Faculdade de Engenharia da Universidade do Porto

Using EEG to evaluate the effects of horse assisted therapy in children with autism spectrum disorders: Definition of the methods and preliminary experiments with an ASD group

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June 2020

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Dissertation carried out within the scope of
Master in Biomedical Engineering

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June 2020

Resumo

Atualmente, o Perturbação do Espectro do Autismo (PEA) é considerado um distúrbio do neurodesenvolvimento caraterizado por *deficits* na comunicação e interação social, além de mudanças comportamentais. Um possível método de intervenção terapêutica para o desenvolvimento de capacidade nas áreas deficitárias de crianças com PEA é a terapia assistida por cavalos. Vários estudos têm demonstrado que a hipoterapia tem um impacto positivo no desenvolvimento dessas crianças; no entanto, o tipo de avaliação feita tem sido apenas qualitativa.

A recolha de dados é realizada de forma observacional do comportamento e estado de concentração da criança durante a terapia e também por meio de entrevistas e preenchimento de formulários sobre comunicação, interação social e comportamento das crianças. Portanto, o fato de os dados serem apenas qualitativos e subjetivos é uma limitação para avaliar a evolução do desempenho de cada criança durante a sessão de terapia.

O objetivo principal desta dissertação é, portanto, contribuir para a validação científica da terapia assistida por cavalos, enquanto método terapêutico para o desenvolvimento das crianças com PEA. Para tal foi construída uma metodologia baseada num sistema de estimulação visual associado à monitorização cerebral EEG (eletroencefalograma), como forma de avaliar parâmetros relacionados com a interação social (em termos de processamento de emoções) e atenção em crianças com PEA. Contudo, a escassez dos dados obtidos nesta tese permite apenas uma avaliação preliminar da metodologia desenvolvida.

Abstract

Autism Spectrum Disorder (ASD) is presently considered a neurodevelopmental disorder categorized by deficits in communication and social interaction, along with behavioural changes. A possible method of a therapeutic intervention for capacity development of children with ASD is horse-assisted therapy. Studies have shown that hippotherapy has a positive impact on the development of these children, however, the type of performed evaluation is merely qualitative.

Indeed, the data collection is performed in an observational way of the behaviour and state of concentration of the child during the therapy and also through interviews and filling of forms on the communication, social interaction and behaviour of the children. Therefore, the fact that the data are only qualitative and subjective is a limitation for assessing the evolution of the child along with the therapy.

The main objective of this dissertation is, therefore, to contribute to the scientific validation of horse-assisted therapy, as a therapeutic method for the development of children with ASD. To that end, a methodology was built based on a visual stimulation system associated with brain monitoring EEG (electroencephalogram), as a way of assessing parameters related to social interaction (in terms of processing emotions) and attention in children with ASD. However, the scarcity of the results obtained in this thesis allows only a preliminary assessment of the developed methodology.

Acknowledgements

First and foremost, I would like to thank my supervisor, Professor Carlos Fonseca[1], for his support. His guidance and insight were invaluable, and I am grateful for his ability to foster innovation and excitement in my work.

I would also like to thank my co-supervisor, Professor Fernando Barbosa[2] for making the Laboratory of Neuropsychophysiology of the Faculty of Psychology of the University of Porto an available place for my theoretical and practice in EEG acquisition and processing of EEG signal, as well I am grateful to his students Rita Passion[3] and Tiago Paiva[4] that help me gaining practice in the choice of the most adequate stimulus task and the best way of conducting my work.

I also have the pleasure to thank my second co-supervisor Professor Ramiro Fernandes[5] for his availability to help in all the things that are made in Vila Real and to the President António Alberto Machado[6] responsible for the Câmara Municipal de Vila Pouca de Aguiar and to Claudia Costa[7] responsible for the horse therapies, for establishing the protocol for the Horse-Assisted-Therapy.

Finally, I am very thankful to the responsible for the children schools, Mrs Director Elisabete Leite[8] and Mrs Director Marina Teixeira[9] for supporting my study with all the availability they could.

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Abbreviations, acronyms and symbols

ANOVA	Analysis of Variance
APA	American Psychiatric Association
APPDA	Associação Portuguesa de Perturbações do Desenvolvimento e Autismo
ASD	Autism Spectrum Disorder
BRIEF	Behaviour Rating Inventory of Executive Functions
CEUP	Committee of Ethics of the University of Porto
Df	Degrees of freedom
DSM	Diagnostic and Statistical Manual of Mental Disorders
EEG	Electroencephalogram
EF	Executive Functions
ERP	Event-Related-Potential
F	Ratio value of statistic tests
HAT	Horse-Assisted-Therapy
ICA	Independent Component Analysis
ID	Intellectual Disability
IDD	Intellectual Development Disorder
IDP	Intellectual Development Profile
IOS	Insistence of Sameness
IQ	Intelligence Quotient
N	Number of subjects
PCA	Principal Component Analysis
QEEG	Quantitative Electroencephalogram
RRB	Restrictive and Repetitive Behaviour
RSM	Repetitive Sensory Motor Action
Sig.	Significance value
TD	Typical Developmental~
VPP	Vertex Positive Potential
Z	Signed- ranks statistic

ε	Estimate value
Hz	Hertz
kΩ	Kilo-Ohms
ms	milliseconds
mV	millivolts
ρ	asymptotic ρ-values
x^2	Chi-Square value

Chapter 1

Introduction

1.1. Motivation

The integration of children with special needs in society and their personal and family well-being is a matter of great relevance and concern for society. In particular, Autism Spectrum Disorder (ASD) is presently considered a neurodevelopmental disorder characterized by deficits in communication and social interaction, along with behavioural changes. Being the target population of this study, children with ASD are faced with several limitations regarding the quality of life itself (communication deficits, unstable behaviour, negative responses to unknown environments). At the family level, in particular, the parents who are the main players in their daily lives are faced with intense family fatigue, lack of leisure time, future ambitions delayed and difficult working life. Also at the level of society, in school and education, they face barriers of learning and discrimination on the part of colleagues due to inappropriate behaviours.

A possible method of a therapeutic intervention for capacity development in the deficit areas of children with ASD is horse-assisted therapy (HAT). Studies have shown that hippotherapy has a positive impact on the development of these children [1], [2]. However, conclusions are based on qualitative evaluation. The data collection is done in an observational way of the behaviour and state of concentration of the child during the therapy and also through interviews and filling of forms on the communication, social interaction ability and behaviour of the children. Summing up, the fact that the data are only qualitative and subjective is a limitation for accurately assessing the performance and concentration of each child, and also an obstacle for taking full advantage of the hippotherapy potential.

1.2. Objective

The main objective of this dissertation is to define an appropriate methodology for the application of a brain monitoring system based in the EEG (electroencephalogram) to measure brain parameters representative of social interaction (in terms of emotion processing) and attention in children with ASD. The methodology will be applied to a small group of ASD children.

This preliminary study will pave the way for a more complete study, towards the full validation of a hippotherapy method. This will involve using the defined methodology to assess the evolution of the children along with the hippotherapy sessions. A larger group of ASD children will be used, together with a control group of age- and sex-matched children, so to ensure statistically valid results.

The proposed solution is thus an added value for professionals who accompany the children during the therapy. It is intended to develop a HAT focused on improving the abilities of children with ASD at the level of social interaction (emotion processing) and attention, with a positive impact on the future performance of individuals with ASD, both at the level of academic and professional integration, as well as social. In a later phase, it is expected that the hippotherapy could be optimized for best results with ASD children.

1.3. Document Overview

This Dissertation consists of four chapters organized as follows.

Chapter 1 provides the motivation for the work to be performed, it exploits the thematic background and justifies the attribution to the fields of biomedical engineering and neuroscience. The objectives of the dissertation beyond the state of the art are justified and highlighted.

Chapter 2 provides the theoretical background on the three main subjects with which this work is concerned: Autism Spectrum Disorder, the application of EEG in the study of ASD and the Horse-Assisted Therapy. Section 2.1 provides a theoretical background about the Autism Spectrum Disorder. Section 2.2 provides some theoretical overview of the Electroencephalography and Event-Related Potentials. Section 2.3 presents the theoretical background on the therapeutic benefits of Horse use in Autism Spectrum Disorders individuals.

Chapter 3 presents the methodology used in this work. Section 3.1 gives the description and selection criteria of the participants and section 3.2 details the recording hardware and software. Section 3.3 presents the stimulation protocol and section 3.4 explains the methods of data analysis used in this work.

Chapter 4 presents the results obtained in this work.

Chapter 5 provides the implications of these results and the limitations of this work.

Chapter 6 concludes the Dissertation as well as future directions for improvements, adaptation and application of the methods for the expected future work using this experimental protocol.

Chapter 2

Literature Review

2.1. Autism Spectrum Disorders

2.1.1. Origin and Historical Evolution of the Concept of Autism

The term autism originates from the Greek word "Autos", which means "self" in conjunction with the term "Ism", synonymous with orientation or state. Autism can be defined as a condition or state of mind of someone who is absorbed in him/herself, unmindful to what happens around him/her. It is then possible to say that an autistic individual is a person who lives only on his/her own as if in a completely isolated world, finding in him/herself all she/he needs to survive [3].

The first scientific work to describe the ASD clinical entity was introduced by Leo Kanner in 1943 with the creation of the designation Autistic disturbances of affective contact. Kanner characterizes a group of children with manifestations of marked social isolation, specific and differentiated from the behaviour observed in most children. This paediatrician has identified the inability to have affective contact with others, great difficulty in using language, anxiety, fears of commonplace misconduct, willingness to keep routine in the same way, and easy arousal for certain objects or topics [4].

In 1944 Hans Asperger identified a group of children, which he described as part of an entity he called Autistic Psychopathy characterized as academically and intellectually normal or above-average but with low and inadequate social interaction [5], being later called Asperger's Syndrome in recognition of his descriptions of these children.

Already in 1979, Wing and Gould completed an epidemiological study and concluded that there was an expanded group of children who did not fit the formal diagnosis of Autism, despite having some difficulty in social interaction, poor communication and lack of interest in activities, and described the concept of "Spectrum" [6].

In the Diagnostic and Statistical Manual of Mental Disorders (DSM) from the American Psychiatric Association (APA), in its Third Edition (DSM-III), the first criterion for the diagnosis of Autism appear with the description of three entities: Infantile Autism, Childhood Onset Pervasive Developmental Disorder and Atypical Pervasive Developmental Disorder [7].

The fourth version of DSM (DSM-IV) was an update of the previous criteria and in this version, Autism was characterized as one of the Global Developmental Disorders, which are subdivided into five types: Autistic Disorder, Rett's Disorder, Childhood Disintegrative Disorder, Asperger's Disorder, and Pervasive Developmental Disorder Not Otherwise Specified [8].

The Autistic Disorder was described by the presence of alterations in three dimensions: the qualitative deficit in social interaction, the communication and restricted and repetitive patterns of behaviours, the interests and the activities.

In 2013, in the fifth revision of the DSM (DSM-V), the current classification, there were significant changes in the definition of Autism, as the clinical entities referred to in DSM-IV are now integrated into the diagnosis of ASD, to overcome some diagnostic difficulties posed by the fact that the distinction between subtypes of Autism in DMS IV is in some cases difficult to establish [9].

2.1.2. Definition, Characteristics and Diagnosis of ASD

Autism Spectrum Disorder is presently considered a neurodevelopmental disorder categorized by deficits in communication and social interaction, along with behavioural changes. At the levels of communication and social interaction, changes are reflected into deficits in socio-emotional reciprocity, the use of nonverbal communicative behaviours (gestures, gaze, body language) and their integration with verbal communication to adjust social interaction. At the behavioural component level, ASD is characterized by repetitive and restricted behaviours, activities or interests that invasively interfere with the normal functioning of the subject [9].

The ASD is usually associated with other neurodevelopmental disorders, such as Intellectual Development Disorder (IDD), language and behaviour, specifically with Attention Deficit Hyperactivity Disorder. Its clinical manifestation arises at a very early age (generally before 2 years of age) and lasts throughout life. Proper diagnosis and recognition of disease are crucial in anticipating the course of its development and in choosing the most appropriate treatment.

On the one hand, this Autism Spectrum nomenclature makes it possible to overcome the difficulties that existed in differentiating sub-types. On the other hand, it puts the most severe and mildest cases of Autism in the same 'category'. It is therefore very important to specify the

level of severity existing in the domains of socialization/communication and repetitive behaviour, which is carried out through the diagnostic criteria for Autism Spectrum Disorder according to DSM V. These are further sub-divided in two large areas or dimensions [9]:

a) Persistent deficits in communication and social interaction in various contexts manifested by the following changes that occur in the present or have occurred in history: a deficit in social and emotional reciprocity; a deficit in nonverbal communicative behaviours used in social interaction; a deficit in establishing and maintaining relationships appropriate to their level of development;

b) Restricted and repetitive patterns of behaviour, interests, or activities manifested by at least two of the following changes that occur in the present or have occurred in past: speech, motor movements and repetitive or repetitive use of stereotyped; excessive resistance to change, excessive adherence to routines, or ritualized patterns of verbal and nonverbal behaviour; fixed and very restricted interests that are abnormal in intensity and focus; hyper or hypo-reactivity to sensory inputs or unusual interest in sensory aspects of the environment.

The succeeding conditions are also required:

c) Symptoms should be present at an early stage of development (but may not fully manifest until social demand exceeds their capabilities, or can be masked by strategies learned later in life);

d) Symptoms cause a significant clinical disturbance in occupational, social, or other important areas of current functioning;

e) These changes are not better explained by a disturbance of intellectual development or global retardation of psychomotor development. Disturbance of intellectual development and disturbance of the Autism spectrum regularly occur in comorbidity, and to make the diagnosis of ASD the media must be under expectations for the overall level of development.

Lastly, the following should as well be defined:

- Severity levels (DSM V criteria) for requirements (a) and (b);

And if there is:

- Comorbidity with disturbed intellectual development;
- Comorbidity with language disorder;
- Association with a medical or genetic condition or an environmental factor;
- Association with another neurodevelopmental disorder, mental or behavioural;
- Association with catatonia.

The severity levels for criteria a) and b) are related to the prerequisite to specify within the Autism Spectrum diagnosis which severity of symptoms happens and the degree of educational

support they need to have. Three severity levels are presented in the DSM V criteria. The quantifiers used by the DSM V criteria should be used to separately specify the level of support needed taking into account the clinical characteristics in the areas of socialization/communication and behaviour. However, these quantifiers should not be used to determine access to existing social, economic and school support. These will be used to better define the therapeutic plan as to the type and frequency of therapies the child needs to have, the intervention objectives to be defined and the need for pharmacological intervention, among other aspects. It should be noted that according to the DSM V proposal, all children who have had a well-established diagnosis according to DSM IV criteria of Autistic Disorder, Asperger's Disorder and Pervasive Developmental Disorder should be diagnosed with Autism Spectrum Disorder by DSM V criteria. Children with marked media deficits but whose symptoms do not meet the criteria for Autism Spectrum Disorder should be framed in the diagnosis of Media Disorder (Pragmatic) [9].

2.1.3. Aetiology

ASD is a neurodevelopmental disorder of multifactorial aetiology. It is widely recognized the contribution of interactions between genetic and environmental aspects to autism phenotypes, yet their specific contributing mechanisms persist badly understood.

Study of the ASD aetiology during the past 25 years emphasizes on genetic aspects, although, the awareness that environmental risks and protective factors are also impacting on the aetiology of ASD together directly and in interaction with genes is well known [10], [11]. All of these aspects are included in a broad and complex field with many current limitations[12].

Equally the timing and the nature of these impacts are critical and can be separated into:

- Preconception environmental risks- Parental age is positively correlated with the risk of ASD. Possible biological mechanisms associated with ageing [13], [14].
- Prenatal (during pregnancy) environmental risks [11], [15]; Maternal drug use [16]; Toxic chemicals [17]; Maternal severe obesity and other metabolic conditions/ hormonal regulation [18]; Maternal immune reactions [19]–[21].
- Prenatal protective factors - Folate supplementary to mother at the start of gestation [22].
- Perinatal risks- Several medical neonatal issues as well as perinatal asphyxia [23].
- Postnatal risks- Severe social scarcity [24];
- Neuroinflammation and encephalitis [10].

2.1.4. Epidemiology

There has been a growth in the incidence of ASD throughout the past 2 decades [25]. The primary reports of ASD manifestation described in 1966 [26] state an incidence of 4,5: 10 000 children aged between 8 and 10 years, whereas the current estimate is at least 1,5: 100 in developed countries [25]. The share with comorbid intellectual disability (ID) verifies a reduction, from about 85 % with intelligence quotient (IQ) < 80 in 1966 [26] to 30 % with ID in 2016 [27].

Increased manifestation of ASD cannot be certainly related to an "epidemic" of ASD due to the effects of changes in screening tools, diagnostic criteria, diagnostic awareness and society at both educational level and the availability of relief services significantly contribute to this increase [25], [28]–[30].

The issues with precision in the diagnostic assessments are also exposed to inexplicable incidence changes and, consequently, the differences in incidence are multi-causal.

Regardless of the considerations above, a true rise of the disorder cannot be ruled out [31].

2.1.5. ASD Neurodevelopmental Profiles

Neurodevelopmental disorders involve a vastly heterogeneous combination of impairments in cognition, communication, behaviour and motor functioning connected with unusual brain development [31].

2.1.5.1. The Social / Communicative Development Profile

The social-communicative intention is demonstrated by the look, gestures and vocalizations. In children with ASD, this purpose is compromised as well as their pre-linguistic skills and reveal very restricted communication patterns, using more communicative functions related to the satisfaction of their needs and protest. The social functions of communication (greeting, commenting and seeking attention) are more limited since their learning implies interaction and social reciprocity. The more severe the communication deficit, the later the language acquisition [31]–[33].

The expression of the social difficulties diverges, from impairments in joint attention and adverse gaze to faces in toddlers [34] to more delicate complications dealing complex social interactions that involve an understanding of other people [35]. Behaviours associated with social cognition intensely change throughout adolescence, paralleled by functional changes in the social areas of the brain [36].

Infants can recognize faces [37] and appears to be further developed due to interactions between the neurobiology of “social brain circuits” and social stimuli [38]. Instant processing of other’s emotions is a prerequisite to respond to other people (Social reciprocity), and studies applying pictures of facial emotional expressions suggest that anomalies in emotion recognition possibly will trigger some of the social issues related with ASD [39]. Some studies demonstrate that high functioning ASD have a normal capability to catalogue elementary facial emotions [40], but show difficulties in recognition of complex emotions [41].

Enhancement in emotion recognition is also seen in ASD development, but the evidence is less clear. Findings support the notion that both children and adolescents with ASD have difficulties recognizing emotions and that this capacity improves with age [42].

Outcomes from studies of face-emotion recognition are unreliable in ASD and the diverging results may be due to the type of paradigm used [43].

Social orienting is a requirement for social development, and the social motivation theory of ASD has freshly gained new interest [44]. The capability to quickly extract and understand emotions (emotional processing) influences social-emotional function and interpersonal reciprocity and thus social motivation [45]. Dissimilarity in disease severity and IQ may contribute to the distinct findings [43].

2.1.5.2. The Intellectual Development Profiles

The intellectual profile of children with ASD is specially categorized by sophisticated skills in the nonverbal cognitive component rather than the verbal cognitive component [46]. Studies found that the domains of intellectual profile that differ in ASD lower performance in relation to Typical Developmental (TD) groups refer to processing speed, divided attention and social understanding/judgment [46]. Categorical thinking was found to be the best performance domain in the ASD groups with an average intellectual profile (total IQ) and the domain that refers to the notion and construction of the whole was the best accomplishment domain for the ASD groups with a profile beneath the average.

The intellectual profile is a strong predictor of prognosis in ASD, so children with an Intellectual Disability Profile (IDP) will have a worse prognosis than children with an average intellectual development [47]. The intellectual profile with a discrepancy between the verbal and nonverbal components (Verbal IQ < Achievement IQ) is strongly associated with a greater deficit in social functioning.

2.1.5.3. Restrictive and Repetitive Behaviour

There are two distinct types of Restrictive and Repetitive Behaviour (RRB): Insistence of Sameness (IOS) and Repetitive Sensory Motor Action (RSM) [48]. RSM exhibit connection with

severity of ASD and intellectual function and remain stable or enhance with age and is less related to the level of language and intelligence. The use of RRB subgroups, principally IOS behaviours, may contribute to having more behaviourally homogeneous subgroups of children with ASD [49].

2.1.6. Executive Function in ASD

Executive function (EF) is referring to a set of higher-level cognitive skills that trigger independent, goal-oriented behaviour [50] needed to complete everyday activities. Higher-order EF is built on three fundamental EFs [51]: Inhibition (self-control/ behaviour inhibition and interference control (selective attention and cognitive inhibition); Working memory; Cognitive flexibility (set-shifting and mental flexibility).

EF is a problematic construct to measure, in part due to the extensive variety of complex interrelated regulatory functions involved. EF is broadly studied in populations of ASD with inconsistent findings [52].

Conventionally, performance founded neuropsychological testing is used for evaluation and is made in highly structured settings, where it is needed to be provided required executive control (planning, organizing, guiding and monitoring), which may be a boundary for the significance of the results for use in real life [52], [53].

The use of questionnaires to rate an individual's every-day real-world self-regulation functioning is an alternative to the testing of EF. One broadly applied instrument is the Behaviour Rating Inventory of Executive Functions (BRIEF) [54] with notable patterns of EF difficulties shown in ASD [55]. The metacognitive features of EF measured by the BRIEF are of great importance for social aptitudes in children and adolescents with ASD [56]. The classification of the scales made by an observer also has limitations influencing their validity [57].

There are huge differences between the result of performance-based test results and BRIEF, and this discrepancy highlights the importance of being conscious of the ecological validity of EF valuations [58]. Performance-based and rating scale measurements provide complementary information on EF [58].

2.1.7. Neurobiology

Understanding the basic neurobiological deficits of ASD is imperative and can form the basis in the development of innovative treatments. ASD is characterized by neurobiological changes that impede the normal functioning of the brain. Functional neuroimaging studies demonstrate that quite a few brain areas have divergent activation [59] with some areas of specific signifi-

cance to ASD. For example, the superior temporal sulcus, the orbital frontal cortex have revealed good correlations between symptom severity and brain structures [60]. The causal mechanisms of these deviants must be elucidated to develop better disease-specific interventions.

Quite a lot of studies support unusual brain development in ASD, both behavioural [61], electrophysiological [62] and neuroimaging [59]. Early neurodevelopmental disorders are reinforced by enlarged brain volume (and augmented head circumference) and dynamic, age-dependent patterns of uncharacteristic structural and functional connectivity [63], [64].

This underlines the importance of critical periods for effective improvements of brain regions and connections disposed of for non-standard development [65]. Studies from the first years of life have mostly described early perturbations to the formation of white matter neurocircuitry [59]. There are also studies reporting enlarged corpus callosum area and thickness in children with ASD beginning at 6 months of age, with diminishing variances by the age of 2 years [66]. Reduced volume of the corpus callosum is constantly found in older children and adults with ASD [67].

Neurobiological variations disturbing basic perception and gating of inputs and attention selectivity may disturb emphasizing and compiling of information, and this way brain development. Attentional processes offer a critical foundation for socio-communicative capabilities, and there are proposals of atypical Attentional Networks as one of the primary impairments associated with ASD [68]. The Salience Network is an intrinsic brain network supposed to modulate attention to internal contrasted with external stimuli and realize enlarged resting-state functional activity between salience network nodes and brain regions involved in primary sensory processing and attention connected with sensory over-responsivity.

2.2. Horse Assisted Therapy for ASD Individuals

The relationship between man and horse has a long-term tie. The value of the horse in man's life is recognized as a resource for a variety of activities: work, walking, sports and war. Horses were traced as detectors of minor changes in human body language, therefore providing a "mirror" for the participant to gain insight into his own psyche [69]. Also, at the physiological level, behavioural and evolutionary biology has shown that there are mechanisms and structures originating both social behaviours in humans and animals, empowering interspecies social relationships to develop, thus affecting human social behaviour [70].

Developing relationships with animals may give children positive interactions and support and provide physical and psychological benefits [71]. The natural attraction of animals engages children in animal-related activities, giving the child the opportunity for positive experiences that can generalize to other environments.

Efficacious interactions with horses provide significant sensory and social stimuli with benefits for psychological, motor, sensory, communication, and social functioning [72].

Therapeutic horse-riding can exercise benefits on emotional, social, and physical domains [71] and these benefits have been demonstrated for the rehabilitation of motor disorders and neurological diseases: the rhythmic equine movements transmitted on patient's body improves balance, coordination, muscle symmetry and posture [73].

Riding for therapeutic and pedagogical purposes is a way of adapted physical activity that uses the horse as a therapeutic tool. It is based on a multidisciplinary approach in health and education that leads to the biopsychosocial progress of people with varied disabilities, thus therapeutic riding can be an innovative rehabilitation practice for children with neurodevelopment disorders who commonly present a mixture of the motor, cognitive, and social disabilities.

In Horse-Assisted Therapy (HAT), the diversity and innovation of activities may engage children with ASD and increase their focussed activities. Finding activities that engage children with ASD in contact with others is essential. Studies have described an increase in social interactions linked to animal-assisted intervention, including horses [72].

Benefits of HAT for children with ASD have been identified, such as improved social motivation [74], scarcer stereotyped behaviours [75], and progresses in social communication and sensory processing during the intervention [76].

Children with ASD have sensory integration or modulation discrepancies [77]. Modulation permits filtering of unconnected stimuli and preservation of an ideal level of arousal that simplifies attention to environmental demands [79] with extended engagement in tasks. The horse's gait and speed can stimulate the vestibular system with either a calming effect through a quiet, constant gait or a warning effect through a fast walk or trot [78]. HAT may embrace on-horse activities that use movement to expand skills and off-horse activities that engender care and relationship building with the animal.

Outcomes of horse-assisted interventions for ASD population contain progresses in diverse areas of functioning known to be impaired in ASD, specifically increased social responsiveness and motivation, language/communication, as well as decreased problems behaviours and stress [76]. HAT has other benefits for ASD children, such as: gaining self-control and self-confidence, improving concentration (especially for those who have difficulty staying on task with activities) and improving socialization, increasing their connection and interaction with the surrounding environment [71], [74].

During HAT sessions the child is motivated to communicate with the therapist and with the horse. Non-verbal ASD children rapidly begin to speak when they are encouraged to use the horse's name or are requested to get the horse moving [71], [79].

Relevant enhancements were described in important domains: socialization, engagement, and shorter reaction time in problem-solving circumstances after having HAT sessions [79].

Child's self-confidence increases once they have formed a sense of competence by learning how to interact and work with their horse. HAT is not only a therapy for the autistic individuals, but has several benefits for children, adolescents and adults who have other intellectual or developmental disabilities [71], [79].

2.3. The Application of Electroencephalography in the Study of ASD

2.3.1. Electroencephalography (EEG)

Hans Berger pioneered the measurement of electrical potential fluctuations in the human scalp in 1924 and coined the term Electroencephalography [68]. The EEG acquires brain activity by measuring the electric potential distributions through the scalp. This potential differences between regions of the head are measured using biopotential sensors that transduce changes of the biopotential at the sensor position originating from ionic currents to an electron-based output signal that is further amplified and processed. The biopotential electrodes rely on direct electrically conductive contact with the biological tissue.

The ease and simplicity of the EEG procedure and its millisecond resolution of brain activity, together with standardized analysis, offer an opportunity for elaborate analysis of brain functions [80]. The typical amplitude range of EEG extends from 0.005 to 0.1 (mV) and the typical frequency range is from 0 to > 650 (Hz) [81].

In the most common gel-based EEG setups it is important to calculate and reduce electrode-skin impedance. Impedance level and particularly the homogeneity of impedance values across all electrode positions were reported to meaningfully affect the level of environmental interference and motion-induced noise [82].

Conventionally, clinical and neuropsychological standards posed a rigid requirement of electrode-skin impedances below 5 - 10 KΩ to minimize the related impedance effects [83]. Yet, state-of-the-art bio-signal amplifiers provide significantly increased dynamic input impedances up to several Giga-Ohm [81], considerably reducing the effect of electrode skin impedance level and homogeneity on the signal quality and noise [84] and thus enabling EEG without skin preparation as well as the use of dry electrodes exhibiting electrode-skin impedances up to the range of a several hundred KΩ.

EEG signal is characterized by the presence or absence of brain wave patterns and the common classification of these is based on the respective frequency band, which is represented in Table 2.1, as well as the associated physiological brain activity. Five main frequency bands can be considered: Delta, Theta, Alpha, Beta and Gamma within a frequency range of 1 to 100 Hz.

Table 2. 1 List of the most common frequency bands with the respective type, associated behavioural, neurotransmitters and locations. Table adapted from [85].

Type/Nomenclature	*Frequency (Hz)*	*Associated Behavioral /Psychological State*	*Neurotransmitter/Hormone*	*Location*
Delta	0-4	Deep rest; Dreamless sleep	Human Growth Hormone; Melatonin	Frontally in Adults; Posteriorly in Children
Theta	4-8	Deeply relaxed	Serotonin; Acetylcholine; Anti-cortisol; Endorphins; Human Growth Hormone;	Thalamic Region
Alpha	8-13	Day dream; calm	Serotonin; Endorphins; Acetylcholine	Posterior Regions
Beta	13-30	Alert; active thinking; anxiety; panic attack; focus; concentration	Adrenaline; Cortisol; Norepinephrine; Dopamine	Frontal and Parietal
Gamma	30-100	Combination of two senses	Serotonin; Endorphins	Somatosensory cortex

A reliable and stable electrode-skin interface is of paramount importance for reproducible signal quality and minimization of artefacts during bioelectric signal acquisition. The most commonly used parameter to assess and ensure enough electrode-skin contact is the interfacial impedance. In-vivo electrode-skin measurements are thus an essential step in the acquisition of EEG signals. Besides, there may be artefacts of different origins, such as bio-signals related to muscle activity, heart rate, and eye blinking are included in the same [86].

Brain complexity, importance and sensitivity are relevant reasons for studying its signals and the EEG has become one of the most used techniques because of its non-invasive nature, its good temporal and spatial resolution, and its low price, especially when compared with other brain imaging techniques. Furthermore, satisfactory signal acquisition does not necessarily require a special laboratory environment [87].

2.3.2. Event-Related Potentials

The Event-Related Potentials (ERPs) are the extracted neural responses from the general EEG associated with specific sensory, cognitive and motor events. The ERPs offers a direct and real-time index of neuronal activity on a millisecond scale as series of scalp-positive and scalp-negative voltage deflections, components that are strict time and phase-locked to the onset of a stimulus or an event.

ERP waveforms consist of a sequence of positive and negative voltage shifts, called peaks, waves, or components. P and N are traditionally used to indicate positive and negative peaks, respectively, and the number indicates the ordinal position of a peak in the signal wave as can be seen in Figure 2.1 (Not always representing the negative peaks up and the positive peaks down, it depends what is chosen by the researcher, in this work the N's and P's are inverted in relation to Figure 2.1). The peak amplitude is measured from the baseline and the latency of a

peak is the instant of time corresponding to the peak maximum amplitude relative to the onset of the stimulation.

ERP is a valuable tool for studying the neuronal activity generated during the processing of new information. With the analysis of ERPs, it is possible, through the exposure of an individual to certain stimuli, internal or external, to identify certain brain activities. Many research works have provided awareness of how ERPs relate to precise cognitive processes in the brain [88]. Three important categories of ERPs are the exogenous, the mesogenous and the endogenous [88]. The exogenous components (very low latency - below 50 ms) depend essentially on the physical properties of the stimulation, that is, its amplitude varies depending on parameters such as intensity, brightness and frequency. The so-called mesogenous (50-250 ms) are affected both by the physical properties of the stimulation and by the cognitive processes recruited to process that stimulus. However, they are always dependent on both the stimulus and the context of the task. Endogenous components (long latency - over 250 ms) are modulated by the cognitive processes allocated to stimulus processing (for example, attention modulates P300).

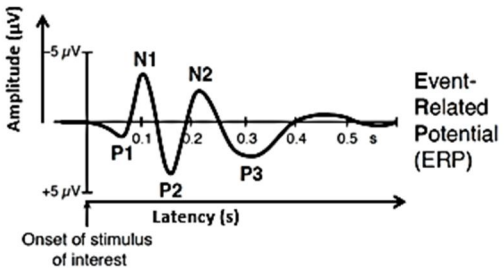


Figure 2. 1: ERP waveform representative of the averaged response to stimuli. Adapted from [89].

The earliest components are the only ones determined by the physical characteristics of the stimuli [90] and the impact of endogenous attention processes rises with post-stimulus time and is changed by attention when discrimination is required.

To see the brain's response to a stimulus, one must record many trials of a stimulus task and average the results together, so that the brain activity is averaged out and the ERPs are obtained. Noise may come from electromyographic or electrocardiographic signals, electromagnetic interference (such as that coming from fluorescent lamps) or patient-related movement. ERPs represent voltage variations extracted from the EEG and are associated with events (sensory, cognitive and motor) and comparing to the background EEG, this a low amplitude activity. The extraction of ERPs is made by averaging multiple repetitions of the same event. The activity related to the event is systematic and the activity related to noise + background EEG is random by reference to the event of interest and so is attenuated by averaging, increasing the signal to noise ratio of the recorded ERPs.

Because of the excellent time-locked resolution, ERPs can reproduce both low-level pathways associated with perception, but also higher-level cognitive processing. ERPs are extensively used to investigate neuronal activity through specific tasks in both healthy participants and subjects with neurodevelopmental and psychiatric disorders [91]. Current reports of relations between specific ERP-components and genes [92] are also promising.

The basic steps of an ERP experiment are EEG collection, artefact rejection and correction, filtering, ERP wave averaging, amplitude and latency quantification, statistical analysis. The analysis of ERPs is made through the grand average, that represents the average of all the subjects for all the electrodes.

2.3.3. Brain Potentials Related with Attention and Emotion Processing

Sensory processing is fundamental to an individual's skill to attend and respond to the environment. Uncommon responses to sensory input are among the most primitive identifiable features of ASD and frequently are observed before a child has received an official diagnosis of ASD [93]. Sensory processing and attention aptitudes often relate, impacting in a straight line a child's capability to sustain engagement in play, social, and academic activities [94]–[96].

Sensory processing can be defined as the adaptive responses/behaviours to sensory experiences, such as visual, auditory, proprioceptive, or vestibular stimuli [94]. Sensory processing problems may manifest as adaptive behaviours that affect daily living activities and communication [95].

Researchers found that hyper-responsivity to sensory stimuli was associated with unusual behaviour, over-focused attention, and exceptional memory; while hypo-responsivity was linked with reduced social skills and communication diminishing [94]. The differences between hypo and hyper-reactive feedbacks were associated with the symptomology of ASD.

Sensory processing can be a mechanism of attention, in that one must selectively attend to explicit/target stimuli while ignoring irrelevant/non-target information [97]. If one is incapable to organize sensory input or filter non-target information, attending to the task in question becomes difficult [98]. On the other hand, if one can respond properly to a task, one must be capable to adapt to the demands of the environment. This environmental adaptation involves the skill to adjust one's states of arousal, which can help to conclude if an individual is a hypo- or hyper-responsive to sensory stimuli.

Researchers have shown that individuals with ASD have explicit difficulties with aspects of attention and filtering out irrelevant sensory stimuli [99].

Many EEG tasks or paradigms are used to evaluate sensory processing and emotion processing such as auditory oddball, visual oddball, Go/No-Go tasks, among others [100], [101]. Furthermore, there are some variations in the use of novel laboratory paradigms (i.e., sensory challenge protocol and multisensory paradigm) and the experimental conditions (i.e., rest, stimulation, and recovery; stimulation only; rest and stimulation only) measured within such paradigms. The various types of paradigms are chosen based on the behavioural parameters

that are intended to be studied, and care must be taken to choose the most appropriate for the respective study. These are applied as a way to study the response of individuals to certain stimuli and can be applied to various parameters (attention, inhibitory control, processing of emotions, etc.).

The EEG paradigms of interest for this study are the oddball visual paradigm to assess attention, and the emotion processing paradigm, also used by many researchers to study attention and emotions in children with ASD [101]–[103]. Visual perception capabilities allow the processing of information by exploring stimuli in the visual field. These processes are essential to the development of quite a lot of specific visual skills, for example, attention, orientation, memory, and spatial imagery [104], [105]. The attention and cognition skills are essential for the development, allowing the individual to accomplish complex spatial tasks and to acquire academic skills. Attention processes may operate even at the early stages of information intake and influence stimulus processing at the larger stage [103]. Study of ERP components by means of Visual Oddball paradigm is a useful way of investigating task-relevant and irrelevant information processing stages as well as selective attention. The P3b is an ERP with a parietal scalp distribution and is the one chosen for analyzing in this study for the attention evaluation due to its validation in this kind of evaluation [103]. In an oddball task, the target stimuli elicit P3b, and it is interpreted as an index of ability to sustain attention to the target.

Individuals with ASD frequently show processing irregularities specific to facial expressions of emotion, including reduced and delayed N170 component in amplitude and latency, respectively, to emotional facial expressions in relation to TD individuals [106]–[108]. Behavioural studies evaluating recognition skills of emotional facial expression in ASD have revealed that individuals with ASD are not as good as at distinguishing emotion when the face stimuli are presented fast, and when the emotional expression is subtle [109].

Studying neurophysiological responses to an emotion processing task can offer significant information such as a task can evaluate potential differences in N170 amplitude and latency responses to angry and happy faces (with direct or averted gaze) across ASD and TD groups [110].

Neurophysiological responses are correlated to an emotional task (N170 component amplitudes and latencies) in individuals with ASD with age and behavioural scores of executive functions, autism traits, intelligence, and social competence [101]. The relevant parameters are the peak amplitude, peak latency and mean amplitude between two fixed latencies of face-sensitive ERP components (N170). These are observed in both infants and adults in response to visual stimuli including faces and it reflects an early stage of visual information on faces [111]. In the ASD literature, the N170 is among the most studied ERPs, is associated with behavioural deficits in face processing, and is considered among the most promising biomarkers of ASD [112], [113]. It should be noted that, although the ERP components that represent attention or the processing of emotions are generally accepted as well as the reporting guidelines, there is no consensus in the scientific community on the methodology to follow to draw conclusions by EEG on ASD. For example, for component N170 some authors [114]–[116] reported enhanced N170

amplitudes in response to averted gaze, other authors reported enhanced N170 amplitudes in response to direct gaze, [117], [118]; however, some authors find no gaze effect at all [119]–[121].

Some authors consider that the vertex positive potential (VPP) is another ERP component that manifests the same brain processes than the N170 component [122]. In fact, it is shown [122] that the amplitude of the N170 and VPP components diverges in an exactly inverse way across reference, the peaks of both components are temporally equal for all reference electrodes, the N170 and VPP components can be accounted for by equal dipolar conformation and show identical functional properties. Due to their value for the evaluation of emotion processing the N170 and the VPP components will be analysed in this work, in a way of comparing the significance of their responses.

2.3.4. Methodology and Data Analysis Guidelines

The need to communicate and validate experimental procedures, materials, and analytic tools in order to facilitate communication and data comparison between authors and readers as well as editors and reviewers resulted in the publication of guidelines to be followed by all players. To guarantee that this research work provides reliable and meaningful information, one will follow the Society for Psychophysiological Research guidelines and recommendations for studies using EEG [123].

The Society for Psychophysiological Research updated and expanded existing publication guidelines and recommendations for reporting in studies using EEG and to follow these guidelines ensures that researches provide key information. The guidelines (Checklist may be found in Annex A) are crucial and can be adapted to each study purpose.

In the statistical analysis, there are two possible ways of analyzing data, namely parametric and non-parametric methods. The application of parametric or non-parametric methods depends primarily on the metric or non-metric nature of the variables. For example, parametric methods with nominal variables are not applied, even if the samples have thousands of participants. Parametric data present a normal distribution such that the variable in question, when plotted, reveals a predictable and symmetrical bell-shaped graph, the so-called Gaussian distribution. Non-parametric tests, on the contrary, have orthodoxly been used for smaller datasets and/or non-normally distributed data [124].

A very popular parametric test is the One-way Repeated Measures Analysis of Variance – ANOVA. It is crucial to assume the degree of similarity of the variance of the differences between combinations of experimental conditions, and this is the assumption of sphericity. If the sphericity is violated the analysis will suffer an increasing probability of error and the statistic value of the test (F-ratio) cannot be related to tabularized values of F-distribution.

There is a test that evaluates if the condition of sphericity has been violated, the Mauchly's test. In case of this test be non-significant, i.e. if the significance value (sig.) is higher than 0.05 the sphericity is not violated, however, if this test is significant, i.e. if the significance value is inferior to 0.05, the assumption of sphericity is violated and a correction to the significance value needs to be performed.

There are three methods to make this correction, the Greenhouse-Geisser (1959), the Huynh and Feldt (1979) and the Lower Bound estimate (the lowest possible theoretical value for the data). If the estimated value (ε) in the Mauchly's test in the Greenhouse-Geisser correction is inferior to 0.75, then the correction method to use should be the Greenhouse-Geisser and if the estimated value is higher than 0.75 the Huynh and Feldt method should be used instead.

The non-parametric analysis equivalent to the repeated measures design (the case of the design of this study) is the Friedman's test [125]. This test has four assumptions that must be met to run the test. The assumptions are to have one group that is measured on three or more occasions the group is a random sample from the population, the dependent variable should be measured at the ordinal or continuous level and samples do not need to be normally distributed [126]. Friedman's test calculates the statistic (x^2) value ("Chi-square"). If the Asymp. Sig. value is higher than 0.05, then there are no significant differences between the conditions, and if this value is lower than 0.05 then the results present significant differences.

Both parametric and non-parametric analysis will be conducted in this study, being aware that the non-parametric analysis is more adequate for the study due to the small sample size and to give an output comparison of both statistical analysis methods.

In both analyses, if there are significant differences it is needed to run a posthoc test to know what conditions differ from each other significantly.

2.3.5. Electrode Montage and Nomenclature

The International Federation of Clinical Neurophysiology [127] adopted the standardization for EEG electrode placement called 10–20 electrode placement protocol. This protocol standardized the physical placements and nomenclature of 21 electrodes on the scalp. With reference points on the skull in the nasion, preauricular points and inion (Figure 2.2 b)), the head is divided into proportional positions to provide suitable coverage of all the brain areas. The division method is based on measuring the distances between Nasion and Inion as well as the preauricular points, dividing them into relative sections of 10 % (at the beginning and end of each line) and 20 % as shown in Figure 2.2 a) correspondingly in sagittal and topographic perspective. Electrodes are positioned at each of the 20 %-line intersections.

The designation of each electrode consists of a letter and a number. The letter denotes the region of the brain where the electrode is positioned (F: frontal, C: central, T: temporal, P:

posterior, and O: occipital), and the number is associated to the cerebral hemisphere (even numbers in the right hemisphere, and odd numbers in the left; Figure 2.2 b)) [128].

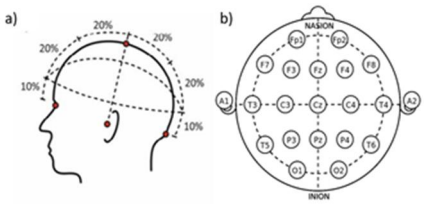


Figure 2. 2: Electrodes Montage based on the 10-20 system: a) Construction principle based on head marks. Adapted from [87]; b) Standard montage of the 21-channel EEG, Adapted from [128].

Cap systems allow for rapid application and reproducible positioning of different electrode types without per-electrode adaptation [87].

The head caps commonly used in the acquisition of EEG for gel-based electrodes are shown in Figure 2.3.

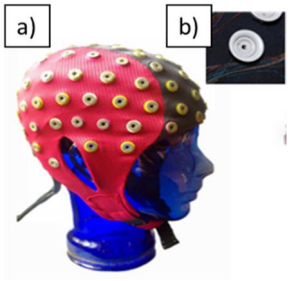


Figure 2. 3: a) EEG head cap with electrodes “already-assembled”; b) Gel-based electrode. Adapted from [129].

2.4. Perspective on the present work

Researchers have demonstrated that HAT is engaging to children with ASD, with the suggestion that HAT can help build attentional and social interaction skills [71], [77], [79]. Even though initial evidence supports the use of HAT for improving attentional skills in children with ASD, there are no known studies studying the impact of an individualized HAT, in a quantitative objectively way, on attentional and emotion processing abilities in children with ASD.

Thus, the further purpose of this study is to establish a test protocol and to collect preliminary EEG according to the established protocol. If the established experimental protocol allows to identify the expected emotion processing and attention parameters in children with ASD then this neurophysiological process may have the potential to be an indicator of treatment impact.

The research questions linked to the potential benefits of this study are:

1. Will EEG measures demonstrate adequate responses in baseline measures of attention and emotion processing in children with ASD to warrant future studies?
2. Will the preliminary experimental protocol be a valid proof of concept so that the established brain monitoring system allows objectively measuring the evolution of children with ASD, in terms of social interaction and attention, during horse assisted therapies?

Chapter 3

Methods

3.1. Participants

The protocol was tested with six children with ASD (1 female) with a mean age of 8.667 years (*SD* = 2.867). Initially, the Portuguese Association for Development Disorders and Autism (APPDA) in Vila Real (where children already have a medical diagnosis of ASD) was contacted, followed by the groupings of schools in the municipality of Vila Real (also considering children with a medical diagnosis of ASD). In all institutions, informative lectures on the study were given, receiving positive feedback from the children's parents, who showed great interest in their children's participation in the study.

The inclusion criterion for participants was having been diagnosed with ASD according to the clinician responsible for their medical history. All diagnoses of ASD were based on the DSM-V according to the clinician responsible for their medical history. The exclusion criteria were: (a) patients with clinical convulsive disorders or EEG reading results suggestive of an active seizure disorder or epileptic encephalopathy (note: patients with occasional EEG spikes were not excluded); (b) doubt expressed by the clinician regarding the diagnosis of ASD; (c) Significant primary sensory disorders, for example, blindness and/or deafness; (d) other concomitant neurological disease processes that may induce alteration of the EEG, for example, hydrocephalus or hemiparesis.

The parents/guardians of the engaged children signed an informed consent previously approved by the Commission of Ethics of the University of Porto (CEUP) (see annexe B) and the Data Protection Commission of the University of Porto (see annexe C).

The protocol designed in this study indicates that the participants with ASD tolerated both preparation and brain testing measures. The chosen environment let the children comfortable and relaxed during the head-cap and gel placing at the electrode sites, the explanation of the tasks procedure and to the completion of the tasks, with exception of two children, one that did not complete the Oddball task and the other did not complete the emotion processing task. A few participants showed initial difficulty to understand how to perform the tasks.

To provide the best environment to the participants, the presence of someone of trust (parents or teachers) was allowed. Moreover, breaks were provided to be attentive to the children needs, engagement and behaviour and in the gel placing it was allowed to the children to be in their parent's or teacher's lap or even to have a tablet, phone to help to relax them. All children tolerated the gel and the gel placing.

3.2. Recording Characteristics and Instruments

3.2.1. Equipment and Specifications

The eego™ system consists of the eego amplifier (eego™ sports 32 pro) and the eego™ software (ANT Neuro, Netherlands). The eego system is typically used in combination with a CE certified, commercially available waveguard cap, that is the one to be used in the signal acquisition. This cap has an active shielding system, allowing to record good-quality EEG in conditions where conventional systems would not ensure a satisfactory signal. Thus, recordings can be done in nearly any environment on nearly any subject. Incorporates an up to 32 of referential DC input channels and a parallel trigger input channel, accessible through high-density connectors. Impedance values can be measured for all electrodes, including the reference and patient ground electrode [129].

The software is part of the Class-IIa medically certified eego system (European Medical Device Directive). All the specifications on the eego™ system can be seen in Annex D.

The waveguard cap is the Waveguard™ Original EEG cap which has 18 (16+2) Ag/AgCl electrodes (Fp1, Fp2, F3, Fz, F4, C3, Cz, C4, P7, P3, Pz, P4, P8, O1, Oz, O8 + ground and Fpz reference). The Waveguard™ Original EEG has different sizes, contributing to fit the subject's head, the size used in this study was M (51-56 cm). Conventional full – head electrode positioning systems are commonly specifically designed cap systems, in which the electrodes are "already assembled". Cap systems implementing electrode numbers up to 256 channels are commercially available.

This head cap is composed of gel-based electrodes. The contact among the Waveguard™ Original electrodes and the subject's head is made by using a conductive gel that is applied with a syringe with a blunt needle.

3.2.2. Electrophysiological Recordings

The EEG data was acquired according to the international 10-20 system using the Waveguard™ Original EEG cap. The conductive gel used was the Gel G005 of high conductivity, 2019© FIAB SpA.

EEG data were sampled at 512 Hz and filtered using a 0.1 – 30 Hz band-pass filter. Impedances were kept under 20 KΩ. The ground electrode was included in the EEG cap as well as the reference electrode (Fpz reference).

3.3. Stimuli Procedures

In all experiments, individuals were asked to sit still in a chair. The task was programmed using E-prime experimental control system (Psychology Software Tools Inc., Sharpsburg, PA, USA).

3.3.1. Visual Oddball Attention Task

An adapted Visual Oddball Task was used as an assessment of selective attention. For the task, subjects were instructed to respond to stimuli bomb images (Figure 3.1), sequentially presented on the screen after a fixation point (variable between 600-1000 ms). One of the stimuli (Figure 3.1 a)) was presented on 75% of the trials (frequent stimuli) and the other stimuli (Figure 3.1 b) was presented on the remaining 25% of the trials and represent the rare stimuli (the target). Participants were told to press the SPACE key on the keyboard when they see the target image on the screen. Each stimulus was presented (in a randomized way) for 1000 ms and the task was divided into two blocks, each one with 100 trials, and a variable break between. The task trial progression is shown in Figure 3.2.

Figure 3. 1: Sample images of both a) frequent and b) rare stimuli.

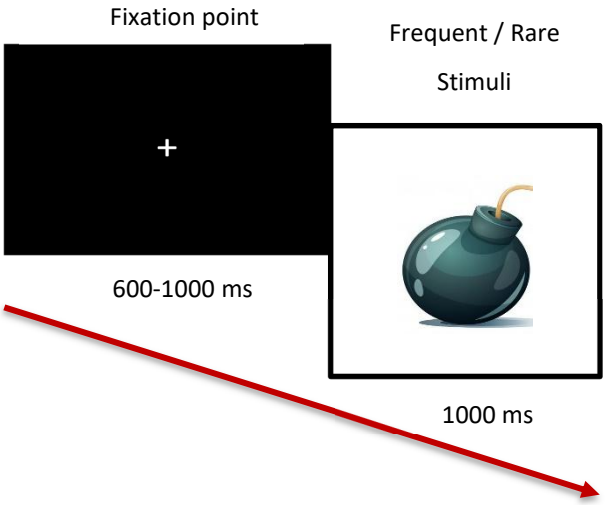


Figure 3. 2: Sample trial progression in the Oddball Task.

3.3.2. Emotion Processing Task

EEG measurements were recorded during a computerized emotion processing task. The stimuli were facial expressions of emotion (joy/neutral/sad and direct /averted gaze), mugs, houses and butterflies (used as targets to kept the children focused on the task, but have no study interest) and were sequentially presented in the centre of a computer screen after a fixation point, in a randomized way. Both mugs and houses were chosen because they are as familiar as faces and in addition to the familiarity factor, houses also have a structure like faces. Each stimulus (1000 ms) was preceded by a fixation point (600 ms). The relevance of face processing [106] and eye gaze processing in the social interaction is undoubted [130], [131] and particularly for eye gaze avoidance and social loss in ASD [132], [133]. The research related to eye gaze processing is not consensual and that is due to the variation of the experimental tasks in the studies [134]. Indeed, the type of experimental task the subjects are asked to complete is one of the most important factors for eye gaze processing. To this end, in this study, it was

investigated how viewing faces with direct or averted gaze affects the participant's performance, during an adapted emotion task to evaluate both the effect of different eye gaze and different emotions.

The task was composed of three blocks and stimuli were presented with equal probability in each block. Each block consisted of 10 random images of each stimulus (mugs, houses, face expressing joy with direct/forward gaze (FJF)), face expressing joy with averted gaze (FJA), neutral face with direct/forward gaze (FNF), neutral face with averted gaze (FNA), face expressing sad with direct/forward gaze (FSF) and face expressing sad with averted gaze (FSA)) and 20 randomly images of the butterflies stimuli to keep the children focused. Subjects received breaks (with the time they need) between the three blocks (each block with 100 trials). Sample images of the used stimuli can be seen in Figure 3.3 and a sample of the trial sequence in this task is represented in Figure 3.4.

Figure 3. 3: Sample images of the stimuli butterflies, houses, mugs and one individual with sad, neutral and joy expressions displaying direct or averted gaze.

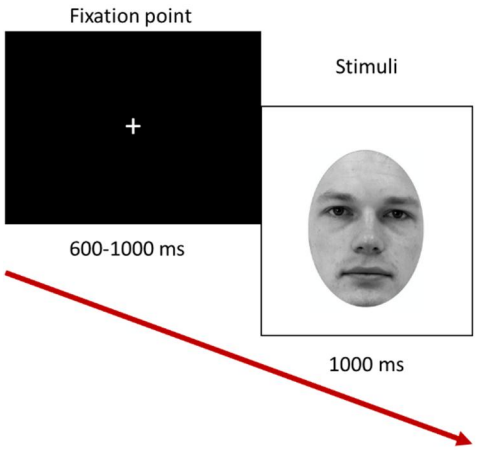


Figure 3. 4: Sample trial progression in with a neutral, direct gaze human face trial in the Emotion Processing task.

The stimuli database was adapted from [135] for mugs, from [136] for houses and both of them were used as valid datasets for research [135], [137].

3.4. Data analysis

The guidelines from the Society for Psychophysiological Research were used [123] to ensure that this research provides meaningful information, as it was described in the Literature Review.

Raw EEG data was pre-processed according to standard procedures using EEGLAB and MATLAB. Continuous EEG recordings were down-sampled to 250 Hz. A band-pass filter 0.1–30 Hz was used and the EEG data was re-referenced into electrodes P7 and P8 (considered the mastoids of this study EEG montage). In the Visual Oddball Attention Task and to an average reference (since the P7 and P8 are the electrodes of interest to the analysis and could not be the references) in the Emotion Processing Task. Records were then segmented in epochs of 1000 ms (200 ms baseline), time-locked to the stimuli. Epochs contaminated by artefacts were manually excluded. Artefact-free epochs were baseline corrected and averaged for ERP responses. The average of accepted epochs was 219 per subject representing 73% of the total epochs in the Emotion processing task, and in the Oddball visual task, it was 143 per subject representing 72% of the total epochs. A p-value/significance value inferior to 0,05 was considered statistically significant in the following analysis.

3.4.1. Event-Related Potentials

The selection of the electrodes of interest was based on previous research showing that the attention ERP components are found over posterior centro-parietal channels, with the P3b component being a midline component, [103] and emotion processing ERP components are found over occipito-central channels [138]. Scalp regions of interest were identified both by visual inspection of grand average and individual data and also corresponded to sites commonly analysed in other attention and emotion processing ERP studies [103], [107], [139], [140].

The peak amplitude, the peak latency and the mean amplitude of the ERP datasets from each subject were calculated, as well as all the studied conditions trials separately for the two stimuli tasks at electrodes Cz and Pz for the P3b component, at electrodes P7 and P8 for the N170 component, and at electrode Fz for the VPP component. P3b latency windows of maximal amplitude were calculated at 200-400 ms, N170 were calculated at 220-320 ms, and VPP were calculated at 150-250 ms. All the chosen time windows were based on the morphology of the obtained ERP waves to calculate the peak amplitude, the peak latency and the mean amplitude of the ERP datasets.

3.4.2. Statistical Analysis

The methods of statistical analysis for both tasks were the One-Way Repeated Measures Analysis of Variance – ANOVA and the Friedman analysis using IBM SPSS 26.0 statistical package, analysing the peak amplitude, peak latency and mean amplitude between two fixed latencies (i.e. in a predefined time-window) at the within-subject level. Each ERP component was analysed for pre-selected channels of interest and time window.

For the ANOVA analysis, the Mauchly's test should be applied to know if the condition of sphericity is met, but due to the small sample size it was not applied, and it was assumed that the sphericity condition is met in all the cases. Also, considering the small sample size, statistical power does not allow to draw robust conclusions. On the other hand, for the Friedman test, the k-related samples model was applied.

The ANOVA and the Friedman design for all dependent ERP variables in the Visual Oddball task for the analysis of the P3b component included within-subject factors Condition (Frequent, Rare) and Electrode (Cz, Pz).

In the Emotion Processing task, for the N170 analysis, both types of statistical analysis were employed with Condition (house, mug, FJA, FNA, FSA, FJF, FNF, FSF) and Electrode (P7, P8) as within-subject factors. For the VPP analysis, the same approach was employed but only with the Condition as a within-subject factor since the analysis was made just in electrode Fz.

Posthoc were applied for any significant results obtained in both methods of statistical analysis.

Chapter 4

Results

4.1. Experimental Results

4.2.1. Visual Oddball Attention Task

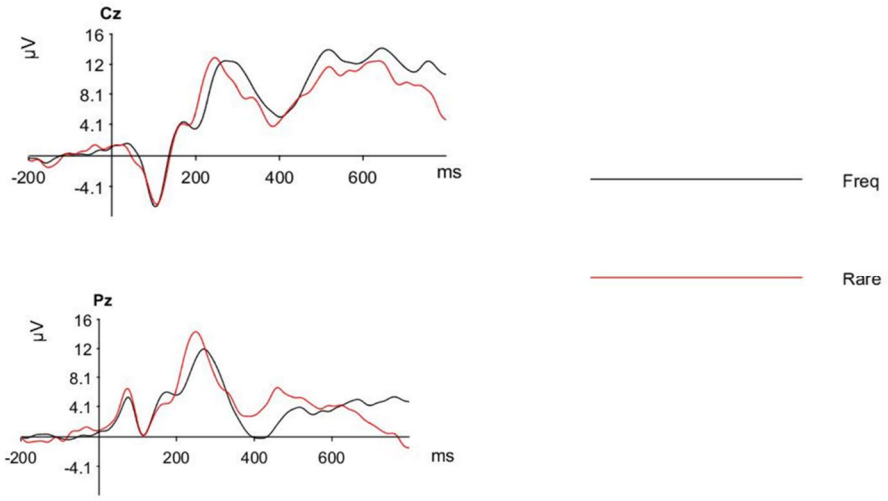


Figure 4. 1. Grand average ERP waves for electrode Cz (above) and Pz (bellow) to Frequent and Rare conditions.

In Figure 4.1 are represented the ERP waves, where the P3b component is noticeable, of the grand average across all ASD participants for the electrodes of interest, Cz and Pz.

4.2.1.1. P3b Peak Amplitude

The results of the repeated measures ANOVA show that there was no significant effect of the Condition type (Frequent, Rare), *F (1, 4) = 0.04, p= .861*, neither of the Electrode site (Cz, Pz), *F*(1, 4) = 0.05, *p* = *.829*, or even of the interaction between the Condition and Electrode, *F*(1, 4) = 1.46, *p* = .294, in the P3b peak amplitude of the ASD participants.

For the Friedman test there was no statistically significant difference in the P3b peak amplitude for the Condition type for electrode Cz, $x^2(1) = 0.20$, *p* = .655, neither for the electrode Pz, $x^2(1) = 1.80$, *p* = .180.

4.2.1.2. P3b Peak Latency

The results of the repeated measures ANOVA demonstrated that there was no significant effect of the Condition type (Frequent, Rare) *F (1, 4) = 2.71, p=.175*, neither of the Electrode site (Cz, Pz) *F (1, 4) = 3.512, p=.134* or even of the interaction between the Condition and Electrode *F (1, 4) = 1.23, p=.331 i*n the P3b peak latency of the ASD participants.

For the Friedman test there was no statistically significant difference in the P3b peak latency for the Condition type for electrode Cz $x^2(1) = 0.20$, p = .655 neither for the electrode Pz $x^2(1) = 1.80$, p = .180.

4.2.1.3. P3b Mean Amplitude

The results of the repeated measures ANOVA for the P3b mean amplitude of the ASD participants revealed that there was no significant effect of the Condition type (Frequent, Rare) *F (1, 4) = 0.004, p=.951*, neither of the Electrode site (Cz, Pz) *F (1, 4) = 0.73, p=.440* or even of the interaction between the Condition and Electrode *F (1, 4) = 4.37, p=0.105*.

For the Friedman test there was no statistically significant difference in the P3b mean amplitude for the Condition type for electrode Cz $x^2(1) = 0.20$, p = 0.655 neither for the electrode Pz $x^2(1) = 0.20$, p = 0.655.

4.2.2. Emotion Processing Task

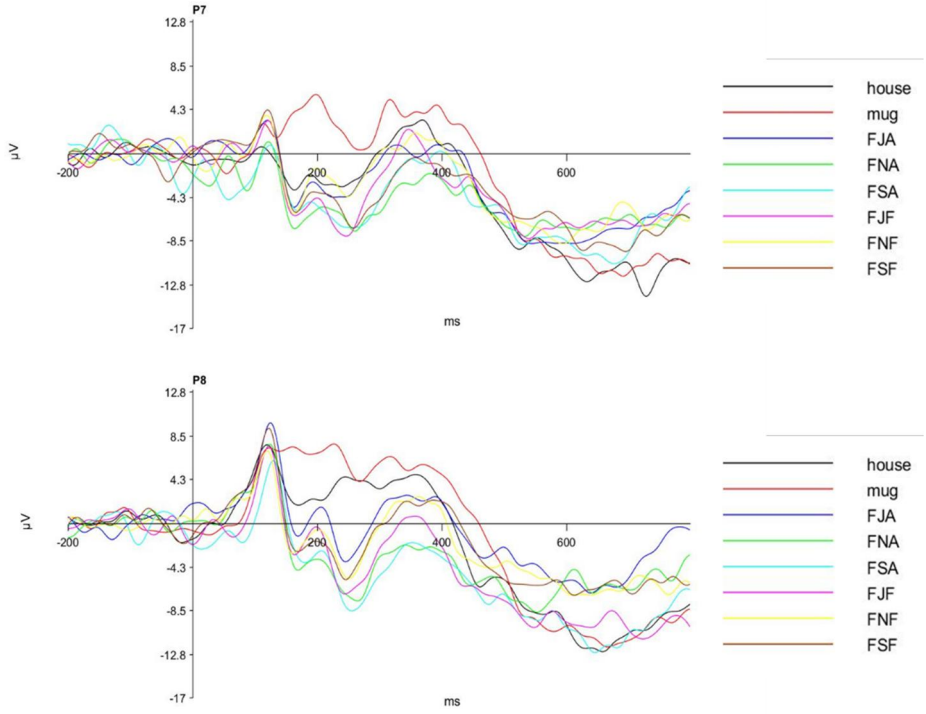


Figure 4. 2.Grand average ERP waves for electrode P7 (above) and P8 (bellow) to the different conditions (house, mug, FJA, FNA, FSA, FJF, FNF, FSF).

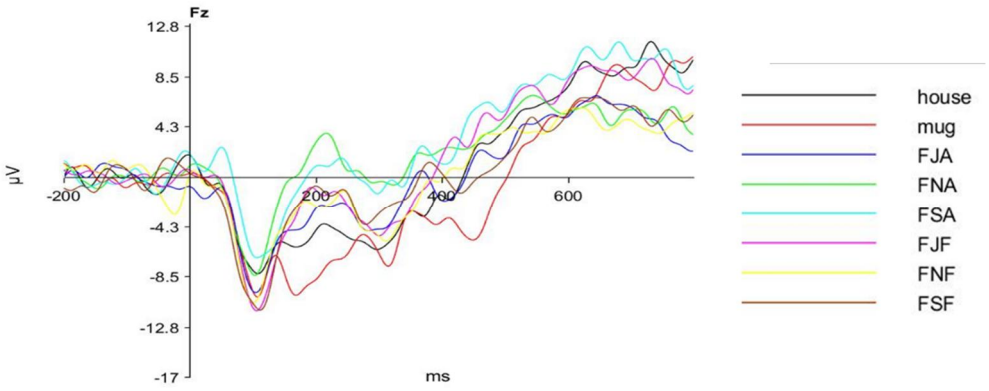


Figure 4. 3. Grand average ERP waves for electrode Fz, to the different conditions (house, mug, FJA, FNA, FSA, FJF, FNF, FSF).

In Figure 4.2 are represented the ERP waves, where the N170 component is visible, of the grand average across the ASD participants for the electrodes of interest P7 and P8, and in Figure 4.3 are represented the ERP waves of the grand average, with the VPP component, across all subjects for the electrode Fz.

4.2.2.1. N170 Peak Amplitude

The results of the repeated measures ANOVA demonstrated that there was significant effect of the condition type (house, mug, FJA, FNA, FSA, FJF, FNF, FSF) *F (7, 28) = 11.48, p<.001*, no significant effect of the Electrode site (P7, P8) *F (1, 4) = 5.53, p=.078* neither of the interaction between the Condition and Electrode *F (7, 28) = 1.55, p=.192* in the N170 peak amplitude of the ASD participants. After running post-hoc tests for the significant differences in the condition type, those differences were notable. The house condition (*M=-1.76; SD=2.43*) presented lower N170 peak amplitudes (non-absolute values) of than all the faces conditions: FJA (*M* = -6.82; *SD* = 2.19; *p* = .049), FNA (*M: -9.79; SD: 1.92; Sig: .007*), FSA (*M: -10.50; SD: 1.88; Sig: .003*), FJF (*M: -9.59; SD: 2.48; Sig<.001*), FNF (*M: -6.46; SD: 2.49; Sig: .007*), FSF (*M: -8.12; SD: 2.01; Sig: .004*). The same happened for mug (*M: 1.51; SD: 1.95*) relatively to the faces FJA (*M: -6.82; SD: 2.19; Sig: .041*), FNA (*M: -9.79; SD: 1.92; Sig: .002*), FSA (*M: -10.50; SD: 1.88; Sig: .001*), FJF (*M: -9.59; SD: 2.48; Sig: .009*), FNF (*M: -6.46; SD: 2.49; Sig: .016*), FSF (*M: -8.12; SD: 2.01; Sig: .022*). The FNF condition (*M: -6.46; SD: 2.49*) also displayed lower values of N170 peak amplitudes than the FSA condition (*M: -10.50; SD: 1.88; Sig: .009*).

For the Friedman test there were statistically significant differences in the N170 peak amplitude for the condition type for the electrode P7 $x^2(7) = 18.60$, p = .010 and for the condition type for the electrode P8 $x^2(7) = 22.67$, p = .002. The Wilcoxon post-hoc tests for the significant differences in the condition type showed that for P8 electrode the houses conditions presented lower values of N170 peak amplitudes than all the faces conditions FJA, FNA, FSA, FJF, FNF, FSF (*Z* = -2.02, *p* = .043, for all houses*faces comparisons); the same happened for mugs relatively to the faces (*Z* = -2.02, *p* = .043, for all mugs*faces comparisons) (except for FJA and FSF conditions); the FNF condition also displayed lower values of N170 peak amplitudes than the FSA (*Z* = -2.02, *p* = .043) and the FJF conditions(*Z* = -2.02, *p* = .043); and the FJA had lower values than the FJF condition. For the electrode P7, the results showed that the house condition presented lower values than the FSF condition (*Z* = -2.02, *p* = .043); the mug condition displayed lower values than all the face conditions (*Z* = -2.02, *p* = .043, for all mugs*faces comparisons); the FJA condition presented lower values than FSA condition (*Z* = -2.02, *p* = .043); and the FNF Condition presented lower values than the FNA (*Z* = -2.02, *p* = .043) and the FSA (*Z* = -2.02, *p* = .043) conditions.

4.2.2.2. N170 Peak Latency

The results of the repeated measures ANOVA demonstrated that there was no significant effect of the Condition type (house, mug, FJA, FNA, FSA, FJF, FNF, FSF) *F (7, 28) = 1.44, p=.230*,

neither of the Electrode site (P7, P8) *F (1, 4) = 0.24, p=.653* or even of the interaction between the Condition and Electrode *F (7, 28) = 1.48, p=.216* in the N170 peak latency of the ASD participants.

For the Friedman test there was no statistically significant difference in the N170 peak latency between conditions for electrode P7 $x^2(7) = 12.13$, p = .096 neither for the electrode P8 $x^2(7) = 12.57$, p = .083.

4.2.2.3. N170 Mean Amplitude

The results of the repeated measures ANOVA for the N170 mean amplitude of the ASD participants revealed that there was significant effect of the Condition type (house, mug, FJA, FNA, FSA, FJF, FNF, FSF) *F (7, 28) = 9.44, p<.001*, no significant effect of the Electrode site (P7, P8) *F (1, 4) = 4.20, p=.110,* neither of the interaction between the Condition and Electrode *F (7, 28) = 2.14, p=.172*. The ANOVA post-hoc tests for the significant differences in the condition type showed that the houses conditions (*M: 1.04; SD: 2.29*) presented lower values of N170 mean amplitudes than all the faces conditions (except for the FJA condition), FNA (*M: -5.71; SD: 1.77; Sig: .006*), FSA (*M: -6.49; SD: 2.24; Sig: .010*), FJF (*M: -5.02; SD: 1.72; Sig: .001*), FNF (*M: -2.58; SD: 2.08; Sig: .002*), FSF (*M: -4.08; SD: 1.40; Sig: .016*). The same happened for mug (*M: 4.47; SD: 1.88*) relatively to the faces FNA (*M: -5.71; SD: 1.77; Sig: .002*), FSA (*M: -6.49; SD: 2.24; Sig: .006*), FJF (*M: -5.02; SD: 1.72; Sig: .008*), FNF (*M: -2.58; SD: 2.08; Sig: .030*), FSF (*M: -4.08; SD: 1.40; Sig: .021*).

The FNF condition (*M: -2.58; SD: 2.08*) also presented lower values of N170 mean amplitudes than the FSA (*M: -6.49; SD: 2.24; Sig: .027*) and FJF (*M: -5.02; SD: 1.72; Sig: .020*) conditions, and the FJA condition (*M: -2.01; SD: 2.05*) displayed lower values of N170 mean amplitudes than FSA condition (*M: -6.49; SD: 2.24; Sig: .040*).

For the Friedman test there was statistically significant difference in the N170 mean amplitude for the different conditions the electrode P7 $x^2(7) = 21.00$, p = .004 and for the condition type for the electrode P8 $x^2(7) = 24.47$, p = .001. The Wilcoxon post-hoc tests for the significant differences in the condition type showed that, for the electrode P7, the house condition displayed lower values than the FNA condition (Z = -2.02, p = .043); the mug condition had lower values than all the face conditions (Z = -2.02, p = .043, for all mug*faces comparisons); and the FNF condition had lower values than the FNA (Z = -2.02, p = .043), FJF (Z = -2.02, p = .043) and FSF (Z = -2.02, p = .043) conditions. For P8 electrode the houses conditions presented lower values of N170 mean amplitudes than all the faces conditions (Z = -2.02, p = .043, for all houses*faces comparisons) (except the FJA); the same happened for mugs relatively to the faces (Z = -2.02, p = .043, for all mug*faces comparisons) (except for FJA); the FJF condition had higher values than the FJA (Z = -2.02, p = .043) and FNF (Z = -2.02, p = .043) conditions; and the FSA had higher values than the FJA (Z = -2.02, p = .043) and FSF (Z = -2.02, p = .043) conditions.

4.2.2.4. VPP Peak Amplitude

The results of the One-Way repeated measures ANOVA demonstrated that there was a significant effect of the Condition type (house, mug, FJA, FNA, FSA, FJF, FNF, FSF) *F (7, 28) = 4.50, p=.002* in electrode Fz in the VPP peak amplitude of the ASD participants. The posthoc tests of ANOVA demonstrated that the house condition (*M: -1.67; SD: 1.86*) presented lower VPP peak amplitude values than FNA (*M: 5.86; SD: 3.90; Sig: .039*), FSA (*M: 5.16; SD: 3.81; Sig: .046*) and FSF (*M: 3.04; SD: 2.90; Sig: .018*) conditions; the mug condition (*M: -5.52; SD: 1.99*) showed lower VPP peak amplitude values than FNA (M: 5.86; SD: 3.90; Sig: .049) and FSA (*M: 5.16; SD: 3.81; Sig: .048*); and that the FJF condition (*M: 2.62; SD: 3.42*) displayed lower values of VPP peak amplitude than FNA (*M: 5.86; SD: 3.90; Sig: .008*) and FSA (*M: 5.16; SD: 3.81; Sig: .010*) conditions.

For the Friedman test there was statistically significant difference in the VPP peak amplitude between conditions $x^2(7) = 16.27$, p =.023 . The post-hoc tests of Wilcoxon demonstrated that the house condition presented lower VPP peak amplitude values than FNA (*Z* = -2.02, *p* = .043), FSA (*Z* = -2.02, *p* = .043) and FSF (*Z* = -2.02, *p* = .043) conditions; the FJF condition displayed higher values of VPP peak amplitude than FNA (*Z* = -2.02, *p* = .043) and FSA (*Z* = -2.02, *p* = .043) conditions; and the FNA had lower values than FJA (*Z* = -2.02, *p* = .043) condition.

4.2.2.5. VPP Peak Latency

The results of the repeated measures ANOVA demonstrated that there was no significant effect of the Condition type (house, mug, FJA, FNA, FSA, FJF, FNF, FSF) *F (7, 28) = 1.13, p=.375* in the electrode Fz in the VPP peak latency of the ASD participants.

For the Friedman test, there was no statistically significant difference in the VPP peak latency between conditions $x^2(7) = 7.86$, p = .345.

4.2.2.6. VPP Mean Amplitude

The results of the repeated measures ANOVA for the VPP mean amplitude of the ASD participants revealed that there was a significant effect of the Condition type (house, mug, FJA, FNA, FSA, FJF, FNF, FSF) *F (7, 28) = 3.82, p=.005* in the electrode Fz. The posthoc tests of ANOVA demonstrated that the house condition (*M: -4.99; SD: 2.34*) presented lower VPP mean amplitude values than FNA (*M: 1.56; SD: 3.65; Sig: .037*) condition; the FJF condition (*M: -2.28; SD: 3.09*) showed lower values of VPP mean amplitude than FNA condition (*M: 1.56; SD: 3.654;*

Sig: .009); and that the FNF condition (*M: -2.61; SD: 3.06*) had lower values of VPP mean amplitude than FNA condition (*M: 1.56; SD: 3.65; Sig: .032*).

For the Friedman test there were statistically significant differences in the VPP mean amplitude for the different conditions $x^2(7) = 14.20$, p = .048. Post hoc analysis with Wilcoxon signed-rank tests was conducted. The Wilcoxon post-hoc tests showed that the house condition had higher VPP peak amplitude values than FNA condition ($Z = -2.02$, $p = .043$); the FJF condition presented higher values of VPP peak amplitude than FNA condition ($Z = -2.02$, $p = .043$); the FJA condition had higher values than the FNA ($Z = -2.02$, $p = .043$) and FSA ($Z = -2.02$, $p = .043$) conditions; and finally that the FNF condition had higher values of VPP peak amplitude than FNA ($Z = -2.02$, $p = .043$) and FSA ($Z = -2.02$, $p = .043$) conditions.

Chapter 5

Discussion

The present study was a pilot study to develop a protocol that evaluates both attention and emotion processing of ASD children given its applicability in the development of a monitoring system for HAT. HAT is engaging to children with ASD, with the suggestion that HAT can help build attentional and social interaction skills [71], [77], [79]. There are no known studies studying the impact of an individualized HAT, in a quantitative objectively way, on attentional and emotion processing abilities in children with ASD.

Thus, the further purpose of this study was to establish a test protocol and to collect preliminary EEG according to the established protocol. The main objective of this dissertation was, therefore, to contribute to the scientific validation of HAT, as a therapeutic method for the development of children with ASD. To this end, a methodology was built based on a visual stimulation system associated with brain monitoring EEG, as a way of assessing parameters related to social interaction (in terms of emotion processing) and attention in children with ASD. The research questions linked to potential benefits of this study are if EEG measures demonstrate adequate responses in baseline measures of attention and emotion processing in children with ASD to warrant future studies and if this preliminary experimental protocol is a valid proof of concept so that the established brain monitoring system allows objectively measuring the evolution of children with ASD, in terms of social interaction and attention, during horse assisted therapies?

5.1. Stimuli Procedures Suitability

The protocol developed in this study showed excellent acceptance from the children.

In what regards the initial instructions, the fact that it took some time for the youngest participants to understand how to perform the task can be, in future work, solved with some kind of more interactive explanation, with actual toys or impressed images for a more comprehensive and efficacious understanding.

Although some participants show more interest for some of the tasks, remaining more attentive during the sequence, providing breaks and congratulate them for what they have done until that break, helped to maintain their engagement.

Furthermore, it is important to note that it was possible to process and analyse results with this protocol, even if they lack statistical power due to the already mentioned limitations, which suggest it can be a starting point for further studies having in mind that it is needed to improve some aspects, especially the sample size.

5.2. Experimental Results

5.2.1. Visual Oddball Attention Task

The study of attention with Visual Oddball tasks was reported by earlier ERP findings [141]–[143], however, the results of this study do not correspond to what was expected from the previous studies.

The present study shows no main effect of Condition, Electrode or interaction between Condition and Electrode for the P3b peak amplitude, peak latency or even mean amplitude (see section 4.2.1). These results may indicate a reduction of the discriminative ability of the ASD participants, i.e, may be indicative of an over-processing network where sensory inputs evoke abnormally large ERPs for stimuli (either Frequent or Rare) at all stages of stimulus processing with signs of a reduction in the selectivity (differentiation) for each stimulus category.

Neural systems of ASD individuals are frequently incorrectly activated [144]. Uncharacteristically improved sensory responses have been described and related to this are deficits in orienting attention and relocating information to higher levels of processing [145]. According to [144] selectivity in ASD arises in an all-or-none way with tiny specificity for the task importance of the stimulus. The P3b component is a centro-parietal wave that has been related to task-relevance and the decision associated character of stimuli [146], thus the lack of significant results also may relate to the task design and the relevance of the differences between the Frequent and the Rare stimuli.

However, more studies are needed to verify if the lack of significant differences in ASD children responses to the stimulus is due to a reduction of the discriminative ability from them, due to the chosen stimuli for the Visual Oddball task design or even due to the small sample size. Other aspects to have in mind are that the statistical analysis with repeated measures ANOVA was just an academic exercise, its applicability would not be possible with such a small

sample size, and the Friedman test was just applied for the significant differences of Condition for each electrode site since this non-parametric analysis does not calculate interactions between factors (Condition*Electrode).

5.2.2. Emotion Processing Task

The relevance of face processing [106] and eye gaze processing in the social interaction is undoubted [130], [131] and particularly for eye gaze avoidance and social loss in ASD [132], [133]. The research related to eye gaze processing is not consensual and that is due to the variation of the experimental tasks in the studies [134]. Indeed, the type of experimental task the subjects are asked to complete is one of the most important factors for eye gaze processing. To this end, in this study, it was investigated how observing faces with direct or averted gaze affects the participant's performance, during an adapted emotion task to evaluate both the effect of different eye gaze and different emotions.

The results of the present study show some similarities between the statistical analysis with parametric and non-parametric methods for the N170 peak amplitude and mean amplitude (see section 4.2.2). In both, almost all the faces had greater N170 peak and mean amplitude than the mugs for both P7 and P8 electrodes, suggesting a trend towards normal face processing. The same happened between the faces and the houses, respectively, except for the P7 electrode site.

Many studies described that N170 latencies elicited in response to objects and faces are shorter in response to faces than objects [147]–[149]. However, in this study, there were no significant differences between faces and objects (mugs or houses) latencies or even between emotion or gaze direction latencies.

Concerning the reaction for different emotions, for the N170 peak amplitude, the FSA condition demonstrates higher peak amplitude (in both parametric and non-parametric methods) and mean amplitude (non-parametric method) values than the FNF, for both P7 and P8 electrode sites (see section 4.2.2) which may indicate that the participants may have a more elicited response to the sadness relatively to the neutral emotion. For the P7 electrode site in the parametric analysis the FSA show higher peak amplitude values than for the FJA, suggesting that the children process better the sadness emotion in comparison to the joy emotion. For the P8 electrode site also in the parametric analysis, the FJF exhibits higher amplitude values than the FNF condition, which may indicate that the participants had an enhanced response to the joy related to the neutral emotion. Individuals with ASD frequently show processing irregularities specific to facial expressions of emotion, including reduced and delayed N170 component in amplitude and latency, respectively, to emotional facial expressions in relation to TD individuals [106]–[108]. Behavioural studies evaluating recognition skills of emotional facial expression in ASD have revealed that individuals with ASD are not as good as at distinguishing positive

emotions from negative emotions [109]. In this study, the results suggested accordance with the previously mentioned.

In the case of gaze direction response, for both methods of analysis and both electrodes sites, the peak amplitudes were higher in the FSA condition related to the FNF as already reported above, which can indicate that the averted gaze is better processed than the direct one. This last assumption is supported by the same result for FNA compared with the FNF, respectively, for the P7 electrode (parametric analysis), however for the electrode P8 (parametric analysis), FJF condition had higher values than FJA, suggesting the opposite, but it can be due to the different emotions presented, i.e, in the joy emotion the children can process better the direct gaze, but in the neutral emotion may process better the averted gaze.

The incoming results from the mean amplitude from the parametric analysis at P8 electrode site and the non-parametric analysis show that the FSA condition displayed higher mean amplitude values than FJA, meaning once again that the sad emotion has a more elicited response than the joy emotion. For both analysis methods and both electrode sites, the FJF condition presents higher mean amplitude values than the FNF conditions, suggesting again that the joy emotion elicited an enhanced response from children than the neutral emotion. In the P7 electrode, the mean amplitudes demonstrate to be higher in the FSF condition in comparison to the FNF condition indicating that, as reported above, that the sadness emotion elicited more brain activity than the neutral emotion.

In what regards the gaze direction effect, in the P7 electrode for parametric analysis, the FNA condition presents higher values than the FNF condition, suggesting that the averted gaze had a higher effect in the participants. The same happened for between the FSA and FSF (P8 electrode in parametric analysis) and between the FSA and FNF conditions (non-parametric analysis) leading to the same suggestions. However, comparably to what occurred in the peak amplitude analysis, in the P8 electrode for the parametric analysis, the FJF had higher values than FJA, indicating that direct gaze had higher effect than averted one, but in the same way, it can be just in the joy condition as happened in the peak amplitude analysis.

Summing up the results of the N170 component in terms of faces*objects effect it may be inferred that the faces elicited a more relevant effect than objects (houses, mugs) in children with ASD; in terms of emotions effect it seems that the children gave more relevance to sadness emotion than to neutral or joy emotions, and to joy than neutral emotion; finally, in terms of gaze direction effect, the averted gaze had a more elicited response for children with ASD than the direct gaze for the different emotions, except in the case of joy emotion, in which the direct gaze had more impact in their reaction.

Both in the parametric and non-parametric analysis of VPP peak amplitude, the house's condition presents lower values than half of the faces conditions (FNA, FSA, FSF), which may indicate that children had a more elicited response to faces than houses, yet not all the faces presented these results and it is important to be careful when making these assumptions. In the non-parametric analysis, the mug condition had lower values than FNA and FSA conditions, making difficult to infer about the effect of mugs compared to all faces. Regarding the emotion

and gaze direction effects on VPP component amplitude, for both analysis methods, the FJF condition presented lower values than the FNA and FSA conditions, suggesting that the neutral and sadness emotions have a more elicited response than the joy emotion and that the averted gaze has better processing than the direct gaze. The FJA presented lower values than the FNA condition, indicating again that the neutral emotion has better processing than joy emotion.

When comparing the mean amplitude between two latencies of the VPP component with both analysis methods, it was noticed that the FNA condition displayed better values than the house condition, leading to the previous conclusion that, i.e, it can't be inferred that the all the faces have a more significant effect than the houses in the children's response. In relation to the emotions and gaze direction, in both analysis methods displayed the FNA condition mean amplitude higher than the FJF and the FNF conditions, suggesting that the neutral faces elicited higher mean amplitudes than the joy faces and that the averted gaze has a better response than the direct one. For the parametric analysis, the FSA condition had higher values of mean amplitudes than the FJA and FNF conditions, leading to the indication that the sadness emotion has better influence in the participant's responses than the joy and neutral emotions, and also to the suggestion that averted gaze has a higher impact in responses than the direct gaze. Finally, also having the FNA condition with higher values than FJA condition, suggests that the neutral emotion has a better influence on the participant's responses than the joy emotion again.

The VPP component analysis should present similar responses to the N170 analysis, once they represent the same brain processes. Both components show significant results just for peak amplitude and mean amplitude. Many studies reported that N170 latencies elicited in response to objects and faces are shorter in response to faces than objects [147]–[149]. However, in this study, there were no significant differences between faces and objects (mugs or houses) latencies or even between emotion or gaze direction latencies either for N170 or VPP component. Analysing the VPP component it can't be inferred that all the faces have a more significant effect than the houses in the children's response antagonizing the results of N170 component. Also, the emotion assumptions of the VPP component are opposite from the N170 for the relationship between neutral and joy faces, but it is important to have in mind, once again, that this study has a lot of limitations and the results can't be scientifically validated.

These study results are in accordance to the literature since it was already reported that sadness emotion is more easily perceived with averted gaze [150] and the joy/happy emotion is perceived more easily with direct gaze [150], [151]. The proposed explanation by the same literature is that the perception of emotions representing signals of behavioural approach and comfort enhance with direct eye gaze and the emotions representing signals of behavioural avoidance are more easily perceived with the averted eye gaze, suggesting that averted and direct gaze are processed differently during emotion processing performed by the same subjects.

Since the results from both methods of analysis are not always coincident, it should only be considered as more reliable the results that are consistent for both analyses, and even just for

the non-parametric, once is the best method considering the small sample size and that the statistical analysis with repeated measures ANOVA was just an academic exercise. Its applicability wouldn't be possible with such a small sample size and the Friedman test was just made for the significant differences of condition for each electrode site since this non-parametric analysis doesn't calculate interactions between factors (Condition*Electrode).

Nevertheless, having results that can be analyzed and some assumptions can be taken is really valuable for this study, since it suggests that the designed protocol and its preliminary application can provide meaningful information on the components of interest and have some significant results, even that in a limited way.

5.3. Limitations

Limitations must be considered when analysing the results of this study. A main limitation is the small sample size. The study was a feasibility study, thus only a small number of participants was enlisted to test the applicability of the technology and the methodology. The absence of a control group is another limitation and it prevents the comparison and discussion of the brain response to patterns between ASD and TD children.

ERP measures can vary based on factors related to the sample composition (age, gender, comorbid conditions and intellectual variation), participants' understanding and amenability with the task instruction. In this study, to better control these factors it was selected a sample of participants around the same age and gender, but the factors of comorbid conditions and intellectual variations were not taken into account, which represents another limitation of the study.

Another limitation is the sparse electrode montage used in this study (18 EEG electrodes). However, also the lack of IQ tests increases the potential confounders to the measurements. Also, the lack of the Autistic Diagnostic Observation Schedule (ADOS) was a limitation, although the availability of clinician diagnosis with DSM-V.

In the data pre-processing there were no rejected channels since the number of channels was already limited and the noise removed was selected with caution. In the data analysis, it was excluded the PCA and the ICA since the data was scarce, the statistical analysis was just for academic exercise having in account the size effect.

There were only a few statistically significant tests, however, due to the small sample size, the results must be taken with caution and are only intended to inform future studies. These results cannot be generalized to larger populations, and the most correct way to use them is to make hypotheses for a more robust study that uses a larger sample size.

Chapter 6

Conclusion and Future Work

This study was conducted as a first step to demonstrate that HAT allows the enhancement of social and intellectual performance of ASD children. The EEG is a low-cost technique to monitor neurophysiological changes of the brain and proved to be a useful tool to study the evolution of the children throughout the HAT.

To this end, a protocol was developed with three moments of evaluation tests before, after five sessions and ten therapy sessions, monitored with EEG. The stimulation tasks were specifically developed for this achievement, contemplating the evaluation of attention and emotion processing in ASD children. EEG measures demonstrate adequate responses in baseline measures of attention and emotion processing in children with ASD to warrant future studies.

The lack of significant results in the Oddball task may indicate a reduction of the discriminative ability of the ASD participants, i.e, may be indicative of an over-processing network where sensory inputs evoke abnormally large ERPs for stimuli (either Frequent or Rare) at all stages of stimulus processing with signs of a reduction in the selectivity (differentiation) for each stimulus category. On the other hand, the results in the Emotion processing task suggested that the faces elicited a more relevant effect than objects (houses, mugs) in children with ASD; in terms of emotions effect it seems that the children gave more relevance to sadness emotion than to neutral or joy emotions, and to joy than neutral emotion; finally, in terms of gaze direction effect, the averted gaze had a more elicited response for children with ASD than the direct gaze for the different emotions, except in the case of joy emotion, in which the direct gaze had more impact in their reaction.

The protocol was pre-tested with six ASD children, and although more experiments are required to draw definitive conclusions, the results not only provide guidance for future studies but also demonstrate the suitability of the designed experimental protocol in the analysis of attention and emotion processing in children with ASD.

In order to continue this study in an investigation of validation and scientific relevance, it is necessary to first define the sample under study. The sample in question would be composed of two groups of subjects, a group with ASD (study group) and a group without ASD with neurotypical development (control group). The characteristics of these groups would be based on the inclusion and exclusion criteria already defined in chapter 3.1, already used in other studies of EEG / ERP with children with ASD [100], [102], [103]. The selection of groups is very important to be able to draw conclusions with statistical significance relevant to the scientific community. The best way to conduct the future study is to select a sample of significant size, considering the number of subjects used in other scientifically validated studies as significant [101], [149], [152], [153].

To this end, further studies need to replicate the approach with a larger sample to prove its efficacy and its usability in a therapeutic environment. It is also important to consider different age groups to find the effects of development on attention and social interaction (in terms of emotion processing) in individuals with ASD. The study needs to be conducted with a control group of age-matched children (with typical development) to allow the evaluation of the between-group differences.

This study was already prepared to be conducted under normal conditions, with both groups of subjects. Initially, the Portuguese Association for Development Disorders and Autism (APPDA) in Vila Real (where children already have a medical diagnosis of ASD) was contacted, followed by the groupings of schools in the municipality of Vila Real (also considering children with a medical diagnosis of ASD). In all institutions, informative lectures on the study were given, receiving positive feedback from the children's parents, who showed great interest in their children's participation in the study. The fact that the entire study was approved by CEUP and by the Data Protection Unit of the University of Porto, work carried out with great advance in order to allow the study to be conducted within the indicated deadlines, was a relevant point for participants' adherence to the study. For the constitution of the control group, school groups were once again contacted to select a group of children with similar characteristics to the study group and framed in a similar educational environment. The continuation of the outlined study was not possible due to the COVID-19 pandemic, making contact with the study subjects impossible.

The continuation of this study would involve some important steps for the success of the same. It would focus on the acquisition of the EEG signals, together with the video recording, according to the following steps (also illustrated schematically in Figure 6.1):

(i) selection of two groups of children, a control group of children who do not have ASD and a group diagnosed with ASD. The ASD group would be subjected to three moments of evaluation of the EEG signal (using the protocol developed in this study) before the start of 10 sessions of HAT (once per week), after 5 sessions of therapy and finally the last moment of evaluation after the 10 sessions of therapy. All the HAT sessions must be carried out in a horse therapy centre. Children would be always accompanied by specialized personnel.

(ii) EEG signals would be acquired for the control group by using a similar set-up, but no therapy would be used;

(iii) acquisition of the EEG signal synchronized with video recording (to monitor children's behaviour), considering non-invasion of the EEG acquisition environment;

(iv) processing and analysing EEG data for each acquisition and comparing the parameters analysed between them in order to understand the impact of HAT in children with Autism in terms of attention and social interaction.

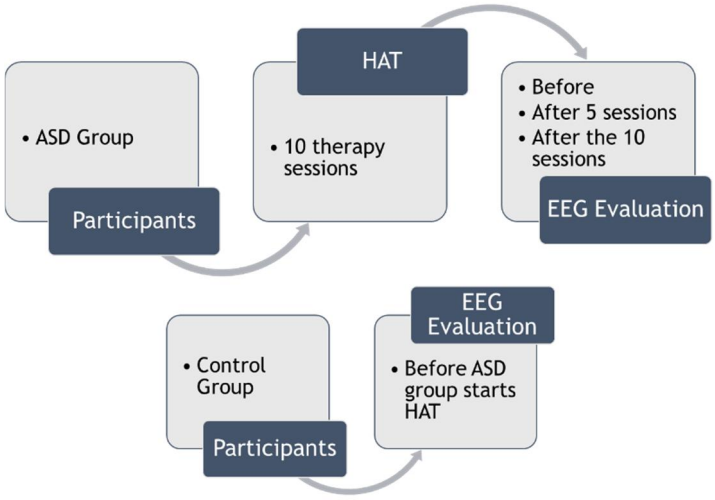


Figure 6. 1. Next steps of the experimental protocol developed in this study.

Choices for the number of HAT sessions and EEG assessment phases were made based on similarities in which other therapies were subjected to an EEG assessment to study the progress of children with ASD. An example of a similar study is the study by Lagasse [100] in which the objective was to study the effect of music therapies on sensory gating and attention on autistic children. Comparing the protocol of this study to that of Lagasse, the level of therapy sessions would be the same number (10), but instead of being twice a week for 5 weeks, in this study, it would be only one therapy session per week, with the protocol lasting 10 weeks.

This was since the HAT Center (Equestrian Center of Pedras Salgadas, Vila Pouca de Aguiar Municipality) with which a draft contract was established for the realization of the therapies has defined a maximum of 10 participants and each one with only one therapy session per week in order to have a weekly slot for all children. Another difference is the fact that the Lagasse study only carries out two evaluation moments, the initial and the final; in the case of this study, three evaluation moments were planned. This was outlined to have a middle ground for comparison since with the increase in the number of therapy time (10 weeks), important developments could be missed and then an intermediate evaluation moment was added.

Making an overview of the expected results after conducting the aforementioned workplan, in the first moment of EEG evaluation with the Oddball task, it would be expected that both TD and ASD groups elicit better responses to rare stimuli, although the ASD group would have

decreased values of peak amplitudes, peak latencies and mean amplitudes than the TD group [143]. At the second moment of EEG evaluation, which would occur after 5 sessions, it would be expected that the results suggested an improvement of the ASD children performance in the Oddball task in terms of increasing the values of the P3b peak amplitudes, peak latencies and mean amplitudes. In other words, improving in a way that would approximate their performance to that gotten from the TD children. For the third and last moment of EEG which would take place after ten sessions, it would be expected that the responses are even better (better attention and focus, better concentration, impact on children's lives) that those observed after 5 sessions and closer to those of obtained for the TD children group [100].

The expected results of the first moment of EEG evaluation for the emotion processing task would be that the ERP component's peak amplitudes, peak latencies and mean amplitudes should have a more elicited response to the different emotions, gaze directions and objects from the TD group than for ASD group [101], [153]. In TD children, the direct gaze should demonstrate enhanced elicited responses than the averted gaze [152], since this group do not face social interaction inhibition. In contrast in ASD children, the averted gaze should have more elicited responses than the direct gaze, as reported in previous studies [153], indicating that in children with ASD the direct gaze does not elicit or elicits reduced brain activity. Similarly to what was observed in the Oddball task, also in the emotion processing, it would be expected that the ASD children would have more elicited responses (higher values of peak amplitudes, peak latencies and mean amplitudes of the N170 and VPP components) after the second and even more improvements after the third moment of EEG evaluation, approaching to the performance of TD children.

All these assumptions should be taken with caution since there is no consensus in the scientific community on the existing differences between ASD and TD groups brain activity. While some authors found differences between these two groups [103], [142], [143], [153], others don't conclude this assumption [119], [121].

Let's now assume that at the end of the study the success would be partial, namely that improvements were observed either at the emotional task or at the Oddball task. If the results show no significant effects in the Oddball task, it could be a good approximation to make adaptations to the task stimuli, to have better engagement from the children, since the lack of results might be related to the task design and the relevance of the differences between the Frequent and the Rare stimuli [146]. The same strategies could be adopted if the lack of results would be at the level of emotion processing tasks. One could, for example, have more adequate emotions or other objects instead of houses and mugs. Also, it would be possible to extend the number of HAT sessions, since the children could need an extended period of therapies to improve their capacity of attention or social interaction.

Furthermore, although the ERP components of this study (P3b, N170 and VPP) can be easily detected at several electrodes, the application of a higher density montage could also bring a higher accuracy to the measurements.

Also, in future studies, it would be advisable to cross-check the EEG information on the benefits of the HAT with another evaluation method, such as IQ test for more rigorous control of factors that can affect the measures. Also, the ADOS is a valuable diagnostic standard in ASD studies that allows testing quantitative associations of biomarkers with autistic severity among ASD individuals and its use in the future can be implemented.

This preliminary experimental protocol could be a valid proof of concept that the established brain monitoring system could allow objectively measuring the evolution of children with ASD, in terms of social interaction and attention, during horse assisted therapies, once improvements would be conducted.

It would be the main goal of this work that the improvement of the protocol developed in this study represents an added value for the professionals who accompany ASD children and to develop a HAT, focused on improving the competencies of children with ASD at the level of social interaction (emotion processing) and attention, with a positive impact on the future performance of individuals with ASD, both at the level of academic and professional integration, as well as social.

Annexe A

Checklist of the Guidelines for EEG studies from the Society for Psychophysiological Research

Appendix

Authors' Checklist

The checklist is intended to facilitate a brief overview of important guidelines, sorted by topic. Authors may wish to use it prior to submission, to ensure that the manuscript provides key information.

Hypotheses	**YES**	**NO**
Specific hypotheses and predictions for the electromagnetic measures are described in the introduction	X	

Participants	**YES**	**NO**
Characteristics of the participants are described, including age, gender, education level, and other relevant characteristics	X	

Recording characteristics and instruments	**YES**	**NO**
The type of EEG/MEG sensor is described, including make and model	X	
All sensor locations are specified, including reference electrode(s) for EEG	X	
Sampling rate is indicated	X	
Online filters are described, specifying the type of filter and including roll-off and cut-off parameters (in dB, or by indicating whether the cut-off represents half-power/half amplitude)	X	
Amplifier characteristics are described	X	
Electrode impedance or similar information is provided	X	

Stimulus and timing parameters	**YES**	**NO**
Timing of all stimuli, responses, intertrial intervals, etc., are fully specified; ensure clarity that intervals are from onset or offset	X	
Characteristics of the stimuli are described such that replication is possible	X	

Description of data preprocessing steps	**YES**	**NO**
The order of all data preprocessing steps is included	X	
Rereferencing procedures (if any) are specified, including the location of all sensors contributing to the new reference	X	
Method of interpolation (if any) is described		
Segmentation procedures are described, including epoch length and baseline removal time period	X	
Artifact rejection procedures are described, including the type and proportion of artifacts rejected	X	
Artifact correction procedures are described, including the procedure used to identify artifacts, the number of components removed, and whether they were performed on all subjects	X	
Offline filters are described, specifying the type of filter and including roll-off and cut-off parameters (in dB, or by indicating whether the cut-off represents half-power/half amplitude)	X	
The number of trials used for averaging (if any) is described, reporting the number of trials in each condition and each group of subjects. This should include both the mean number of trials in each cell and the range of trials included	X	

Measurement procedures	**YES**	**NO**
Measurement procedures are described, including the measurement technique (e.g., mean amplitude), the time window and baseline period, sensor sites, etc.	X	
For peak amplitude measures, the following is included: whether the peak was an absolute or local peak, whether visual inspection or automatic detection was used, and the number of trials contributing to the averages used for measurement	X	
An a priori rationale is given for the selection of time windows, electrode sites, etc.	X	
Both descriptive and inferential statistics are included	X	

Statistical analyses	**YES**	**NO**
Appropriate correction for any violation of model assumptions is implemented and described (e.g., Greenhouse-Geisser, Huynh-Feldt, or similar adjustment)		
The statistical model and procedures are described and results are reported with test statistics, in addition to p values	X	
An appropriate adjustment is performed for multiple comparisons		
If permutation or similar techniques are applied, the number of permutations is indicated together with the method used to identify a threshold for significance		

Figures	YES	NO
Data figures for all relevant comparisons are included	X	
Line plots (e.g., ERP/ERF waveforms) include the following: sensor location, baseline period, axes at zero points (zero physical units and zero ms), appropriate *x*- and *y*-axis tick marks at sufficiently dense intervals, polarity, and reference information, where appropriate	X	
Scalp topographies and source plots include the following: captions and labels including a key showing physical units, perspective of the figure (e.g., front view; left/right orientation), type of interpolation used, location of electrodes/sensors, and reference		
Coherence/connectivity and time-frequency plots include the following: a key showing physical units, clearly labeled axes, a baseline period, the locations from which the data were derived, a frequency range of sufficient breadth to demonstrate frequency-specificity of effects shown		

Spectral analyses	YES	NO
The temporal length and type of data segments (single trials or averages) entering frequency analysis are defined		
The decomposition method is described, and an algorithm or reference given		
The frequency resolution (and the time resolution in time-frequency analyses) is specified		
The use of any windowing function is described, and its parameters (e.g., window length and type) are given		
The method for baseline adjustment or normalization is specified, including the temporal segments used for baseline estimation, and the resulting unit		

Source-estimation procedures	YES	NO
The volume conductor model and the source model are fully described, including the number of tissues, the conductivity values of each tissue, the (starting) locations of sources, and how the sensor positions are registered to the head geometry		
The source estimation algorithm is described, including all user-defined parameters (e.g., starting conditions, regularization)		

Principle component analysis (PCA)	YES	NO
The structure of the EEG/MEG data submitted to PCA is fully described		
The type of association matrix is specified		
The PCA algorithm is described		
Any rotation applied to the data is described		
The decision rule for retaining/discarding PCA components is described		

Independent component analysis (ICA)	YES	NO
The structure of the EEG/MEG data submitted to ICA is described		
The ICA algorithm is described		
Preprocessing procedures, including filtering, detrending, artifact rejection, etc., are described		
The information used for component interpretation and clustering is described		
The number of components removed (or retained) per subject is described		

Multimodal imaging	YES	NO
Single-modality results are reported		

Current source density and Laplacian transformations	YES	NO
The algorithm used and the interpolation functions are described		

Single-trial analyses	YES	NO
All preprocessing steps are described		
A mathematical description of the algorithm is included or a reference to a complete description is provided		

Annexe B

Opinion of the Ethics Committee of the University of Porto

PORTO
COMISSÃO DE ÉTICA

PARECER N? 74/CEUP(2019)

PARECER DA COMISSÃO DE ÉTICA DA UNIVERSIDADE DO PORTO SOBRE O PROJETO:

Uso da eletroencefalografia (EEG) na avaliação dos efeitos das terapias assistidas por cavalos em crianças com Perturbação do espetro autista TEA

SUBMETIDO POR:

Patrícia Gaspar Brás

INSTITUIÇÃO DE ORIGEM

Faculdade de Engenharia da Universidade do Porto (FEUP)

Relatora: Profa. Doutora Paula Pinto de Freitas outubro

2019

TÍTULO DO PROJECTO: Uso da eletroencefalografia (EEG) na avaliação dos efeitos das terapias assistidas por cavalos em crianças com Perturbação do espetro autista TEA.

Investigador Principal /Responsável: Professor Doutor Carlos Fonseca do Dep. Eng ª Metalúrgica e de Materiais; Faculdade de Engenharia da Universidade do Porto, orientador de tese da estudante do Mestrado em Engenharia Biomédica, Patrícia Gaspar Brás.

Fundamentação do estudo: Um método de intervenção terapêutica alternativo para o desenvolvimento de capacidades nas áreas de mais dificuldade das crianças com TEA é a terapia assistida por cavalos. Estudos mostraram que a hipoterapia tem um impacto positivo no desenvolvimento destas crianças, contudo, o tipo de avaliação feito nestas terapias é apenas qualitativo. A recolha de informação é feita de uma forma observacional do comportamento e estado de concentração da criança durante a terapia e também através de entrevistas e preenchimento de formulários sobre a comunicação, interação social e comportamento das crianças. Assim sendo, o facto de a informação ser apenas qualitativa e subjetiva é uma limitação para avaliar o desempenho e a concentração de cada criança durante a sessão de terapia

O objetivo do estudo é aplicar um sistema de monitorização cerebral com eletroencefalograma (EEG) que vai permitir a recolha de informação de forma quantitativa e o seu enquadramento na resposta de cada criança aos estímulos recebidos durante a terapia. Para uma monitorização estímulo-resposta eficaz, o objetivo é gravar audiovisuais de cada sessão sincronizados com o sinal EEG adquirido durante a sessão de terapia.

Plano de trabalho:

A tarefa 1 é dedicada à aquisição de informação bibliográfica necessária para o desenvolvimento desta tese. Vai focar-se, nomeadamente, nos seguintes tópicos: analise da resposta EEG de crianças com TEA, de forma a identificar as posições dos elétrodos no couro cabeludo cujos sinais refletem o estado de concentração das crianças; A referência [4] representa um bom ponto de partida para este estudo. Além disso a mestranda vai adquirir treino prático na aquisição de sinal EEG.

A tarefa 2 vai ser dedicada ao desenvolvimento da montagem para aquisição de sinais. Em particular, o sinal EEG terá de ser sincronizado com um sinal vídeo de forma a conseguir-se identificar os estados de concentração/desconcentração por parte da criança.

A tarefa 3 vai focar-se na aquisição de sinais EEG, juntamente com a gravação dos vídeos correspondentes. Vai incluir os seguintes passos: (i) seleção de dois grupos de crianças, um grupo controlo e um grupo de crianças com TEA; (ii) o grupo de controlo será sujeito a um momento de avaliação EEG e o grupo com TEA será sujeito a avaliação ao longo das sessões de hipoterapia (10 sessões); (iii) processamento do sinal EEG para avaliar os benefícios da terapia assistida por cavalos para crianças com TEA.

Pedidos de Esclarecimento e Respostas do Investigador Principal, Professor Doutor Carlos Fonseca:

QUESTÃO 1: Gostaríamos de saber se a monitorização cerebral com EEGQjá foi testada numa população de crianças normotípicas e a sua tolerabilidade ao equipamento que agora pretendem utilizar em crianças com PEA. O mesmo no que respeita à sincronização entre o EEG e a gravação audiovisual durante uma sessão de hipoterapia. Dito de outro modo, as tarefas 1 e 2 estão já suficientemente desenvolvidas e testadas numa população normotípica?

RESPOSTA: Como informação complementar os autores gostariam de informar que as crianças com PEA serão recrutadas na Associação Portuguesa para as Perturbações do Desenvolvimento e Autismo de Vila Real- APPDA de Vila Real, à qual foi proposto este estudo.

Para além disso, os voluntários serão acompanhados durante o estudo por um técnico da Associação APPDA de Vila Real e por um técnico do Centro Hípico. A técnica a usar nas tarefas 1 e 2, nomeadamente a eletroencefalografia (EEG), tem sido amplamente utilizada para estudos em populações com PEA e populações normotípicas, conforme pode ser atestado pelas referências [1-41, embora nunca tenha sido testada num contexto de validação da terapia com cavalos. São raras as referências a intolerância relativamente à técnica. Tipicamente, os voluntários são colocados perante tarefas que exigem concentração enquanto é realizado o registo EEG. No caso presente, para além desta tarefa, o participante estará em cima de um cavalo, especialmente usado para terapia com crianças e conduzido a passo pelo tratador, enquanto é realizado o registo EEG. Já o registo vídeo é menos frequente, embora tenha demonstrado ser de grande utilidade para validar o estado de concentração/desconcentração da criança [31, razão pela qual os autores gostariam de adotá-lo.

QUESTÃO 2: Agradecíamos informação mais detalhada relativamente à operacionalização da tarefa 3, passo a passo:

- constituição do Grupo normotípico e Grupo de crianças com PEA bem como ao processo de seleção e caracterização dos grupos (faixas etárias, diagnóstico de PEA, níveis funcionais .....

- Adicionalmente gostaríamos de saber se está prevista a exclusão ou interrupção da participação das crianças no estudo e quais os critérios.

RESPOSTA: 1) O grupo de voluntários normotípicos será constituído por aproximadamente o mesmo número de elementos que o grupo de voluntários com PEA. Serão recrutados participantes de duas faixas etárias (3-12 anos e 16-20 anos), de forma a avaliar também a influência da idade na resposta à terapia [1,4]. Todos os voluntários diagnosticados com PEA, independentemente do grau ou nível funcional, serão considerados à partida elegíveis, salvo opinião contrária do técnico da APPDA. Contudo, os referidos dados serão considerados na avaliação dos resultados. Abaixo indicam-se os critérios de exclusão.

2) Os grupos de participantes normotípicos e portadores de PEA serão sujeitos a um momento de monitorização EEG durante a realização de uma tarefa simples mas que desafie e incentive momentos de concentração [2]. Esta tarefa será proposta e acompanhada pelo técnico da APPDA de Vila Real. Em seguida, o grupo com PEA será sujeito a 10 sessões de terapia assistida por cavalos, com monitorização EEG (não necessariamente em todas as sessões), de forma a verificar a evolução da capacidade de concentração ao longo da terapia. Por fim, será feita uma última monitorização no grupo com PEA, fora do ambiente de terapia, consistindo na repetição da tarefa inicialmente proposta acompanhada de registo EEG. Os resultados serão comparados com os obtidos inicialmente e com os da população normotípica, de forma a aferir a eficácia da terapia.

3) Todas os elementos pertencentes ao grupo com PEA terão de estar devidamente enquadrados na Associação APPDA de Vila Real com acompanhamento a vários níveis, nomeadamente, psicológico e fisioterapêutico.

4) Todo o tratamento dos dados recebidos vai ser realizado com técnicas de processamento de sinal baseado no Software MATLAB E EEGLAB.

5) A interrupção da participação poderá ter lugar em qualquer momento do estudo, de acordo com a vontade do participante ou dos respetivos encarregados de educação. Tal está explícito no termo de consentimento que virá a ser proposto.

6) Os critérios de inclusão/exclusão, tanto para a população normotípica como para a população com PEA estão detalhados abaixo, baseando-se em estudos anteriores [2,3,5]. Critérios de inclusão de participantes com PEA- voluntário diagnosticado com PEA por parte do clinico responsável pelo seu histórico médico. Critérios de exclusão de participantes com PEA-distúrbios convulsivos clínicos ou resultados de leituras de EEG sugestivos de um distúrbio convulsivo ativo ou encefalopatia epilética (nota: pacientes com picos de EEG ocasionais não estão excluídos); dúvida expressa pelo clínico referente ao diagnóstico de PEA; desordens sensoriais primárias significativas, por exemplo, cegueira e / ou surdez; outros processos de doenças neurológicas concomitantes que podem induzir a alteração do EEG, por exemplo, hidrocefalia ou hemiparesia. Critérios de inclusão de elementos no grupo normotípico- são consideradas normais pelos pais na vida que fazem; na mesma faixa etária dos elementos com PEA que participam no estudo. Critérios de exclusão de voluntários normotípicos- diagnosticados com doenças, distúrbios ou suspeitas de condições neurológicas ou psiquiátricas, por exemplo, atraso no desenvolvimento global, disfasia do desenvolvimento, Perturbação de défice de atenção e défice de atenção com Perturbação de hiperatividade, distúrbio convulsivo clínico ou leitura de eletroencefalograma (EEG) sugerindo um distúrbio convulsivo ativo ou encefalopatia epilética (Nota: Indivíduos com picos raros de EEG não foram excluídos);

Referéncias:

[1] L. Billeci, F. Sicca, K. Maharatna, F. Apiceila, A. Narzisi, G. Campatelli, S. Calderoni, G. Pioggia, F. Muratori, "On the application of quantitative EEG for characterizing autistic brain : a systematic review," Front. Hum. Neurosci. (2013) 7:442.

[2] L. Billeci, A. Tonacci, G. Tartarisco, A. Narzisi, S. Di Palma,D. Corda, G. Baldus, F. Cruciani, S.M. Anzalone, S. Calderoni, G. Pioggia, F. Muratori, "An Integrated Approach for the Monitoring of Brain and Autonomic Response of Children with Autism Spectrum Disorders during Treatment by Wearable Technologies," Front. Neurosc. 10 (2016) 276.

[3] L. Robert, R. Rodri, and C. Beltra, 'tQEEG Spectral and Coherence Assessment of Autistic Children in Three Different Experimental Conditions," J Autism Dev Disord 45 (2015) 406-424.

[4] E. A. Lushchekina, E. D. Podreznaya, V. S. Lushchekin, and V. B. Strelets, "A Comparative EEG Study in Normal and Autistic Children," Neuroscience and Behavioral Physiology, 42(3) (2012) 236-243

[5] F. H. Duffy and H. AIS, "A stable pattern of EEG spectral coherence distinguishes children with autism from neuro- typical controls - a large case control study", BMC Medicine 2012, 10:64 (2012) 2-18.

Curriculum dos Investigadores envolvidos: evidenciam formagäo especializada e participacäo em investigacäo e publicacöes no åmbito em que se desenvolve o estudo.

Beneficios e Riscos: näo se preveem beneficios diretos imediatos para os participantes e os riscos potenciais, em particular para as pessoas com TEA, estäo acautelados, nomeadamente pelo envolvimento dos respetivos terapeutas, técnicos da APPDA-Vila Real.

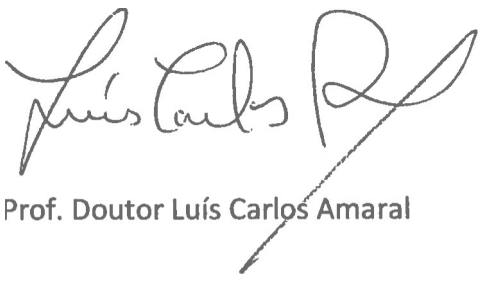
Conclusäo: Em conformidade com o exposto e considerando näo haver qualquer impedimento de caracter ético, entendeu a CEUP dar parecer positivo å implementação deste projeto.

Universidade do Porto, 22/10/2019

A Relatora

Prop. Doutora Paula Pinto de Freitas

O Presidente da CEUP



Prof. Doutor Luís Carlos Amaral

Annexe C

Opinion of the Data Protection Unit of the University of Porto

U.PORTO	Reitoria da Universidade do Porto	DATA: 08/11/2019

Nome	Carlos Fonseca / Patrícia Brás
Nº Mecanográfico	239252 / 201809052
Unidade Orgânica	Faculdade de Engenharia da Universidade do Porto (FEUP)
Título	Uso da eletroencefalografia (EEG) na avaliação dos efeitos das terapias assistidas por cavalos em crianças com transtorno do espetro autista
Ticket Nº	2019042615001774

Sumário do Pedido

No âmbito de uma dissertação de mestrado em Engenharia Biomédica, pretendem os requerentes validar a equitação terapêutica, ou Hipoterapia, como terapia benéfica para crianças com Transtorno do Espetro do Autismo (TEA). O estudo em causa envolve:

- analisar sinais eletroencefalográficos (EEG) de um grupo de crianças com TEA, registados enquanto estas frequentam sessões de Hipoterapia;
- sincronizar esse registo com gravação de vídeo das sessões para melhor monitorizar e enquadrar a resposta de cada criança aos estímulos recebidos durante a terapia;
- comparar o registo EEG com o de um grupo controlo de crianças não diagnosticadas com TEA.

A recolha de dados (EEG e vídeo) das crianças com TEA será efetuada ao longo de um total de 10 sessões de Hipoterapia, durante as quais serão sempre acompanhadas por profissionais habilitados, tanto na área da equitação como para lidar com TEA. Por sua vez, o grupo de controlo será sujeito a um único momento de avaliação EEG.

Atenta a natureza sensível dos dados pessoais objeto de tratamento neste estudo, bem como o caráter vulnerável dos titulares de dados visados, foi realizada uma Avaliação de Impacto sobre a Proteção de Dados, nos termos do art.º 35.º/1 do Regulamento Geral sobre a Proteção de Dados e do art.º 6.º do Regulamento 1/2018 da Comissão Nacional de Proteção de Dados.

Síntese do parecer da Encarregada da Proteção de Dados

Uma vez analisado o pedido de autorização submetido, e tendo-se verificado na AIPD, a inexistência de riscos elevados para os direitos, liberdades e garantias dos potenciais participantes no estudo, somos do parecer que poderá ser realizado o tratamento de dados pessoais acima descrito, uma vez que os requerentes cumpram a implementação de todas as medidas referidas no documento supramencionado.

Decisão Reitoral

Uma vez analisado o pedido em questão e tendo em consideração o parecer da Encarregada da Proteção de Dados da Universidade do Porto com a referência P-10/2019:

☒ Autorizo

☐ Não Autorizo

O Reitor

António de Sousa Pereira

Annexe D

Datasheet of Eego™ System

eego™sports product range

System components	eego sports 8 (ES-234)	eego sports 32 (ES-230) eego sports 32 pro (ES-231)	eego sports 64 (ES-232) eego sports 64 pro (ES-233)
eego amplifier	8-channel eego amplifier, 2 kHz, CE Class IIa medical device	32-channel eego amplifier, 2 kHz, CE Class IIa medical device	64-channel eego amplifier, 2 kHz, CE Class IIa medical device
eego software recording, review & export	✓	✓	✓
waveguard eeg cap – sizes B, I, C, S, M, L	One 8-channel waveguard cap (free choice of size)	One 32-channel waveguard cap (free choice of size)	One 64-channel waveguard cap (free choice of size)
asa pro advanced EEG/ERP analysis	3-month trial	3-month trial	3-month trial
Small Windows tablet	✓	✓	✓
eego sports backpack	✓	✓	✓
EEG starter kit	✓	✓	✓
eego sports suitcase	✓	✓	✓
Trigger adaptors	DB25 and BNC	DB25 and BNC	DB25 and BNC
Bipolar channels	–	2 (only for ES-231)	2 (only for ES-233)
Auxiliary channels	–	4 (only for ES-231)	4 (only for ES-233)
Auxiliary sensor kit	–	only for ES-231	only for ES-233
Bipolar channel upgrade (unshielded, up to 24)	–	optional for ES-231	optional for ES-233
Sensebox	–	only for ES-231	only for ES-233
Network event compatible (LSL)	✓	✓	✓
Warranty	eego amplifier: 2 years waveguard cap: 1 year	eego amplifier: 2 years waveguard cap: 1 year	eego amplifier: 2 years waveguard cap: 1 year
Support	6 months of free remote support & software updates	6 months of free remote support & software updates	6 months of free remote support & software updates

* optional exchange for laptop or PC.

An extensive list of available product options and accessories as well as additional remote and on-site trainings is available upon request.

ANT Neuro b.v., Enschede, The Netherlands,
tel. +31 53 43 65 175, fax. +31 53 43 03 795,
internet: www.ant-neuro.com, e-mail: info@ant-neuro.com

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eego™sports

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