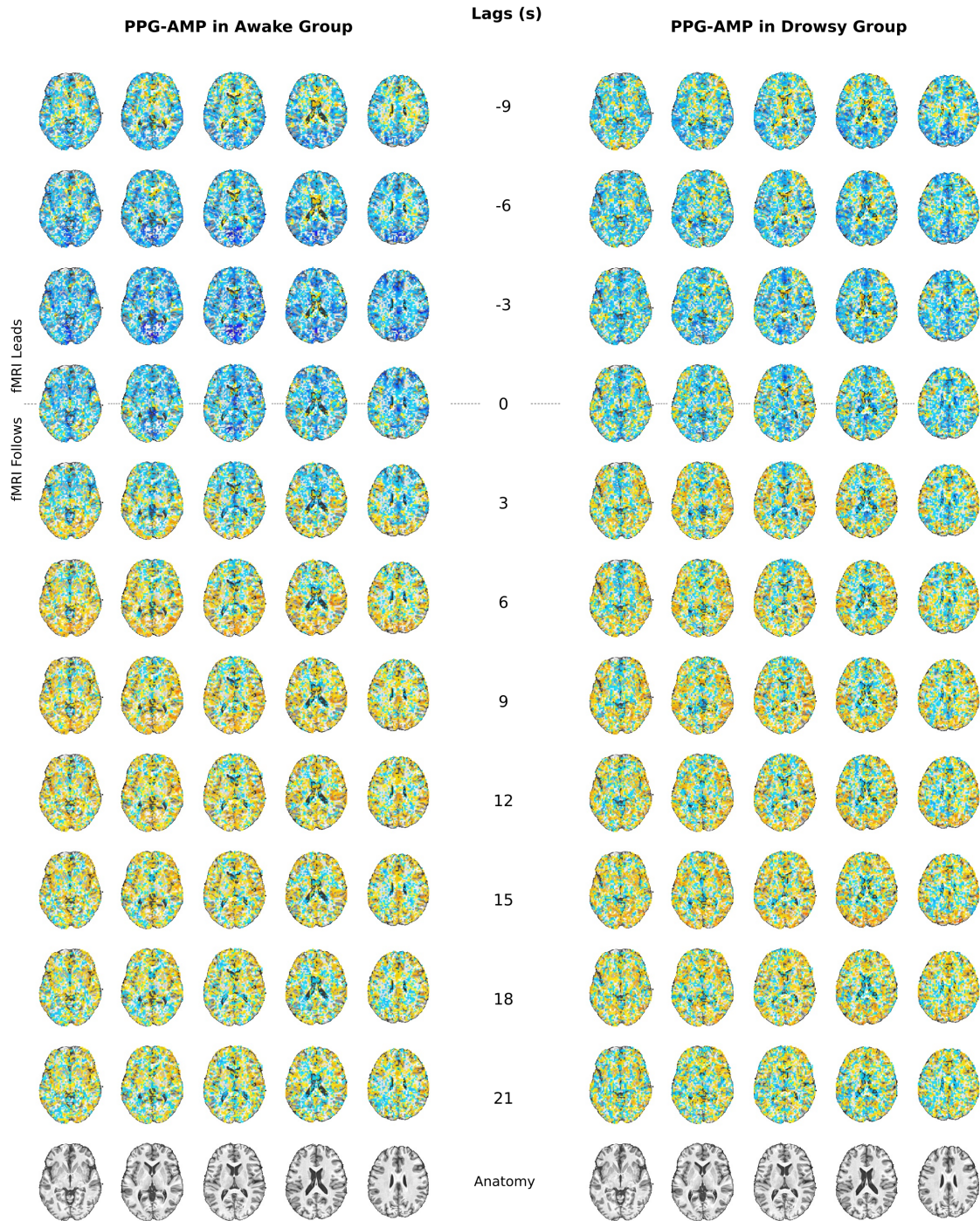


Figure 4.12: Voxel-wise correlation group averages of the PPG amplitude signal with whole brain for *comprehensive* group, left for awake, right for drowsy subjects.

## CSF Oscillations and Respiratory Variation

In CSF & RV correlations (Figure 4.7), we see a significantly different behavior at 9 seconds positive lag, with awake subjects of the *comprehensive* group having a peak  $r = 0.12$ . In the analysis of the *boundary* group, the peak for the drowsy group is more apparent, and seemingly shifted towards 0 second lag with  $r = 0.09$ .

Figure 4.14 highlights the brain's correlation patterns at a lag of approximately 12 seconds. In the awake group, stronger positive correlations emerge at later lags (9-12 seconds), extending to other ventricles, including the lateral ventricles, compared to the drowsy group. Additionally, the awake group exhibits generally higher voxel-wise positive correlations in sensory regions at earlier lags, particularly around 0 to 3 seconds.

## 4.4 fMRI and EEG Vigilance Metrics

Finally, the previously acquired EEG vigilance metrics are taken into analysis and the time-lagged correlation matrices between fMRI and EEG metrics are calculated. Figure 4.15 shows results for Olbrich's frontal vigilance metric correlations with the brain, and Figure 4.16 shows the correlations of occipital vigilance metric with the brain.

Frontal vigilance metric showed a much greater, clearer difference between the awake and drowsy groups. In Figure 4.15 a general negative correlation in awake group can be easily seen especially in 3 and 6 seconds lags. The significant differences are in middle cingulate ( $p = 0.025$  for 3 seconds lag), primary auditory cortex ( $p = 0.004$  for 3 seconds lag,  $p = 0.051$  for 6 seconds lag), angular gyrus ( $p = 0.018$  for 0 seconds lag) and primary motor cortex ( $p = 0.028$  for 0 seconds lag). On the other hand, with the occipital metric, no particular significant correlation is observed within this study.

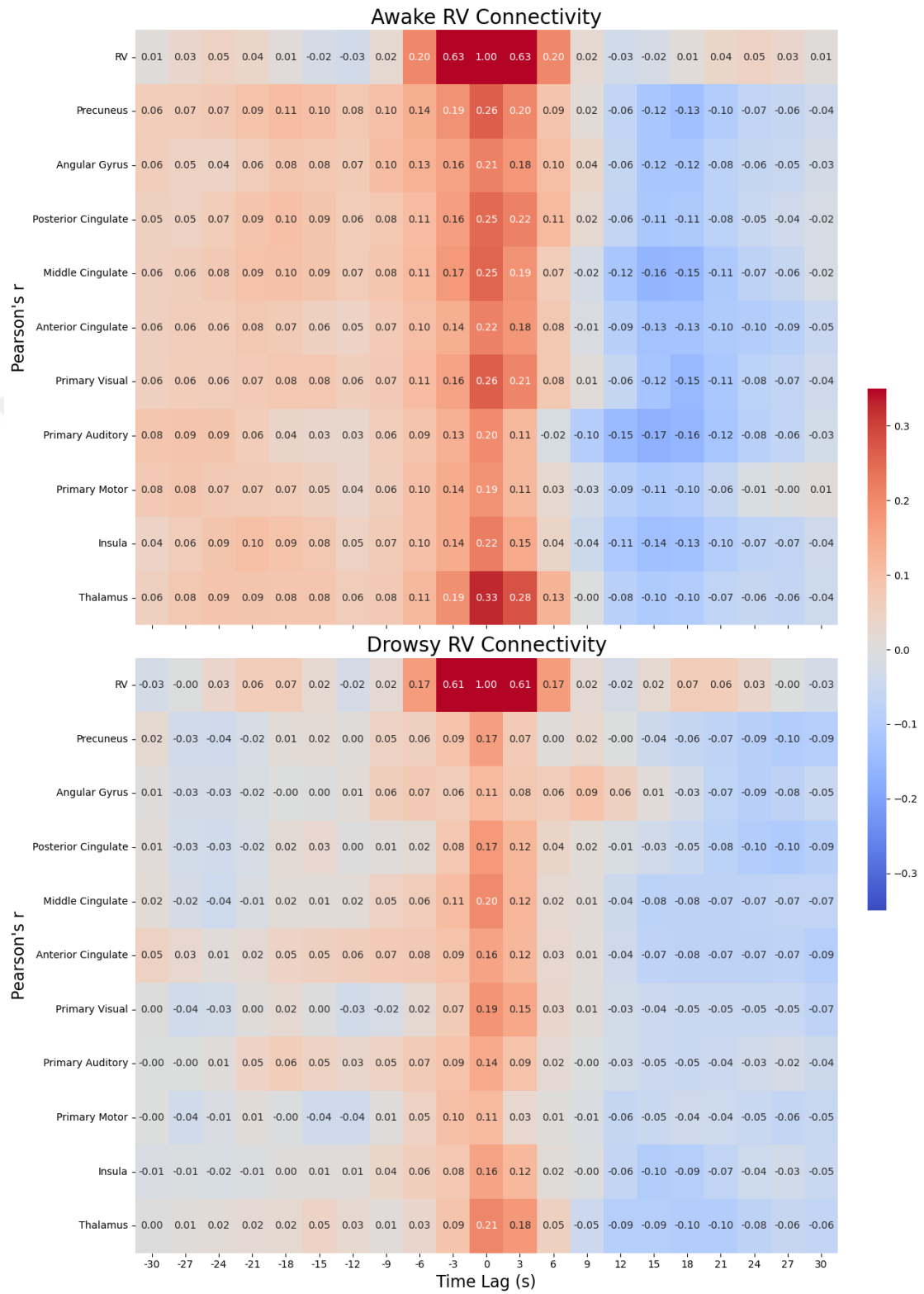


Figure 4.13: Time-lagged correlation matrix of the respiratory variation signal with various ROI in *comprehensive* group. First row auto-correlation, x-axis represents time-lags.



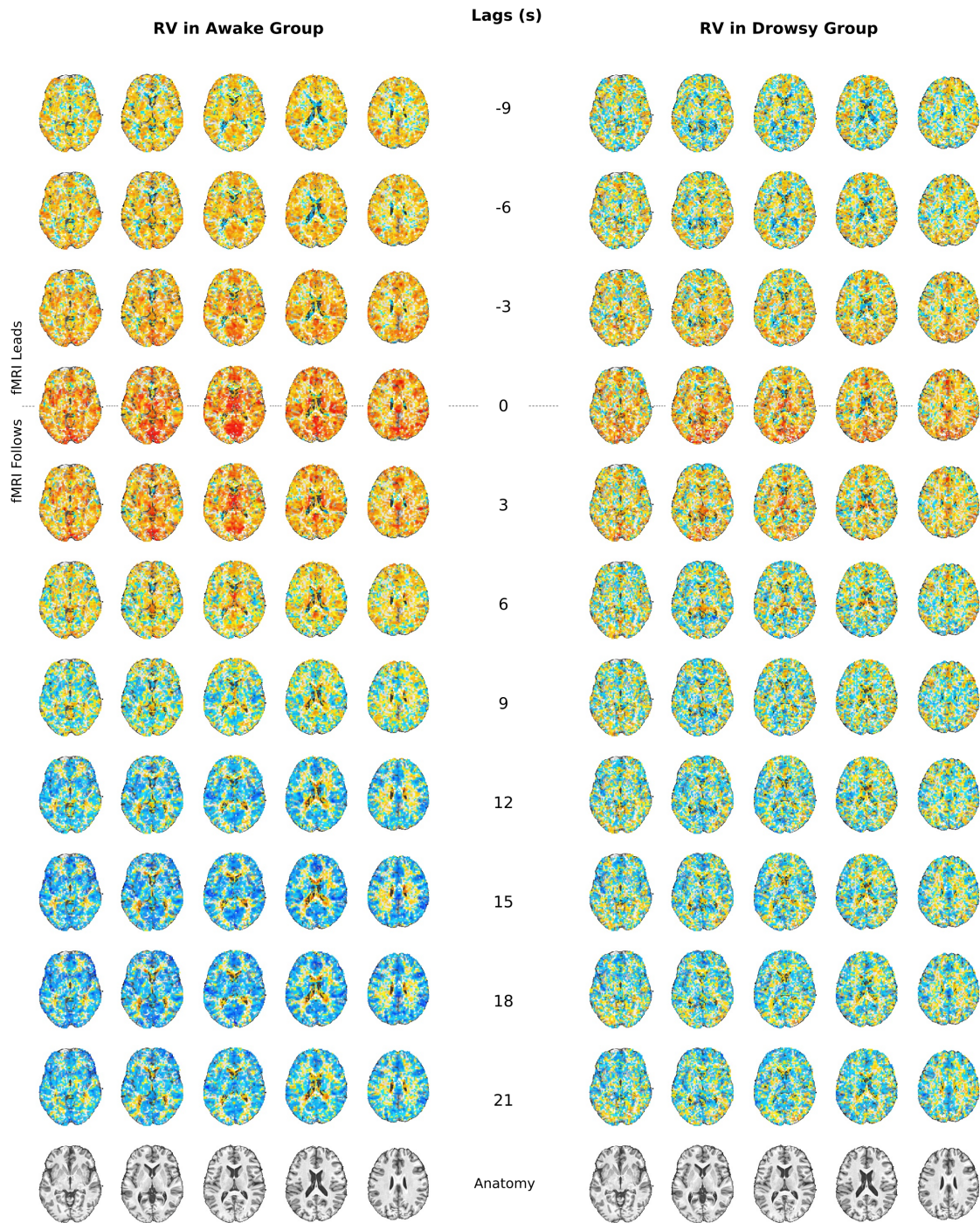


Figure 4.14: Voxel-wise correlation group averages of the respiratory variation signal with whole brain for *comprehensive* group, left for awake, right for drowsy subjects.

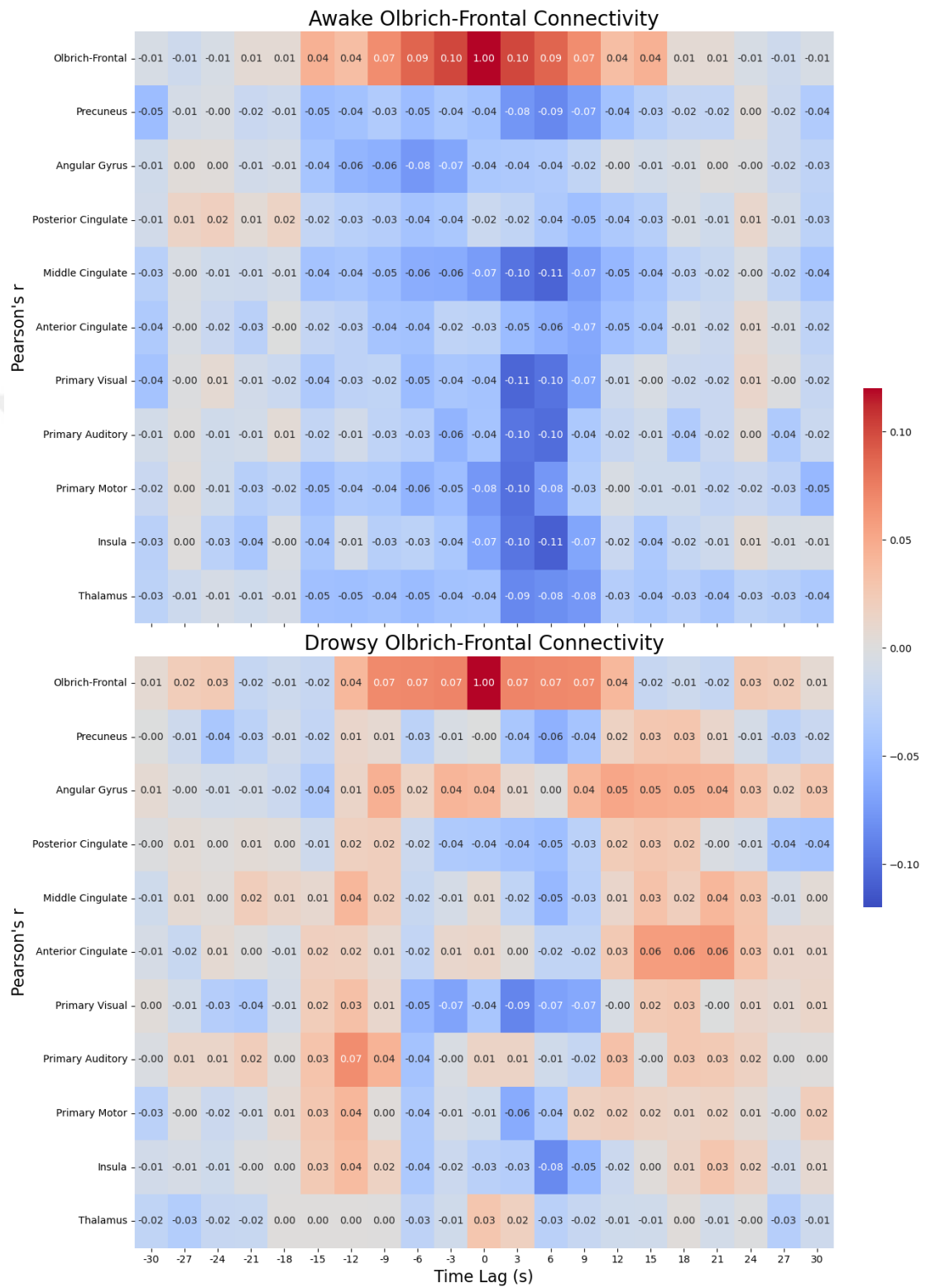


Figure 4.15: Time-lagged correlation matrix of the Olbrich's frontal metric with various ROI in *comprehensive* group. First row auto-correlation, x-axis represents time-lags.



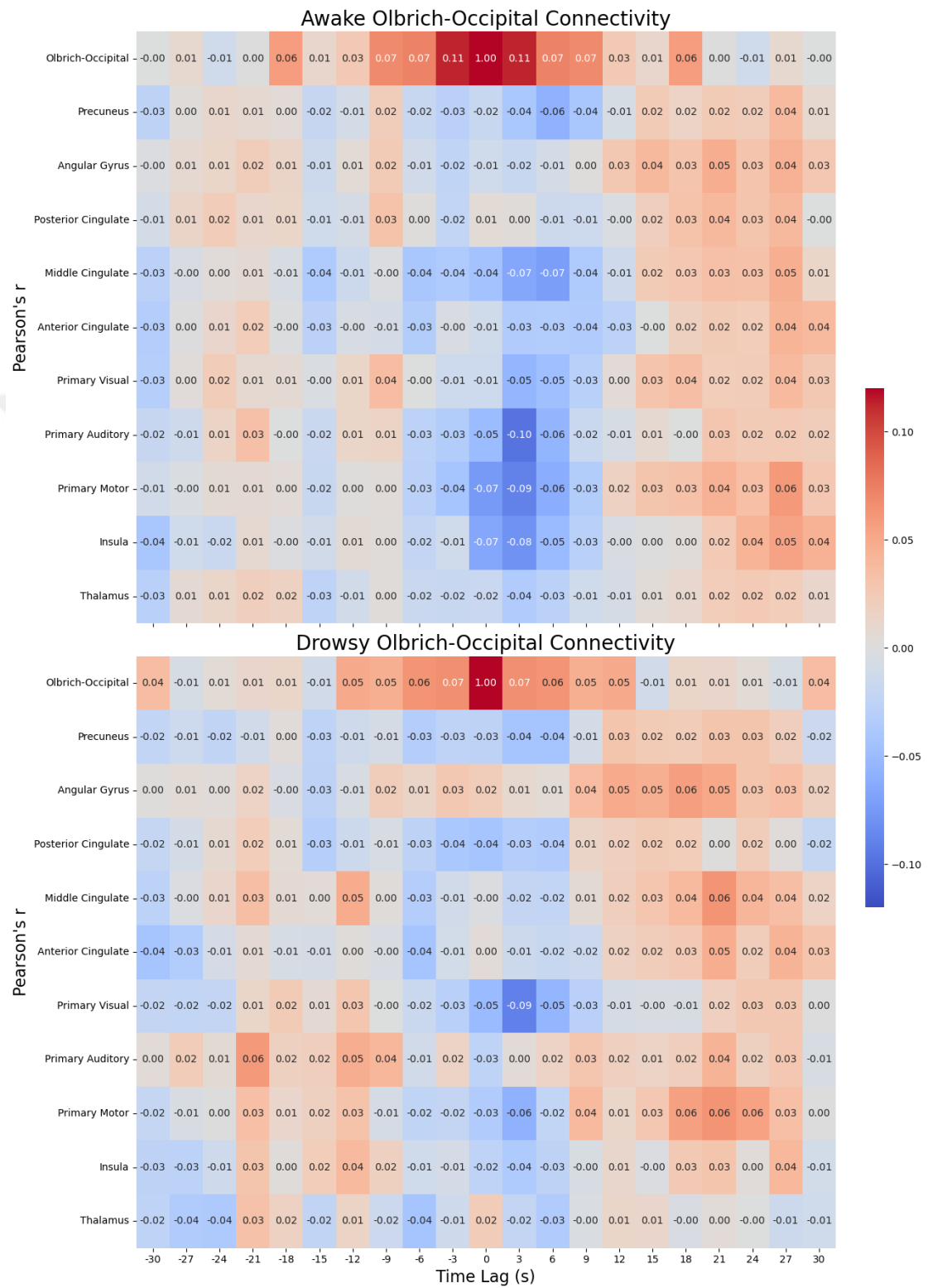


Figure 4.16: Time-lagged correlation matrix of the Olbrich's occipital metric with various ROI in *comprehensive* group. First row auto-correlation, x-axis represents time-lags.

## 5. DISCUSSION

The primary objective of this study was to identify vigilance states during fMRI by applying previously established EEG-based vigilance metrics to resting-state datasets from our experiments and to investigate how systemic physiological changes relate to vigilance-related fluctuations in fMRI. By examining electrophysiological, fMRI, and systemic physiology signal changes, we aimed to gain deeper insights into how drowsiness influences the activity of different brain regions and the autonomic nervous system. Vigilance states were identified by first calculating vigilance metrics from the preprocessed EEG data. These states were further characterized by analyzing and comparing the spatial and temporal relationships between physiological signals and BOLD activity.

### Vigilance Classification

We used EEG data from occipital channels (O1, O2, O3, O4) to differentiate between states of wakefulness and drowsiness. Specifically, we focused on the power of the alpha frequency band (8-12 Hz), which is known to be associated with relaxed wakefulness and a reduced level of cognitive engagement. The total power spectrum was calculated over the range of 2-12 Hz for each time point of the subject's scan, and a threshold-based method was applied to classify each time point. For each time point, if the alpha power exceeded 50% of the total power (2-12 Hz), the subject was classified as awake. This is based on the observation that alpha power typically increases during resting wakefulness. If alpha power was below this 50% threshold, the subject was classified as drowsy. This allowed for the classification of brain states on a moment-to-moment basis during the scan. The algorithm is constructed such that a decrease in the "EEG vigilance" would mean an increase in drowsiness.

Our approach yielded 22 awake and 15 drowsy runs. We expected to see some inter-individual differences between the participants' ability to sustain vigilance, and

the frequency with which the drowsiness events would occur. These inter-individual differences between higher and lower vigilance events are reflected on the classification of awake and drowsy subjects. We propose that the reason behind the imbalance between groups is the discomfort of the scanner environment. The MRI is a noisy and uncomfortable environment for many participants, which is reflected as a higher number of participants being more awake, probably being more anxious and finding it harder to relax and become drowsy in the scanner. Despite the uncomfortable scanner environment and our relatively shorter scan time, we were able to observe drowsiness, further proving our point to account for vigilance fluctuations in every fMRI scan.

### **fMRI and Heart Rate**

The correlations between anterior cingulate cortex (ACC), insula, auditory and visual areas and HR was both stronger and positive at 0 lag in drowsy group. The stronger coupling between these brain regions and HR might be reflecting a synchronization between heart and brain in more drowsy states. In alert states, fMRI signal in ACC and insula reflect the neural activity associated with higher attentional engagement. However due to reduced attentional state in drowsiness, systemic physiology related influences may be more pronounced in the fMRI signal in drowsy states, explaining the positive correlation between these regions and HR. Similarly with visual and auditory regions, without a sensory stimulus and in a drowsy state, the fMRI signal from these regions are more coupled to changes in HR, reflecting more of physiology-related changes than the cognitive processes. In the awake group, most regions exhibit the strongest negative correlation with the HR signal at a 9-second lag. A positive lag indicates that the HR signal precedes the fMRI signal, suggesting that changes in HR may influence the fMRI signal after a delay. These findings are consistent with previous reports on increased autonomic effects on fMRI signal in lower vigilance states during longer (25 mins) and eyes-closed scans [30], implying that accounting for physiology-related changes in the fMRI data become more critical in when transitions between wakefulness states occur, even in 6 minutes eyes-open studies like ours.

### **fMRI and PPG-AMP**

As discussed in results and shown in Figure 4.9., in both precuneus and angular gyrus, correlations at lag 0 with the awake group are either negative (precuneus) or around 0 (angular gyrus). This means that a change in the precuneus activity would occur synchronously with a change in the PPG-AMP in the same direction (either increase or decrease) in the drowsy group, but in the opposite direction in the awake group. Additionally compared to the drowsy group, the awake group showed more negative correlations in posterior areas of the brain, especially in the visual cortex. These findings imply the ability of PPG-AMP to reflect differences in coupling in awake and drowsy conditions. If PPG-AMP reliably varies between awake and drowsy states, and these variations correspond to the differences in neural activity (e.g., negative correlations in posterior areas), it suggests that PPG-AMP captures critical aspects of alertness-related brain activity.

## **fMRI and RV**

In the awake group, significantly higher correlations observed between DMN and RV is indicative of the active role of DMN in maintaining physiological regulation and supporting systemic processes during the resting awake state. On the other hand in the drowsy state, the control of breathing might be more independent from neuronal processes related to attentional or cognitive processing, and more coupled to other autonomic regulatory systems. During NREM sleep, Ozbay et al. 2019 reported voxel-wise correlation maps between PPG-AMP and fMRI showing positive correlations in the gray matter at 0 lag [10]. Consistently in our findings for the drowsy group, positive correlations in precuneus and angular gyrus, which belong to gray matter areas, might be reflecting the transition into light sleep.

## **CSF and Physiology**

As mentioned in earlier chapters, 4th ventricle is taken as a representative signal of the CSF. We observed that 4th ventricle BOLD signal and RV positively correlated in positive lags in the awake group. We argue that respiration triggered sympathetic vasoconstriction and subsequent CSF inflow in the fourth ventricle is the reason behind

this correlation behavior. Vasoconstriction will force CSF flow into the brain from the 4th ventricle. The sequence goes like this: in about 6 seconds after an increase (or decrease) in RV, BOLD signal in the fourth ventricle increases (or decreases) too, correlating with the RV signal positively. The awake group exhibiting this signal pattern is consistent with the previous findings that CSF inflow did not require sleep, and occurs due to autonomic events [8].

## EEG Vigilance Metrics

Consistent with the findings of Olbrich et al., we reported negative correlation between anterior cingulate cortex and EEG-vigilance (frontal metric), especially nuanced in the awake group [13]. We observed that frontal metric revealed a greater disparity between the two vigilance groups; awake and drowsy. We also noted that between the frontal vigilance metric and several brain regions, correlation amplitudes are overall higher for the awake group than the drowsy group. On the other hand, drowsy group exhibited more transient dynamics in frontal-brain correlations. Since the metric itself also represents increased or decreased vigilance, normally the correlations between the vigilance metric and brain regions would be expected to vary in a similar manner for both drowsy and awake groups. However, the disparity in the correlation behavior between the awake and drowsy groups might be reflecting changes in the coupling between electrophysiological activity and hemodynamic response in different vigilance states. Previous literature also reported flexibilities in electrophysiological and hemodynamic coupling, observed during different sleep stages [8], or leading to different behavioral outcomes [74].

## Limitations

Further studies extending to bigger sample sizes would be valuable for increasing the accountability of the results, which would also increase the *boundary* group size that represent the edge-cases we have analyzed. Future work could also experiment with different TR values; as discussed in previous chapters, we have utilized a 3 second TR value, but a shorter TR would allow averaging the EEG data in shorter intervals

which would increase the temporal resolution. Another limitation of this study is that the cohort predominantly consisted of males, primarily due to the unavailability of an EEG cap suitable for the average female head size during most of the study. However, with additional budgetary support, a smaller cap was recently purchased, allowing for the inclusion of two new participants, who were female, to expand the diversity of the cohort. Finally, eye camera data could not be used within this study since the setup and synchronization have taken a while to be reliable enough for uninterrupted data to be collected. Therefore, a limitation of this study is the lack of pupil size data for many of the subjects, which could be a valuable addition in future research to further investigate the relationship between pupil dynamics and brain activity.

## 6. CONCLUSION

The study of brain function during rs-fMRI has provided valuable insights into neural connectivity, brain network organization, and the relationship between brain activity and behavior. Increasingly, it has become clear that vigilance plays a significant role in the variation observed in resting-state fMRI signals. Despite extensive evidence highlighting the influence of vigilance, many functional neuroimaging studies still neglect this factor. The primary aim of this study was to investigate how vigilance states influence rs-fMRI and physiological signals in a healthy young population. We hypothesized that vigilance states significantly impact rs-fMRI results, even during short eyes-open resting-state scans, and that these states could be reliably detected using multimodal measurements, including EEG, fMRI, and systemic physiological markers. Our findings support these hypotheses, shedding light on the complex interplay between vigilance states, brain connectivity, and their interactions with systemic physiology.

This thesis employed EEG-fMRI and systemic physiology signals recorded simultaneously during 6-minute eyes-open resting-state sessions, allowing us to capture natural transitions toward drowsiness while avoiding the confounds of sleep-like paradigms. Vigilance classification strategies, based on EEG power analysis, time-lagged cross-modal correlations, and functional connectivity matrices, were developed to elucidate subtle transitions in vigilance states. Methodologically, this study contributed several key advancements, such as integrating cerebrospinal fluid (CSF) signals and systemic biomarkers like PPG-AMP into vigilance detection. The differences observed between awake and drowsy states in CSF and PPG-AMP dynamics underscore the potential of these signals as biomarkers for vigilance detection. Furthermore, the application of time-lagged correlation analyses enriched our understanding of spatio-temporal dynamics linked to varying vigilance states.

Significant findings include correlations between respiration and CSF signals,

and between heart rate and sensory areas, illustrating their interactions during vigilance shifts. Notably, the relationship between default mode network regions and respiratory volume further highlights the influence of systemic physiology on brain connectivity. Our results also uncovered unique dynamics in PPG-AMP signals, particularly in regions like the insula and primary visual cortex, as well as DMN regions. These findings emphasize the need to consider both neural and systemic physiological signals when studying vigilance transitions. Additionally, we observed reduced brain connectivity in drowsy states compared to wakefulness, even during short-duration scans, underscoring the impact of vigilance transitions on rs-fMRI outcomes.

The broader implications of these findings extend to our understanding of how vigilance influences neural activity and rs-fMRI metrics. These insights not only enhance scientific knowledge of brain connectivity but also pave the way for potential clinical applications, such as monitoring vigilance-related conditions like sleep deprivation, stress, and fatigue. Future research could explore applying these methodologies to clinical populations or aging studies, where vigilance fluctuations may have different implications. Investigating the long-term effects of sustained vigilance changes on systemic physiology and brain connectivity could further deepen our understanding. Additionally, incorporating additional biomarkers, such as blood pressure or eye-tracking metrics, could refine vigilance state classification.

In conclusion, our findings demonstrate that vigilance levels influence the functional integration of brain networks and the coupling between the brain and systemic physiology. We recommend that vigilance levels be systematically assessed, even in short resting-state sessions, to improve the interpretation of fMRI results in future studies. The methodologies and insights developed in this thesis contribute both to theoretical advancements and practical applications, expanding the potential of fMRI in understanding brain function and its interactions with systemic physiology.



## REFERENCES

1. Seitzman, B. A., A. Z. Snyder, E. C. Leuthardt, and J. S. Shimony, "The State of Resting State Networks," *Topics in Magnetic Resonance Imaging*, Vol. 28, pp. 189–196, Aug. 2019.
2. Wang, C., J. L. Ong, A. Patanaik, J. Zhou, *et al.*, "Spontaneous eyelid closures link vigilance fluctuation with fMRI dynamic connectivity states," *Proceedings of the National Academy of Sciences*, Vol. 113, pp. 9653–9658, Aug. 2016.
3. Chang, C., Z. Liu, M. C. Chen, X. Liu, *et al.*, "EEG correlates of time-varying BOLD functional connectivity," *NeuroImage*, Vol. 72, pp. 227–236, May 2013.
4. Liu, T. T., and M. Falahpour, "Vigilance Effects in Resting-State fMRI," *Frontiers in Neuroscience*, Vol. 14, p. 321, Apr. 2020.
5. Liu, T. T., "The role of vigilance in resting-state functional MRI," in *Advances in Resting-State Functional MRI*, pp. 175–205, Elsevier, 2023.
6. Tagliazucchi, E., and H. Laufs, "Decoding Wakefulness Levels from Typical fMRI Resting-State Data Reveals Reliable Drifts between Wakefulness and Sleep," *Neuron*, Vol. 82, pp. 695–708, May 2014.
7. Gu, Y., F. Han, and X. Liu, "Arousal contributions to resting-state fmri connectivity and dynamics," *Frontiers in Neuroscience*, Vol. 13, p. 1190, 2019.
8. Picchioni, D., P. Ozbay, H. Mandelkow, J. A. de Zwart, *et al.*, "Autonomic arousals contribute to brain fluid pulsations during sleep," *NeuroImage*, Vol. 249, p. 118888, 2022.
9. Fultz, N. E., G. Bonmassar, K. Setsompop, R. A. Stickgold, *et al.*, "Coupled electrophysiological, hemodynamic, and cerebrospinal fluid oscillations in human sleep," *Science*, Vol. 366, pp. 628–631, Nov. 2019.
10. Ozbay, P. S., C. Chang, D. Picchioni, H. Mandelkow, *et al.*, "Sympathetic activity contributes to the fMRI signal," *Communications Biology*, Vol. 2, p. 421, Nov. 2019.
11. Gonzalez-Castillo, J., I. S. Fernandez, D. A. Handwerker, and P. A. Bandettini, "Ultra-slow fmri fluctuations in the fourth ventricle as a marker of drowsiness," *NeuroImage*, Vol. 259, p. 119424, 2022.
12. Oken, B., M. Salinsky, and S. Elsas, "Vigilance, alertness, or sustained attention: physiological basis and measurement," *Clinical Neurophysiology*, Vol. 117, pp. 1885–1901, Sept. 2006.
13. Olbrich, S., C. Mulert, S. Karch, M. Trenner, *et al.*, "EEG-vigilance and BOLD effect during simultaneous EEG/fMRI measurement," *NeuroImage*, Vol. 45, pp. 319–332, Apr. 2009.
14. Knaut, P., F. Von Wegner, A. Morzelewski, and H. Laufs, "EEG-correlated fMRI of human alpha (de-)synchronization," *Clinical Neurophysiology*, Vol. 130, pp. 1375–1386, Aug. 2019.
15. Schneider, M., P. Hathway, L. Leuchs, P. G. Samann, *et al.*, "Spontaneous pupil dilations during the resting state are associated with activation of the salience network," *NeuroImage*, Vol. 139, pp. 189–201, Oct. 2016.

16. Schwalm, M., and E. Rosales Jubal, "Back to Pupillometry: How Cortical Network State Fluctuations Tracked by Pupil Dynamics Could Explain Neural Signal Variability in Human Cognitive Neuroscience," *E-neuro*, Vol. 4, pp. 0293–16.2017, Nov. 2017.
17. Duyn, J. H., P. S. Ozbay, C. Chang, and D. Picchioni, "Physiological changes in sleep that affect fMRI inference," *Current Opinion in Behavioral Sciences*, Vol. 33, pp. 42–50, June 2020.
18. Xie, T., and N. Ma, "Tracking vigilance fluctuations in real-time: a sliding-window heart rate variability-based machine-learning approach," *Sleep*, p. zsa199, 2024.
19. Fourcade, A., F. Klotzsche, S. Hofmann, A. Mariola, *et al.*, "Linking brain–heart interactions to emotional arousal in immersive virtual reality," *Psychophysiology*, Vol. 61, no. 12, p. e14696, 2024.
20. Chang, C., J. P. Cunningham, and G. H. Glover, "Influence of heart rate on the BOLD signal: The cardiac response function," *NeuroImage*, Vol. 44, pp. 857–869, Feb. 2009.
21. Joliot, M., S. Cremona, C. Tzourio, and O. Etard, "Modulate the impact of the drowsiness on the resting state functional connectivity," *Scientific Reports*, Vol. 14, p. 8652, Apr. 2024.
22. Tagliazucchi, E., F. von Wegner, A. Morzelewski, S. Borisov, *et al.*, "Automatic sleep staging using fmri functional connectivity data," *NeuroImage*, Vol. 63, no. 1, pp. 63–72, 2012.
23. Altmann, A., M. S. Schröter, V. I. Spoormaker, S. Kiem, *et al.*, "Validation of non-rem sleep stage decoding from resting state fmri using linear support vector machines," *NeuroImage*, Vol. 125, pp. 544–555, 2016.
24. Wong, C. W., V. Olafsson, O. Tal, and T. T. Liu, "The amplitude of the resting-state fmri global signal is related to eeg vigilance measures," *NeuroImage*, Vol. 83, pp. 983–990, 2013.
25. Chang, C., D. A. Leopold, M. L. Schölvinck, H. Mandelkow, *et al.*, "Tracking brain arousal fluctuations with fMRI," *Proceedings of the National Academy of Sciences*, Vol. 113, pp. 4518–4523, Apr. 2016.
26. Poudel, G. R., C. R. Innes, P. J. Bones, R. Watts, *et al.*, "Losing the struggle to stay awake: Divergent thalamic and cortical activity during microsleeps: Neural Activity During Microsleeps," *Human Brain Mapping*, Vol. 35, pp. 257–269, Jan. 2014.
27. Goldman, R. I., J. M. Stern, J. Engel, and M. S. Cohen, "Simultaneous EEG and fMRI of the alpha rhythm:," *NeuroReport*, Vol. 13, pp. 2487–2492, Dec. 2002.
28. Horovitz, S. G., M. Fukunaga, J. A. de Zwart, P. van Gelderen, *et al.*, "Low frequency bold fluctuations during resting wakefulness and light sleep: A simultaneous eeg-fmri study," *Human Brain Mapping*, Vol. 29, no. 6, pp. 671–682, 2008.
29. Fukunaga, M., S. G. Horovitz, P. van Gelderen, J. A. de Zwart, *et al.*, "Large-amplitude, spatially correlated fluctuations in bold fmri signals during extended rest and early sleep stages," *Magnetic Resonance Imaging*, Vol. 24, no. 8, pp. 979–992, 2006.
30. Gold, B. P., S. E. Goodale, C. Zhao, H. Pourmotabbed, *et al.*, "Functional mri signals exhibit stronger covariation with peripheral autonomic measures as vigilance decreases," *Imaging Neuroscience*, Vol. 2, pp. 1–25, 09 2024.

31. Han, F., J. Chen, A. Belkin-Rosen, Y. Gu, *et al.*, “Reduced coupling between cerebrospinal fluid flow and global brain activity is linked to alzheimer disease–related pathology,” *PLoS Biology*, Vol. 19, no. 6, p. e3001233, 2021.
32. Campbell, M. C., J. M. Koller, A. Z. Snyder, C. Buddhala, *et al.*, “Csf proteins and resting-state functional connectivity in parkinson disease,” *Neurology*, Vol. 84, no. 24, pp. 2413–2421, 2015.
33. Lleo, A., E. Cavedo, L. Parnetti, H. Vanderstichele, *et al.*, “Cerebrospinal fluid biomarkers in trials for alzheimer and parkinson diseases,” *Nature Reviews Neurology*, Vol. 11, no. 1, pp. 41–55, 2015.
34. Lim, J., and D. F. Dinges, “A meta-analysis of the impact of short-term sleep deprivation on cognitive variables,” *Psychological Bulletin*, Vol. 136, pp. 375–389, May 2010.
35. Birn, R. M., J. B. Diamond, M. A. Smith, and P. A. Bandettini, “Separating respiratory-variation-related fluctuations from neuronal-activity-related fluctuations in fMRI,” *NeuroImage*, Vol. 31, pp. 1536–1548, July 2006.
36. Birn, R. M., K. Murphy, and P. A. Bandettini, “The effect of respiration variations on independent component analysis results of resting state functional connectivity,” *Human Brain Mapping*, Vol. 29, no. 7, pp. 740–750, 2008.
37. Tong, Y., L. M. Hocke, and B. B. Frederick, “Low frequency systemic hemodynamic noise in resting state bold fmri: characteristics, causes, implications, mitigation strategies, and applications,” *Frontiers in Neuroscience*, Vol. 13, p. 787, 2019.
38. Chang, C., and G. H. Glover, “Time–frequency dynamics of resting-state brain connectivity measured with fmri,” *NeuroImage*, Vol. 50, no. 1, pp. 81–98, 2010.
39. Richiardi, J., H. Eryilmaz, S. Schwartz, P. Vuilleumier, *et al.*, “Decoding brain states from fmri connectivity graphs,” *NeuroImage*, Vol. 56, no. 2, pp. 616–626, 2011.
40. El-Baba, M., D. J. Lewis, Z. Fang, A. M. Owen, *et al.*, “Functional connectivity dynamics slow with descent from wakefulness to sleep,” *PloS One*, Vol. 14, no. 12, p. e0224669, 2019.
41. Falahpour, M., C. Chang, C. W. Wong, and T. T. Liu, “Template based prediction of vigilance fluctuations in resting-state fmri,” *NeuroImage*, Vol. 174, p. 317–327, July 2018.
42. Zhang, S., S. E. Goodale, B. P. Gold, V. L. Morgan, *et al.*, “Vigilance associates with the low-dimensional structure of fmri data,” *NeuroImage*, Vol. 267, p. 119818, Feb. 2023.
43. Iber, C., “The aasm manual for the scoring of sleep and associated events: Rules,” *Terminology and Technical Specification*, 2012.
44. Brown, R. W., Y.-C. N. Cheng, E. M. Haacke, M. R. Thompson, *et al.*, *Magnetic Resonance Imaging: Physical Principles and Sequence Design*, Hoboken, New Jersey: John Wiley & Sons, Inc, 2014.
45. Buxton, R. B., *Introduction to Functional Magnetic Resonance Imaging: Principles and Techniques*, Cambridge: Cambridge University Press, 2nd ed., 2009.
46. Ogawa, S., T.-M. Lee, A. S. Nayak, and P. Glynn, “Oxygenation-sensitive contrast in magnetic resonance image of rodent brain at high magnetic fields,” *Magnetic Resonance in Medicine*, Vol. 14, pp. 68–78, Apr. 1990.

47. Ogawa, S., and T.-M. Lee, "Magnetic resonance imaging of blood vessels at high fields: *In vivo* and *in vitro* measurements and image simulation," *Magnetic Resonance in Medicine*, Vol. 16, pp. 9–18, Oct. 1990.
48. Seitzman, B. A., C. Gratton, T. O. Laumann, E. M. Gordon, and others, "Trait-like variants in human functional brain networks," *Proceedings of the National Academy of Sciences*, Vol. 116, pp. 22851–22861, Nov. 2019.
49. Dierker, D., J. L. Roland, M. Kamran, J. Rutlin, *et al.*, "Resting-state Functional Magnetic Resonance Imaging in Presurgical Functional Mapping," *Neuroimaging Clinics of North America*, Vol. 27, pp. 621–633, Nov. 2017.
50. Barks, S. K., L. A. Parr, and J. K. Rilling, "The Default Mode Network in Chimpanzees (Pan troglodytes) is Similar to That of Humans," *Cerebral Cortex*, Vol. 25, pp. 538–544, Feb. 2015.
51. Raichle, M. E., A. M. MacLeod, A. Z. Snyder, W. J. Powers, *et al.*, "A default mode of brain function," *Proceedings of the National Academy of Sciences*, Vol. 98, pp. 676–682, Jan. 2001.
52. Harmony, T., "The functional significance of delta oscillations in cognitive processing," *Frontiers in Integrative Neuroscience*, Vol. 7, 2013.
53. Klimesch, W., H. Schimke, and J. Schwaiger, "Episodic and semantic memory: An analysis in the EEG theta and alpha band," *Electroencephalography and Clinical Neurophysiology*, Vol. 91, pp. 428–441, Dec. 1994.
54. Majumder, S., B. Guragain, C. Wang, and N. Wilson, "On-board Drowsiness Detection using EEG: Current Status and Future Prospects," in *2019 IEEE International Conference on Electro Information Technology (EIT)*, (Brookings, SD, USA), pp. 483–490, IEEE, May 2019.
55. Foxe, J. J., and A. C. Snyder, "The Role of Alpha-Band Brain Oscillations as a Sensory Suppression Mechanism during Selective Attention," *Frontiers in Psychology*, Vol. 2, 2011.
56. Engel, A. K., and P. Fries, "Beta-band oscillations — signalling the status quo?," *Current Opinion in Neurobiology*, Vol. 20, pp. 156–165, Apr. 2010.
57. Mulert, C., and L. Lemieux, eds., *EEG - fMRI: Physiological Basis, Technique, and Applications*, Cham: Springer International Publishing, 2022.
58. Goldman, R. I., J. M. Stern, J. Engel, and M. S. Cohen, "Acquiring simultaneous EEG and functional MRI," *Clinical Neurophysiology*, Vol. 111, pp. 1974–1980, Nov. 2000.
59. Hill, R. A., K. H. Chiappa, F. Huang-Hellinger, and B. G. Jenkins, "EEG during MR imaging: Differentiation of movement artifact from paroxysmal cortical activity," *Neurology*, Vol. 45, pp. 1942–1943, Oct. 1995.
60. Debener, S., K. J. Mullinger, R. K. Niazy, and R. W. Bowtell, "Properties of the ballistocardiogram artefact as revealed by EEG recordings at 1.5, 3 and 7 T static magnetic field strength," *International Journal of Psychophysiology*, Vol. 67, pp. 189–199, Mar. 2008.
61. Yan, W. X., K. J. Mullinger, G. B. Geirsdottir, and R. Bowtell, "Physical modeling of pulse artefact sources in simultaneous EEG/fMRI," *Human Brain Mapping*, Vol. 31, pp. 604–620, Apr. 2010.

62. Charlton, P. H., D. A. Birrenkott, T. Bonnici, M. A. F. Pimentel, *et al.*, “Breathing Rate Estimation From the Electrocardiogram and Photoplethysmogram: A Review,” *IEEE Reviews in Biomedical Engineering*, Vol. 11, pp. 2–20, 2018.
63. Cox, R. W., “AFNI: Software for Analysis and Visualization of Functional Magnetic Resonance Neuroimages,” *Computers and Biomedical Research*, Vol. 29, pp. 162–173, June 1996.
64. Allen, P. J., G. Polizzi, K. Krakow, D. R. Fish, *et al.*, “Identification of EEG Events in the MR Scanner: The Problem of Pulse Artifact and a Method for Its Subtraction,” *NeuroImage*, Vol. 8, pp. 229–239, Oct. 1998.
65. Allen, P. J., O. Josephs, and R. Turner, “A Method for Removing Imaging Artifact from Continuous EEG Recorded during Functional MRI,” *NeuroImage*, Vol. 12, pp. 230–239, Aug. 2000.
66. Chang, C., C. D. Metzger, G. H. Glover, J. H. Duyn, *et al.*, “Association between heart rate variability and fluctuations in resting-state functional connectivity,” *NeuroImage*, Vol. 68, pp. 93–104, 2013.
67. Ozbay, P. S., C. Chang, D. Picchioni, H. Mandelkow, *et al.*, “Contribution of systemic vascular effects to fMRI activity in white matter,” *NeuroImage*, Vol. 176, pp. 541–549, Aug. 2018.
68. Pollreisz, D., and N. TaheriNejad, “Detection and Removal of Motion Artifacts in PPG Signals,” *Mobile Networks and Applications*, Vol. 27, pp. 728–738, Apr. 2022.
69. Utevsky, A. V., D. V. Smith, and S. A. Huettel, “Precuneus Is a Functional Core of the Default-Mode Network,” *The Journal of Neuroscience*, Vol. 34, pp. 932–940, Jan. 2014.
70. Damoiseaux, J., C. Beckmann, E. S. Arigita, F. Barkhof, *et al.*, “Reduced resting-state brain activity in the “default network” in normal aging,” *Cerebral Cortex*, Vol. 18, pp. 1856–1864, Aug. 2008.
71. Klimesch, W., “EEG alpha and theta oscillations reflect cognitive and memory performance: a review and analysis,” *Brain Research Reviews*, Vol. 29, pp. 169–195, Apr. 1999.
72. Chen, X., C. Hsu, D. Xu, J. Yu, *et al.*, “Loss of frontal regulator of vigilance during sleep inertia: A simultaneous EEG-fMRI study,” *Human Brain Mapping*, Vol. 41, pp. 4288–4298, Oct. 2020.
73. Roth, B., “The clinical and theoretical importance of eeg rhythms corresponding to states of lowered vigilance,” *Electroencephalography & Clinical Neurophysiology*, 1961.
74. Zhao, C., D. Li, J. Guo, B. Li, *et al.*, “The neurovascular couplings between electrophysiological and hemodynamic activities in anticipatory selective attention,” *Cerebral Cortex*, Vol. 32, no. 22, pp. 4953–4968, 2022.