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Souto

**Sinais EEG durante controlo por realidade virtual:
perspetiva e incorporação na atividade
sensório-motora**

**EEG signals during virtual reality control: gaze
direction and embodiment on sensorimotor activity**



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Documento apresentado à Universidade de Aveiro, para cumprimento dos requisitos necessários à disciplina de Projeto de terceiro ano da Licenciatura em Engenharia Biomédica, sob a orientação do Doutor Miguel Santos Pais Vieira, Professor no Departamento de Ciências Médicas e Investigador no Instituto de Biomedicina, iBIMed, da Universidade de Aveiro.

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palavras-chave

Banda Teta, C4, Direção da perspetiva, Eletroencefalograma, Exoesqueleto, Incorporação, Realidade Virtual

resumo

A integração entre a realidade virtual e a monitorização neurofisiológica abre novas possibilidades para aprofundar a compreensão da sensação de incorporação (*embodiment*) e da interação entre humanos e máquinas, especialmente em contextos de reabilitação e tecnologias assistivas. Este projeto investiga como a introdução de um exoesqueleto virtual nos membros inferiores e a direção do olhar, podem influenciar a atividade cerebral e a percepção de incorporação em ambientes virtuais imersivos. Durante a experiência, os participantes visualizaram um avatar em primeira pessoa, expostos a quatro condições experimentais que combinavam a presença ou ausência do exoesqueleto (com Exo vs. sem Exo) com duas direções do olhar (para a Frente vs. para Baixo). A atividade elétrica do cérebro foi registrada através de 16 canais de eletroencefalografia (EEG), com especial atenção aos elétrodos C3 e C4 — regiões associadas ao processamento sensório-motor. Para analisar a modulação da atividade neuronal, foi calculada a densidade espectral de potência (PSD), com ênfase nas bandas de frequência teta, alfa e beta.

As análises estatísticas, incluindo testes não-paramétricos e ANOVA de medidas repetidas, revelaram efeitos neuronais mais consistentes na banda teta, especificamente no elétrodo C4. Tanto a presença do exoesqueleto como a direção do olhar para baixo estiveram associadas a um aumento da potência na banda teta, embora se tenha verificado uma variabilidade individual significativa.

As avaliações subjetivas, obtidas através de questionários, corroboraram os dados eletrofisiológicos, indicando uma maior percepção de incorporação e de atribuição de peso nas condições com exoesqueleto, sobretudo quando os participantes olhavam para baixo. Os resultados obtidos evidenciam a complexa interação entre os estímulos perceptivos e a dinâmica neuronal, destacando a relevância das oscilações na banda teta como possível biomarcador da modulação da atividade cerebral em ambientes virtuais.

Esta investigação contribui para o avanço do campo da neurorreabilitação e das tecnologias imersivas, ao estudar o impacto da introdução de um exoesqueleto virtual e da perspetiva visual na atividade cerebral e na percepção de incorporação. Para além disso, estabelece bases sólidas para estudos futuros com amostras maiores, maior número de elétrodos e métricas neurocognitivas mais abrangentes, com o objetivo de aprofundar o conhecimento e otimizar a utilização da realidade virtual em contextos de reabilitação.

keywords

C4, Embodiment, Electroencephalogram, Exoskeleton, Gaze Direction, Theta Band, Virtual Reality

abstract

The integration of virtual reality with neurophysiological monitoring offers new opportunities to enhance embodiment and assess human-machine interaction, particularly in rehabilitation and assistive technology contexts. This project investigates how the presence of a virtual lower-limb exoskeleton and variations in gaze direction influence neural activity and perceived embodiment during VR exposure. Participants viewed a first-person avatar under four conditions combining exoskeleton presence (Exo vs. No Exo) and gaze direction (Forward vs. Downward). Electroencephalographic data were recorded from 16 channels, processed and analysed, with a primary focus on the C3 and C4 electrodes associated with sensorimotor processing. Power spectral density was computed and analysed, particularly in theta, alpha, and beta frequency bands to assess neural modulation.

Statistical analyses, including non-parametric tests and repeated-measures ANOVA, revealed that the most consistent neural effects occurred in the theta band at electrode C4. Specifically, both the presence of the exoskeleton and downward gaze direction were associated with increased theta activity, though individual variability was considerable.

Subjective assessments, as a result of questionnaires, further supported the neural findings, indicating higher perceived embodiment and weight attribution in conditions involving the exoskeleton, particularly when looking downward. These results underscore the complex interplay between perceptual input and neural dynamics and highlight the relevance of theta oscillations as a potential biomarker for the modulation of neural activity in a virtual reality environment.

This research contributes to the growing field of research on immersive technology and neurorehabilitation by elucidating how exoskeletons and visual perspective affect brain activity and body ownership. It also lays the groundwork for future studies with larger samples, more electrodes, and expanded neurocognitive metrics to better understand and optimize virtual embodiment in rehabilitation and assistive applications.

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List of Acronyms

Acronyms

ALS	Amyotrophic Lateral Sclerosis
BCI	Brain-Computer Interface
BMI	Brain-Machine Interface
ECoG	Electrocorticography
EEG	Electroencephalography
ERPs	Event-related Potentials
fMRI	Functional Magnetic Resonance Imaging
ICA	Independent Component Analysis
MEG	Magnetoencephalography
MI	Motor Imagery
MRI	Magnetic Resonance Imaging
PSD	Power Spectral Density
SCI	Spinal Cord Injury
SMA	Supplementary Motor Area
STD	Standard Deviation
STS	Superior Temporal Sulcus
VR	Virtual Reality

Chapter 1

Introduction

1.1 Motivation and Framework

The integration of virtual reality (VR) with neurophysiological monitoring is rapidly emerging as a promising approach to enhance embodiment in digital environments and assess human-machine interaction, specially in rehabilitation and assistive technologies [1–3]. In recent years, lower-limb exoskeletons have been increasingly adopted in clinical and non-clinical settings to support individuals with mobility impairments [3–6]. However, the subjective experience of embodiment (described in Section 2.5) remains underexplored, particularly from a neurophysiological perspective. Exploring the neural mechanisms underlying embodiment in VR simulations with exoskeletons can offer valuable insights to enhance the design and functionality of these systems [3–5].

Despite growing interest in electroencephalogram-based (EEG-based) analysis of motor-related brain activity, few studies have examined how specific visual and auditory feedback influence embodiment and neural engagement during passive observation of avatars with or without exoskeletons [1]. Moreover, the impact of gaze direction on these neural signatures is not well understood. By examining brain responses across four VR conditions — analysing the influence of gaze perspective and the effects of exoskeleton presence — alongside subjective assessments of embodiment, this study aims to contribute to a better understanding of neural processing in immersive environments involving exoskeletons.

The experimental design involved presenting participants with a virtual avatar viewed in first-person perspective, which either wore a lower-limb exoskeleton or did not, while participants directed their gaze forward or downward. These four conditions were systematically varied to isolate the effects of exoskeleton visualization and gaze direction on participants' brain activity.

EEG data were recorded using a 16-channel system configured according to the 10-20 standard, with primary focus on C3 and C4 electrodes corresponding to the left and right sensorimotor cortices. Preprocessing techniques, including Independent Component Analysis (ICA) and frequency-domain normalization, were applied to reduce artifacts and improve signal quality, enabling more robust comparisons across conditions [7]. Power spectral density (PSD) in the delta, theta, and alpha bands served as the primary metric for assessing changes in neural activity associated with embodiment.

To complement the neural data, participants completed a embodiment questionnaire after each

trial to capture subjective experiences of body ownership, agency, and self-location [2,8]. Statistical analysis using non-parametric tests and repeated-measures analysis of variance (ANOVA) enabled evaluation of condition-specific and hemispheric differences in brain activity [9].

Together, this framework provides a novel avenue for quantifying the neural impact of exoskeleton-based avatar representations in immersive VR, offering both theoretical and practical contributions to neuroergonomics, virtual rehabilitation, and embodied cognition research [4,10].

1.2 Aims and Objectives

The primary aim of this study was to investigate EEG signals during VR control, specifically focusing on the neural activity of users interacting with a virtual exoskeleton, as well as comparing the effects of two different gaze directions (forward and downward). This allowed for an in-depth analysis of how the presence or absence of the exoskeleton and the variation in perspective influenced neural activity during VR interactions.

The study started by applying preprocessing techniques using the EEGLAB toolbox in MATLAB R2023a to prepare the data for further analysis [11,12]. Subsequently, an exploratory data analysis of the C3 and C4 channels (S1 and M1 sensorimotor cortex) was conducted using MATLAB R2023a to examine the general structure and characteristics of the EEG signals [12]. The PSD of the data was then computed, and the mean and standard deviation values were analysed using bar graphs and spectral plots within the relevant frequency range. Statistical analysis was performed using non-parametric tests to evaluate differences in spectral power across frequency bands and experimental conditions. Finally, ANOVA and correlation analyses were carried out to assess the effects of the presence or absence of the virtual exoskeleton and the perspective condition on neural activity in the C3 and C4 regions, which correspond to the left and right sensorimotor cortices, respectively. These regions were selected due to their well-established involvement in body representation and motor processing, particularly in tasks involving embodiment or motor simulation. The hypothesis was further focused on C4, as it showed more consistent and statistically significant results, whereas no significant effects were observed in C3.

Table 1.1: Postulated hypotheses

Hypothesis	Description
<i>Effect of Exoskeleton Presence</i>	
H1	There is a difference in theta band PSD values at electrode C4 between the exoskeleton and non-exoskeleton conditions.
H2	There is a difference in alpha band PSD values at electrode C4 between the exoskeleton and non-exoskeleton conditions.
H3	There is a difference in beta band PSD values at electrode C4 between the exoskeleton and non-exoskeleton conditions.
<i>Impact of Gaze Direction</i>	
H4	There is a difference in theta band PSD values at electrode C4 between the forward and downward viewing direction.
H5	There is a difference in alpha band PSD values at electrode C4 between the forward and downward viewing direction.
H6	There is a difference in beta band PSD values at electrode C4 between the forward and downward viewing direction.
<i>Interaction Between Exoskeleton Presence and Gaze Direction</i>	
H7	There is a difference in theta band PSD values at electrode C4 between the viewing direction and the presence of exoskeleton.
H8	There is a difference in alpha band PSD values at electrode C4 between the viewing direction and the presence of exoskeleton.
H9	There is a difference in beta band PSD values at electrode C4 between the viewing direction and the presence of exoskeleton.
<i>Perceptual Measures</i>	
H10	Exoskeleton presence and/or gaze direction influence perceived embodiment.
H11	Exoskeleton presence and/or gaze direction influence perceived weight.

The decision to focus the analysis exclusively on the C4 electrode is grounded in its neurophysiological relevance to motor activity and sensorimotor integration, particularly concerning the right hemisphere's involvement in controlling the contralateral (left) side of the body. Previous literature often highlights C4 as a reliable site for capturing EEG signals related to motor control [13, 14].

Chapter 2

Literature Review

This chapter outlines the theoretical framework underpinning the present project, focusing on key concepts, methodologies, and tools associated with EEG-based brain-machine interfaces (BMIs) and their application in neurofunctional rehabilitation.

2.1 EEG: Basic Principles and Use in Neuroscience

Electroencephalography (EEG) is a non-invasive technique for recording the brain's electrical activity by measuring voltage fluctuations produced by ionic currents within neuronal populations [15–18]. EEG signals are acquired via electrodes placed on the scalp, which capture the summed post-synaptic potentials generated primarily by large ensembles of pyramidal neurons - whose apical dendrites are aligned perpendicular to the cortical surface - in the cerebral cortex [15]. Analysis of these recordings reveals oscillatory patterns across defined frequency bands (delta, theta, alpha, beta, gamma), each reflecting coordinated activity of neural assemblies and correlating with particular cognitive or behavioral states [19].

EEG is widely used in both clinical and research settings. It is the primary method for detecting epileptic activity, such as spikes and seizures, and plays a crucial role in polysomnography for diagnosing sleep disorders [17, 20]. It is also important in BMIs, enabling communication for individuals with motor impairments [20]. EEG contributes significantly to cognitive and behavioural neuroscience by aiding in the understanding of neural mechanisms underlying attention, memory, perception, and sensory processing [15, 17]. Moreover, it is used to study conditions such as autism spectrum disorders, schizophrenia, and depression by analysing alterations in neural oscillations and connectivity [17, 20].

EEG has distinct advantages over other neuroimaging techniques, including high temporal resolution (millisecond-level precision), non-invasiveness, and affordability [15, 21]. However, it has some limitations, such as limited spatial resolution due to signal distortion by the skull and scalp. In contrast, intracortical EEG, or electrocorticography (ECoG), involves electrodes placed directly on the brain's surface or within the cortex and offers much higher spatial and temporal resolution - though it is highly invasive. EEG is also susceptible to artefacts from muscle movements, eye blinks, and environmental noise, and it presents challenges in isolating deep brain structures due to the use of surface electrodes [15, 18, 22]. Despite these limitations, EEG remains a cornerstone

in neuroscience research and clinical diagnostics because of its ability to provide real-time insights into brain function.

In addition to EEG, several other neuroimaging techniques are commonly used in both clinical and research settings, each offering unique strengths and weaknesses. For example, magnetoencephalography (MEG) offers a higher spatial resolution than EEG by measuring the magnetic fields generated by neural activity [23]. This allows for better localization of brain activity, especially in deeper regions such as the hippocampus [23]. However, MEG is more expensive, less portable, and has a lower temporal resolution than EEG [23]. It also tends to be more sensitive to noise from the surrounding environment, limiting its applicability in certain settings [23].

Functional Magnetic Resonance Imaging (fMRI), on the other hand, excels in its ability to provide spatial resolution (on the millimeter scale), making it ideal for identifying the precise locations of brain activity [24]. However, fMRI is limited by its poor temporal resolution, it is not capable of tracking brain activity in real-time like EEG [24]. Additionally, fMRI is a costly technique that requires participants to remain still within the magnetic resonance imaging (MRI) scanner, making it unsuitable for certain populations, such as children or patients with movement disorders [24].

Despite EEG limitations, its real-time nature, affordability, and ability to capture dynamic brain activity on the millisecond scale keeps it as one of the most used tools in both clinical practice and research [20]. With ongoing advancements, including the use of machine learning algorithms and the integration of EEG with other neuroimaging techniques like fMRI and MEG, EEG is becoming increasingly versatile and informative. These innovations help to overcome some of EEG's spatial limitations and enhance its utility in both basic neuroscience and clinical applications, particularly in neuroprosthetics, cognitive neuroscience, and brain-machine interfaces [20].

EEG signals are categorized into different frequency bands, each associated with specific cognitive and physiological states [16]. Delta waves (0.5–4 Hz) are associated with deep sleep and certain pathological states, while theta waves (4–8 Hz) are linked to drowsiness, meditation, memory processes and visualization. Alpha waves (8–13 Hz) are prominent during relaxed wakefulness, particularly in the occipital region, whereas beta waves (13–30 Hz) are related to active thinking, problem-solving, and motor activity [16, 25, 26]. Gamma waves (30–100 Hz) are associated with high-level cognitive functions such as perception and consciousness. It is important to recall that this frequency limits are somewhat conventional rather than absolute [16, 27]. This classification is based on observed brain activity patterns in EEG studies [16].

EEG signals are captured using conductive electrodes attached to the scalp, following standardized placement systems such as the 10-20 system. The signals are amplified, filtered, and digitized for further analysis, to examine patterns, spectral content, and event-related potentials (ERPs). ERPs are time-locked electrical responses of the brain to specific sensory, cognitive, or motor events. One of the most well-known ERP components is the P300, a positive peak that occurs approximately 300 milliseconds after a stimulus and is often linked to attention and decision-making processes [19, 28].

The first human EEG rhythm recorded through the intact scalp was the alpha rhythm [15]. Since this discovery, alpha and other EEG rhythms have been extensively studied due to their relevance in both clinical and cognitive neuroscience. Among these, the alpha rhythm has proven to

be particularly significant, showing strong associations with a range of neurological and psychiatric conditions, including depression [29], schizophrenia [30, 31], and Alzheimer's disease [32]. It also plays a crucial role in processes related to visual perception [33].

Understanding the alpha rhythm is particularly important in power spectral density (PSD) analysis, which was applied in this study, where it can appear as a clear peak in the 8–13 Hz range. The presence, amplitude, and location of this alpha peak can provide insight into cortical activity and brain maturation [34]. For instance, a reduction in alpha power or peak frequency has been associated with cognitive decline in ageing and dementia [25, 35].

Similarly, the theta rhythm, typically ranging from 4 to 8 Hz, plays a key role in cognitive processing, particularly in memory and attention-related tasks. Increased theta activity has been linked to working memory performance and is often observed during meditative or drowsy states. Alterations in theta power have also been reported in neurodegenerative conditions and psychiatric disorders, making it a relevant marker for cognitive functioning [26].

2.1.1 Neuroscientific Basis

Motor imagery (MI) is a cognitive process in which an individual mentally simulates a movement without physically performing it. Previous studies have demonstrated that MI activates the same neural pathways as actual movement execution, particularly in the primary motor cortex (M1, responsible for the initiation and control of voluntary movements), premotor cortex (involved in the planning and preparation of movements), supplementary motor area (SMA, important for the coordination and sequencing of movements), and parietal regions (associated with spatial awareness and sensorimotor integration) [36].

Even in individuals with spinal cord injury (SCI, where physical movement is impaired, the brains' motor regions can still be engaged through MI [14]. This is thought to occur due to the mirror neuron system that fires both when an action is performed and when it is merely observed or imagined. By activating these networks, MI training can help maintain motor representations in the brain and promote functional reorganization - an essential aspect of neuroplasticity [3]. When an individual engages in MI, brain regions responsible for movement continue to generate neural activity. This can prevent cortical atrophy (degeneration of unused motor areas) and promote synaptic plasticity, potentially facilitating recovery of function. Motor imagery practice has been shown to strengthen connectivity between motor and sensory areas, which can be crucial for functional recovery, even in individuals with motor impairments [3].

2.2 Virtual Reality in the Context of Neurofunctional Rehabilitation

Virtual reality has become a powerful tool in the field of neurofunctional rehabilitation, offering new possibilities for patients recovering from neurological injuries [3, 5]. By immersing individuals in virtual environments, VR enables them to engage in motor, sensory, and cognitive tasks that are designed to facilitate rehabilitation and promote neuroplasticity—the brain's ability to reorganize itself after injury [2, 3, 5].

In neurofunctional rehabilitation, VR presents several advantages over traditional therapeutic methods [5]. One of the most significant benefits is its ability to provide an immersive and engaging environment for patients [5]. Traditional rehabilitation exercises often involve repetitive tasks, which can be monotonous and demotivating. VR addresses this challenge by offering interactive, game-like experiences where patients can perform rehabilitation tasks in a simulated, dynamic environment [3,5,37].

Motor rehabilitation is one of the key areas where VR has shown considerable promise [4,5,37]. Patients who have experienced a stroke or other neurological impairments often struggle with regaining motor function [4]. Traditional physical therapy can be time-consuming and limited in its capacity to engage patients. VR, however, allows for the creation of immersive environments in which patients can practice specific movements, such as reaching, grasping, or walking, in a virtual space [3–6]. This method of rehabilitation is particularly effective because it can provide immediate feedback and adapt to the patient's progress, making the therapy more dynamic and personalized [6].

A 2022 study examined the effectiveness of motor imagery training in conjunction with immersive VR for SCI rehabilitation [2]. This case report explored how embodiment comfort levels were affected when a patient was engaged in VR-based MI training [2].

The participant in the study experienced a spinal cord injury that resulted in limited mobility. Through motor imagery training combined with immersive VR, the participant was asked to visualize and mentally simulate movements like walking or performing specific tasks, even though these actions were not physically possible due to the injury. The immersive VR environment was designed to replicate real-world experiences and allowed the participant to engage in tasks such as walking through virtual spaces, all within a safe, controlled environment.

This study demonstrated that the ability to simulate real-life movements in a virtual setting while incorporating sensory feedback (such as tactile feedback, using vibrating sleeves) can significantly improve the embodiment experience, leading to better rehabilitation outcomes [2]. The psychological and emotional benefits of this process are particularly significant, as patients with SCI often experience a loss of confidence and control after their injury [3,10].

2.3 Neurophysiological and Cognitive Perspectives on Human Gait

Gait, encompassing both walking and running, is a fundamental component of human systemic organization, integrating general navigation and specific interactions with environments [38]. The cognitive mechanisms underlying gait can be broadly divided into two interacting processes - bottom-up and top-down processes. Bottom-up mechanisms integrate sensory and motor inputs into neural circuits that directly coordinate the execution of locomotor patterns . In parallel, top-down mechanisms arising from higher-order brain regions modulate and prioritize this sensory-motor information, contributing to the formation of internal representations, goal maintenance, and adaptive control of gait-related behaviours [38–40].

Although the rhythmic and repetitive act of stepping typically occurs with minimal conscious awareness of its complex neural underpinnings, the brain continuously orchestrates a multitude

of cognitive and motor operations that do not require focused attention on locomotion itself [39]. Nevertheless, dual-task paradigms have demonstrated that gait is not an entirely automatic process, it imposes demands on attentional resources, motivational states, and executive functions [39]. Such cognitive involvement is essential for the evaluation and adjustment of gait under varying internal and external conditions, except in rare circumstances such as sleepwalking or certain epileptic states where these controls may be bypassed [39].

These cognitive demands become particularly evident when gait is challenged by environmental complexity or concurrent cognitive tasks, revealing the intricate interplay between motor control and higher-order cognitive functions [39]. Furthermore, ageing and neurological disorders, such as Parkinson's disease or Alzheimer's disease, often impair these top-down regulatory mechanisms, leading to gait disturbances that increase the risk of falls and reduce mobility [41]. Thus, studying gait from a cognitive neuroscience perspective not only advances our understanding of human motor behaviour but also provides critical insights for developing interventions to preserve mobility and quality of life.

2.4 Neural Correlates of Directional Eye-Gaze Perception

Eye-gaze perception plays a fundamental role in how humans process visual information and interact with their environment [42]. From a neurocognitive standpoint, the ability to detect and interpret gaze direction involves a specialized and distributed network of brain regions, particularly those implicated in visual attention, face processing, and social cognition [43]. This system supports the rapid evaluation of gaze cues, which serve not only to orient attention but also to infer intention and contextual relevance in both social and spatial domains [44].

Key components of this network include the superior temporal sulcus (STS), which is consistently engaged during the perception of eye direction and dynamic facial cues [42]. A 2007 study demonstrated that distinct gaze directions are encoded by separate neural populations within the STS, indicating a fine-grained specialization for processing spatial aspects of gaze [44].

Another study identified shared cortical regions involved in gaze perception, attentional shifts, and oculomotor control, suggesting that gaze cues are tightly integrated with neural systems responsible for directing eye movements and shifting spatial attention [43]. This supports the idea that gaze perception is not a passive visual process, but one that actively prepares the observer for action by modulating sensorimotor circuits [43].

2.5 Embodiment: Concepts and Relevance

Embodiment is a multifaceted concept used across a variety of disciplines, with its meaning varying depending on the context. At its core, embodiment refers to the subjective experience of one's body as inherently belonging to oneself, and the process through which external devices — such as prosthetics, virtual avatars, or exoskeletons — are incorporated into this bodily self-perception [1, 2, 8]. This incorporation expands the boundaries of what one experiences as part of their own body [1, 2, 8].

In neuroscience, BMIs, and neurorehabilitation, embodiment is understood as the extent to which a user perceives an artificial or virtual body part as an integral part of their own body

[1, 2]. This experience involves several interrelated but distinct components: the feeling of being located within a body (self-location), the sense of control over one’s movements (agency), and the perception that the body or its parts belong to oneself (body ownership) [2, 8, 45]. Impairments in any of these components can alter the overall sense of embodiment, as seen in certain neurological conditions and injuries [1, 8, 46].

At the neural level, embodiment arises from the integration of multisensory information across a distributed network involving sensory, motor, and associative brain areas [8]. Visual, tactile, and proprioceptive feedback are especially important in shaping embodiment, with congruent and synchronous sensory signals enhancing both the sense of ownership and control over artificial limbs or virtual bodies [1, 2]. For example, seeing a prosthetic move in exact alignment with one’s intended movement strengthens the feeling that the device is part of the body.

In the data used in this study, the direction of participants’ gaze was varied to manipulate the type of visual feedback. When participants looked downward, they had direct visual access to the virtual avatar’s legs and, where applicable, the lower-limb exoskeleton used during the task. This downward gaze was intended to enhance the alignment between visual information and proprioceptive signals coming from the body and the assistive device. By increasing this multisensory congruence, the downward gaze was expected to strengthen embodiment. This effect is particularly relevant for users without prior experience using an exoskeleton, as seeing the device move in real-time helps promote a stronger sense of ownership and agency [42, 43]. Enhancing embodiment in this way may facilitate motor learning, increase user motivation, and ultimately improve rehabilitation outcomes [2].

2.6 Signal Acquisition: Brain-Machine Interfaces

Brain-Computer Interfaces (BCIs) or Brain-Machine Interfaces (BMIs) represent a groundbreaking advancement in neuroscience and technology, enabling direct communication between the brain and external devices [46, 47]. These systems operate by detecting and interpreting brain signals, often acquired through EEG, and converting them into executable actions by computers or assistive devices [46].

BMI are particularly significant in the medical field, offering hope for individuals with motor impairments, such as those affected by spinal cord injuries, amyotrophic lateral sclerosis (ALS), or stroke [2, 3, 47]. By bypassing damaged neural pathways, BMIs may enable individuals to regain control over prosthetic limbs, wheelchairs, or even digital communication platforms.

A relevant factor in BMI development is the role of sensory feedback, which enhances the user’s sense of embodiment and control. Research has shown that incorporating real-time sensory feedback such as tactile, visual, or auditory stimuli can significantly improve performance in brain-controlled tasks [1, 2].

Despite these advancements, several challenges persist. The accuracy and reliability of BMIs depend on the quality of neural signal acquisition and interpretation. EEG-based BMIs, while non-invasive and practical, suffer from the disadvantages of EEG usage mentioned earlier. Additionally, the physiological state of the individual can have a significant impact on performance. For example, on a more stressful day, the brain activity patterns may be markedly different, leading to a completely altered performance of the BMI [1].

Chapter 3

Materials and Methods

This chapter describes the materials and equipment used in order to obtain the signals, as well as the methods and techniques applied to analyse them.

3.1 Subjects

The signals used in the study were collected at the Catholic University of Porto between October and December 2023. The university's ethics committee provided approval for the research. Thirteen participants, ranging in age from 20 to 47 years (average age 28.75 ± 9.27), contributed to the study. None of the participants reported any acute or chronic illnesses, particularly neurological conditions, brain injuries, or epilepsy. Only twelve participants (of thirteen) were included in the analysis, as the signals from participant 004 contained excessive noise in the channels of interest, making them unsuitable for analysis. The participant group consisted of seven males ($7/12 = 58.33\%$) and five females ($5/12 = 41.67\%$). All participants had completed high school, with seven holding bachelor's degrees ($7/12 = 58.33\%$), two possessing master's ($2/12 = 16.67\%$) and two doctoral degrees ($2/12 = 16.67\%$). Six of the participants had prior experience with lower-limb exoskeletons ($6/12 = 50.00\%$), and eleven had significant experience with virtual reality gaming ($11/12 = 91.67\%$). Eleven of the participants were right-handed ($11/12 = 91.67\%$), with one ambidextrous participant (both left- and right-handed). Recruitment was carried out through the research professional networks and all participants provided their informed consent prior to participating.

3.2 Experience Set Up and Procedure

The neural data acquisition setup utilized a 16-channel EEG system (actiCAP; Brain Products GmbH, Gilching, Germany) configured according to the 10-20 system, as shown in Figure 3.1. The recording system comprised electrodes, an amplifier (V-AMP, Brain Products GmbH, Gilching, Germany), an impedance box, and an electrolyte gel for signal conduction. Real-time neural data recording was conducted using BrainVision Recorder software (Version 2.2.1, Brain Products GmbH, Gilching, Germany).

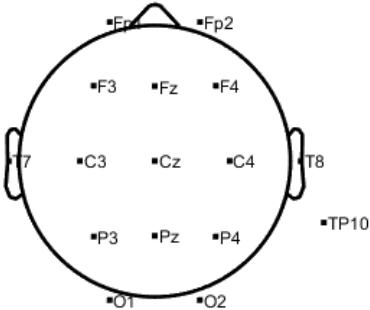


Figure 3.1: EEGLAB electrode placement according to the 10-20 system of 16 electrodes. Image by the author.

The VR system employed in the study was the HTC Vive Pro Eye, composed by a headset with integrated headphones, two handheld controllers, and a motion-tracking system [48].

Participants were immersed in a VR environment designed to replicate a realistic urban street setting, featuring houses on one side, a stone wall on the other, and a paved walkway. This environment was selected to provide a neutral yet engaging scenario for participants. The virtual avatar, depicted as a casually dressed male, exhibited a walking motion modelled after motion capture data from an ExoAtlet I user [49]. This ensured that the gait remained consistent across conditions, regardless of whether the avatar appeared to be wearing a lower-limb exoskeleton.

An adapted questionnaire was administered to evaluate participants' sense of embodiment [2]. This questionnaire assessed three key dimensions of embodiment - body ownership, sense of agency, and self-location - through a series of questions. Two additional items were included to explore participants' perception of avatar weight and their overall self-assessed embodiment.

Each experimental session was conducted with the presence of at least two investigators. Participants first provided informed consent and completed a demographic and background questionnaire. Each individual was assigned a unique identifier ranging from 001 to 013. Following this, the EEG cap was fitted, and electrolyte gel was applied to optimize signal quality. Impedance levels were maintained at $50\text{ k}\Omega$ to ensure a good electrical connection between the scalp and electrodes while minimizing noise and signal distortion. The EEG cap was connected to an amplifier, which was then linked to a computer running BrainVision Recorder. A plastic cover was placed over the EEG cap before the VR headset was positioned.

Participants were instructed to read a phrase displayed in the VR environment to verify headset fit and visual clarity. Signal quality was further assessed by instructing participants to blink, chew, and close their eyes momentarily. If these actions produced expected neural responses, participants were handed the VR controllers, representing their avatar's hands. The experiment then initiated.

During each trial, the virtual avatar continuously walked forward for three minutes. Participants remained stationary, facing forward or downward depending on the assigned condition, while minimizing extraneous movements to prevent EEG signal artifacts. The four conditions were presented in a rotating order across participants. This counterbalancing helped mitigate potential biases in neural recordings and embodiment responses by preventing learning effects.

In conditions where participants were instructed to look forward, they initially glanced downward for the first three steps to allow investigators to confirm the avatar's stepping pattern. When

the avatar appeared to be wearing an exoskeleton, auditory feedback mimicking the sound of the real ExoAtlet I in motion was incorporated to enhance immersion.

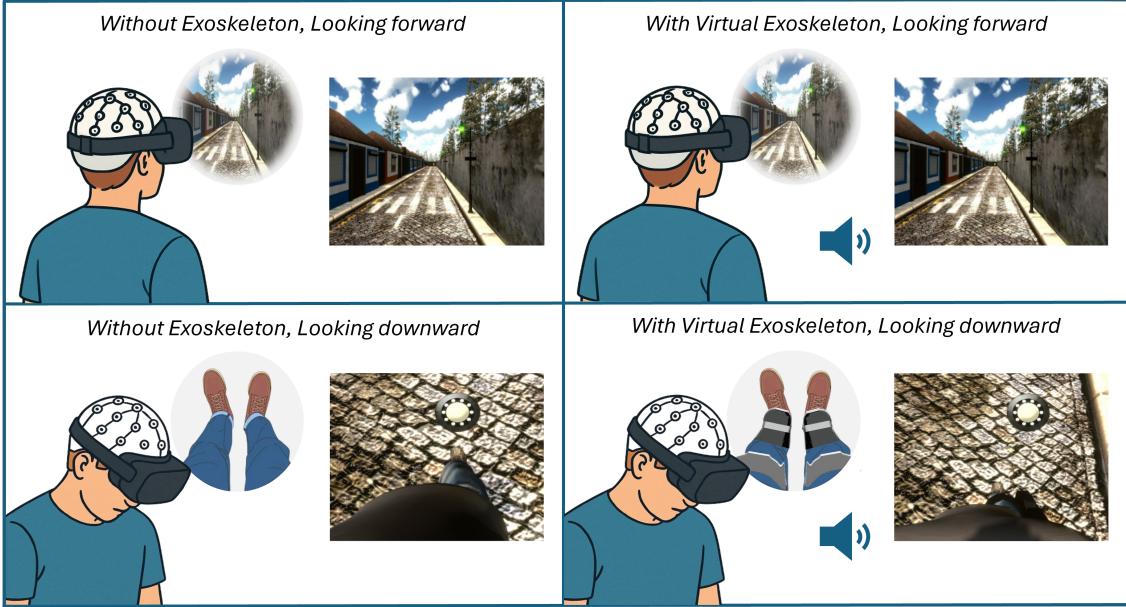


Figure 3.2: Experimental Conditions and Virtual Environment Representation: Corresponding first-person views of the virtual environment in each condition: looking forward or downward, with or without the exoskeleton. In conditions featuring the exoskeleton, a symbolic representation of auditory feedback indicates the simulated sound of the ExoAtlet I during motion. Image by the author.

Upon completing each task, participants removed the VR headset and completed the embodiment questionnaire. Afterwards, the headset was repositioned, signal quality was reassessed, and the next trial began. Once all four experimental conditions were completed - referred to as no exoskeleton, looking forward; no exoskeleton, looking downward; exoskeleton, looking forward; and exoskeleton, looking downward - (exoskeleton in the avatar), as seen in Figure 3.2, the EEG electrodes and the VR equipment were removed, marking the conclusion of the session.

3.3 Data Pre-Processing

The preprocessing of the recorded EEG data was conducted using the EEGLAB toolbox in MATLAB R2023a, a widely recognized tool for EEG signal analysis [11, 12]. A total of 52 datasets were processed, corresponding to four conditions per participant, with data collected from 13 individuals. Due to excessive noise in channels FP1, FP2, C3, and O2, along with minor disturbances in other electrodes, the dataset from Subject 004 had to be excluded from the analysis. Given that C3 is a critical electrode for this study, its compromised signal made the data unsuitable for further processing. Thus, the results focused on the analysis of signals from 12 subjects (001 to 013, except 004). The preprocessing pipeline followed a structured approach to ensure data quality and minimize artefacts, as shown in Figure 3.3.

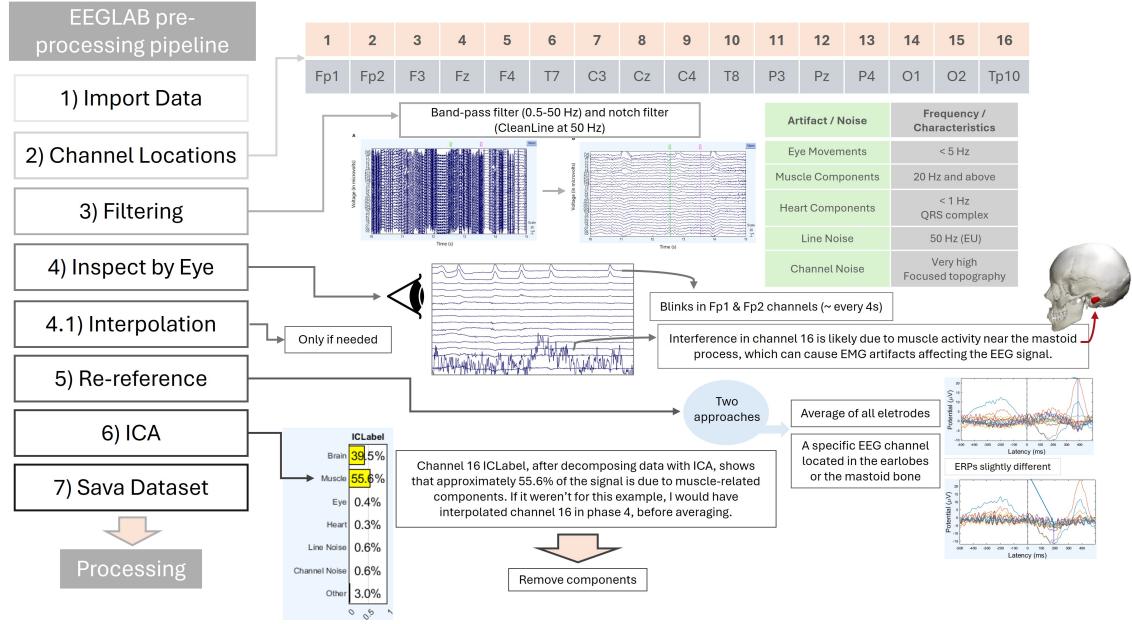


Figure 3.3: EEGLAB pre-processing pipeline: The image illustrates the EEGLAB pre-processing pipeline, which consists of seven phases. Image by the author.

The raw EEG data were imported into EEGLAB using the BIOSIG interface, allowing for seamless handling of the recorded signals. The channel location of the electrodes was employed to correctly define their positions.

To enhance signal quality and remove unwanted frequency components, a band-pass finite impulse response (FIR) filter (using the default settings in EEGLAB) was applied, restricting the frequency range between 0.5 Hz and 50 Hz. This filtering approach preserves physiologically relevant EEG activity while eliminating slow drifts and high-frequency noise [27,50]. Additionally, a CleanLine notch filter was implemented at 50 Hz to remove power line interference [27,50]. The use of FIR filtering ensured that phase distortions were minimized, preserving the integrity of neural oscillatory activity [27,50].

Following filtering, the data were visually inspected to identify channels with excessive noise, artefacts, or signal loss. Electrodes exhibiting significant disturbances were interpolated using EEGLAB's default spherical spline interpolation method to restore spatial consistency in the dataset. Only the electrodes that exhibited excessive noise or signal dropout were interpolated on a case-by-case basis for each EEG recording. This selective interpolation approach ensures that only necessary corrections are made while preserving as much of the original signal integrity as possible. Similar methodologies have been employed in previous studies to maintain data quality without introducing artificial distortions [7,50,51]. Interpolating only the affected electrodes helps to retain the spatial characteristics of the EEG signal while minimizing the impact on overall neural activity patterns, which is crucial for analyses such as Independent Component Analysis (ICA) and power spectral density estimation.

The EEG signals were then re-referenced using the common average reference method. This approach enhances signal quality by reducing common noise across electrodes, thereby improving the reliability of subsequent analyses [52].

To isolate and remove artefacts, ICA was applied. This technique decomposes the EEG signal into independent components that represent different sources. Components corresponding to non-neuronal sources, such as eye blinks, muscle activity, cardiac interference, and residual line noise, were identified and removed by excluding their contributions during signal reconstruction [52, 53]. The remaining components, representing brain activity and other non-artefactual signals that could not be confidently classified as noise (identified as 'Other'), were retained. After artefact removal, the preprocessed EEG data were saved for further analysis.

3.4 Data Processing and Exploratory Analysis

The processing stage involved the extraction of several features from the EEG signals, namely the power spectral density (PSD), standard deviation (STD), median, mean, maximum, and minimum values. Each electrode (C3 and C4) was analysed separately for each frequency band (delta, theta, alpha, beta, and gamma) and across the four experimental conditions. These electrodes are positioned over the left and right sensorimotor cortices, respectively, and are commonly investigated in EEG research due to their involvement in motor control, attentional modulation, and hemispheric asymmetries [54]. The EEG data were sampled at a frequency of 500 Hz. These features were computed using Welch's method (`pwelch`) in MATLAB with Hanning windows of 4 seconds length and 50% overlap—a widely used approach for estimating the power of a signal at different frequencies [55]. PSD represents the distribution of signal power across frequency components, providing insights into brain activity within specific frequency bands (e.g., delta, theta, alpha, beta, and gamma). The PSD of the C3 and C4 electrodes was estimated and expressed in $\mu\text{V}^2/\text{Hz}$. The extracted features can vary based on electrode location, age, and cognitive state, but they provide a general framework for comparison across conditions.

EEG signals are inherently non-stationary, meaning their spectral properties can vary over time due to changes in mental state and cognitive load. To account for this temporal variability, frequency-domain normalization over time is often employed [32]. This technique involves tracking how the relative power in different frequency bands evolves during the recording period, which is especially useful in studies involving prolonged tasks. The primary goal of frequency-domain normalisation over time is to reduce inter- and intra-subject variability by focusing on the relative distribution of power across frequency bands rather than on absolute power levels [32]. This is particularly advantageous when comparing EEG data across individuals or conditions where baseline amplitude differences may obscure true functional changes. So, to allow for meaningful comparisons across individuals and reduce inter-subject variability, frequency-domain normalisation was applied:

$$P_{\text{normalised}} = \frac{\sum_{f=f_1}^{f_2} P(f)}{\sum_{f=f_L}^{f_H} P(f)} \quad (3.1)$$

where $[f_L, f_H] = [0.5, 50]$ Hz defines the full frequency range considered, and $[f_1, f_2]$ corresponds to the limits of the selected frequency sub-band [32].

This form of normalisation also facilitates group-level statistical analyses and visualisation of frequency profiles across subjects, by putting all participants on a common scale [32]. Similar methods have been used in attention and working memory studies to monitor changes in alpha

and theta power throughout cognitive tasks [26].

3.5 Embodiment Questionnaire

To evaluate the sense of embodiment and perceived weight of the virtual legs across conditions, participants completed a 9-item embodiment questionnaire, a perceived weight rating, and a self-assessed embodiment classification. The embodiment questionnaire was adapted from previous work by the BMI Research Lab group [2], which in turn was originally based on the avatar embodiment questionnaire [8]. For the perceived weight measure, participants rated how heavy the virtual legs felt in each condition. To account for individual differences and the counterbalanced experimental design, the weight rating from each participant’s first condition was treated as a baseline (zero), and the ratings from the remaining conditions were interpreted relative to this initial reference.

To further analyse participants’ sense of embodiment, the scores from the 9-question questionnaire were combined into a single embodiment score per participant and per condition. The original response range from -3 to $+3$ was rescaled to a 1–7 scale to facilitate interpretation. Additionally, scores from negatively phrased questions (e.g., “I felt like the virtual legs were someone else’s”) were reversed so that higher values consistently reflected stronger embodiment. The resulting total embodiment score ranged from 9 to 63.

3.6 Statistical Analysis

Statistical analysis was conducted in MATLAB complemented with GraphPad Prism [12, 56].

To assess the effect of condition, a combination of non-parametric tests and ANOVA was employed. To compare PSD values between two conditions, the Wilcoxon matched-pairs signed-rank test was used, with each pair representing the same subject under both conditions. This test is a non-parametric alternative to the paired t-test, suitable when the assumption of normality is not met or when dealing with small sample sizes [57]. It evaluates whether the median difference between paired observations is zero and is commonly applied in EEG studies where PSD distributions may be skewed [57].

In addition, the difference between brain hemispheres was also evaluated using a C3–C4 relative power index. This index enables the quantification of hemispheric asymmetry, offering insights into lateralized cognitive processes and functional dominance across different frequency bands and experimental conditions [58].

To further examine the interaction between multiple conditions and frequency bands, a repeated-measures two-way ANOVA was applied. This parametric test enables the assessment of within-subject differences across multiple conditions [59]. ANOVA was chosen because it controls for individual variability and allows for the evaluation of both main effects and interaction effects between multiple factors simultaneously [60]. Although it requires the assumption of normally distributed data, it has been reported to be robust to moderate deviations from normality, particularly when sample sizes are approximately equal and contain at least 10–15 observations [60, 61]. In addition, a previous EEG study demonstrated that ANOVA can be applied to PSD data when variances are approximately equal across groups and the data are symmetrically distributed, even

if not perfectly normal [60, 62]. Moreover, [63] discussed the widespread use of ANOVA in EEG research, noting that it can be applied even when the assumption of normality is moderately violated, particularly when data are approximately symmetric and variances are homogeneous. Although formal normality tests were not conducted in this study, the features analysed (power spectral density values) are commonly treated as approximately normally distributed in EEG research. Based on this and prior evidence of ANOVA's robustness, the test was deemed appropriate for evaluating main effects and interactions between conditions.

In reporting the statistical outcomes, the Wilcoxon signed-rank test yields the test statistic W , representing the sum of signed ranks of differences between paired conditions. For the ANOVA, the F -statistic quantifies the ratio between explained and unexplained variance. The degrees of freedom (df) are presented as two values: the first corresponds to the effect of interest (e.g., condition), and the second to the residual error. For example, $df = 1, 44$ indicates 1 degree of freedom for the main effect and 44 for the residual error. p -values reflect the probability of observing the result under the null hypothesis, with $p < 0.05$ usually considered statistically significant. When p exceeds this threshold, results are marked as not significant (n.s.).

Given the number of comparisons involving both hemispheres and multiple frequency bands, a Bonferroni correction was applied to control for the increased risk of Type I error (false positives). As a result, the significance level for ANOVA analysis was adjusted, reflecting the division of the alpha level (threshold p for statistical significance) by the number of primary comparisons. This conservative approach ensures greater statistical rigour in the interpretation of condition and hemisphere effects. For the Wilcoxon tests, a standard threshold of $p < 0.05$ was maintained.

Despite the use of non-parametric statistical tests, such as the Wilcoxon signed-rank test, the mean and standard deviation of the PSD values are reported in Table 4.1 rather than the median and inter-quartile range. This choice was made to facilitate interpretation and comparison across conditions, especially given the exploratory nature of the study and the small sample size [64]. The mean provides a clearer representation of the central tendency when examining group-level trends, particularly for visual comparisons across multiple frequency bands and conditions [64]. In addition, the standard deviation offers insight into the spread and variability of the data, which is critical to understanding the individual differences [64]. Although medians can be more robust to outliers, the relatively homogeneous distribution of PSD values between subjects in this data set made the mean a suitable and informative descriptive measure.

Chapter 4

Results

The results section is divided into three parts: (i) the analysis of the PSD in commonly known frequency bands (delta, theta, alpha, beta, and gamma) under different experimental conditions and the statistical analysis of the data and (ii) the questionnaire results analysis.

4.1 Results

As indicated in the Methods section, PSD values were calculated in different frequency bands - delta, theta, alpha, beta, and gamma - providing a comprehensive measure of brain electrical activity. The four experimental conditions included the presence or absence of visual and auditory stimuli (exoskeleton) and the direction of gaze. Both factors are expected to modulate the brain's neural responses, and the PSD values provide insight into these variations.

Figure 4.1 illustrates the variability between two subjects PSD at electrode C4 under the four experimental conditions. These two individuals, participants 003 and 007, were specifically selected for comparison due to their similar demographic characteristics (both male and in their 40s), yet markedly different EEG spectral profiles.

The left panel shows bar graphs highlighting differences in spectral power profiles between participants. The right panel presents PSD continuous curves across frequency, with each line representing a different experimental condition. It provides a detailed overview of the PSD patterns at electrode C4, comparing both frequency band averages and full spectral profiles across participants and experimental conditions. The general shape of the PSD curve follows the expected pattern in EEG data, with a prominent peak in the delta range and a gradual decrease in power as frequency increases, consistent with the characteristic distribution of neural activity. However, beyond this general trend, the figure also highlights substantial intersubject variability, both in the overall power distribution and in the modulation of specific frequency bands by the experimental conditions. Participant 003 exhibits relatively low alpha power across all conditions when compared to participant 007, suggesting a distinct neural profile. Moreover, the condition-specific curves in the right-hand plots often overlap substantially, especially in participants like 007, indicating that, for some individuals, the experimental manipulations had a limited or subtle effect on the overall spectral shape. Additionally, participant 007 shows a pronounced peak in the alpha band, in contrast to the PSD spectrum of participant 003.

Intersubject Average PSD Graphic Comparison Between Subjects at Electrode C4

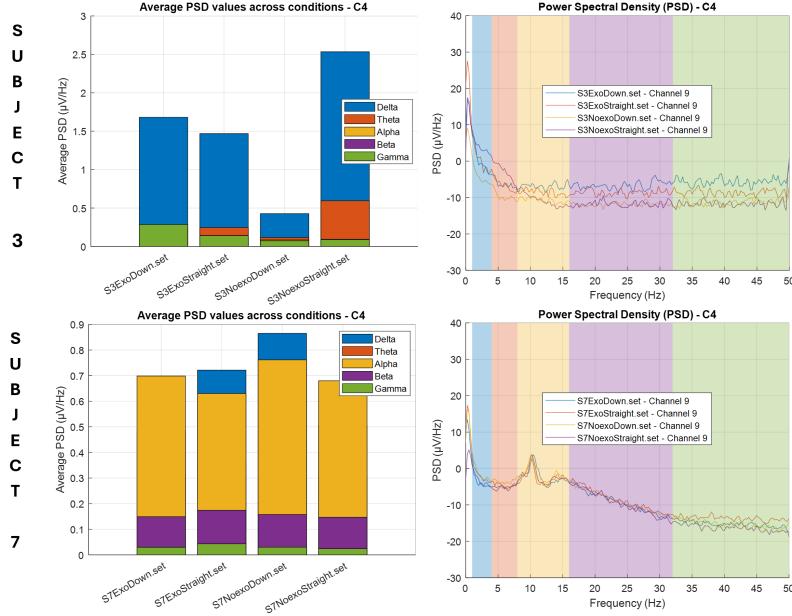


Figure 4.1: Intersubject average PSD comparison at electrode C4. Left: Bar graphs depict average PSD values within Delta, Theta, Alpha, Beta, and Gamma frequency bands for each subject under four experimental conditions (Virtual Exo and Looking Down, Virtual Exo and Looking Straight, No Exo and Looking Down, No Exo and Looking Straight). Right: Frequency spectra show PSD across the full frequency range (0–50 Hz). Image by the author.

Table 4.1 shows the average power spectral density values and standard deviations (expressed in $\mu\text{V}^2/\text{Hz}$) for the experimental conditions “No Exo” and “Virtual Exo” combined with gaze directions “Looking down” and “Looking straight.” The data are presented for electrodes C3 and C4 across five frequency bands. This table provides a detailed overview of the mean cortical electrical activity in each frequency band under the different conditions, allowing comparison of the effects of virtual exoskeleton use and gaze direction on brain activity.

Table 4.1: Average PSD and standard deviation values ($\mu\text{V}^2/\text{Hz}$) for the No Exo and Virtual Exo, Looking down and Looking straight conditions across Delta, Theta, Alpha, Beta and Gamma frequency bands.

Electrode	Condition 1	Condition 2	Delta	Theta	Alpha	Beta	Gamma
C3	Virtual Exo	Looking down	0.0291 ± 0.0115	0.0077 ± 0.0026	0.0058 ± 0.0029	0.0030 ± 0.0011	0.0017 ± 0.0009
	Virtual Exo	Looking straight	0.0282 ± 0.0119	0.0066 ± 0.0026	0.0056 ± 0.0027	0.0032 ± 0.0010	0.0020 ± 0.0011
	No Exo	Looking down	0.0250 ± 0.0093	0.0070 ± 0.0025	0.0062 ± 0.0030	0.0032 ± 0.0007	0.0023 ± 0.0019
	No Exo	Looking straight	0.0276 ± 0.0124	0.0066 ± 0.0020	0.0063 ± 0.0036	0.0030 ± 0.0010	0.0020 ± 0.0009
C4	Virtual Exo	Looking down	0.0280 ± 0.0096	0.0076 ± 0.0022	0.0064 ± 0.0032	0.0030 ± 0.0009	0.0017 ± 0.0010
	Virtual Exo	Looking straight	0.0261 ± 0.0109	0.0065 ± 0.0012	0.0061 ± 0.0027	0.0034 ± 0.0010	0.0020 ± 0.0011
	No Exo	Looking down	0.0245 ± 0.0076	0.0078 ± 0.0015	0.0065 ± 0.0027	0.0033 ± 0.0007	0.0020 ± 0.0013
	No Exo	Looking straight	0.0236 ± 0.0122	0.0072 ± 0.0017	0.0064 ± 0.0036	0.0034 ± 0.0012	0.0022 ± 0.0012

The C3-C4 relative power analysis did not yield statistically significant results across the sample. However, for the delta band, a comparison of gaze direction (looking down vs. straight) revealed a potential tendency toward increased C3–C4 asymmetry when participants looked down at their legs, in the absence of the exoskeleton ($\text{Median} \pm \text{IQR}(\text{Down}) = -0.0008 \pm -0.0041$; $\text{Median} \pm \text{IQR}(\text{Straight}) = 0.0014 \pm -0.0018$; $W = -44.00$; $p = 0.0923$; n.s.).

Similarly, individual Wilcoxon matched-pairs signed-rank tests conducted for each frequency band across C3 and C4 electrodes generally revealed non-significant differences. A near significant effect was observed in the theta band at electrode C4 ($\text{Median} \pm \text{IQR}(\text{Exo}) = 0.0074 \pm 0.0008$; $\text{Median} \pm \text{IQR}(\text{NoExo}) = 0.0081 \pm 0.0017$; $W = -48.00$; $p = 0.0652$; n.s.). When subject 001 – who was the only participant identified as ambidextrous – was excluded from the analysis, the Wilcoxon test for theta at C4 reached statistical significance ($\text{Median} \pm \text{IQR}(\text{Exo}) = 0.0074 \pm 0.0005$; $\text{Median} \pm \text{IQR}(\text{NoExo}) = 0.0083 \pm 0.0011$; $W = -60.00$; $p = 0.0087$).

Of note, in the theta band at the electrode C4, the two-way ANOVA revealed a significant effect of gaze ($F = 5.396$; $df = 1, 44$; $p = 0.0249$), specifically showing an increase in power when subjects were able to see their own legs, independent of exoskeleton use. This effect was even more robust after removing subject 001 ($F = 7.047$; $df = 1, 40$; $p = 0.0096$). These results suggest that visual perspective modulates theta activity in the right sensorimotor cortex. No significant differences were found for Interaction ($F = 0.6923$; $df = 1, 44$; $p = 0.4099$; n.s.) nor for Exo ($F = 0.1202$; $df = 1, 44$; $p = 0.7305$; n.s.).

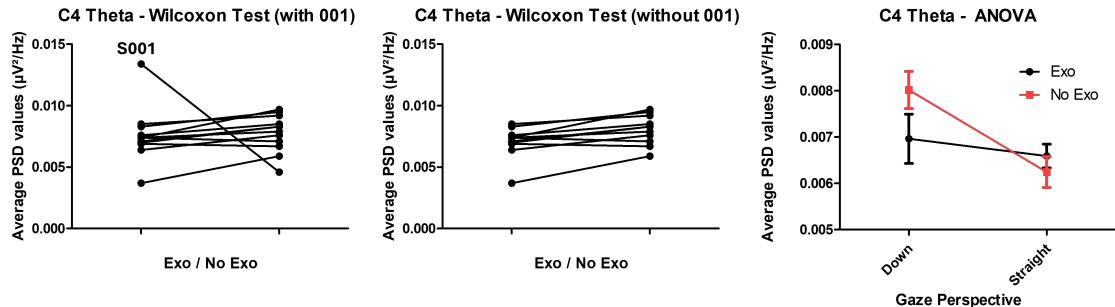


Figure 4.2: Left: The Wilcoxon matched-pairs test comparing theta power in Exo vs. No Exo conditions under downward gaze revealed an overall consistent increase in theta power, as indicated by individual participant lines trending upward — except for subject 001. Middle: Wilcoxon matched-pairs test comparing theta power in Exo vs. No Exo conditions after excluding the ambidextrous subject (001). Right: Results from a two-way ANOVA showing average theta PSD in electrode C4 for downward and straight-ahead gaze conditions, without subject 001.

The findings in the graphs shown in Figure 4.2 converge to highlight a robust modulation of theta band activity at electrode C4.

4.2 Embodiment Questionnaire and Perception of Weight

Figure 4.3 presents box plots illustrating the distribution of embodiment and weight scores across the four experimental conditions. This visualization provides a comparative overview of the central tendency and variability within each condition, facilitating an intuitive assessment of differences and trends in the data.

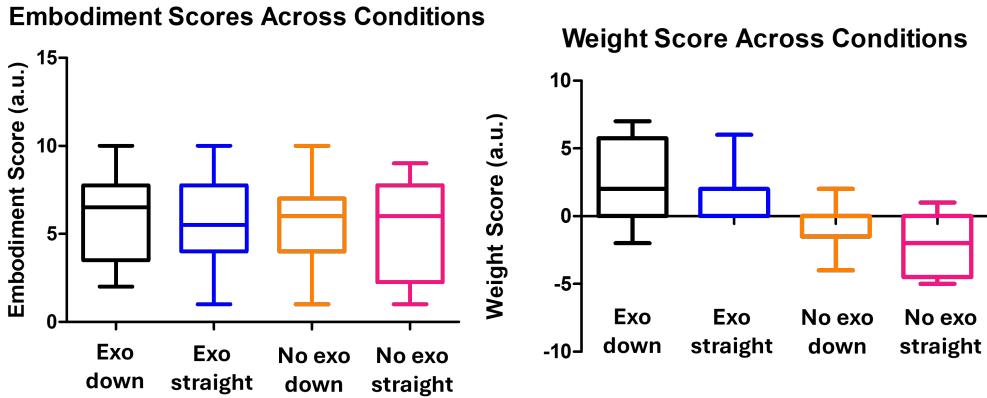


Figure 4.3: Left: Box plot showing self-assessed embodiment scores across the four experimental conditions. Right: Box plot showing weight scores across the four experimental conditions.

Descriptive statistics were calculated for the main outcome measures derived from the embodiment questionnaire. These include the mean score of the 9-item questionnaire (originally ranging from -3 to 3, rescaled to a 1 to 7 scale for interpretability), a single-item self-reported embodiment rating (a single-item score ranging from 1 to 10), and the perceived weight score (a single-item score ranging from -10 to 10). For each experimental condition, the minimum, maximum, mean, and standard deviation (STD) were computed. Table 4.2 summarizes these statistics. All results are presented in arbitrary units (a.u.).

Table 4.2: Descriptive statistics for embodiment and weight scores across the four experimental conditions

Condition	n	Min	Max	Mean	Std
<i>Mean Scores of the 9-item Questionnaire</i>					
Exo Down	12	1	7	4.194	1.613
Exo Straight	12	1	7	4.194	1.431
No Exo Down	12	1	7	4.296	1.433
No Exo Straight	12	1	7	4.185	1.473
<i>Embodiment Self-assessed Scores</i>					
Exo Down	12	2	10	6.083	2.597
Exo Straight	12	1	10	5.583	2.499
No Exo Down	12	1	10	5.333	2.461
No Exo Straight	12	1	9	5.167	2.703
<i>Weight Scores</i>					
Exo Down	12	-2	7	2.417	2.842
Exo Straight	12	0	6	1.250	2.046
No Exo Down	12	-4	2	-0.500	1.607
No Exo Straight	12	-5	1	-1.917	2.100

Participants' self-assessed embodiment scores, as reflected in Figure 4.3 (left) and summarized in Table 4.2, exhibited some variation across experimental conditions but generally clustered around mid-to-high values on the 1 to 10 scale. The highest mean embodiment rating was observed in the Exo Down condition (*Mean* = 6.08), followed by Exo Straight (*Mean* = 5.58), No

Exo Down (*Mean* = 5.33), and No Exo Straight (*Mean* = 5.17).

The two-way ANOVA revealed a highly significant effect of exoskeleton presence on weight perception ($F = 42.28$; $df = 1, 88$; $p < 0.0001$), and a non-significant effect of gaze direction ($F = 2.541$; $df = 3, 88$; $p = 0.0615$; n.s.). As for the interaction between exoskeleton presence and gaze direction, the results were not significant ($F = 0.02380$; $df = 3, 88$; $p = 0.9950$; n.s.).

Furthermore, a two-way ANOVA was conducted on the total embodiment scores (derived from the sum of the 9-question questionnaire), with gaze perspective and exoskeleton presence as within-subject factors. The results revealed no significant effect of gaze perspective ($F = 0.01063$; $df = 1, 44$; $p = 0.9184$; n.s.), no significant effect of exoskeleton presence ($F = 0.01530$; $df = 1, 44$; $p = 0.9021$; n.s.), and no significant interaction between this two factors ($F = 0.01530$; $df = 1, 44$; $p = 0.9021$; n.s.).

Chapter 5

Discussion

5.1 Spectral Power Analysis

Figure 4.1 reinforces the conclusion that EEG responses are not uniform and that participant variability plays a major role in shaping neural outcomes in immersive VR contexts. While group effects — such as the theta modulation observed at C4 — offer insights into shared neural mechanisms, this figure reminds that neural activity is highly variable across individuals.

The results, presented in Table 4.1, indicate that the effects of the experimental conditions (Virtual Exo vs. No Exo, and Looking Down vs. Looking Straight) on PSD values were not consistent across frequency bands and participants (though individual variability is not fully represented in the table). Specifically, while some participants exhibited increased PSD values — particularly in the Delta and Theta bands under the 'Looking Down with Virtual Exo' condition — others demonstrated either decreases or no significant changes. This variability was observed across both C3 and C4 electrode sites.

For example, in the Delta band, the Virtual Exo condition generally produced higher PSD values when participants were looking down with a virtual exoskeleton compared to looking straight with no exoskeleton. However, the differences were small and did not indicate a clear and consistent pattern across subjects. The Theta band exhibited similar trends, with slightly higher PSD values when participants were "Looking down" in both conditions, but again, the variations were not large.

In contrast, the Alpha, Beta, and Gamma bands indicated little to no variation between the conditions.

These findings, therefore, suggest that while some individuals did show an increase in PSD values in specific frequency bands, these effects were not consistent across all participants. These results emphasize the complexity of the neural response to experimental conditions and suggest that future research should explore the individual factors that might contribute to these differences in neural activity, such as cognitive state, neuroanatomical differences, level of engagement, fatigue or mental workload and sensitivity to external stimuli.

This study was based on a small sample size of only twelve subjects, which limits the generalizability of the findings. Given the relatively small group, the observed variability in PSD values across participants may not fully reflect broader trends that could emerge in a larger sample.

While the results provide some insights into the potential effects of the experimental conditions on brain activity, caution is needed when interpreting these findings. The variability observed in the data could be influenced by individual differences, which might be less pronounced in a larger and more diverse group. Therefore, further research with a larger sample size would be necessary to confirm these results and provide a more comprehensive understanding of how these conditions affect brain activity.

5.2 Embodiment Questionnaire and Perception of Weight

The relatively larger standard deviations within all conditions (2.5 to 2.7) indicate substantial individual differences in perceived embodiment, which is common in subjective measures of virtual body ownership and agency.

In addition, participants tended to report higher weight scores in the presence of exoskeleton (Exo conditions, especially Exo Down), with medians above zero and wider interquartile ranges, suggesting greater perceived heaviness when the virtual exoskeleton was present. This could indicate that the visual presence of an exoskeletal structure led participants to attribute more mass or effort to the avatar's movements, potentially due to associations with mechanical bulk or increased physical realism.

In contrast, the No Exo conditions (both down and straight) resulted in lower and more negative weight scores, indicating that participants perceived the avatar as lighter or less physically demanding when no augmentation was visible. Interestingly, No Exo Straight condition showed the lowest median weight estimation and the tightest distribution, which may reflect a diminished sense of embodiment or reduced cognitive association with physical load when both exoskeleton and downward gaze were absent.

Comparing embodiment scores to weight perception reveals a partially convergent pattern: conditions with the exoskeleton generally elicited higher weight estimations, while embodiment scores showed a more nuanced distribution. Specifically, the Exo Down condition yielded the highest embodiment scores, supporting the idea that the combination of visual augmentation and downward gaze strengthens the sense of body ownership. However, the No Exo Down condition displayed a slightly higher median embodiment score than Exo Straight, indicating that the absence of an exoskeleton does not necessarily diminish embodiment, especially when combined with a downward perspective. This suggests that gaze direction may also play a role in modulating embodiment independently of visual augmentation.

These findings imply that while the presence of an exoskeleton can enhance perceived weight and, in some cases, embodiment, other contextual cues — such as gaze perception — can also influence the subjective integration of the avatar's body. Thus, the relationship between embodiment and weight perception is not strictly linear, and different sensory or attentional factors may modulate each component independently.

The two-way ANOVA on weight perception scores confirmed these observations statistically. There is a highly significant main effect of exoskeleton presence ($F = 42.28; df = 1, 88; p < 0.0001$), showing that participants consistently rated weight perception higher when the virtual avatar wore the exoskeleton, regardless of gaze direction. The effect of gaze direction approached a tendency to significance but did not reach the conventional threshold. No significant interaction between

exoskeleton presence and gaze direction was found.

In contrast, the two-way ANOVA on total embodiment scores revealed no significant effects. Neither gaze perspective, exoskeleton presence, nor their interaction significantly influenced embodiment ratings. It is important to note that the embodiment questionnaire relies on subjective self-assessment, which may be influenced by individual interpretation and response styles. Such subjectivity can contribute to variability and inconclusiveness in the results, as reflected by the lack of statistically significant effects.

5.3 Statistical Analysis

The shift in statistical significance observed when subject 001 was excluded from the analysis underscores the potential impact of individual variability in handedness on hemispheric EEG patterns. Prior literature has documented that handedness can influence lateralization in EEG activity, particularly in tasks involving motor planning or attentional modulation, with ambidextrous individuals often showing reduced or atypical hemispheric asymmetries compared to right- or left-handed individuals [65]. Ambidextrous individuals are known to exhibit more bilateral or atypical patterns of brain lateralization, which may dilute or mask effects driven by more lateralized participants [65].

Moreover, the two-way ANOVA results for the theta band at electrode C4 indicate that visual perspective modulates theta activity in the right sensorimotor cortex, potentially reflecting spatial attention or postural embodiment effects—both of which are sensitive to orientation and vestibular input [66]. The significance of this effect across both models (with and without subject 001) strengthens its interpretation, while the enhancement of the effect following subject exclusion highlights the confounding influence of individual differences in small-sample EEG studies.

The Wilcoxon paired test comparing theta power at C4 between Exo and No Exo conditions under downward gaze showed a trend toward increased theta activity when the exoskeleton was absent. This pattern is visually evident in Figure 4.2 (left), where most individual subject lines trend upward from Exo to No Exo. When subject 001 was excluded, this trend reached statistical significance ($p = 0.0087$), further supporting the idea that the exoskeleton modulates cortical oscillations, but this modulation may be more complex than a simple linear effect.

In the ANOVA analysis, while the overall interaction effect did not reach statistical significance after correction for multiple comparisons, a trend emerged suggesting that theta power was influenced by both gaze direction and the presence of a virtual exoskeleton. Specifically, theta power was reduced during straight-ahead gaze compared to downward gaze, and this reduction was more pronounced when the virtual exoskeleton was not visible.

These findings demonstrate that theta band activity at electrode C4, corresponding to the right sensorimotor cortex, reflects condition-related effects, with statistically significant results emerging even when the ambidextrous participant is included. The persistence and enhancement of these effects across multiple statistical models reinforce the interpretation of a robust lateralization in theta oscillatory dynamics, suggesting that right-hemispheric sensorimotor regions may be particularly sensitive to visual perspective and embodiment-related cues in immersive virtual environments.

5.4 Conclusion on Hypotheses

Theta band activity at C4 showed some differences between conditions, with reduced power during straight-ahead gaze compared to downward gaze, particularly without the exoskeleton. The two-way ANOVA revealed a significant effect of visual perspective on theta activity at C4, suggesting that H4 is supported. The exoskeleton presence, on the contrary, was not supported, and thus H1 is not supported. Moreover, alpha and beta bands showed no significant variation across exoskeleton conditions (H2 and H3) or gaze directions (H5 and H6), and therefore these hypotheses are not supported.

Regarding interaction effects, theta band activity showed some condition-specific variation when both exoskeleton and gaze direction were considered (H7), though not statistically robust, suggesting partial support. No interaction effects were found in alpha or beta bands (H8 and H9 not supported).

In terms of subjective experience, embodiment scores were higher with the exoskeleton, especially in the downward gaze condition, but not significantly (H10 not supported). In contrast, perceived weight was consistently and significantly higher with the exoskeleton, particularly when looking down, strongly supporting H11.

In the context of studies on brain-machine interfaces and neural activity modulation in response to exoskeletons and gaze direction, the results of this study suggest that the presence of the exoskeleton and gaze direction may have a substantial effect on how the brain processes sensory and motor information, which is shown in the theta band in electrode C4. Previous research has demonstrated that exoskeletons can influence neural activity, particularly in sensorimotor regions [50, 67], and similar effects have been observed in studies examining gaze direction and its impact on attentional processes [67, 68]. The findings in this study align with these observations, although individual variability in responses was significant as the number of subjects was low, indicating that the neural mechanisms underlying these effects might be more complex than initially assumed.

5.5 Future Directions

Looking ahead, future research should aim to build on these findings by incorporating larger participant samples to better understand the factors influencing embodiment and motor processing in VR. Additionally, exploring other neural frequency bands, such as the mu rhythm, and investigating other potential influences such as cognitive states, sensory feedback, and user experience with exoskeletons and VR, could provide further insights. The use of more advanced neuroimaging techniques, along with a deeper exploration of the role of attention and motor control in the embodiment process, could help clarify the neural mechanisms underlying these effects. Moreover, adding more electrodes would allow analysis of larger brain networks, which is particularly important as neural patterns related to motor processing, control, and sensory integration are often distributed across multiple brain regions rather than localized to a single area.

Chapter 6

Conclusions

As BMIs and neurotechnologies evolve, their integration into neurorehabilitation and immersive VR environments presents promising opportunities. However, important questions remain regarding how virtual assistive features — such as exoskeletons — and gaze perspective impact users' sense of embodiment and underlying brain dynamics. This study investigated these effects by examining how gaze direction and the presence of a lower-limb virtual exoskeleton influenced perceived embodiment, weight attribution, and neural activity.

The most notable findings emerged in the theta frequency band at electrode C4, where gaze direction showed a statistically significant effect. These results suggest a meaningful role for right-hemisphere sensorimotor theta oscillations. The consistency and enhancement of these effects across multiple statistical models reinforce the interpretation of robust lateralization in theta activity, indicating that right-hemispheric sensorimotor regions may be particularly sensitive to visual perspective and embodiment-related cues in immersive virtual environments.

In contrast, no significant changes were found in the alpha and beta bands. Despite this, the findings emphasize the importance of considering individual variability in EEG responses and contribute to a more nuanced understanding of how immersive VR and distinct stimuli modulate brain activity.

These insights may contribute to the design of more effective VR-based neurorehabilitation tools, where visual perspective and virtual body augmentation are used to enhance sensorimotor engagement. However, the limited sample size and variability across participants suggest caution in generalizing the results. Future research should explore these effects with larger and more diverse populations, and investigate how long-term exposure to such virtual environments influences neuroplasticity and functional outcomes.

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