Brain activity and physiological modulation in conditions mimicking fMRI resting-state studies

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Thesis to obtain the Master of Science Degree in

Biomedical Engineering

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November 2022

This is the first day of my life...

- Bright Eyes

Preface

The work presented in this thesis was performed at the Evolutionary Systems and Biomedical Engineering Lab (LaSEEB), within the Institute for Systems and Robotics (ISR), Instituto Superior Técnico, University of Lisbon, during the period March-October 2022, under the supervision of Professor Gina Caetano and Professor Patrícia Figureiredo.

Declaration

I declare that this document is an original work of my own authorship and that it fulfills all the requirements of the Code of Conduct and Good Practices of the Universidade de Lisboa.

Acknowledgments

First of all, I want to thank my supervisors Professor Gina Caetano and Professor Patrícia Figueiredo, as well as MSc Inês Esteves. I feel deeply grateful for their guidance, patience and teachings throughout these months. And also to Professor Athanasios Vourvopoulos and everyone at LaSEEB for making it such a great environment. It was a pleasure to work here.

To all the friends, colleagues and LaSEEB members that were kind enough to provide their valuable time and heads and participated in this study.

To my parents and brother, who not only have to put up with me, but have always invested in my education and made sure I had everything I needed to succeed.

To Albifas for being like a second family to me for so many years and a constant source of support and friendship no matter the times.

To all the close friends I made at IST and shared so many dinners, hangouts, coffees at Bdb, trips and talks that made these 5 years the best that they could have been.

To Beatriz, for listening, answering, supporting, laughing, crying, encouraging, singing, cooking, dancing and nonsensing, waiting and growing with me.

To BCNR, Maria, Conor and Alex for inspiring and accompanying me in my daily commutes and making life more beautiful.

Resumo

A aquisição de imagens médicas, em específico de ressonância magnética funcional (fMRI), são feitas em condições específicas de postura, neste caso horizontal. Estudos recentes têm revelado que a postura induz alterações na espessura do líquido cefalorraquidiano e que a estimulação dos barorreceptores altera a atividade cerebral, assim como a sua medição através de EEG. Esta tese propôs investigar a influência das condições ecológicas em aquisição de imagens médicas, nomeadamente os efeitos da postura e intensidade de luz no sinal de EEG e em indicadores fisiológicos medidos através de electrocardiograma (ECG), fotopletismografia (PPG) e respiração. Um protocolo experimental onde os participantes alternaram entre combinações diferentes de luz e postura foi usado para o efeito. Diferenças significativas devido à postura foram encontradas nas potências das bandas Delta e Theta do EEG, que foram maiores na posição deitada, indicando inibição cortical nessa condição. Por outro lado, a potência da banda Alfa foi menor, o que contradiz estudos anteriores, enquanto que a potência do Theta/Beta ratio foi significativamente maior na posição horizontal. O fator luz afetou significativamente apenas as bandas Alfa e Teta. A banda Alfa aumentou enquanto Teta diminuiu em potência com as luzes desligadas, devido a um menor estado de alerta nesta condição. Os sinais fisiológicos mostraram maior função parassimpática na postura deitada, bem como maior atividade dos barorreceptores. Estes resultados confirmam conculsões de estudos anteriores relativamente à inibição cortical e maior atividade parassimpática quando posturas horizontais são assumidas, bem como o maior estado de alerta com o aumento da intensidade da luz. No entanto, outros resultados, em concreto quanto ao efeito da postura na banda Alfa, contradizem estudos anteriores. Isto mostra a necessidade de mais estudos no âmbito destes efeitos e dos seus mecanismos fisiológicos subjacentes.

Palavras-chave: EEG, Postura, Luz, Sistema Nervoso Autónomo, HRV, Reflexo barorreceptor

Abstract

Functional magnetic resonance (fMRI) acquisitions are mainly made while the patient is lying down face up, in the supine position. On the other hand, other neuroimaging modalities (e.g. EEG and MEG) as well as human daily activity, are mostly carried out in upright postures, either sitting or standing. This yields a generalization problem when transferring imaging studies' results to other conditions and contexts. In fact, evidence has shown that posture induced changes in cerebrospinal fluid (CSF) thickness and baroreceptor stimulation modulate cerebral activity, as well as its measurements on the scalp EEG. This thesis aimed at further investigating the influence of imaging acquisition ecological conditions in electroencephalography (EEG) and physiological signals (ECG, PPG and respiration), through an experimental protocol where subjects shift between different states of posture and room lighting. Significant differences dues to posture were found in the EEG Delta and Theta band powers, which were higher in the supine position, indicating cortical inhibition in this condition. On the other hand alpha power was lower, which contradicts previous findings, while Theta/Beta ratio was significantly larger in the supine position. Light only affected the Alpha and Theta bands significantly. Alpha decreased while theta increased in power with the lights on, due to alertness. Physiological signals showed higher parasympathetic function in supine, as well as higher baroreceptor activity. These results confirm previous findings regarding cortical inhibition and parasympathetic activity when horizontal postures are assumed as well as the higher alertness with increased light intensity. However other findings, regarding the effect of posture on alpha band contradict previous studies. This shows the need of further investigation exploring these effects and their underlying physiological mechanisms, as well as standardization of experimental conditions between studies, such as duration of acquisition.

Keywords: EEG, Posture, Light, Autonomic nervous sytem, HRV, Baroreflex, CSF

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List of Acronyms

ANOVA	Analysis of Variance
ANS	Autonomous Nervous System
aR	Autonomic Regulation
CAML	Lisbon Academic Medical Center
CHULN	Northern Lisbon Academic Hospital Center
CNS	Central Nervous System
CSF	Cerebrospinal fluid
ECG	Electrocardiogram
EC	Eyes Closed
EEG	Electroencephalography
EO	Eyes Opened
EPSP	Excitatory Post-Synaptic Potential
FDR	False Discovery Rate
FIR	Finite Impulse Response
fMRI	Functional Magnetic Ressonance Imaging
fNIRS	Functional Near Infrared Spectroscopy
HADS	Hospital Anxiety and Depression Scale
HDBR	Head Down Bed Rest
HF	High frequency
HRV	Heart Rate Variability
HDT	Head-down Tilt
HTF	High Transition Frequency
HUT	Head-up Tilt
IAB	Individual Alpha Band
IAF	Individual Alpha Frequency
ICA	Independent Component Analysis
IPSP	Inhibitory Post-Synaptic Potential
ISR	Institute for Systems and Robotics
LaSEEB	Evolutionary Systems and Biomedical Engineering Lab
LF	Low frequency
MEG	Magnetoencephalography
PNS	Parassympathetic Nervous System
PPG	Photoplesthymography
SNS	Sympathetic Nervous System
TF	Transition Frequency
VLF	Very Low frequency

Chapter 1

Introduction

1.1 Context and Motivation

The majority of human activity while awake is performed in an upright posture. Often in neuroimaging studies and exams, these require the patients to lie horizontally, either due to technical or more conceptual reasons. Such is the case of Functional Magnetic Resonance Imaging, one of the most widely employed methods to analyze dynamic brain activity and function. However, the geometry of the scanner requires that the subjects must lie facing up, in the termed supine position, and usually with their eyes opened.

It was only recently that researchers started to question the ecological validity of fMRI acquisitions (Raz et al. [2005], Thibault and Raz [2016]). There is ample evidence that posture and other factors relevant in neuroimaging can significantly influence measured brain activity.

Despite being possible, acquisition of fMRI in upright postures isn't comparable to the standard grade since vertical scanners can only generate low magnitude magnetic fields. To overcome this constraint, brain activity has been measured and compared across different postures through the use of electroencephalography (EEG). It is portable and has a high temporal resolution in the order of the milisecond, making it an optimal technique for this purpose. Other modalities such as magnetoencephalography (MEG) or functional near infrared spectroscopy (fNIRS) have also been employed, although to a much lesser extent.

EEG studies comparing measurements done in supine and more vertical stances have showed that spectral power, coherence and localization of activity generators can significantly change between these conditions. Yet, there doesn't seem to be a consensus regarding which EEG bands are altered, how their power changes and what are the brain regions that are affected the most.

1.2 Background

This section's contents are based on (Schomer and Lopes da Silva [2017]) ”Niedermeyer's Electroencephalography”.

1.2.1 Electroencephalography

1.2.1.1 Introduction to EEG

Electroencephalography is one of the oldest but still widely relevant neurophysiological measurements. It is a technique used for the recording of electrical brain activity by electrode's placed on the scalp's surface. It has a superior temporal resolution, making it a go-to approach for the study of dynamic processes involved in neuronal processing.

The most popular clinical use for EEG is in epilepsy and seizure evaluation and diagnosis. Epileptic patients show specific and common patterns in the the EEG during seizures, giving rise to characteristic structures in the obtained waveform such as spikes and discharges. Evoked potentials and event-related potentials, obtained by averaging the EEG waveform, can also be of relevant clinical interest regarding the evaluation of somatosensory, visual and auditory function.

The signal is generated primarily by pyramidal cells in the cerebral cortex that are perpendicularly oriented to the brain's surface. The potentials they produce have very low voltages and from their source to the tip of the electrodes it has to pass through layers of cerebrospinal fluid (CSF), skull and skin. Moreover, other physiological and nonphysiological sources of electrical activity, namely movement of the eyes and head and power supply noise, have amplitudes of much larger orders of magnitude. So, the activity picked up by the electrodes can be largely corrupted by other sources than the desired. Fortunately, modern signal processing tools and methods are able to isolate the EEG signal with great quality, removing almost all artifacts and noise sources from the obtained data.

The electroencephalogram is then a real-time graphical representation of the variation of voltage across time. The display usually consists of multiple plots, each in respect of a specific electrode. EEG activity is mostly rhythmic but some characteristic structures, such as the k-complex or the spindles captured during sleep, can also be present. In order to better interpret what is seen in the EEG plots it is important to first understand the neurophsyiological processes underlying the observed activity.

1.2.1.2 Celullar neurophysiology

Neurons are the basic units of the central nervous system (CNS) which contains about 100 billion of these cells. They are composed of the cell body, or soma, where organelles such as the nucleus, mitochondria and Golgi complex are contained. From the soma come up several processes that can be divided into two groups according to their function. Dendrites branch out to multiple smaller ramifications and recieve the electrochemical signals propagated from other cells. Every neuron also contains an axon, a longer process that is responsible for the propagation of the nervous impulse. Axons communicate with other cells through the synapses, either targetting dendrites or the bodies of other neurons.

Glial cells are another component of the CNS. They consist of support cells that are located between the neurons and although they are not directly involved in information processing, they assist synapse formation and regulate the chemical environment that surrounds the neurons.

The transmission of information between the neurons takes place at the synapses, where the terminal of an axon meets the post-synaptic membrane of the soma or dendrite of another cell. Between these two structures there is, in most cases, a gap of extracellular space called synaptic cleft, through which chemical compounds responsible for signalling activity, the neurotransmitters, are released from the presynaptic terminals of the axons and trigger responses in the posts-ynaptic membrane of the target cells.

Cell membranes and their properties play an important role in the propagation of electrical signals in the CNS. The neuron membrane's resting potential is of about negative 60 to 70 milivolts and is maintained by an equilibrium of ionic exchanges between the cell and the external medium through ionic channels. Neurotransmitters act by disturbing this resting potential which leads either to an excitatory or an inhibitory effect. When channels that allow the influx of positive ions are opened, this causes the local depolarization of the cell membrane which triggers an excitatory post-synaptic potential (EPSP) when a threshold is reached. On the other hand, when positive charges are transported to the exterior of the cell or negative ones influx to the interior, there is a depolarization of the membrane and an inhibitory post-synaptic potential (IPSP) is triggered when a threshold is reached. A neuron has multiple synapses and may experience several EPSPs or IPSPs over a brief time period, which if summated result in a certain depolarization level that triggers the opening of Na+ ion channels throughout the axon, spreading the depolarization, which is known as an action potential. When the action potential reaches the terminal of the neuron an IPSP or EPSP is triggered in the post-synaptic membrane and the neuronal signal is propagated.

The ionic exchanges responsible for the propagation of EPSPs or IPSP develop potential gradients not only along the membrane's intracellular space but also in the extracellular space. These ionic currents in the extracellular space, mainly those arising from synaptic activity, give rise to extracellular field potentials. Synchronous activity in multiple afferent fibers generate the wave potentials in the superficial generators, the pyramidal cells, summating and thus making up the EEG signal that can be recorded.

1.2.1.3 EEG rhythms

The essential components of the EEG signal, besides evoked potentials, are the periodic waves or oscillations. Due to their periodic nature, their analysis is done in the frequency space by dividing the EEG spectrum into distinct bands. This way, the normal adult EEG is divided in the following way: Delta band (lower than 3.5Hz), Theta band (4-7.5 Hz), Alpha band (8-13 Hz), Beta band (14-30 Hz) and Gamma band (above 30Hz). Additional subdivisions can also be commonly found across literature and studies, such as breaking down the Beta band into low and high Beta. The different frequencies are associated with distinct states of processing and cognition, with some being more prominent during sleep or wakefulness than others or when the eyes are closed or open.

Theta band frequencies are scarce in the waking adult EEG, with no rhythmic activity being detectable. In younger children it is an important indicator of drowsiness and sleep states. Just as Delta activity, it is correlated to cholinergic function and central cholinergic pathways. Some pathologies can be responsible for the presence of large Theta power in adults.

The Alpha rhythm is perhaps the most central and studied EEG band. It comprises the frequencies between 8 and 13 Hz and is characterized by round or sinusoidal waveforms. It occurs during wakefulness and is clearly an expression of the posterior brain, being detected over occipital, parietal and posterior temporal regions. It is most prominent when the eyes are closed and in states of physical relaxation or reduced mental effort. Opening of the eyes, afferent stimuli or higher alertness lead to its reduction, suppression or complete blockage. It is related to the mu rhythm, which has similar frequency and amplitude characteristics, but distinct topography and underlying physiologic meaning. The 10-Hz is related to motor and somatosensory cortex functions and is mostly detected in precentral and postcentral regions.

Due to interpersonal variability, namely regarding age, memory capabilities and brain volume, the use of individualized Alpha bands (IAB) has been suggested in Klimesch [1999]. It is characterized by the central individual Alpha frequency (IAF), that corresponds to the peak in the spectrum within the Alpha range. The lower limits, that marks the separation between Theta and Alpha bands, is the transition frequency (LTF) and is defined as being below the IAF. The upper limit, the high transition frequency (HTF), is defined as being 2Hz above the IAF. This way the Alpha band is also divided into two sub-interval, the lower Alpha between TF and IAF and upper Alpha between IAF and HTF.

The Beta band consists of activity within the 14-Hz and 30-Hz range. Rhythmic activity can be found over frontal and central regions. It appears to increase in amplitude in states of drowsiness, but mostly due to attenuation of other bands. Its absence can be an indicator of abnormality.

1.2.1.4 EEG acquisition

EEG is acquired through a system of electrodes placed on the subjects scalp that are connected through wires to an amplifier. The electrodes are made of a conductive material in order to pickup the small detectable scalp voltages. Skin oils and other substances can be resistant to current, hence why cleaning of the skin must be performed before the placement of the electrodes. Normally an electrolyte solution is also applied to increase the conductivity and thus signal detection. Since the picked up brain voltages are of very small amplitude, amplifiers are required to magnify the signal.

The localization of the placement of the electrodes is one of the most determinant practical aspects of EEG. The spatial distribution of brain activity is linked to it's function and meaning. High rhythm activity can different meanings based on their spatial distribution. So, routine EEG recording follows a standard 25 scalp electrode position configuration. It is called the International 10-20 system and it was developed to ensure standardized methodology amongst EEG studies and recordings. For purposes that might need higher accuracy, other systems exist such as the 10-10 or 10-5 systems. In order to reduce localization errors a minimum of 64 electrodes would be needed, which is commonly used in research, as well as 128 or even 256 channels (Acharya and Acharya [2019]).

Not only the number of channels, but the type of montage is also an important technical aspect of

EEG recording. There are two main types; bipolar and referential, or monopolar. In bipolar setups, the channels are usually arranged in chains where each channel corresponds to the difference in potential between two adjacent electrodes. They are useful for detecting localized activity and can be less affected by artifacts. On the other hand, in referential montages each electrode uses a single reference. In the common reference setup, all channel use a referential electrode that ideally does not pickup activity, however that is not possible to achieve. The most common practices are using the mastoid bone as reference or the Cz channel. The common average montage is also commonly used and consists in summing all the electrodes' outputs, averaging them and using the obtained signal as the reference.

1.2.1.5 Artifacts

The sensitivity of the EEG recording makes it easily susceptible to external extracerebral interferences which are given the name of artifacts. These can often significantly alter the waveform's characteristics, thus leading to unreadable data or wrong interpretations of brain activity. Having the ability to identify and classify the different types of artifacts is therefore of major importance when working with EEG.

EEG artifacts are sorted into two classes: physiological and nonphysiological. The former are inherent to the patient's activity, i.e, any type of electric potentials other than the brain's that can be picked up by the scalp electrodes, such as head muscles, blinks or eye movement. In turn, nonphysiological artifacts are related to interferences in the recording equipment (wires, electrodes) or noise from nearby electronics. Unlike physiological, nonphysiological artifacts are more variable and appear in less interpretable structures thus leading to significant distortion of the EEG signal, sometimes even making the data unreadable. It is then of upmost importance to verify the recording setup before data acquisition in order to avoid these issues.

Physiologic artifacts are unavoidable and appear in all EEG recordings. Within the human body there are several other sources of electric activity that can be picked up by the electrode cap. One of the most common artifacts derived from eye-related movements, which occur when participants are awake with their eyes open, and that lead to distinct patterns in the recorded data. The cornea and the retina generate a dipole that gives rise to a large symmetrical potential that varies depending on the speed and orientation of the movement. Opening and closure of the eyes, and so also blinks, generate an artifact produced by the upward rolling of the eyes as they close, which moves upward the positive dipole generated by the cornea, also referred to as the Bell effect. Eye fluttering in its turn create what can seem as anomalous activity due to it's relatively high frequency. On the other hand, lateral eye-movements appear mainly on the F7 and F8 lead electrodes, with lateral gaze producing a typical structure characterised by a quick rise and slower and more gradual fall. Myogenic artifacts are another very common type of physiological artifacts and are generated by muscle activity, mainly facial muscles such as the frontalis and temporalis. They characteristically appear as spiky high-frequency noise. Frontalis activity is related to voluntary eye closure and originates potentials similar to railroad tracks. The temporalis muscles are involved in jaw clenching and bruxism, originating spikes that resemble small interictal discharges.

1.2.2 Posture and brain activity

The interest in studying the ecological validity of brain imaging research settings, specifically in the context of fMRI acquisitions, has only recently emerged. Decades of evidence mainly concerning the effect of posture on the CNS have led to researchers and experts on the field to start questioning whether acquisitions made in a lying position can be generalized and compared to the brain's activity during everyday tasks and contexts.

A large contribution to what we know today on the differences in brain activity due to postural change comes from studies motivated from a rather different standpoint. Towards the end of the 20th century, researchers started to seek to understand how spaceflight affected neuronal activity, more specifically, the influence of zero, or near zero, gravity conditions. Considering this and the fact that measurement of brain activity during spaceflight isn't feasible, researchers developed protocols known as microgravity simulation, where subjects assume a head down horizontal position, with the head tilted down 6 degrees. This simulates the zero gravity state felt in space missions and allowed the surge of studies regarding the connection of such conditions to brain activity. Vaitl and colleagues (Vaitl et al. [1996]) compared long-duration EEG measurements done on twenty subjects while they assumed a 6º head-down tilt (HDT) and a 6º head-up tilt (HUT), each for 23 hours. Spectral analysis showed power increase in HDT in the Delta and Theta ranges, being more accentuated in the latter, which are indicators of cortical inhibition. Furthermore, Schneider et al. [2008] also compared EEG activity between upright and both bed rest and tilted (9º head-down) positions and found overall decreases in the high frequency range, above 18Hz, when moving from seated to supine. More recently, it has also been shown that simulated microgravity leads to inhibition of startle reflex (Messerotti Benvenuti et al. [2011]), an important index of learning and brain plasticity, and also dampened emotional response (Messerotti Benvenuti et al. [2013]). In a head down bed rest (HDBR) study, where one group assumed a horiozntal position with a 6º head-down tilt, Spironelli and Angrilli [2011] measured the effects of several degrees of pain stimulation on EEG evoked potentials. Compared with the upright position, the HDBR group revealed a modulation of evoked potential components, with results suggesting a reduction in processing of nociceptive information in the somatosensory cortex. Brauns et al. [2021] also studied whether the duration of the bed rest condition affected the resting state cortical activity by analysing EEG power from long-duration acquisitions. They found that Theta, Delta, Alpha and Beta activity decreased when HDBR was assumed and remained low for the duration of the study, increasing when upright posture was assumed. The changes occurred immediately after the posture transition leading to believe that the duration doesn't induce further changes in measured EEG activity.

The underlying neuronal and physiological mechanisms for the modulation of brain activity remain under debate, lacking a general consensus, yet, one of the most widely accepted contributing factors is baroreceptor activity under the different experimental conditions (Lipnicki [2009], Vaitl and Gruppe [1991]). For instance, the comparison between measurements done preflight and after long-duration spaceflight missions showed that several indicators of autonomic control of circulation were altered (Fritsch-Yelle et al. [1994]). Specifically, changes in carotid baroreceptor cardiac reflex function such as reduction in slope, operational point and range were found. Furthermore, an increase in blood flow in the upper body, specifically in thoracic blood volume and hydrostatic pressure, was found after long-duration spaceflight in both the HDT position and in the transition from seated to lying down. This stimulates arterial and cardiopulmonary baroreceptors, materializing in reduction in baroreflex materializing in a decrease of sympathetic activity such as lowering of the heart rate. Up until the beginning of the second half of the 20th century it had already been shown that baroreceptor stimulation in the CNS induces certain patterns of electrical cortical activity in animals. The first studies with human subjects only arrived later, towards the end of the century. Cole [1989] conducted an experiment to test the hypothesis that reduced baroreceptor firing, through the assumption of an upright posture, induced an increase in EEG cortical activity. EEG and physiological measurements were done on subjects when they assumed two different postures, 40º head-up tilt and the supine positions. Results showed that subjects showing increased heart rate, caused by reduced baroreceptor firing during tilt, also revealed increased arousal, with the prominence of high frequency bands. Rau et al. [1993] conducted a study in which directed stimulation of baroreceptors was performed in order to verify previous findings. The results demonstrated the expected changes in autonomic functioning, such as reduction of heart rate, and once again cortical inhibition, manifested in slow cortical potentials. Moreover, measurements of EEG evoked potentials have demonstrated that baroreception is also linked to dampened pain and emotional processing (Angrilli et al. [1997], Mini et al. [1995]).

Brain shift is also regarded as a major mechanism responsible for posture-induced differences in brain scalp measurements. Postural transitions lead to a redistribution of the cerebrospinal fluid (CSF) and change of it's layer thickness. For example, Alperin et al. [2005] used quantitative fMRI done in both supine and upright positions to study the changes of CSF. The results showed a significant increase in intracranial blood flow and CSF volume in the supine posture compared with the seated upright position. The relevance of the CFS layer thickness in EEG measurements derives from it's high conductivity, which in turn may induce big changes in EEG signal amplitude with only small changes in thickness. For instance, CSF can be one hundred times more conductive than bone and about ten times more conductive than gray and white matter, making it a relevant factor affecting the electrical fields measured in the scalp. Several studies have aimed to investigate its influence on the basis scalp EEG acquisitions (Ramon et al. [2004], Wendel et al. [2008]), as well as with simulations that include the CSF effect in realistic head models. It was found that small variations in the CSF layer thickness can have significant changes in scalp potential amplitudes and current density.

Amidst all the growing evidence, researchers started to gradually take a more critical look into the environment in which imaging acquisitions are made and question how valid and comparable the obtained results are. In a review paper, Raz et al. [2005] do a critical analysis regarding the ecological validity of fMRI acquisitions mentioning the important influence of posture but also psychological stressors, with the latter not being within the scope of this thesis. This was perhaps one of the first published works where this concern is explicitly expressed.

It was during the last decade that a growing amount of studies started to specifically use protocols that measure brain activity in supine posture, assumed in fMRI and other imaging methods, and compare with measurements performed in supine or HDT position. In a 2016 study, Spironelli et al.

[2016] aimed at investigating the effects of horizontal position on cortical brain activity, specifically on low-frequency EEG power. The experimental protocol consisted in measuring EEG at four different time points during the course of two hours on two groups. One group remained in the seated position (SP) throughout the whole study, while the other was initially seated (first measurement), transitioned to bed rest (BR) in supine position (second measurement), remained in the same position for two hours (third measurement), after which assumed a sitting position again (fourth measurement). Power analysis was done for the Delta and Alpha bands. Regarding Delta amplitude, SP showed no modulation across the experiment while BR suffered significant increase in T1 and T2, where supine position was assumed. As for the Alpha band, in contrast to Delta, BR showed modulation (increase) but only in T1 with the amplitude having decreased over the 2 hours. This revealed that the Alpha frequency band of EEG might be more sensitive to rapid postural changes than to stable posture. The following year, the authors applied the same protocol but this time the aim was to analyze the high-frequency EEG activity (Spironelli and Angrilli [2017]). The targeted bands were high-Beta (20-35 Hz) and gamma (35-65 Hz). Results showed significant power decreases for both bands in the BR group at the two time points when supine position was assumed (T1 and T2). An additional and rather interesting observation was that when the BR group assumed the horizontal position, the left frontal asymmetry that both groups exhibited when seated was lost. So, results show similar behaviour to what had previously been seen in HDBR subjects in microgravity studies, supine position seems to inhibit cortical stimulation with low frequency being more prominent than high-frequency components. In a previous study, Chang et al. [2011] measured EEG and autonomic function, through HRV, on subjects while lying supine and sitting upright. Their analysis was done on the whole EEG power spectrum and results showed significant increase of Delta (just as Spironelli et al. [2016]) and Theta, and significant decrease in Beta, gamma (similar to Spironelli and Angrilli [2017]) and omega (45-100Hz) in supine comparing with upright. However, regarding Alpha, where Spironelli et al. [2016] saw significant increase transitioning from supine to upright, the results showed no changes across different postures. The influence of supine on pain processing was also studied (Fardo et al. [2013]) by comparing pain-related EEG potentials in upright and supine positions after different degrees of electrical pain stimulation. Source analysis results revealed reduced anterior and posterior slow wave amplitudes, indicating overall inhibition of the fronto-parietal network that is related to late phases of the processing of pain.

In another study, Rice et al. [2013] took a different and interesting approach comparing brain activity in two different lying postures: supine (facing up) and prone (facing down). Not only EEG evoked potentials were measured but MRI was used to quantify CSF layer thickness. The authors also ran multidipole model simulations to assess the influence of CSF thickness on EEG activity. Overall EEG signal power increased from prone to supine and the CSF thickness layer decreased by an approximate amount of 30%. Simulating a CSF layer thickness increment from 2mm to 3mm led to predicted reductions in scalp potential power up to 40%. The authors found a direct correlation between CSF and EEG spectral power, with results pointing toward a significant influence of cerebrospinal fluid on measured scalp potentials.

Several studies have also compared brain activity measurements across different degrees of verticalization starting from supine position. Zhavoronkova et al. [2012] measured power and coherence across the whole EEG spectrum in subjects transitioning between supine, sitting upright and standing postures. They observed increases in high frequency components, namely Alpha, Beta and gamma with verticalization. There was also widespread increase in coherence, most prominent in the right hemishpere, and more accentuated in high frequency bands. Thibault et al. [2014] also measured EEG power across different postures with the addition of a 45º inclined position. Moreover, other factors were also considered such as eyes open or closed and performance of a mental task or no task. Results indicated increases in Beta and gamma with increased verticalization regardless of whether eyes were open or closed and a mental task was performed or not. Two years later the same authors did a study base don MEG measurements where they intended to asses whether brain activity captured in this way would yield the same effects as those seen with EEG. MEG was measured in the same postures as in the previous study and compared. Unlike in EEG, only high gamma frequencies showed significant changes, and only in the left hemisphere. In a following study (Lifshitz et al. [2017]) where it was applied the same protocol but instead used a source localization approach, the authors found overall greater power in high frequency components ranging from Beta to gamma across the parietal-occipital cortex in upright posture compared to supine. Interestingly they also found a decrease in power in prefrontal regions across all bands although in different degrees, when upright posture was assumed. Common to both studies was the finding that the contrast between supine and 45º reclined postures is similar to those when comparing to upright, but in a lower degree, and that there aren't significant differences between the EEG power between 45º incline and sitting posture.

In summary, there seems to be a convergence in the results indicating significant changes in cortical EEG features due to posture, with the contrast lying more heavily between upright and horizontal postures, whether it's the canonical position assumed during fMRI and other neuroimaging exams - the supine position - or other bed rest postures. Nevertheless there are contradictory results regarding how and which bands, sources and regions of the brain are affected.

1.2.3 Posture and autonomic nervous function

The human body has a capacity to adjust its internal functions in response to external conditions. This is precisely the role of the autonomous nervous system (ANS): maintain internal equilibrium, also defined as homeostasis (Hawkins [1955]), through non-voluntary control of functions such as circulatory, digestive or respiratory regulation. The ANS is divided into three parts: the sympathetic nervous system, related to the "flight-or-flight" body response; the parasympathetic nervous system, related to activating functions during rest; and the enteric nervous system, which controls the gastrointestinal tract. It is then expected that body posture triggers a shift in demands regarding cardiac activity or respiration as responses to changes such as blood volume or pressure, as already mentioned in the previous section.

Evidence indicates that vertical posture promotes sympathetic control over the ANS, while horizontal posture (supine) leads to a prominence of parasympathetic activity. In McLaughlin et al. [1978] it was shown that basal levels of heart rate, skin resistance, blood pressure and finger volume are significantly influenced by posture. Heart rate and diastolic pressure were both significantly lower in horizontal posture, while finger volume, measured through PPG, and skin resistance were higher. In a more recent study and with a considerably larger sample size (157 healthy subjects compared to the 20 of the previous study), blood pressure levels were compared between three different postures: supine, sitting and standing (Eşer et al. [2007]). It was found that verticalization tends to decrease blood pressure, and results showed that supine posture exhibited significantly higher values of both diastolic and systolic pressure than the other two body positions.

Contrarily to what was believed for many years, the healthy heart's rhythm in resting-state is highly irregular due to the dynamic interactions between the body's systems that maintain equilibrium (Shaffer et al. [2014]). So, another way to evaluate autonomic nervous system function is through heart rate variability (HRV). This is a designation used to encompass several time and frequency measures that can be calculated from the variation in length of consecutive heart beats. Besides statistical metrics based on time properties such as standard deviation or root mean square of successive differences between beats, spectral analysis similar to EEG also yields useful information. For instance, the HRV's spectrum is often divided into the high-frequency (HF), low-frequency (LF) and very-low frequency (VLF) bands. The LF band is an indicator of baroreceptor activity and is a reflection of both SNS and PNS. The HF on its hands, reflects vagal modulation (PNS) due to the respiratory cycle. Respiration contributes for fluctuation in heart ate, for instance during inspiration HR is faster, and during expiration it slows down. Thus, the power ratio between the two bands, LF/HF ratio, is often used to measure SNS and PNS activity ratio (Shaffer and Ginsberg [2017]).

So, HRV is a useful tool to measure ANS modulation due to posture. It was precisely what Watanabe et al. [2007] did, by comparing different HRV metrics, along with heart rate and pressure, across supine, prone and sitting postures. Comparison between sitting and prone position revealed significant increase in LF/HF ratio in the sitting position, indicating dominance of sympathetic function. Heart rate was significantly higher while blood pressure was lower.

1.2.4 Light and brain activity

To what concerns the effect of light on the brain and its dynamics, namely on the activity captured by EEG, the works developed have mainly focused on daylight and nighttime contrasts. Nevertheless, some interesting insights can be taken concerning the influence that light exerts on the circadian rhythms, and thus its consequence on hormone balance and nervous system regulation, namely alertness.

As Vandewalle et al. [2009] says ”Light is not only for vision” as it is also fundamental for the regulation of cognition and alertness in the human body. The circadian rhythm, influenced by natural daylight, plays an important role in the modulation of cognitive tasks related to attention and memory (Blatter and Cajochen [2007], Carrier and Monk [2000]). In Cajochen et al. [2002], results found that during wakefulness and throughout the day, EEG activity modulates according to performance and alertness, with Alpha and Beta peaking in the second part of the day. Vandewalle et al. [2006] measured neural correlates of awareness in response to an auditory oddball task after and before exposure to bright light, through functional magnetic ressonance imaging (fMRI). Responses in the thalamus showed improvement in alertness after light exposure.

In Badia et al. [1991], EEG Beta power was used as an index of alertness. In the study, subjects were exposed to continuous or alternating periods of bright and dim light while EEG and other behavioral and psychophysiological measured were registered. Results showed that, as expected, alertness was higher in the bright light conditions, which manifested through higher Beta power. Kuller and Wetterberg [1993] did an experiment where the impact of two types of light at two differing intensities was observed. Two fluorescent lamps were used which they called ”daylight”, warmer, and ”white”, more agressive and visually tiring than the former. A broad spectrum EEG analysis was performed and differences between conditions were seen in multiple bands. Independently of light type, Delta band power was significantly lower in higher illumination contexts. Theta increased under the ’daylight’ lamp but no effect due to light intensity was seen. Alpha increased in lower intensity light conditions, and so did Beta as well. In a different study, Cajochen et al. [2000] exposed subjects to increasing degrees of illuminance and found that Alpha and Theta power decreased with light intensity. They correlated this decrease in EEG activity with a significant increase in subjective alertness.

1.3 Objectives

This thesis proposes to further contribute to the evidence regarding the effects of posture and light on the brain, factors which are relevant and variable in research settings. Previous findings have not been coherent between studies regarding some of the EEG frequency bands, and the mechanisms underlying the modulation aren’t completely understood yet.

So, through the use of an experimental design that combines both factors light and posture, an EEG spectrum-wide power analysis will be made in order to compare different conditions. Autononmic regulation will also be measured and compared among trials, through the use of ECG, PPG and respiration signals. Correlation between brain and physiological signals will also be calculated to evaluate if or how their modulations relate.

1.4 Thesis Outline

This thesis is then divided in five chapters. The first presented an introduction to the theoretical concepts of EEG and a literature review on past studies focusing on EEG and autonomic function modulation due to posture and light. In the second chapter the methodology adopted will be described, specifically the experimental design and recording setup and the data and statistical analysis. In the third, the results obtained are presented. In the fourth chapter follows the discussion of the findings, comparing the results to what has previously been done and a critical view

Chapter 2

Methods

The following chapter describes all the steps of the adopted methodology. First, descriptions of the experimental setup and design used for the data acquisition are given. Then follow all the details of the data analysis, comprised by data preprocessing, EEG bandpower analysis, physiological signal (ECG, PPG and respiration) feature extraction and correlation with brain activity. The last section details the approach regarding the statistical analysis.

2.1 Participants

A total of 20 healthy subjects participated in the study. All were subject to the same recording conditions (there was no division in groups) with the trial order having been randomized. It was assured that all orders occurred with the same frequency, nonetheless sample size wasn't large enough to have repetition and so all participants were subject to different trial sequences.

All the procedures and possible exclusion criteria were previously explained. At any time during the recording session subjects were free to leave if desired, which was also made clear beforehand. Informed written consents were signed at the beginning of the experiment. In order to screen possible exclusions, participants were asked to fill-in two self-reported health questionnaires. One consisted in the Hospital Anxiety and Depression Scale (HADS) Zigmond and Snalth [1983], a short 14 general question form that assigns the final score to the Normal, Borderline or Clinical range of depression and anxiety. The other was an adapted the self-reported Autonomic Regulation (aR) short questionnaire Kröz et al. [2008] which consists of 20 questions regarding different aspects of general health, namely circulatory and rest/activity regulation. The final score is a weighted sum of the answers which can be correlated to several conditions that alter autonomic regulation. A big portion of participants were students from Instituto Superior Técnico, Universidade de Lisboa. There were no rejections due to compliance with the exclusion criteria. A summary of the group demographics is available in Table 2.1, illustrated below.

Table 2.1: Demographic details of participants

Age	23.6 $\pm 1.1 (22 - 27)$
Sex	10 Female (50%), 10 Male (50%)
Education	1 Secondary School (5%), 10 Degree (50%), 9 Masters' Degree (45%)
Handedness	18 Right (90%), 1 Left (5%), 1 ambidextrous (5%)

Experimental procedures were approved by the Ethics Committee of the Northern Lisbon Academic Hospital Center (CHULN) and Lisbon Academic Medical Center (CAML). No form of monetary compensation was given for the participation in the study.

2.2 Equipment and Recording Setup

All data was acquired at the Evolutionary Systems and Biomedical Engineering Lab (LaSEEB), within the Institute for Systems and Robotics (ISR) of Instituto Superior Técnico, in Lisbon. Both brain activity (EEG) and physiological signals (ECG, PPG and respiration) were acquired. EEG was recorded with LiveAmp EEG amplifier (BrainProducts GmbH, Germany), and using actiCAP's 32 active electrodes system in a configuration following the international 10-20 system: Fp1, Fz, F3, F7, FT9, FC5, FC1, C3, T7, Fcz, CP5, CP1, Pz, P3, P7, O1, Oz, O2, P4, P8, TP10, CP6, CP2, Cz, C4, T8, FT10, FC6, FC2(Figure 3.1). Cotton swabs were used to clean all electrode sites with isopropyl alcohol before electrode placement. Then, in order to keep impedances below 10 kΩ, an electrolyte gel was carefully applied at electrode site with a blunt syringe.

Physiological signals were captured using LiveAmp's auxiliary inputs. ECG recording was done through a 3-electrode triangulation setup, according to Eidnhoven's triangle: positive and negative leads placed on either side of the chest, just below the clavicles, and the ground on the lower left rib. The ribs were selected since bone tissue is electrically more neutral and the influences of muscle and the respiration band are diminished. The PPG sensor was placed on the left index finger for all subjects. A respiration belt was also used to monitor the changes in chest diameter due to breathing cycles. It was set either on the chest or just below it, depending on the subject's apparent breathing type (abdominal or thoracic), with timely adjustments being made throughout the experiment's duration in order to optimize signal quality.

Digital recording of the raw data was executed with BrainVision Recorder software (BrainProducts, GmbH, Germany) and with a 500 Hz sampling frequency.

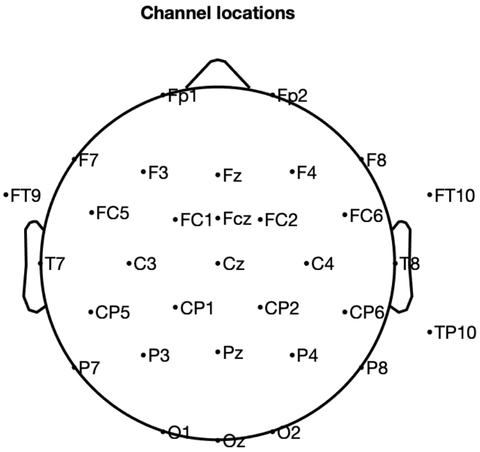


Figure 2.1: EEG electrode locations used in data acquisition.

2.3 Experimental protocol

Subjects underwent one single session of data acquisition, with each session being composed of four different blocks of continuous recording. As seen in Chapter 1, this study aimed at investigating the influence of ecological factors on the EEG and physiological signals, namely light conditions and posture. Light was explored between being off and on, while posture was varied by recording while subjects were either sitting or lying down. Thus, four unique combinations of the varying factors were tested, each being fixed throughout each block: lights on and sitting on a chair (sitON), lights on and lying supine (layON), lights off and sitting on a chair (sitOFF), lights off and lying supine (layOFF). Figure 3.2 illustrates the design of the experiment. Each block had a duration of 11 minutes. The first four consisted of a calibration period with alternating 1-minute periods of eyes closed (EC) and opened (EO). The last 7 minutes consisted of a resting-state period with the eyes open.

Figure 2.2: Schematic representation of recording session comprising four trials (layOFF, layON, sitOFF, sitON)

After the informed consent was signed, sessions started with participants being asked to fill two questionnaires: the HADS and aR forms, already detailed above. After all preparation was done, recording started in accordance with a predefined randomized block order. While sitting down on a chair, participants had a computer monitor before them with a black cross displayed over a grey background. As for the supine condition, subjects lied down on a soft yoga mattress carefully placed below a black cross which had been marked on the ceiling. Participants were asked to remain as still as possible and center their eyes on the marks to prevent excessive artifacts due to muscle and eye movement. Throughout the duration of the experiment, audio cues were played to indicate closure and opening of eyes and also the end of the trial. Blood pressure and heart rate were measured at the start and end of each session with a cuff placed on the left arm.

2.4 Data analysis

All data was analyzed and pre-processed offline using Matlab (R2019b version, MathWorks, Inc., Massachussetts, United States). Scripts were developed using built-in functions as well as EEGLAB, a Matlab toolbox specifically designed for EEG analysis Delorme and Makeig [2004].

2.4.1 Pre-processing

2.4.1.1 EEG

For each subject's session, four files were generated and saved in BrainVision's file format as *.vhdr*. These contained all the channels' continuous time series and markers indicating the beginning and end of the trial as well as EO/EC timestamps. These were then imported to Matlab where a preprocessing script was run.

First, data was trimmed according to time markers in order to only use the 11-minute trial length. Then, the continuous raw data was bandpass filtered with EEGLAB's linear finite impulse response (FIR) filter, which uses a Hamming windowed sinc. For the low cut-off, the chosen value was 1Hz as is common practice. This not only removes linear trends but also ensures a better Independent Component Analysis decomposition (Klug and Gramann [2021]). Since the goal of the experiment was to study EEG bands up to Beta (15-30Hz), only the lower end of the EEG spectrum was needed and a high-cutoff of 40 Hz was chosen. This not only cleans out the high-frequency noise but also removes the need to Notch filter the 50 Hz power line noise.

The next step was re-referencing the channels. It is common to use the average of all electrode signals as the reference, however this method is less robust to outliers like bad channels (da Cruz et al. [2018]). It can also lead to spreading of noisy data to other channels. To overcome these problems the biweight estimate of the mean were chosen as the re-referencing method instead, performing better regarding outliers and having a lower sampling variability (Hoaglin et al. [1983]). It is a weighted average obtained by first computing the median and median absolute deviation. Weights decrease in a nonlinear fashion the further the points are from the center of the distribution, with extreme values having a given weight of zero. A weighted mean is then calculated with the assigned weights.

To further remove noise and artifacts, Independent Component Analysis (ICA) was run on the data. As discussed previously, one of the major drawbacks of EEG regarding its interpretation is that the signal that is seen in one electrode can have multiple sources, both neuronal and non-neuronal. The most used solution is to apply ICA to the recorded signals, which allows to separate the different sources into their own time series and subtract those that correspond to artifacts and noise. The algorithm assumes that brain activity and noise can be treated as linearly independent, returning a number of ICs (independent components) that are statistically independent from each other. Each IC has its characteristic time series and topography, thus they can be interpreted as the signals one would record if the electrodes were directly placed on the sources. After identifying the components related to noise, these are subtracted from the original data leaving only artifact-free activity arisen from brain sources.

So, ICA was run using EEGLab's *pop runica* function, having chosen the 'runica' algorithm for the decomposition among the various options that are available. Then, ICALabel was run on the obtained components. ICALabel is an EEGLab plugin that classifies independent components according to their source (muscle, eyes, line noise, brain, etc.) and scores them with a percentage probability (as shown in Figure 3.3). Components that were classified as having a probability larger than 90% of being originated from noise sources were automatically removed. Then the topographies of the remaining ICs were visually analyzed and classified according to guidelines (Chaumon et al. [2015]) and those related to artifact sources were rejected.

Finally, after outliers were identified and rejected data was trimmed according to eyes state, yielding 5 different time series for each session: two eyes open and eyes closed periods relative to the first 4 minutes of calibration, and one final 7-minute eyes opened resting state period.

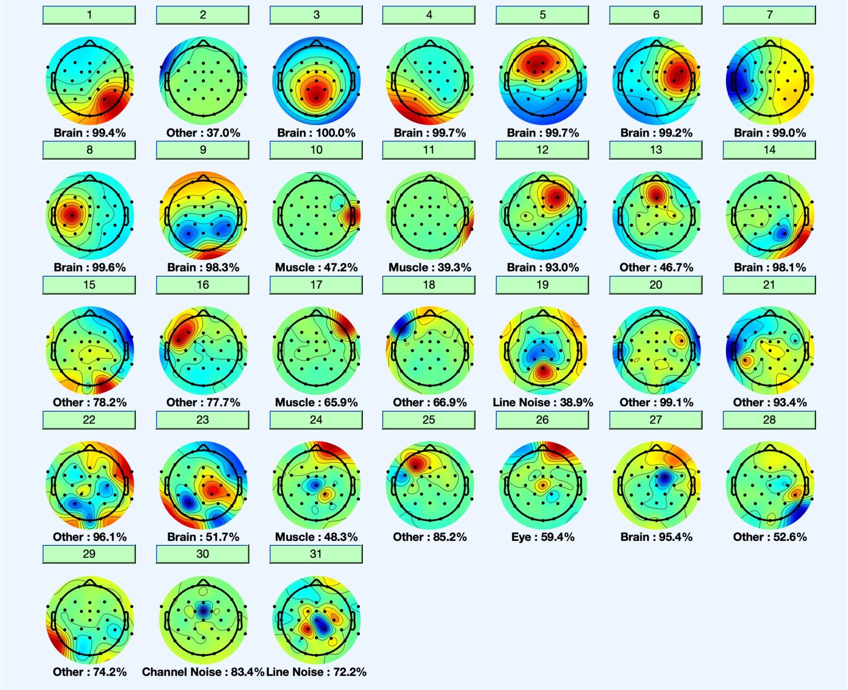


Figure 2.3: Independent component topographies classified by EEGLab's ICA Label

2.4.1.2 Physiological signals

Regarding the preprocessing of physiological signals, the approach was similar to the one chosen for EEG. A simple and conservative pipeline was designed.

First, the raw data was imported to EEGLab and the signals were filtered. For this purpose, the means of the ECG, PPG and respiration were subtracted from each respective channel data and filtering was applied with different cut-offs according to the specificities of the signal in question.

For the ECG, MATLAB's *lowpass* and *highpass* functions were used, which make use of minimum-order filters with a 60db stopband in order to reduce the delay inserted by the filter. A value of 40Hz was chosen as the high-cutoff frequency and 0.5Hz as the low-cutoff. The PPG signals had an overall worse quality then the cardiac activity, therefore a more robust high-pass filtering was required for the cleaning. A 6th order zero-phase Butterworth with a 10Hz cut-off was applied. There was no difference among the several filter orders that were compared (see Figure 3.4). Matlab's *highpass* function with 0.5 cut-off was then applied. As for the respiration, considering most of the signal's power is concentrated on the very low frequencies the signal was simply filtered using MATLAB's *highpass* function with a cut-off of 0.05Hz.

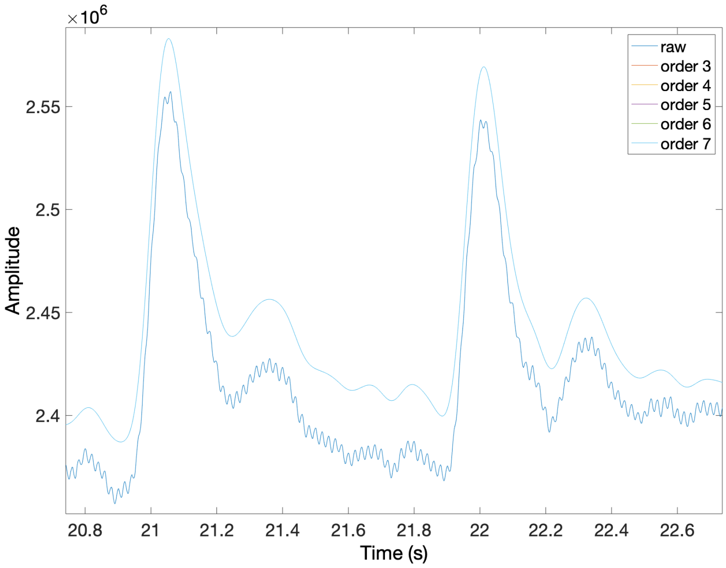


Figure 2.4: Comparison between different Butterworth filter orders for PPG cleaning

And lastly, as was done for the EEG, data was trimmed acccording to eyes state periods. For the analysis where the experimental conditions were compared only the last 7-minute resting state period was considered. The initial four minutes of alternating eyes state were used for IAB computation.

2.4.2 EEG analysis

2.4.2.1 Individual Alpha Bands

The main goal of this study was to evaluate the effects of different recording ecological conditions on EEG bandpowers. Regarding the Alpha band, IAB was used instead of the conventional fixed band limits for reasons that have been previously discussed in Section 1 (Klimesch [1999]). Although the main motivation for the use of individualized ranges is due to intersubject variation, IAB was computed for each separate dataset, resulting in four different intervals for each subject.

IAB was then computed using the mean of the O1 and O2 channels' first four minutes of recording (two minutes EC and two minutes EO). The trimmed data was fed into the *iabfeatures* function. First both EC and EO series are decomposed into the frequency domain by Welch's power spectral density method (Welch [1967]), with a 1024 sample window and a 50% overlap. The Individual Alpha Frequency (IAF) is defined as the spectral peak of the EC's decomposition within the standard 8-12 Hz range. Then, the lower and higher transition frequencies were computed according to the following:

- If IAF $<$ 10Hz then:

$$LTF = IAF - (1 - abs(IAF - 10)/10) * 2;$$

$$HTF = LTF + 4$$

- If IAF $>$ 10Hz then:

$$HTF = IAF + (1 - abs(IAF - 10)/10) * 2;$$

$$LTF = HTF - 4$$

2.4.2.2 Bandpowers

After carefully cleaning all the EEG data and the IAB were defined for each dataset, bandpowers were extracted for the four typical bands within the 0-30Hz frequency range. The following fixed intervals were used:

- **Delta**: 1.5 to 4 Hz
- **Theta**: 4 to 6.5 Hz.
- **Beta**: 14 - 30 Hz.

Theta's upper and Beta's lower limits were adapted to avoid possible IAB overlaps.

Regarding the extraction of the bandpowers, each of the 32 channel's preprocessed data was decomposed using the wavelet transform and time-frequency estimates were obtained. The choice of decomposing the signals into the time-frequency domain as opposed to the simpler frequency decomposition with the Fourier Transform lied on the fact that, although the recording's duration is relatively short, it is of interest to evaluate spectral power changes across time. Additionally, time-based correlation with physiological signal features could also be computed, as will be detailed in a following section. A Morlet wavelet with a factor of R = 7 and 100 frequency bins in the 1 - 30 Hz range was used. The convolution coefficients and EEG power matrices derived from each channel's decomposition were stored. From these, mean absolute bandpowers were obtained by calculating the mean of all frequency bins within each band's interval and across the whole duration of each trial. Then, relative powers were obtained by dividing the absolute bandpowers by the total power (in the considered 0Hz to 30 Hz) in the whole spectrum. Additionally, Theta/Beta ratio was obtained.

2.4.3 Physiological signals

MATLAB scripts were developed for the processing of physiological signals and both time and frequency based features were extracted.

2.4.3.1 ECG

The electrocardiogram's morphology is characterized by the QRS complex, a combination of several wave deflections. One heart beat generates a single complex, whose highest peak corresponds the R wave's peak, the R peak. So, R peaks were detected in order to derive R-R intervals and thus get the heart rate and heart rate variability. After the peaks were automatically located, manual inspection with an interactive function int was done (Figure 2.5).

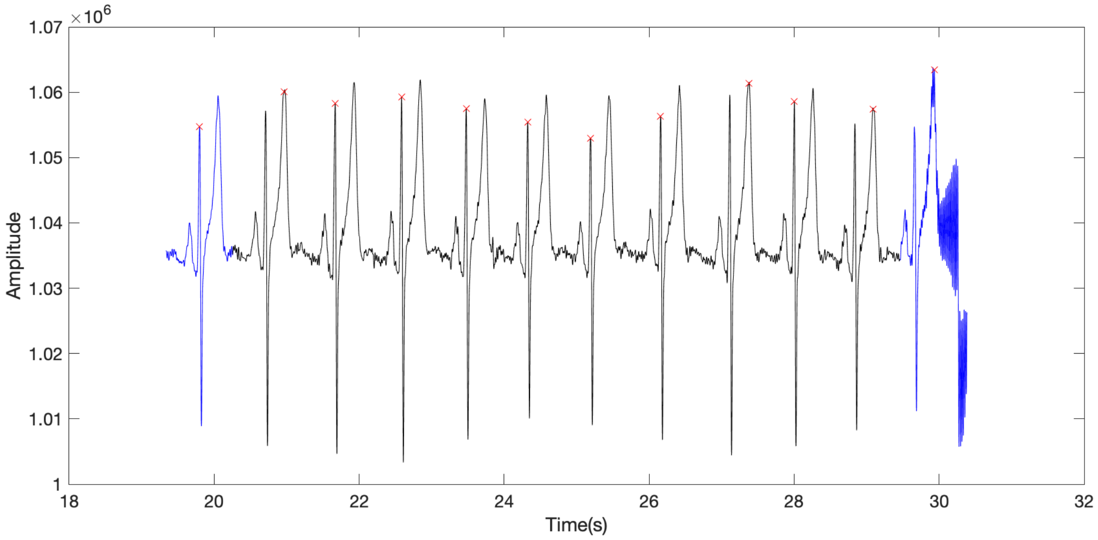


Figure 2.5: *interactiveQRS* function display window, used to manually correct R peaks

With the corrected R-peaks, Heart rate was derived by:

$$HR = 60/RRI$$

, where RRI is the interval, in seconds, between consecutive peaks.

Having defined the R to R intervals, these were used to derive heart rate variability (HRV) measures, both time and frequency-based. For this purpose, functions within a MATLAB tool application (Vollmer [2019]) were used. All the computed metrics can be found in Table 3.3.

2.4.3.2 PPG

The PPG is a non-invasive method that measures blood volume changes. Although it has been widely applied for blood oxygenation estimation, it can also be used as an indirect indicator of blood pressure (BP). The time delay between the ECG and PPG waveforms is called Pulse Arrival Time (PAT) and, although not completely accurate (Kachuee et al. [2016]), has been used as a proximal estimate due to its' straightforward measurement (Mukkamala et al. [2015]). This delay is calculated as the interval between the R peak of the ECG and different features of the PPG wave (Rajala et al. [2017]). Previous works have used the foot, dicrotic notch and the derivative peak to build linear models (Kachuee et al. [2016], Sun et al. [2016], Shin and Min [2017]) in order to get accurate BP calculations, however, it was not within the scope of this work to get such an accurate measure. Not only this, but the quality of the signal recording wasn't consistent, with many subjects' measurements being significantly noisy even after the preprocessing, which would make it rather difficult to identify certain features. So, PAT was defined as the time interval between the R peak and the PPG's highest peak, thus being designated as PATp.

2.4.3.3 Respiration

The respiration signal's waveform is very simple and rather easy to interpret. It can be seen as somewhat sinusoidal and is composed by a positive deflection, which is a response to the increase in thoracic volume due to inspiration, and a negative deflection reflecting the decrease in thoracic volume during exhalation.

Therefore, two wave features were identified: the highest point of the wave, the peak, and the lowest, the foot. With this, inhale periods were calculated as the time difference between a peak and its' preceding foot. In a similar fashion, exhale periods were defined as the difference is were also n time between a foot and its' preceding peak. Respiration rates (RR) were also computed in the following manner:

$$RR = 60/RPI$$

,where RPI corresponds to the time interval between two successive peaks.

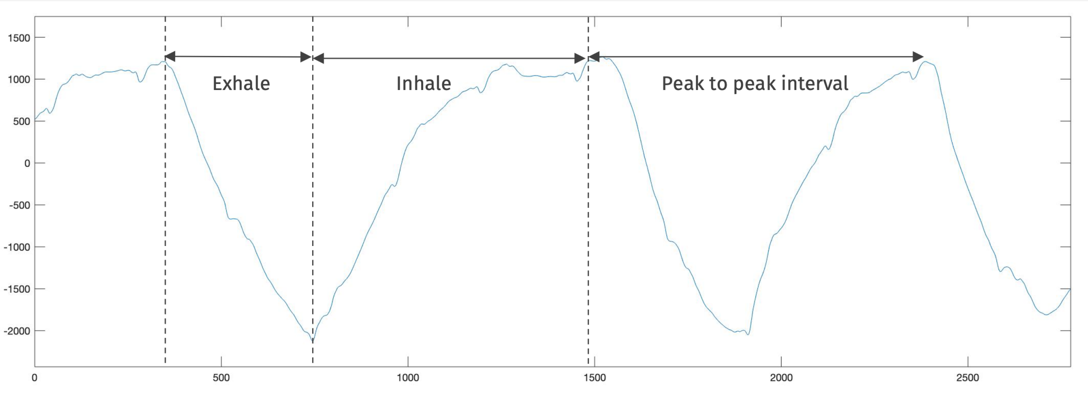


Figure 2.6: Respiration feature definition through identification of peaks and foots.

2.4.3.4 Summary

In sum, temporal and spectral information was extracted from the physiological signal recordings. Indicators of cardiac activity, blood pressure and respiratory performance were obtained in order to evaluate function of the autonomous nervous system across the different experimental conditions. The following table gathers all the computed features.

Table 2.2: Physiological time and frequency-based measures.

	Metric	**Description**
ECG	Heart rate	Derived from R peak to R peak intervals.
PPG	Pulse Arrival Time	Time distance between ECG's R peak and PPG's peak.
Respiration	Respiration rate	Derived from peak to peak interval.
	Exhales	Time interval of exhale. Calculated as time distance from waveform's foot to its following peak.
	Inhales	Time interval of inhale. Calculated as time distance from waveform's peak to its following foot.
	RVT	Respiratory Volume Time
Heart rate variability	SDNN	Standard deviation of consecutive NN intervals
	RMSSD	Root mean square of successive RR interval differences
	pNN50	Percentage of successive RR intervals that differ by 50 ms
	TRI	Triangular index
	TINN	Baseline width of RR interval histogram
	SD1	Standard deviation of distance of each point from y=x axis in Pointcaré fitted ellipse
	SD2	Standard deviation of distance of each point from y=x+average R-R interval axis in Pointcaré fitted ellipse
	VLF	Relative power of very low frequency band (0.0033-0.04Hz)
	LF	Relative power of low frequency band (0.04-0.15Hz)
	HF	Relative power of high frequency band (0.15-0.4Hz)

2.4.4 EEG and physiological data correlation

As seen in Section 1, autonomic nervous system function and physiological responses are related. There have also been found correlation between EEG activity and HRV measures. In this work, one of the aims was to evaluate that relationship.

For this purpose, Pearson correlation coefficients were calculated. In a first approach, correlation between the means of bandpowers and physiological signal features were obtained. For each subject, the mean power in each frequency band was calculated for the whole resting state period in each trial. Mean values of physiological features were similarly obtained and compared.

Additionally, having done a time-frequency decomposition of the EEG it was possible to correlate the evolution of the bandpowers and physiological features through the duration of the recording. This way it was possible to capture events that otherwise would be overlooked if only mean values were considered. There was, however, an issue regarding sampling frequency differences. While the EEG was acquired with a 500 Hz frequency, the physiological signal features' resolution varies depending on the case. To overcome this, the EEG decompositions were downsampled to a 250 Hz frequency and the physiologic signal features were resampled to the same sampling frequency using spline cubic interpolation. Correlations were then calculated between each channel time-series and the corresponding trial vector for each physiological feature.

2.5 Statistical Analysis

All the statistical data analysis was done in JASP software (Version 0.16.3, JASP Team, 2022) with *csv* files generated in MATLAB. Non-parametric Friedman tests (Friedman [1937]) were used for the different analyses.

The analysis of EEG bandpowers was done in different dimensions. First, the mean power at each frequency band was averaged across all channels for each condition, which yielded four mean values for each subject, at each band (layOFF, layON, sitOFF, sitON). The objective was to evaluate whether the main effects posture and light would be significant when considering the whole brain as a distribution. Then, channel-specific inspection was made to understand which brain regions were the most affected by the changes in the experimental conditions.

First, descriptive statistics were calculated for each variable in each dataset. Mean values and standard deviations were obtained and Shapiro-Wilk tests were also run. The Shapiro-Wilk tests whether data is normally distributed. Relying on measures of skewness and kurtosis, it returns the test statistic W, ranging between 0 and 1. Values close to 1 indicate normality of the data, while low values close to 0 lead to rejection of a normal distribution (Shapiro and Wilk [1965]).

For visualization purposes, boxplots were also obtained. The boxplot is an easily interpretable way of displaying data. It can be seen as having two features: the box and the ”whiskers”. The box has a midline that indicates the median of the data, while the upper and lower limits correspond to the third and first quartiles, respectively. The third and first quartiles are the medians of the upper and lower halves of the data, respectively. The whisker lines indicate the lowest and highest data points excluding outliers. The outliers are points that significantly diverge from the rest of the data, and the way they are defined can vary. The MATLAB function used in this work defines outliers as points that distance more than $1.5 * IQR$. The IQR is the Interquartile range and corresponds to the difference between the third and first quartiles. The whiskers are then drawn as lines that extend 1.5 times that distance above the third quartile or below the first. Any points outside this range are classified are outliers. After obtaining the plots, outliers were identified and subjects with data points with such a classification were excluded from the analysis in question.

To compare the EEG powers and physiological signal features across the different experimental conditions and evaluate whether the factors posture and light influence the variables, non-parametric Friedman tests were used. Although Shapiro-Wilk tests returned high W values and close to one, the sample sizes (N = 20, and in some cases where outliers were excluded N = 18) weren't large enough to confidently assume normality. So instead of doing analysis of variance, two-way repeated measures Friedman tests were applied. These are a non-parametric alternative to the analysis of variance (ANOVA) when the data distribution is unknown. The data is first organized in tables where each column corresponds to a specific condition's vector of values for the variable in question, and the rows represent subjects. The test evaluates the difference in the means of the samples in a rank-based manner.

Regarding the channel-wise analysis, in order to correct for multiple comparisons p-values were adjusted using false discovery rate (FDR) according to Storey's approach (Storey [2002]).

Chapter 3

Results

The following chapter describes the results obtained in the three domains of the data analysis: EEG bandpowers; physiological signal features; correlation between brain and physiological signals activity. The main objective of the analysis was to assess the influence of the varying experimental conditions regarding light and posture on the multiple signal properties chosen.

3.1 EEG bandpowers

3.1.1 Individual Alpha Band

The individual Alpha bands were defined for each subject per trial. Spectra for the EO and EC conditions, spanning the first four minutes of each experimental condition, were estimated to infer on possible changes in the IAF (Figures depicting the spectra and estimates of LTF, IAF and HTF can be found in Annex 1).

As mentioned in Section 2.4.2.1, the IAB is defined by three frequencies: LTF, IAF, and HTF. The distributions of the LTF, IAF and HTF across the sample of participants are depicted in Figure 3.1 as boxplot representations, respectively. Visual inspection allows to see that there isn't significant modulation due to change in posture or light. A Friedman test was applied, with the null hypothesis that the variables LTF, IAF, and HTF had no significant diferences between the experimental conditions. All p-values were larger than 0.05, and hence no significant differences were found between experimental conditions.

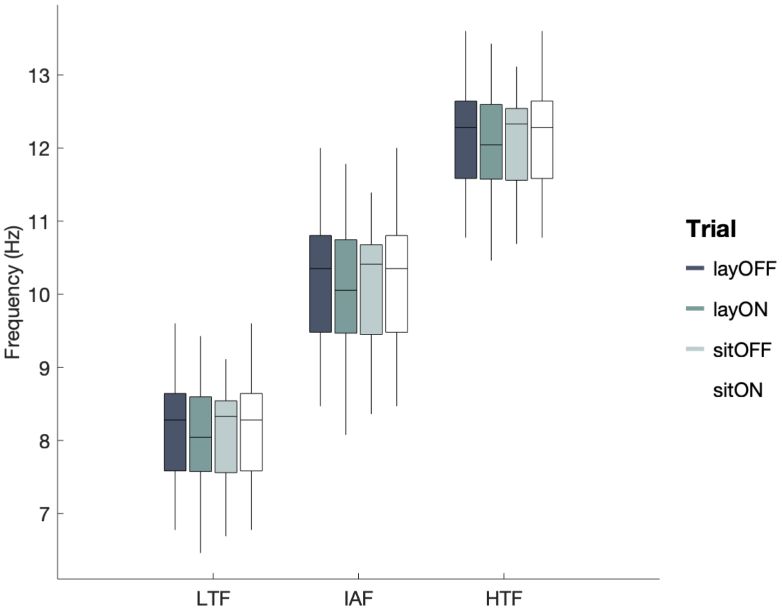


Figure 3.1: IAB frequency limits boxplots (LTF, IAB, HTF). Each plot represents the respective distribution across subjects.

3.1.2 Power spectral density

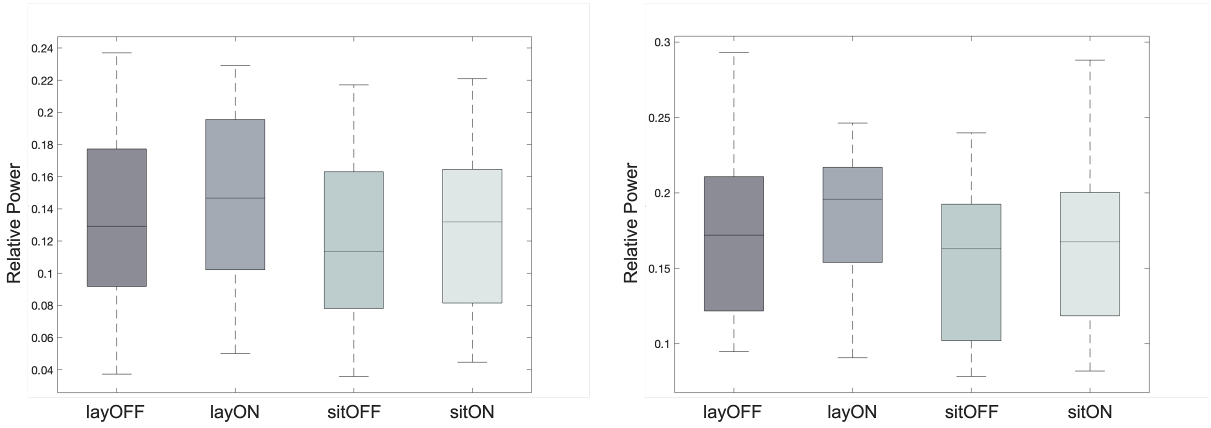
Overall

Having defined the IAB for each participant, it was possible to proceed with the spectral power density computation. First, overall relative bandpowers were compared. For each experimental condition and each frequency band, the mean band power and the Theta/Beta ratio were estimated across all EEG channels per participant. The objective was to consider the whole brain as a single distribution in order to verify whether position or luminance factors had a significant effect on the overall brain activity levels. Boxplots representing the distribution of participants' mean power in each frequency band are displyed in Figure 3.2, showing the effects of the position and light factors on measured values. Regarding the effect of posture, Friedman tests showed significant changes in Delta ($p = 0.007, W = 0.302$), Theta ($p < 0.001, W = 0.490$) and Alpha ($p = 0.016, W = 0.203$) relative powers and in Theta/Beta ratio ($p = 0.048, W = 0.203$). The boxplots show that Delta and Theta powers and the Theta/Beta ratio were larger when subjects were in the supine position than when sitting down. On the other hand, Alpha power was smaller in the supine position. As for the effect of light, only the Theta band showed a significant difference ($p = 0.016, W = 0.360$) between lights on, where power was larger, and lights off.

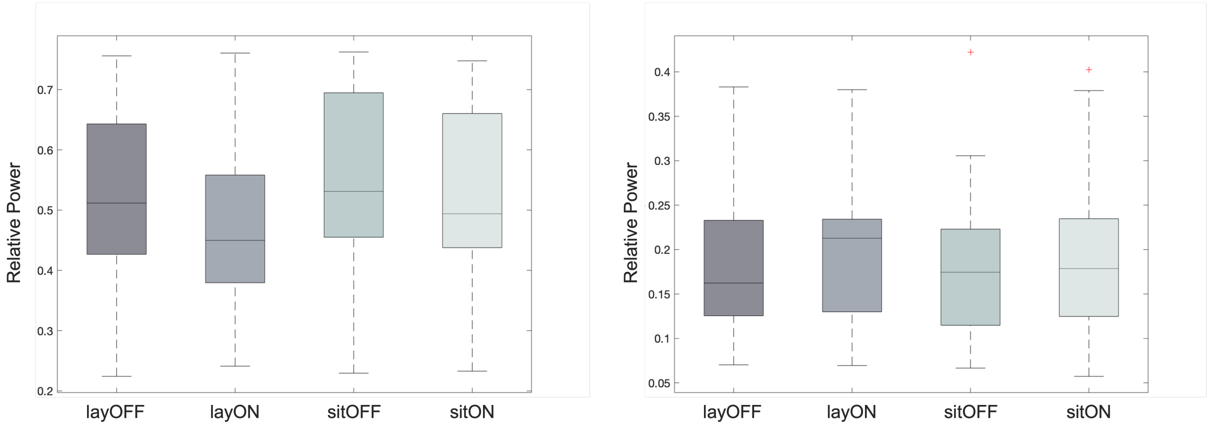
Concerning the Beta band no significant result was found, with the boxplot showing very similar distributions for the four experimental conditions.

Channel-specific results

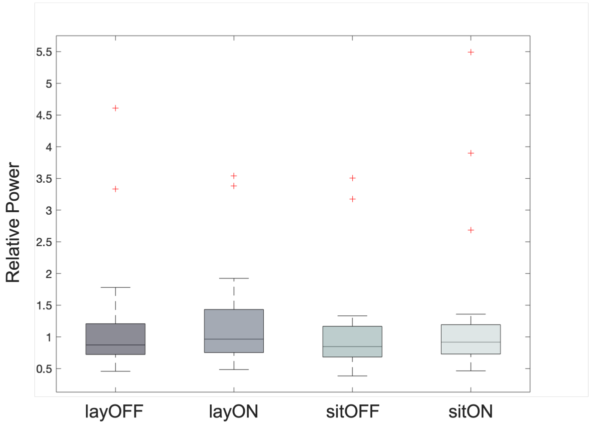
Next, having found that significant modulations were found widespread through the scalp, a channel-wise analysis was done in order to find which channels and brain regions were the most affected. Figure



(a) Delta (b) Theta



(c) Alpha (d) Beta



(e) Theta/Beta ratio

Figure 3.2: Overall relative bandpower boxplots: a) Delta, b) Theta, c) Alpha, d) Beta, e)Theta/Beta ratio.

3.3 illustrates the topographic plots of the average relative power at each frequency band, per condition. In a general sense, the results obtained in the previous section are visible. Delta and Theta are overall larger in *lay* (supine position) than *sit* (sitting position) conditions, while Alpha has the opposite behaviour. All bands show characteristic topographies common to all trials.

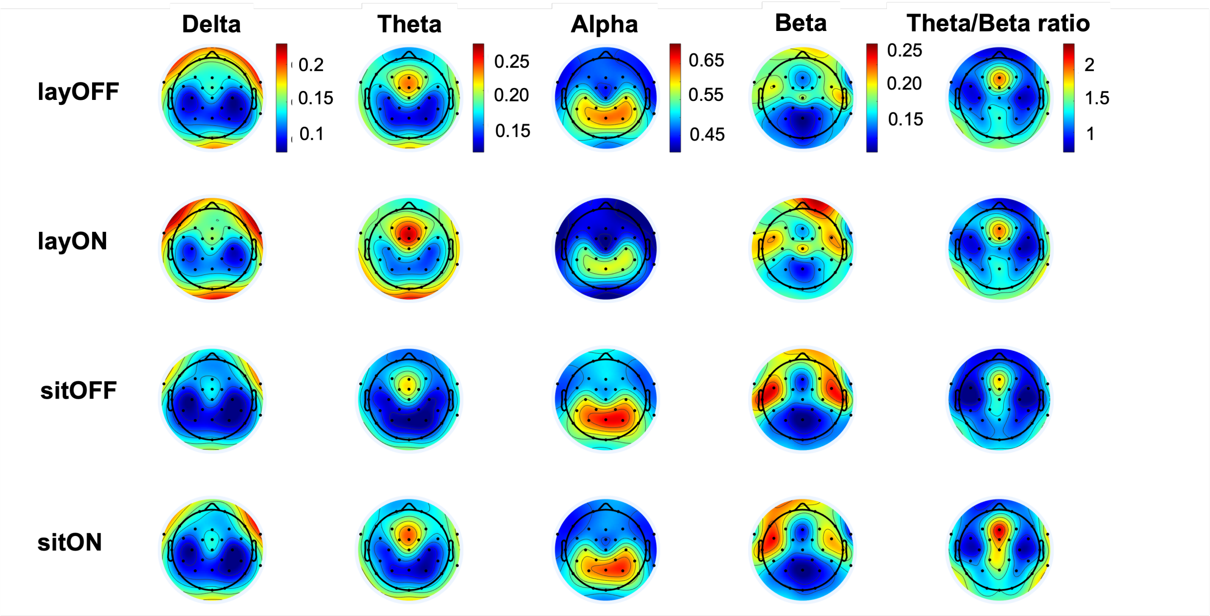


Figure 3.3: Average topographic distributions of channel relative bandpowers: Delta (left), Theta (center-left), Alpha (center), Beta (center-right), Theta/Beta ratio (right). Rows represent trials: layOFF (first), layON (second), sitOFF (third), sitON (fourth).

To understand where changes were most prominent, percentual difference between trial pairs were calculated at each channel and the topographies were plotted, as seen in Figures 3.4 and 3.5. In order to see the effect of posture, the difference between layOFF and sitOFF was done as well as between layON and sitON (Figure 3.4). With the lights ON, there was a big rise in Delta and Theta power in the center-left posterior region when lying down compared with the sitting position. The Beta band shows mixed results, with some scalp locations suffering increases while others showed decrease in power when in the supine position. Overall, the posture factor exhibited notably larger differences (highest value was 80 %) than the light factor (highest values were $\pm 15\%$).

In order to see the effect of light, the difference between sitOFF and sitON was done as well as between layOFF and layON (Figure 3.5). The Theta band showed a widespread decrease in power with he lights off, while Delta showed mixed results, some electrode locations increased in power while others decreased. The Alpha band showed the largest differences, with an increase in power with the lights off in both postures. Overall, the posture factor exhibited notably larger differences (highest value was 80 %) than the light factor (highest values were $\pm 25\%$).

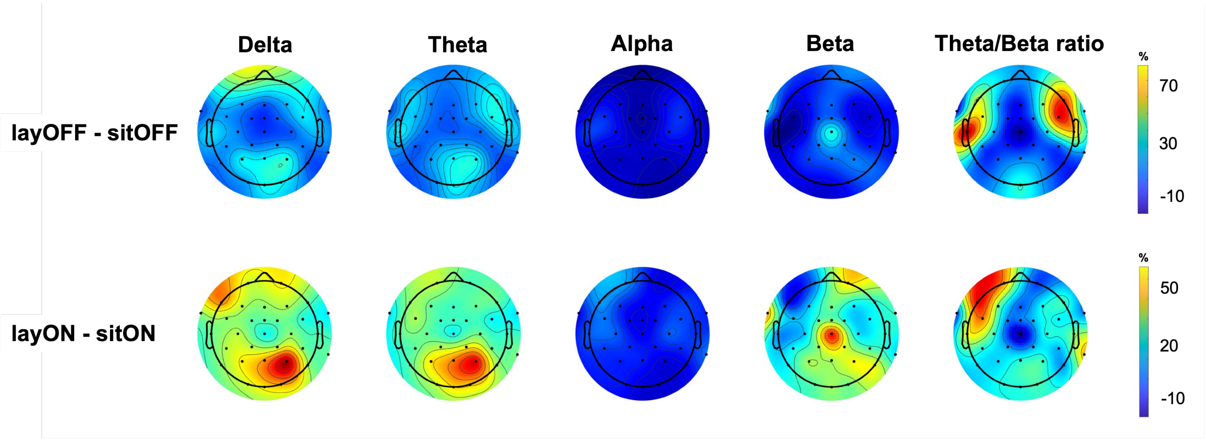


Figure 3.4: Percentual difference in mean relative power between trials regarding position factor. Top row shows difference between layOFF and sitOFF, the lower row shows between layON and sitON.

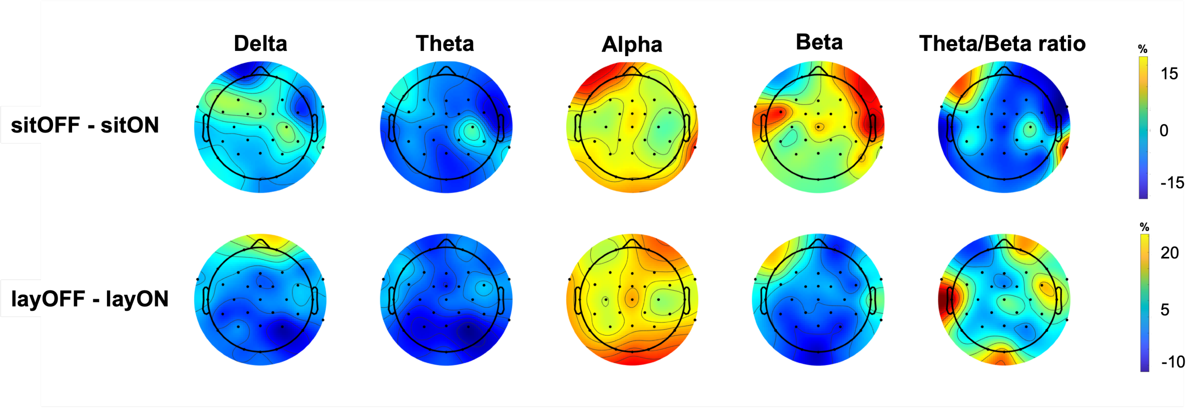


Figure 3.5: Percentual difference in mean relative power between trials regarding light factor. Top row shows difference between sitOFF and sitON, while lower shows between layOFF and layON.

Channel-wise Friedman tests were done for each frequency band and p-values after false discovery rate were represented across topographic maps. Figure 3.6 illustrates the distributions for each frequency band concerning the posture factor. The Delta band stands out as the only one where all channels showed significant differences ($p < 0.05$). The outer edges of the brain and the center, specifically the Cz channel, showed the highest values and thus the least significant differences between positions. The T7, CP5 and P4 channels had the most significant results ($p - value < 0.001$). The Theta band had a rather non uniform topography, with the lowest p-values corresponding to the T7, P4 and F4 channels. Regarding the Alpha band, the most significant differences were exhibited across the center-anterior and the posterior regions. The lowest scores corresponded to the FC1 and P4 channels (p-values=0.016). The Beta band exhibited a uniform distribution of low scores throughout the anterior-center and posterior regions. Multiple channels showed p-values¡0.01 and the Fz, FC1, Fcz and Cz electrodes exhibited the most significant differences (p-values=0.0028). No significant p-value was obtained for any channel concerning the Theta/Beta ratio. The lowest scores corresponded to the F7, T7, Oz and O2 channels.

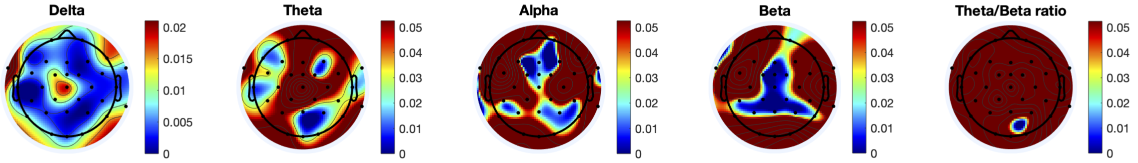


Figure 3.6: Posture effect FDR corrected p-values per channel

The same was done for the light factor (Figure 3.7). The Delta and Beta bands and Theta/Beta ratio didn't show significant values in any channel. With respect to the Theta band, multiple channels (Fz, C3, Fcz, CP5, CP1, Oz, O2, P4, TP10, FT10, F8) exhibited borderline scores (p-values=0.0465). The Alpha band stands out as all the channels showed signficant p-values (< 0.05). The lowest value (equal to 0.0125) is shared by multiple channels widespread through the brain. The least significant differences can be seen in the center-right region as the C4 and T8 channels revealed the highest results (p-values=0.0280).

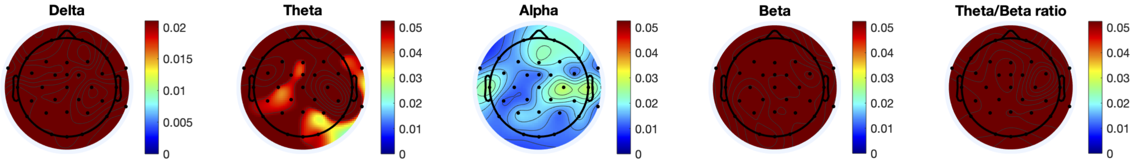


Figure 3.7: Light effect FDR corrected p-values per channel

3.2 Physiological signals

The mean physiological signal features from all subjects were compared between the four trials, in a similar fashion to what was previously done with EEG frequency and powers. This way it was possible to assess if the trial-specific distributions differed and if posture and light factors played a role in the obtained contrasts.

Friedman tests revealed that only posture influenced the results, with no significant values having been obtained with light as a factor.

Concerning the posture factor, no significant results were obtained for the respiration features (Inhales, Exhales, RVT and respiration rate) nor for the HRV measure SDNN. As can be observed in Figure 3.8, the boxplots for each of these variables depict distributions that do not differ and show similar medians acorss the four experimental conditions.

On the other hand, the remaining features showed significant results with notable contrasts. Friedman tests FDR corrected p-value for HR yielded a very significant value ($p-value < 0.001$) and the boxplot (Figure 3.9a) showing that the heart rate was larger in the sitting position compared to supine. Analogous results were obtained with the PATp ($p-value < 0.001$), which revealed higher values when participants were sitting down. For the HRV measures in the temporal domain, RMSSD and PNN50 reached significance ($p-values < 0.01$), showing higher values when participants were in supine position. In the spectral features, the low-frequency components, VLF and LF, showed both ($p-values < 0.05$) and higher powers in sitting posture while HF ($p-values < 0.01$) revealed higher powers in supine position. The ratio LF/HF was very significant ($p-values < 0.001$) and distributions for the sitting position were higher than lying down.

Table 3.1: Physiological signals Friedman test results

	Position	**Light**
Heart Rate	$p < 0.001$	$p = 0.671$
PATp	$p < 0.001$	$p = 0.322$
Respiration Rate	$p = 0.943$	$p = 0.777$
Inhale duration	$p = 0.943$	$p = 0.257$
Exhales duration	$p = 0.723$	$p = 0.257$
HRV - SDNN	$p = 0.120$	$p = 0.480$
HRV - RMSSD	$p = 0.009$	$p = 0.248$
HRV - pNN50	$p = 0.008$	$p = 0.546$
HRV - SD1	$p = 0.017$	$p = 0.080$
HRV - SD2	$p = 0.861$	$p = 0.186$
HRV - LF/HF ratio	$p > 0.001$	$p = 0.322$

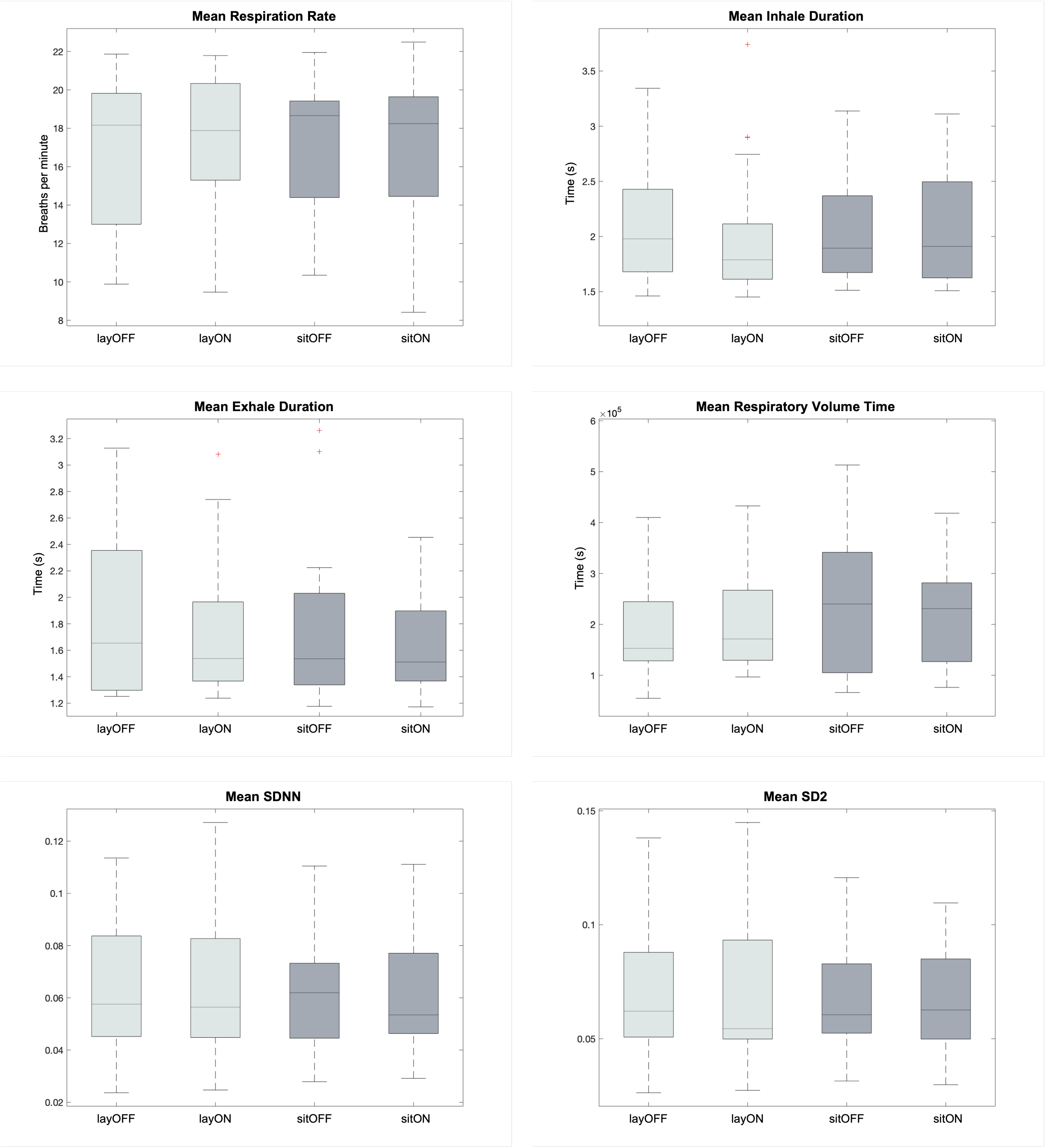


Figure 3.8: Boxplots of non-significant features: respiration (rate, inhales, exhales and RVT), SDNN and SD2.

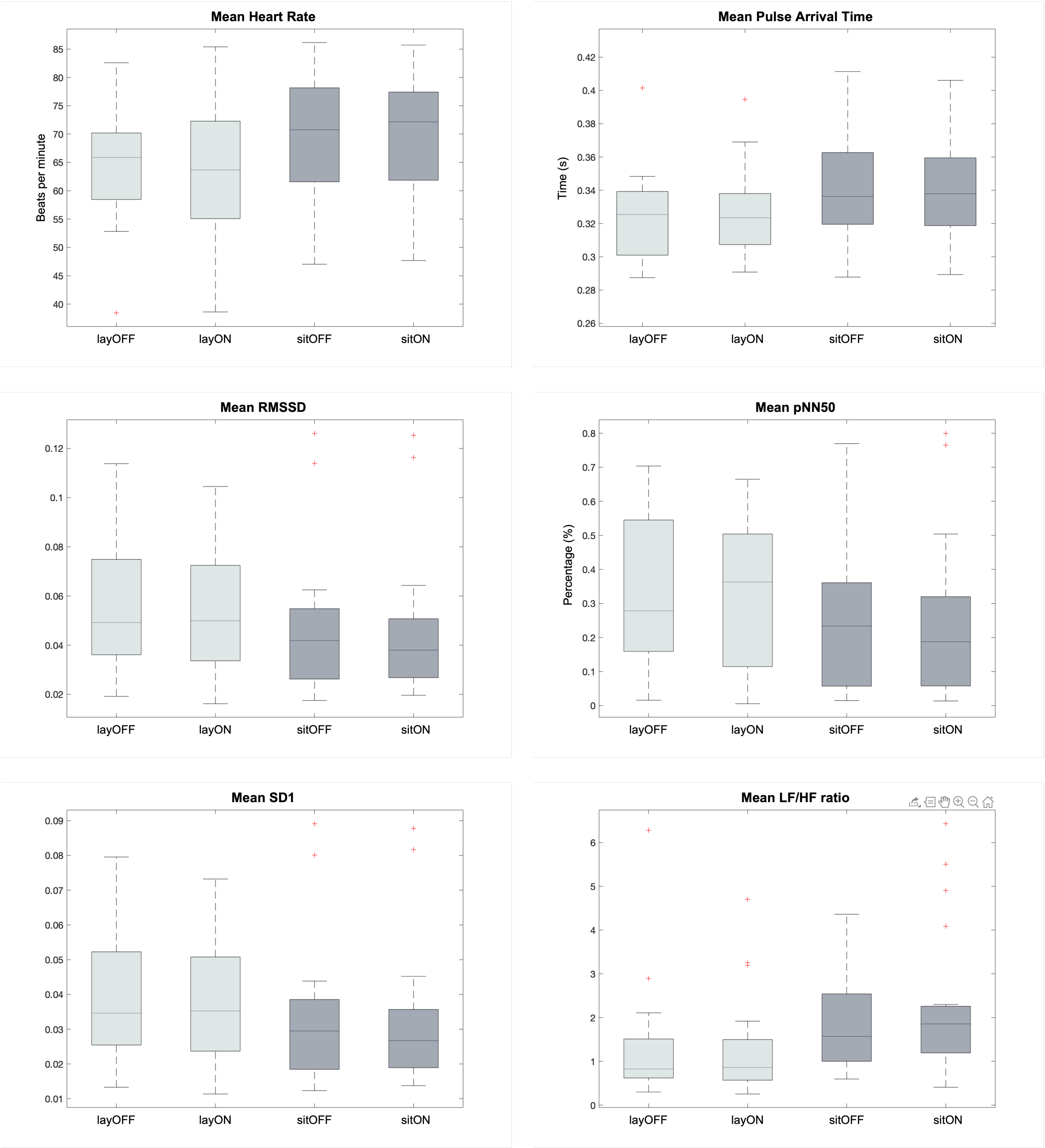


Figure 3.9: Boxplots of significant features: a) HR, b) PATp, c) RMSSD, d) pNN50, e) SD1, f) LFHF

3.3 Brain and physiological activity correlation

The final portion of the data analysis made use of the time-frequency decomposition of the EEG data, in the EO resting condition, to compare the temporal modulation for each experimental condition, per participant brain activity and physiological signal features. The vectors containing each physiological signal feature were interpolated to match the EEG data number of samples. Pair-wise Pearson correlation coefficients were calculated between every feature vector and a select number of EEG channel's power-time series. The chosen channels were those which revealed significant differences in the bandpower analysis: Fz, FC1, T7, Fcz, CP5, P4, Cz and F4.

No strong correlations were found. The biggest share of the obtained coefficients were close to zero, meaning that there was no correlation between the two variables. The remaining few which were considered significant only indicated moderate correlations (the biggest coefficient value was $\rho = 0.4934$). Out of the 20 participants, only 8 showed acceptable results, but there were no visible trends concerning the pairs of EEG/physiological variables or channels or trials. The channel T7 had the highest number of significant correlations with physiological features,and HR and multiple HRV variables (SD1, SD2, pNN50, RMSSD) were the most prominent features. As for the frequency band powers, the Beta band revealed as the one that correlated the most with the other variables, with Delta and Alpha following. There were no relevant results involving the Theta band.

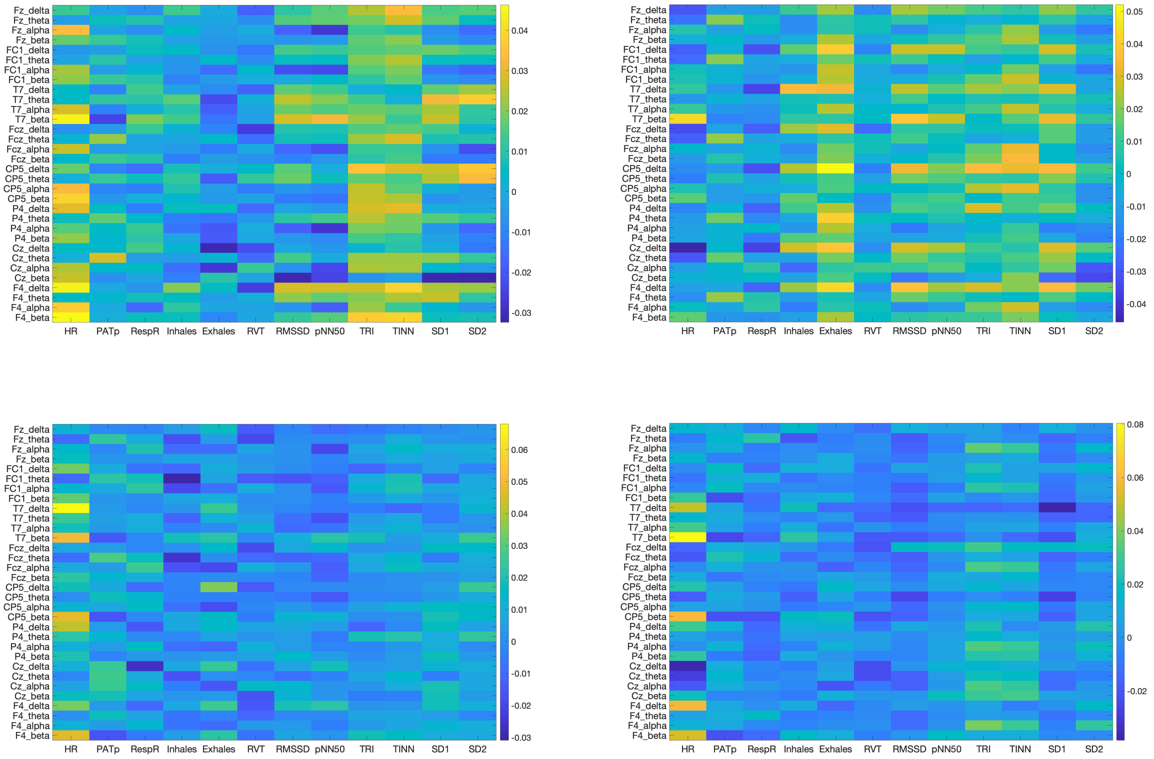


Figure 3.10: Average Pearson correlation heatmaps, per trial: a) layOFF, b) layON, c) sitOFF, d) sitON

Chapter 4

Discussion

In the next pages the results presented in the preceding section are analyzed and compared to the findings of previous works. Three different perspectives are discussed: the changes observed in brain activity, the change in autonomic nervous system function and the possible relationship between the two.

4.1 EEG bandpower

4.1.1 Posture

The main goal of the analysis was to evaluate the effect of the ecological conditions, posture and light, on brain activity recorded through a specifically designed EEG protocol.

Unlike previous studies, individual Alpha bands were used instead of the broader fixed frequency range of 8 to 13 Hz. In fact, the boxplot distributions show that there is variability within subjects with the lower limit of the band fluctuating between a little less than 7 Hz up to just below 10 Hz, while the upper limit fluctuates between below 11 Hz and above 13 Hz.

Results showed that Delta relative power was significantly higher in the supine position comparatively to upright. Thus, this further contributes to the evidence already found in previous works that horizontal postures lead to higher low-frequency powers (Chang et al. [2011], Zhavoronkova et al. [2012], Spironelli et al. [2016]) and contradicts findings from Brauns et al. [2021] which indicate otherwise. Although the main concept is common to all the studies, the experimental setup design varies, namely in what concerns the duration of the acquisition. Whereas for this work, a 7-minute period was chosen, others measured EEG activity in shorter periods (60-70 seconds) and notably longer periods too (2 hours or more). This consistency among results shows that the modulation in mean Delta power is independent of the acquisition run time, conclusions to which Spironelli et al. [2016] and Brauns et al. [2021] also arrived when comparing the powers at different time points within their long-duration experiments. Hence, the differences found in this study cannot be attributed to short-term transitional mechanisms.

The channel-specific analysis indicated that all channels showed significant changes in Delta power due to posture, with the percentual difference maps indicating that Delta increased in all channels in the supine position. This differs from the findings of Chang et al. [2011], which only found significant differences in the right hemisphere, but is coherent with Spironelli et al. [2016] and Zhavoronkova et al. [2012]. The parietal region (mainly T7, P4 and CP5 which had the most signficant changes) showing the most prominent differences is coherent with the literature. The upmost frontal region also showed high percentual differences, however, the FP1 and FP2 channels are easily corrupted by noise and so were not considered for analysis.

The eyes factor must also be taken into consideration when interpreting the results. In Chang et al. [2011] EEG was acquired with the subjects having the eyes closed. In this study, as well as in Spironelli et al. [2016], subjects had their eyes opened throughout the duration for resting-state comparison. In Zhavoronkova et al. [2012] both eyes states were studied, however significant differences were only found in EC. In fact, the Delta rhythm in healthy adults is most prominently observed during late deep stages of sleep (Steriade et al. [1993]) and is related to inhibition of several cortical and subcortical regions (Spironelli et al. [2016], Hofle et al. [1997]). When visible in the resting wake adult it is often reported as a marker of abnormal brain lesions due to tumors, infarction, contusion or infection (Spironelli and Angrilli [2009]). Thus, the higher powers clearly indicate that horizontal posture leads to inhibition, just as reported in several previous works and according to what is expected through everyday experience: that rest is more easily associated with lying down than sitting upright.

Theta relative power results were similar to those found for Delta: increase in supine position relative to sitting. However, only select regions suffered significant differences. Again, this was coherent with findings by Chang et al. [2011] and Zhavoronkova et al. [2012] and contradicts Brauns et al. [2021]. As discussed in Spironelli et al. [2016], contrarily to Delta and other frequency bands, the interpretation of Theta by itself isn't unanimous among experts. In Vaitl et al. [1996], comparison between upright and head-down tilt posture showed increases in Theta and Delta in the horizontal group, changes which were associated with cortical inhibition. Klimesch [1999], Sauseng et al. [2004] attribute Theta synchronization to memory performance. A study done with sleep-deprived subjects Caldwell et al. [2000] also compared EEG activity between supine and standing upright, and results suggested that vertical posture attenuates Delta and Theta, which leads to higher alertness levels. High Theta powers have also been reported as an indicator of pathological psychological conditions.

By Figure 3.4 it is visible that, although the highest powers are seen in the frontal region (Figure 3.3), the brain locations with the highest percentual differences between power in both postures are the parietal and occipital, similar to Delta. It thus seems that the increases in power of both frequency bands when subjects assumed the supine positions are related and point to the cortical inhibition that previous works have reported.

Regarding the Alpha band, it is a much more complex case than its' lower and higher frequency counterparts. Prior evidence is not coherent, with some studies reporting increases of Alpha power in supine position relative to sitting (Spironelli et al. [2016], Zhavoronkova et al. [2012]), while Brauns et al. [2021] found the exact the opposite, lower power in supine, and Chang et al. [2011] didn't find any significant differences due to posture.

Across all four trials, topographical maps showed the Alpha's characteristic antero-posterior contrast, with Alpha power appearing most prominently in posterior sites. The regions where the highest percentual differences between lay and sit conditions occurred coincided precisely with the ones that showed significant changes dues to the posture factor: parietal and center frontal regions. Across these regions the relative power of Alpha was significantly lower in supine than in sitting posture. These findings further contribute to the incoherent evidence on the effect of posture on the Alpha band.

A possible explanation could lie on the duration and setup of the acquisition. Spironelli et al. [2016] results further showed that 120 minutes after the sitting-supine transition and accompanying Alpha power increase, the relative power in posterior sites significantly decreased, indicating that Alpha activity might be sensitive to the postural transition. This could explain why in Brauns et al. [2021], where the long-term effects of bed rest were studied, horizontal posture were associated with decreases in Alpha power. Here the acquisition was much longer, with the first time point of power measurement corresponding to several hours after the transition, thus registering the decrease. On the other hand, Zhavoronkova et al. [2012] only measured one minute of EEG activity in the sitting position about 3 to 5 minutes (not specified exact amount) after subjects were lying down. Here the time interval between the two conditions could have been insufficient and the measured decrease of power associated to the supine/sitting transition could be originated by the same mechanism that led to the increase reported by Spironelli et al. [2016] after sitting/supine change. This hypothetical transitional effect was controlled in our study through the randomization of trial order and time interval between the upright-supine transition and start of recording. Further, the 7-minutes of recording could additionally contribute to a sufficiently long duration such that the effect caused by the transition wasn't visible and thus lower powers were found in supine posture, similar to the long-term effects reported by others. However, this hypothesis is contradicted by findings in an older study where orthostatic changes in the EEG were evaluated (Ivanova [1988]). Resting-state EEG power was measured in supine position and then immediately after a passive transition to a 70 degree tilt and again after a duration of 15 minutes. Increases in posterior power of Alpha was seen in both time points. The Alpha band is in fact complex and there is no unanimity among different studies regarding its relationship with posture. A better understanding of the underlying physiological mechanisms involved in this modulation could help answer the doubts regarding this frequency band.

It must also be taken into the consideration that in this study, the individual Alpha bands were used. Results showed that subjects display different range of peak Alpha power, justifying the use of IAB. This can also be a source of the differences between this study and previous findings, which used the fixed frequency range for all subjects.

Regarding the Beta frequency band, it has a non-uniform modulation across the brain. By visually inspecting the topographic maps in Figure 3.3, and in a general sense, it seems that the supine position induces higher powers in the posterior regions and lower powers in the anterior regions when comparing with the sitting position. However, the percentual differences between trials with different posture (Figure 3.4) shows that there is almost widespread increase in power when subjects were lying down, with the center region standing out as having the largest increase. Only some left central/anterior regions suffered a decrease in power, which nevertheless were much lower percentually than the the increase sin the remaining locations. Friedman tests showed that indeed the center electrodes had the most significant change in power between the two positions.

These findings, however, are contradictory to what was expected. Previous works all found that Beta power, as well as other high-frequency activity, is lower in supine position relative to sitting or upright posture. This is thought to be caused by the cortical inhibition that horizontal body position induces, as already discussed for the previous frequency bands.

Finally, the Theta/Beta ratio (TBR) was also considered. Over the last couple of years it has become an important marker of neurological conditions, specifically Attention-deficit/Hyperactivity Disorder (Clarke et al. [2019], Picken et al. [2020], Lin et al. [2021]), and successfully used as a neurofeedback index in the treatment of the disease (Enriquez-Geppert et al. [2019]). The ratio of slow brain wave power relative to high frequency activity, indicated through the Theta/Beta ratio, is related to cortical arousal. Thus, it is thought that ADHD symptoms derive from inhibition caused by cortical under-arousal, which in itself is manifested through the abnormally high levels of TBR that patients reveal.

TBR was then included in the analysis as a possible indicator of the cortical inhibition that previous studies have found to be related to the supine position. No significant differences across postures were found in any channel, however Figure 3.3 and 3.4 point towards a higher value of TBR in the supine position compared to sitting down when the lights were OFF, and mixed findings with the lights ON. Although the highest values are seen in central/frontal region, the percentual difference plots show that temporal regions had the biggest difference between the two postures. The relationship between posture and this index then remains to be further clarified. It seems logical that the odd results obtained regarding the Beta band power affected the modulation in TBR and its interpretation.

4.1.2 Light

Regarding the effect of light on the EEG spectral powers, not many significant results were found.

Of the four bands considered, and the Theta/Beta ratio, only Theta and Alpha showed significant differences between the lights OFF and ON conditions. The percentual difference topographies (Figure 3.5) and boxplot (Figure 3.2) show similar behaviour when contrasting the light factor in both sitting and supine, with a widespread increase in Alpha power in lights OFF compared to lights ON.

Although the Theta band showed only significant differences in a few channels (and with high p-values, close to 0.05) and the Delta band didn't show any significant difference, results point towards a decrease of power in both bands in a lower lighting environment (Delta showed positive percentual differences in the frontal electrodes, which are easily corrupted by noise and weren't considered in the analysis).

It has been seen that exposure to high light intensities leads to decrease of Delta and Theta power (Chang et al. [2013]) but also to decrease of power in the Alpha range (Kuller and Wetterberg [1993], Cajochen et al. [2000]). This is due to the fact that better lit environments lead to an effect of higher alertness in the human body.

The Beta band has also been used as an index of alertness (Badia et al. [1991]), and its behaviour was the opposite of the other bands since activation and higher alertness are related to increase in power of the higher frequencies. In this study, results don't point toward a clear trend. As already discussed when addressing the posture factor, there seems to be an interaction between the two factors which doesn't allow to make any concrete conclusions. The percentual difference results revealed a higher tendency to increase of power with the lights OFF when subjects were sitting down. However when comparing both lighting conditions in the lying position, some regions show increase power but the majority show a decrease. Technical aspects also can't be discarded in this case, with possible interference of muscle activity and artifacts in the Beta frequency range leading to the unexpected behaviour shown in the results.

Having said, the effect of light in the results isn't clear. Theta power decreased in lower lighting conditions. However, the significant increase in supine position of the Alpha band, characteristic of less alert states, indicates the reduction of arousal that would be expected in such conditions.

4.2 Autonomic response

Beside the impact on brain activity, another domain in which the effect on ecological conditions was studied was the physiological responses of the autonomic nervous system. This was done through the comparison of different time and spectral features from the ECG, PPG and respiration signals.

Respiration properties like inhale and exhale periods and respiration rate did not suffer any significant modulation across trials. Everyday experience could lead to the assumption that a horizontal posture would show lower heartbeats and longer inhales and exhales due to an increase in relaxation. This is somewhat visible in the results relative to the layOFF trial (Figure 3.8), however the distribution isn't significantly different from the remaining as all medians roughly coincide. And on the other hand, the layON trial showed very similar results to the sitting condition trials.

The significant changes were visible in features concerning cardiac activity and only posture effects were seen. From the ECG, heart rate and heart rate variability statistical and spectral metrics were extracted, as well as the pulse arrival time which relates the waveform captured in the electrocardiogram with the PPG. Pulse arrival time has been used as a practical, and most importantly continuous, index of blood pressure (Mukkamala et al. [2015]). However it does have limitations and has been shown to work best in the identification of trend changes in hyper and hypotensive patients (Escobar-Restrepo et al. [2018]). The results in this project showed a significant decrease of pulse arrival time in the supine position. Considering the results in Escobar-Restrepo et al. [2018], which showed moderate negative correlations between PAT and both diastolic and systolic blood pressures, this could indicate that blood pressure was higher in the lying down trials. Such trend is within what was expected since less upright postures are related to increased blood pressure (Eşer et al. [2007]).

Regarding heart rate, it was significantly lower in the *lay* trials comparatively with sit. This also goes along to what was expected. The influence of posture on heart rate has been well established (Chizh [2016], Watanabe et al. [2007]). The transition to a lesser upright posture leads to deactivation of the autonomic nervous system, as a response to situational demands (McLaughlin et al. [1978]).

Several heart rate variability measures also had significant modulation across trials. RMSSD, which reflects beat-to-beat variance of heart rate, was significantly higher in supine position than sitting. It is seen as the main time-based indicator of vagally-mediated changes within HRV. Thus results point toward higher parasympathetic function when subjects were lying down. A related measure is the pNN50, which was also significantly higher in the supine position. It is highly correlated to spectral high frequency (Umetani et al. [1998]) and, along with RMSSD, is an indicator of parasympathetic activity. Regarding the non-linear metrics SD1 was also significantly higher in supine posture, while for SD2, although differences were not statistically significant, the boxplot also shows higher values in supine position. Both are correlated to baroreflex sensitivity while the first is a predictor of diastolic BP (Shaffer and Ginsberg [2017]). Concerning the spectral information, low-frequencies (VLF and LF) were significantly lower in supine, while HF was significantly higher. While the physiological correlates of VLF are not well understood, such is not the case for LF and HF. The HRV's low-frequency band is related to baroreflex function (Goldstein et al. [2011]) while high frequency reflects parsympathetic activity and is inhibited in states of stress and anxiety.

4.2.1 Posture and the Brain/ANS responses

Possible explanations for the results found for the EEG bandpowers may be justified to what was seen in the physiological features, and mainly HRV.

The physiological signals showed that the assumption of the supine position led to increased vagal, thus parasympathetic, activity, which manifested in cardiac slowing and decreased heart rate as well as modulation of HRV. The horizontal posture then clearly, and as expected, leads to an overall less aroused state and to cortical inhibition expressed through the observed increases in Delta and Theta relative power.

The question on which physiological mechanisms produce the differences seen in brain activity between the two postures still hasn't been answered. The modulation in the HRV metrics SD1, SD2 and low-frequency power give insight into what is considered as one of the mechanisms involved. The higher levels of SD1 and SD2 indicate high baroreceptor sensitivity in supine posture, and the lower LF power indicates decreased baroreflex activity. When a horizontal position is assumed thoracic baroreceptors are stimulated leading to them inhibiting baroreflex. As a consequence, sympathetic activity is decreased and parasympathetic activity increases. It is believed that this is related to the cortical inhibition materialized by higher Delta/Theta power, as studies have shown that direct stimulation of baroreceptors produce this effect on EEG (Cole [1989], Angrilli et al. [1997]).

By correlating the physiological features and EEG activity it was hoped that this relationship could shine some light on the physiological basis responsible for the effects that posture produces on neural activity. However, results were not significant, but hopes of future studies focusing on this aspect could lead to important findings.

Chapter 5

Conclusions

The presented work aimed to help answer some of the questions that in recent years have been made towards the ecological validity of imaging studies. More specifically, and through the use of EEG and physiological signals, the objective was to evaluate how the factors posture and light would affect these signals when recorded in varying conditions.

Concerning posture, several significant contradictions were seen in the EEG powers. Delta and Theta were higher in supine position than sitting, indicating that horizontal posture leads to cortical inhibition, with this being a possible effect of reduced baroreflex. Lower heart rate as well as modulation of HRV measures, such as higher RMSSD, SD1, SD2 and LF indicated higher parasympathetic activity in supine. Contrarily to what has mostly been seen in previous studies, the Alpha band, whose power is reduced due to lower levels of arousal, showed a significant decrease in supine.

As for the light factor, the Alpha and Theta bands showed significant modulation. Alpha power increased when the lights were off, result of the lower state of alertness that such an environment induces on the nervous system. On the other hand, Theta power decreased which was unexpected.

It was shown that brain activity is clearly modulated by the two factors. However they both affect the neural dynamics in different ways. While the presence of light leads participants to a more alert state, posture induces a physiological response in cardiac mechanisms and changes in CSF distribution. Understanding these underlying mechanisms might help unravel the uncertainties that still exist regarding the modulation of certain EEG frequency bands.

5.1 Achievements

The first main contribution of this work consisted in the experimental design used. While a significant number of literature that explores the effect of light and posture on these signals is available, to what was possible to evaluate, no previous study has combined these two factors, that are variable within imaging acquisition environments. The results are coherent with previous findings, with the exception of the unclear modulation of the Alpha band due to posture.

5.2 Future Work

Regarding future work, the data collected in this study can still be further analysed. Different aspects of the EEG activity could be explored, such as functional connectivity or microstate analysis. The expansion of the sample size could also help achieve more significant statistical results

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Appendix A

Individual Alpha bands spectra

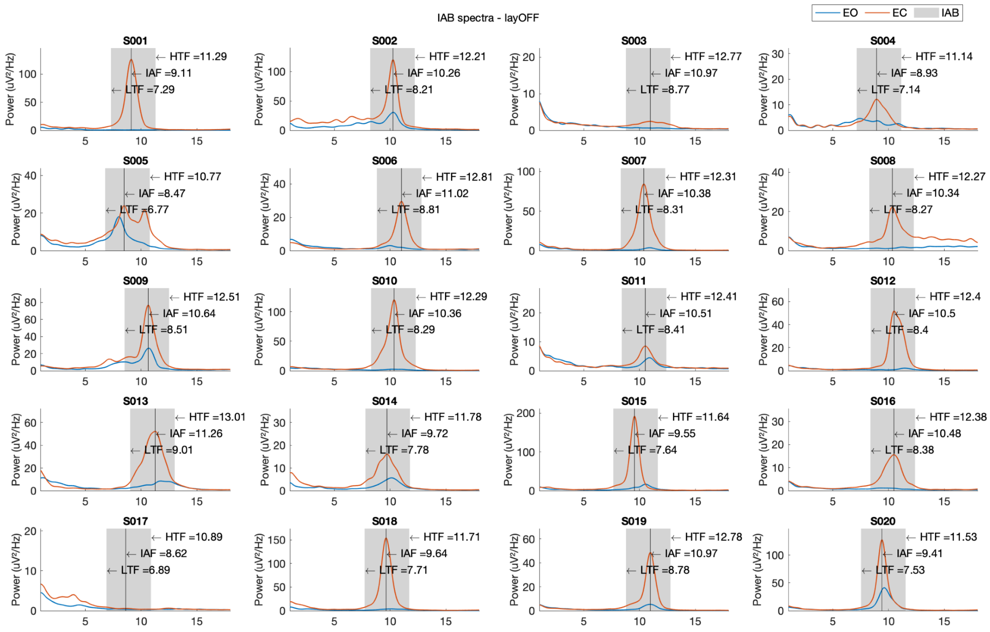

Figure A.1: IAB eyes closed and eyes opened overlapping spectra per subject in supine posture and lights off condition.

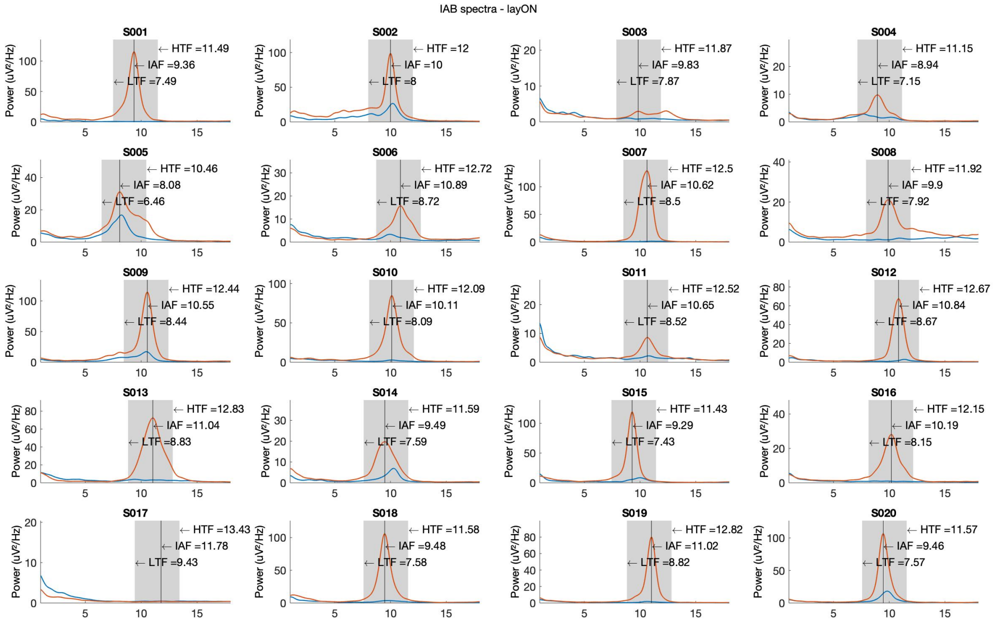

Figure A.2: IAB eyes closed and eyes opened overlapping spectra per subject in supine posture and lights on condition.

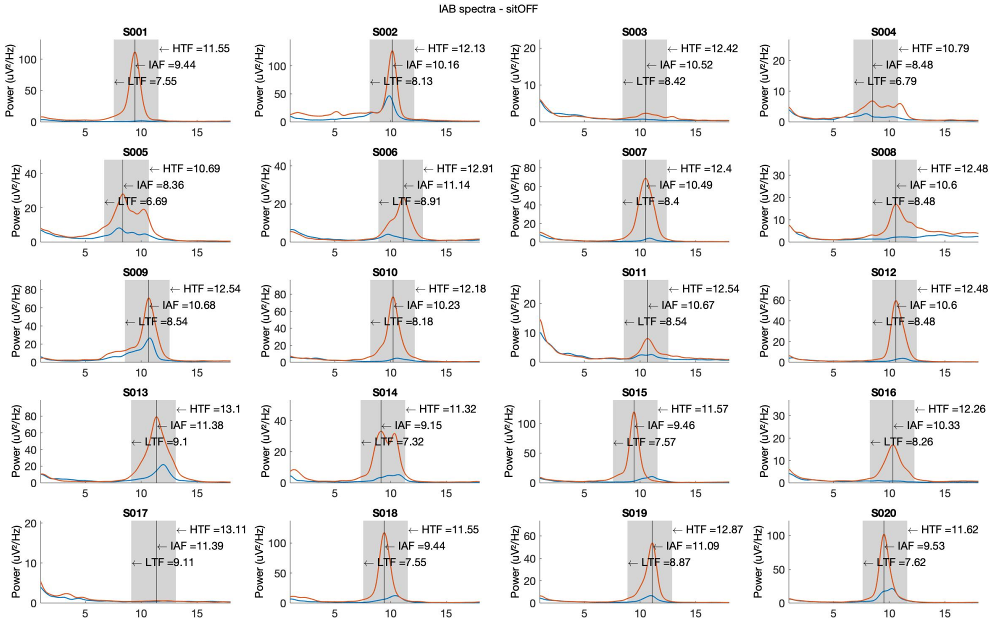

Figure A.3: IAB eyes closed and eyes opened overlapping spectra per subject in sitting position and lights off condition.

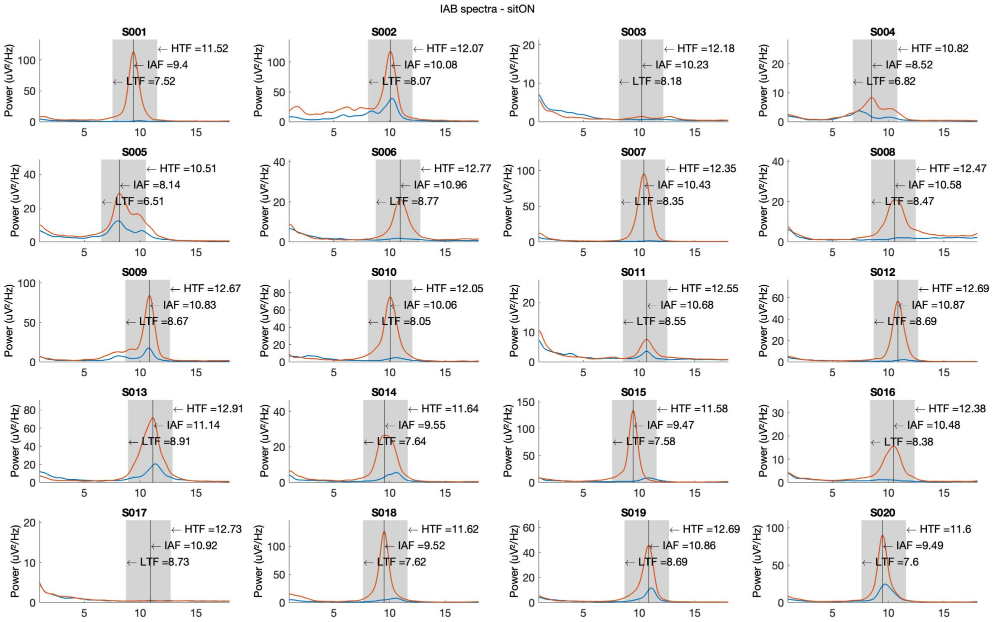

Figure A.4: IAB eyes closed and eyes opened overlapping spectra per subject in sitting position and lights on condition.