Investigating the effects of neuromodulatory training on autistic traits: a multi-methods psychophysiological study.

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Declaration

I declare that this thesis, 'Investigating the effects of neuromodulatory training on autistic traits: a multi-methods psychophysiological study.', represents my own work, except where otherwise stated. None of the work referred to in this thesis has been accepted in any previous application for a higher degree at this or any other University or institution. All quotations have been distinguished by quotation marks and the sources of information specifically acknowledged.

Submitted by Tania K. García Vite

Signature of Candidate:

Date:

Table of Contents

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Abstract

Autism spectrum disorder (ASD) is characterized by noticeable difficulties with social interaction and communication. Building on past research in this area and with the aim of improving methodological perspectives, a multi method approach to the study of ASD, mirror neurons and neurofeedback was taken. This thesis is made up of three main experiments: 1) A descriptive study of the resting state electroencephalography (EEG) across the spectrum of autistic traits in neurotypical individuals, 2) A comparison of 3 EEG protocols on MNs activation (mu suppression) and its difference according to self-reported traits of autism in neurotypical individuals, and 3) Neurofeedback training (NFT) on individuals with high autistic traits. In chapters 3 and 4 we employed simultaneous monitoring of physiological data. For chapter 3 EEG and eye-tracking was used, In the case of chapter 4, EEG and eye-tracking as well functional near infrared spectroscopy (fNIRS). Overall the findings revealed differences in mu rhythm reactivity associated to AQ traits. In chapter 2, the rEEG showed that individuals with high AQ scores showed less activation of frontal and fronto-central regions combined with higher levels of complexity in fronto-temporal, temporal, parietal and parieto-occipital areas. In chapter 3, EEG protocols that elicited Mu reactivity in individuals with different AQ traits suggested that as the AQ traits become more pronounced in neurotypical population, the event related desynchronization (ERD) in low alpha declines. Chapter 3 was also the basis for the choice of pre/post assessment for chapter 4. In chapter 4 the multi-method physiological approach provided parallel physiological evidence for the effects of NFT in sensorimotor reactivity, namely, an increase in ERD in high alpha, higher levels of oxygenated haemoglobin and changes to the amplitude and frequency in the microstructure of mu for participants who underwent active training as opposed to a sham group.

Foreword

Autism spectrum disorder (ASD) is characterized by persistent deficits in social communication and interaction across multiple contexts (American Psychiatric Association, 2013a). Many underlying mechanisms have been proposed as being responsible for the symptomatology of ASD. Among the neural systems that have been associated to this disorder are the limbic system, the face processing system and the mirror neuron system.

Sensorimotor reactivity is associated to the mirror neurons, these neurons fire both when an individual observes and executes an action (Giacomo Rizzolatti & Craighero, 2004). Although mirror neuron activity cannot be measured directly, different neuroimaging techniques have been employed to measure their reactivity in an indirect manner (Braadbaart et al., 2013; Lepage & Théoret, 2006; Jaime A. Pineda, 2005; Yin et al., 2016). Chief among them and of particular interest to this thesis is electroencephalography (EEG) which measures the electrical activity at the scalp of underlying synchronous neuronal postsynaptic potentials (Angelakis et al., 2007). The EEG index of sensorimotor and mirror neuron reactivity is the Mu rhythm (Braadbaart et al., 2013; Lepage & Théoret, 2006; Muthukumaraswamy et al., 2004). This rhythm is an EEG oscillation originally described within the alpha frequency bandwidth of 8-13 Hz, and it is recorded over the sensorimotor cortex (Niedermeyer, 1997).

Sensorimotor reactivity is among the neural traits that have been described as abnormal in ASD. In individuals with ASD, abnormal mu modulation has been identified and associated with abnormalities in the motor system as well as social function difficulties (Bernier et al., 2007; Bernier et al., 2014; Cooper et al., 2013; de Vega et al., 2019; Lepage & Théoret, 2006; Oberman et al., 2005; Ruysschaert et al., 2014). For example, when observing a grasping action

executed by strangers, individuals with ASD exhibit reduced desynchronization of mu compared to control participants (Oberman et al., 2008).

With regards to therapeutic interventions, several lines of research are currently underway intending to improve the quality of life of people with ASD. These interventions range from pharmacological to cognitive and behavioural (Baghdadli et al., 2002; Coben et al., 2010; Lam et al., 2006; McDougle, 1998; Zhang et al., 2016). The choice of therapy will depend greatly on the severity of ASD as well as the comorbid disorders that the individual may have. One intervention that is both non-invasive and has had positive results in the treatment of autistic symptomatology is neurofeedback (Casanova & Sokhadze, 2019; Coben & Padolsky, 2016). Neurofeedback is a form of operant conditioning where a participant is taught to enhance specific brainwave patterns by monitoring and receiving feedback of physiological parameters in real time (Hammond, 2011). The use of neurofeedback as a clinical tool in the study and treatment of several pathologies has slowly grown, based on the positive results found in past research (Kaur et al., 2019; D. Vernon et al., 2003a).

With that in mind this thesis aims to tackle two main objectives:

1) Building on past research in this area and improving methodological issues in order to obtain more objective information, a multi method approach to the study of mirror neurons was taken. In order to address the attentional bias that has been suggested as responsible for differences in sensorimotor activity when studying ASD populations, the experiments employed simultaneous monitoring of physiological data; in the case of chapter 4 EEG and eye-tracking, and in the case of chapter 5, EEG and eye-tracking as well as EEG and functional near infrared spectroscopy (fNIRS). This, with the intention of obtaining a

broader physiological picture of the neural underpinnings of sensorimotor reactivity, autistic traits and neurofeedback.

2) To investigate the possibility that training healthy individuals with different levels of autistic traits to enhance the mu rhythm in their EEG via neurofeedback protocols can influence their sensorimotor reactivity.

To achieve these purposes, in addition to the theoretical overview chapter that encompasses the three main topics of the project (ASD, mirror neurons and neurofeedback; Chapter 1) this thesis is comprised of the following experimental chapters:

Chapter 2: Resting state EEG (rEEG) in autistic traits.

An observational/descriptive study of the rEEG across the spectrum of autistic traits in neurotypical individuals. The first experimental chapter is based on the premise that differences in sensorimotor reactivity exists intrinsically in the brain according to different levels of autistic traits. This chapter aimed to explore individual differences of oscillatory and nonlinear measures of the rEEG based on the self-reported level of autistic traits in individuals. Two minutes of rEEG activity was recorded on individuals that previously filled out the Autism-Spectrum Quotient (AQ) which is a self-administered short scale that measures the degree to which an adult of normal IQ exhibits traits associated with the autistic spectrum (Simon Baron-Cohen et al., 2001). Higher scores equal a higher level of autistic traits. The AQ was answered by an initial sample 291 from which high and low scoring individuals were selected to take part in the study. High and low group scores were selected by taking one standard deviation above and below from the mean score among

the sample (M=18, SD=6). Group differences and correlation to AQ traits were identified and described in both oscillatory and nonlinear measures.

Chapter 3: EEG protocols on hMNS activation (mu suppression) and its difference according to self-reported traits of autism in neurotypical individuals.

Following the group differences that were identified as associated to AQ traits, the following chapter focused on understanding functional changes in the EEG and the role that mirror neurons may have in goal-oriented movements and how these may vary depending on individual differences of AQ traits. In order to achieve this, we compared 3 different protocols that have been previously associated with the hMNS in terms of anatomy as well as mu-suppression (in sensorimotor alpha and low beta bands) in high and low AQ individuals. This was measured through simultaneous EEG and eye-tracking in order to control for the level of attention afforded to the different protocols between groups. Given that the ultimate purpose of this thesis was to test the effect of neurofeedback on autistic traits while controlling for some of the methodological limitations described previously in the literature, another crucial goal of this chapter was to identify which of the three protocols would serve as the best physiological pre- and post-training assessment for the neurofeedback study.

Chapter 4: Neurofeedback training on individuals with high autistic traits.

Continuing on the findings from the EEG protocols on mu rhythm suppression, the neurofeedback training (NFT) protocol was assessed though pre/post EEG and eye-tracking using the selected protocol from the previous chapter. Haemodynamic changes throughout the NFT using near-infrared spectroscopy (NIRS) were monitored as an added physiological measure of assessment.

On this occasion, the study focused on individuals with high AQ traits which were randomly divided into neurofeedback training and a sham training group. All individuals underwent 8 sessions, naïve to their training group allocation, and were monitored with EEG as well as NIRS over the right sensorimotor cortex. The premise of this final study was that by increasing the power of mu through NFT this would also translate to an increase in the suppression of mu by increasing the neuronal population firing and therefore influence their sensorimotor reactivity.

Following the experimental chapters, a final discussion chapter (Chapter 5) is included with the main conclusions and remarks of the overall project.

1. Theoretical overview

1.1. Autism

1.1.1. Definition and classifications

Autism Spectrum disorder (ASD) refers to a series of persistent deficits in social communication and social interaction across multiple contexts, including deficits in social reciprocity, nonverbal communicative behaviours used for social interaction, and skills in developing, maintaining, and understanding relationships (American Psychiatric Association, 2013a). These symptoms (difficulties with social development, communication and strong narrow interests/repetitive behaviour]) are traditionally known as the Autistic Triad. However, the domains in which the processing of social and emotional information arise also include face recognition, joint attention, responses to the emotional displays of others, and lack of eye contact (Back, Ropar, & Mitchell, 2007; Fakhoury, 2015; Fan, Chen, Chen, Decety, & Cheng, 2013; Pelphrey, Morris, & McCarthy, 2005; Schultz, 2005; Senju, 2013). Atypical responses to sensory stimuli have also been reported in vision, hearing, touch, the vestibular system, hearing, smell and taste (Baum et al., 2015; Iarocci & McDonald, 2006; Kern et al., 2007; Lane et al., 2014; Leekam et al., 2007; Robertson & Simmons, 2013; Tavassoli et al., 2014). Such sensory responses are described in terms of hyper and hypo sensitivities. For example, in the case of auditory and visual stimulation, noises and lights can cause discomfort because they appear exacerbated to an individual with ASD (Baron-Cohen, Ashwin, Ashwin, Tavassoli, & Chakrabarti, 2009; Tavassoli et al., 2014). Whereas, in the case of temperature or painful stimuli, a hyposensitivity has been reported, that is, the threshold for pain is higher in ASD than in neurotypical individuals (Bird & Cook, 2013).

In terms of comorbid psychiatric and medical conditions that individuals with ASD present, the

most common conditions that have been reported are: epilepsy, bowel/gastrointestinal disorders, sleep disorders, cranial anomalies, schizophrenia, attention deficit hyperactivity (ADHD), anxiety and other mood disorders (Kohane et al., 2012; Mazurek et al., 2014; Ming et al., 2008) as well as alexithymia (Bird & Cook, 2013; Fan, Chen, Chen, Decety, & Cheng, 2014).

The diagnostic and statistical manual of mental disorders in its fifth edition (DSM-V) includes ASD as part of the neurodevelopmental disorders, that is to say, disorders that have an onset in early stages of development. In the case of ASD, the initial appearance of symptoms has been reported around 1 to 3 years of age, however the degree of severity, alongside their I.Q. level and socioeconomic status can affect the age at which they receive diagnosis (Mazurek et al., 2014). Although traditionally the symptoms regarding ASD were considered as a triad and Autism was considered as one of the 5 pervasive development disorders (along with Asperger´s Disorder, Rett´s Disorder, Childhood Disintegrative Disorder and Pervasive Developmental Disorder Not otherwise specified) (American Psychiatric Association, 2000), newer editions of the DSM have since described diversity regarding both symptoms and their severity in favour of a spectrum based perspective. The DSM-V classifies the severity of ASD into 3 levels depending on the amount of support that the individual requires in order to interact and fulfil day-to-day activities. These are: level 1 (requiring support); level 2 (requiring substantial support); level 3 (requiring very substantial support). A more detailed description of each level can be found in Table 1.1.

Clinical features in ASD are also manifested with heterogeneity among the spectrum. At the low functioning end of ASD, individuals tend to experience more difficulties with verbal and non-verbal language, have lower IQs, experience oversensitivity towards sensory stimulus, and present inflexibility toward change, as well as self-injurious behaviours (Gabriels et al., 2005). On the other side, individuals with higher functioning autism often present an IQ that is at or above the

neurotypical population, and although they experience less difficulties with verbal and non-verbal language, they still display social communication and interaction difficulties, may have problems understanding metaphors, and/or display abnormal attention to eyes and mouth when socializing (Bar-Haim et al., 2006; Bellini, 2004; Boraston & Blakemore, 2007; Meyer & Minshew, 2002). In terms of comorbidities, higher degrees of severity in autistic symptoms also tend to present more medical comorbidities while lower degrees reflect more psychiatric comorbidities (Ming et al., 2008). For example, an individual with high functioning autism is more prone to developing an obsessive compulsive disorder that may stem from either the patterns of repetitive behaviours that are characteristic of ASD symptoms or from experiencing high levels of anxiety as a result of social difficulties (Gillott et al., 2001). In addition, in individuals with a higher severity of ASD traits there is a higher incidence of epilepsy and gastrointestinal abnormalities (Ming et al., 2008). Such diversity in comorbidities and clinical features can also impact the initial diagnosis of ASD, in some cases causing delay or masking other symptoms (Mazurek et al., 2014). For these reasons, the causes and courses involving this disorder are assorted and have given rise to several theories that have attempted to explain its core features and provide different insights in terms of treatment. The following section will focus on such past research.

Table 1.1 severity of ASD according to the DSM-V (American Psychiatric Association, 2013)

Severity level	Social communication	Restricted, repetitive behaviours
Level 3 "requiring very substantial support"	Severe deficits in verbal and nonverbal social communication skills cause severe impairments in functioning, very limited initiation of social interactions and minimal response to social overtures from others. For example, a person with few words of intelligible speech who rarely initiates interaction and, when he or she does, makes unusual approaches to meet needs only and responds to only very direct social approaches.	Inflexibility of behaviour, extreme difficulty coping with change, or other restricted/repetitive behaviours markedly interfere with functioning in all spheres.
Level 2 "requiring substantial support"	Marked deficits in verbal and nonverbal social communication skills; social impairments apparent even with supports in place; limited initiation of social interactions; and reduced or abnormal responses to social overtures from other. For example, a person who speaks simple sentences, whose interaction is limited to narrow special interests, and who has markedly odd nonverbal communication.	Inflexibility of behaviour, difficulty coping with change or other restricted/repetitive behaviours appear frequently enough to be obvious to the casual observer and interfere with functioning in a variety of contexts. Distress and/or difficulty changing focus or action.
Level 1 "requiring support"	Without supports in place, deficits in social communication cause noticeable impairments. Difficulty initiating social interactions, and clear examples of atypical or unsuccessful responses to social overtures of others. May appear to have decreased interest in social interactions. For example, a person who is able to speak in full sentences and engages in communication but whose to-and-fro conversation with others fails, and whose attempts to make friends are odd and typically unsuccessful.	Inflexibility of behaviour causes significant interference with functioning in one or more contexts. Difficulty switching between activities. Problems of organization and planning hamper independence.

1.1.2. Brief overview of Autism theories

The study of autism dates back to the 1940s, described in an almost parallel form by Leo Kanner (1943) and Hans Asperger (1944). Both published papers where they described cases of children with autistic symptomatology (a term originally coined by Bleuler Schirmer, a Swiss psychiatrist that described characteristics of individuals with schizophrenia (Lyons & Fitzgerald, 2007)). Since their pivotal studies, a growing number of researchers have been dedicated to the study of this disorder. Throughout history there have been multiple theories that have attempted to explain the key features and causes of autism, each with its own perspective. Many theories have since been discredited. One example is the "refrigerator mother" theory (Bettelheim, 1967). This theory stated that Autistic symptoms were a result of a lack of emotion in parenting style. However, with the advancement of technology new research techniques have allowed a surge of theories that focus more on cognitive and cerebral aspects of individuals with ASD.

Cognitive theories propose that the causes of ASD can be reduced to a few underlying mental processes (Simon Baron-Cohen, 2009). Some theories have been modified as a result of the advancement of research in the field. For example, in the case of Mindblindness, it was originally proposed that individuals with ASD did not have a Theory of mind (ToM), (the ability to put oneself in another person´s state of mind and in this way understand their behaviours and feelings) it was later modified as a delay in the development of ToM when studies showed that the absence of ToM was not universal within the ASD population (Baron-Cohen, 1995). Another example is the Empathising-Systemising (E-S) theory (Baron-Cohen, Wheelwright, Lawson, Griffin, & Hill, 2002) which proposes that ASD can be described as a deficiency in empathy combined with an exacerbation of systemizing traits. It was also later modified, evolving into the Extreme male brain theory (Baron-Cohen, 2002) where E-S theory is applied to the psychometric profile of each sex. ASD is then viewed as a manifestation of an extreme of the male cognitive profile.

Connectivity theories focus on the connections between different brain areas or within neural assemblies that can result in ASD symptomatology (Baron-Cohen et al., 2000; Coben & Myers, 2008; Happé & Frith, 2005). For example, Courchesne, Redcay, Morgan, & Kennedy (2005) describe ASD as a consequence of both over connectivity within frontal cortex and under connectivity of the frontal cortex with other areas in the brain. This in turn impedes the normal functioning and communication with other brain regions.

In terms of genetic theories, the Zhao et al. (2007), propose that ASD is the result of a genetic alteration that occurs for the first time in one family member, which is usually passed on to the offspring by females who are more resistant to the mutation. This also explains how male offspring have a higher probability of manifesting the ASD, as they are less resistant to this mutation.

On the other hand, neuroscientific theories that focus on anatomical and functional features of brain activity. These theories can be broadly classified into structural, functional reactivity and connectivity based. Structural theories focus on the differences in anatomical structures of the brain between neurotypical populations and individuals with ASD (Amaral et al., 2008). Functional reactivity theories focus on specific brain rhythms (mainly the mu rhythm, which will be covered in detail later in the chapter), and the differences in reactivity of these rhythms between ASD and the neurotypical population (Fox et al., 2016). These theories will be covered specifically in future sections.

1.1.3. Broader Autistic Phenotype (BAP) and Autistic traits in the normal developing population.

Given the heterogeneity in the spectrum of behavioural and cognitive traits, research has also looked to the role of genetic factors in the development of ASD. In this sense, studies have focused on twin and family members of individuals with ASD. What researchers began to observe were a variety of behaviours that were qualitatively similar to the ASD symptomatology but didn't necessarily reach the severity or criteria to establish a diagnosis. For example, Happé, Briskman, & Frith, (2001) compared cognitive styles in parents of children with autism and dyslexia to those of neurotypical children and found that in the parents of autistic children there was an enhanced performance in tasks that were related to local processing. Such performance and attention to detail, however, was an advantage in the parents as it did not impede global processing of information as in the case of ASD according to the weak central coherence theory (WCC) (Rajendran & Mitchell, 2007). These behavioural and cognitive traits in relatives of individuals with ASD are known as the broader Autism phenotype (BAP) (Dawson & Webb, 1989; Le Couteur et al., 1996; Piven, Palmer, Jacobi, Childress, & Arndt, 1997) and can offer another approach in genetic studies of ASD as well as information about the neural circuitry involved (Losh et al., 2009).

Results from research on the BAP sparked interest in other populations that could also manifest ASD-like traits. Recent studies have focused on the presence of autistic traits in the general adult population. What has been found is a normal distribution of autistic traits (Baron-Cohen, Wheelwright, Skinner, Martin, & Clubley, 2001; Constantino et al., 2003; Wakabayashi, Baron-Cohen, Wheelwright, & Tojo, 2006). Exact individual markers have not yet been defined causally, and the degree to which each person exhibits these traits varies among individuals. However, sex-

linked biological factors, expertise in certain skills (scientific, mathematical) as well as particular personality traits such as high levels of neuroticism and low levels of agreeableness and extraversion (Austin, 2005; Wakabayashi, Baron-Cohen, & Wheelwright, 2006) have been considered as indicative of higher or lower autistic traits (Baron-Cohen, Richler, Bisarya, Gurunathan, & Wheelwright, 2003).

Research with both the BAP and autistic traits in the general population can provide the basis for obtaining phenotypes associated to ASD and their neural underpinnings (Losh et al., 2009). This perspective has led to a new model of autism, not as a clear cut disorder, but rather as a different cognitive style; a spectrum where autistic traits can be viewed in a continuum, and that can be studied quantitatively (Wakabayashi, Baron-Cohen, & Wheelwright, 2006). Robertson and Simmons, (2013) for example, investigated the link between ASD and sensory sensitivity in the general population, finding a positive correlation between number of autistic traits and the frequency of sensory processing problems. EEG activation differences in the pre-motor cortex and supplementary motor area have also been found between neurotypical individuals with high and low traits of autism (Puzzo et al., 2010). Cooper, Simpson, Till, Simmons, and Puzzo (2013) also found differences in activation of human mirror neurons modulated by emotional facial according to individual differences in the level of autistic traits. Another study by Puzzo, Cooper, Cantarella, Fitzgerald, and Russo (2013) focused on the effect of rTMS over the inferior parietal lobule (IPL) on EEG sensorimotor reactivity of neurotypical individuals with different degree of self-reported traits of autism. The authors found that active rTMS over the IPL modulates the oscillatory activity of the low beta frequency of a distal area, and that this modulation changes according to the degree of self-reported traits of autism.

Studying ASD from this perspective also brings about several benefits in terms of conducting the studies themselves and diminishes the possibility of encountering certain methodological limitations or confounds that could alter results (Cooper et al., 2013). First of all, working with autistic traits within the typically developed adult population allows for a larger sample size in the potential participants of each study, which leads to more robust and generalizable findings. Also, the use of medications that could cause effects in task performance is also diminished in the typically developed population. Thirdly, because the participants do not manifest a diagnostic ASD symptomatology, a wider range of research instruments and techniques can be applied without causing discomfort to the participants during the data collection. For example, individuals with diagnosed ASD could find wearing an EEG cap extremely uncomfortable or find it very difficult to sit still for the duration of an experiment.

Thus, more research into the clinical phenotyping of individuals with autistic traits at the extreme ends of behavioural traits as well as those with a diagnosis is needed to identify measures of severity in different domains of performance (Oberman et al., 2013) and the degree to which alterations in neural systems have a causal role.

1.1.4. Neural systems implicated

ASD brain-based and genetic mechanisms have been the increasing focus of research in the past years. To date, neither a specific core mechanism nor an individual gene has been found responsible for the symptoms that distinguish ASD (Grove et al., 2019; Levy et al., 2011; Loparo & Waldman, 2015a). What has instead been generally accepted thus far, is that several genes seem to be involved as a risk factor for various components of the symptomatology (Dawson & Webb, 1989). In terms of functional neuroanatomy, studies have also shown a diverse set of neural

systems involved (Amaral et al., 2008; Brambilla et al., 2004; Stanfield et al., 2008). In this sense, it is imperative that the systems that normally mediate social functions and regulate repetitive behaviours and patterns are understood (Schultz, Romanski, et al., 2000). Recently, three corresponding neural systems have been emphasized as interconnected cortical and subcortical networks that play a role in the deficits observed in ASD (Ameis & Catani, 2015; Catani et al., 2013). These are: the limbic system, the face processing system and the mirror neuron system.

1.1.4.1. The limbic system

The limbic system is composed of cortical and subcortical structures that provide the neural basis for many instinctive and emotional aspects of behaviour as well as memory (Binder et al., 2009). It has several pathways that project to the hypothalamus and it also receives input from the neocortical association areas. This involves the limbic system in linking complex goal-directed behaviour to more primitive, instinctive behaviour and internal homeostasis. (Crossman & Neary, 2014).

The amygdala is an important part of the limbic system. It has been the focus of research in ASD because it influences both drive-related behaviours and associated emotions (Baron-Cohen et al., 2000). In people with high functioning autism, the neuropsychological profiles have traits similar to those with amygdala damage, such as impairments in the facial recognition of expressions of fear, perception of eye gaze and recognition memory for faces (Amaral et al., 2008). By using quantitative magnetic resonance image analysis techniques, Howard et al. (2000) found a significant increase in global amygdala volume in this population. These results support the role of developmental malformation of the amygdala in social-cognitive impairments associated with ASD.

More recently, research focus has been on the amygdala and its connections with particular systems in the temporal and frontal cortices, in which abnormalities lead to social deficits and repetitive behaviours (Schultz, 2005; Schultz, Romanski, et al., 2000). In a review by Catani et al. (2013), he provides a model describing parallel fronto-temporal limbic circuits involved in emotion, behaviour and memory that are associated to ASD: 1) the temporo-amygdala-orbitofrontal circuit; 2) the dorso-medial limbic circuit;

1.1.4.1.1. Temporo-amygdala- orbitofrontal circuit.

Responsible for integrating visceral and emotional input with cognition and behaviour. Some tasks associated to this circuit that have been found in studies with humans, include comprehension of single words, the processing of faces, as well as regulating outcomes of actions (Catani et al., 2013). In ASD populations, a dysfunction has been found in this circuit through fMRI studies. For example, during a task where participants had to guess what a person might be feeling or thinking by looking at gazes (expression in the eyes) individuals with ASD did not have activation of the amygdala despite being able to activate frontotemporal regions (Baron-Cohen et al., 1999). The dysfunction of different components in this circuit suggest a possible explanation for differences in cognition that involve socio-emotional factors as well as deficits in regulation of behaviours that are classically associated to ASD symptoms (Bachevalier & Loveland, 2006).

1.1.4.1.2. Dorso-medial limbic circuit

Comprised of ventromedial prefrontal cortex, precuneus, posterior cingulate cortex and the temporal parietal junction. These regions have an enhanced activity when conducting specific goal-directed behaviours following a synchronous deactivation of the default mode network

described by Raichle et al. (2001). That is, going from a resting but awake state to executing specific tasks associated with: working and autobiographic memory, mentalising (understanding the intentions of other people), prospective thinking, and attention to activities that are driven by sensory stimulation (Amodio & Frith, 2006). Damage to this circuit results in sensory alterations such as abnormal threshold to pain and olfactory perception, as well as impaired expression of emotions and attention difficulties (Catani et al., 2013). These sensory alterations and difficulties with affective communication and attention coincide with symptoms and traits that have been described in ASD (Hamilton et al., 2007; Iarocci & McDonald, 2006; Kern et al., 2007). A summary of the networks, their functions and the resulting disorders from lesions to the areas can be found in fig. 1.1 and a detailed description of the figure can be found in table 1.2.

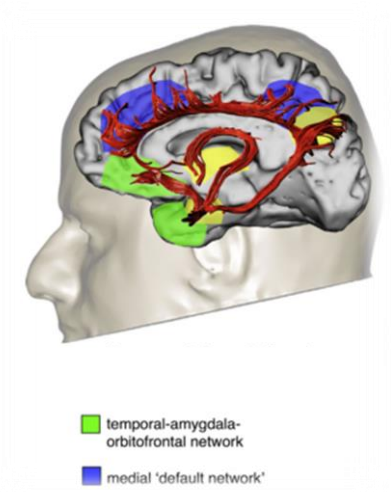

Fig. 1.1. Model proposed by Catani et al. (2013) for the functional-anatomical division of the limbic system into partially overlapping networks, their associated functions and the resulting disorders from lesions to the areas.

Table 1.2 Model proposed by catani et al. (2013) for the functional-anatomical division of the limbic system into 3 partially overlapping networks, their associated functions and the resulting disorders from lesions to the areas.

Network	Function	Disorder
Temporo-amygdala orbitofrontal	▪ Behavioural inhibition ▪ Memory for temporally complex visual information ▪ Olfactory –gustatory-visceral function ▪ Multimodal sensory integration ▪ Object-reward association learning ▪ Outcome monitoring	▪ Alzheimer's disease (advanced) ▪ Semantic Dementia ▪ Kluver-Bucy syndrome ▪ Temporal lobe epilepsy ▪ Geschwind's syndromes ▪ Psychopathy ▪ Bipolar affective disorders
Dorsomedial default network	▪ Pain perception ▪ Self-knowledge ▪ Attention ▪ Mentalizing ▪ Empathy ▪ Response selection and action monitoring ▪ Autobiographical memory ▪ Person perception	▪ Depression ▪ Autism ▪ Schizophrenia ▪ Obsessive compulsive disorder ▪ Mild Cognitive Impairment ▪ Alzheimer's disease (early) ▪ Attention Deficit Hyperactivity Disorder ▪ Anxiety

1.1.4.2. The face processing system

The face processing system comprised of the superior temporal sulcus, the fusiform face area (inferior temporo-occipital cortex) and the amygdala is another of the neural systems described as altered in individuals with ASD (Ameis & Catani 2015).

Lesions to these areas result in alterations to recognising affective facial expressions (Philippi et al., 2009). Deficits in processing information from faces in ASD has been widely documented (Back et al., 2007; Bar-Haim et al., 2006; Ensenberg et al., 2016; Leppänen, 2016; Moore et al., 2012; Nickl-Jockschat et al., 2014; Pelphrey et al., 2007; Schulte-Rüther et al., 2014; Schultz, 2005; Spezio et al., 2007). For example, Pelphrey, Morris, Mccarthy, and Labar (2007) compared fMRI scans of individuals with high functioning autism while they observed dynamic and static facial expressions and found reduced activity in social brain regions including the amygdala, posterior superior sulcus and fusiform gyrus. In the next three sections we will take a closer look as to the functions of each area and their contributing role in the identification and processing of faces.

1.1.4.2.1. The superior temporal sulcus

The superior temporal sulcus (STS) plays a role in processing gaze shifts in social contexts where the information could convey relevance in terms of intentions, and exhibits a different response in individuals with autism (Pelphrey, Morris, & McCarthy, 2005). It shows a preferential response to those stimuli that are meaningful for the individual and has been linked as the underlying mechanism not only for social interaction but also for the development of language (Redcay,

2008). This area also responds to biological motion and a diminished activation has been found on PET studies with ASD individuals during mentalization tasks (Ameis & Catani, 2015).

The STS has also been proposed as an extended part of the human mirror neuron system. Although the neurons located here are not considered to be mirror neurons per se, (which fire both when observing and performing an action), they are important in action understanding. They support the functional role of mirror neurons by reacting to actions associated to the eyes and head in addition to images that depict biological motion (Pineda, 2008). The human mirror neuron system and its association to ASD will be explained more in depth in the following section. However, it is important to emphasize that the role of the STS in social perception (assessing social information through eye gaze and facial expression) makes any dysfunction in this area exceedingly detrimental to the ability to interact socially, and thus, very important in the study of ASD.

1.1.4.2.2. The fusiform face area

Located in the inferior temporo-occipital cortex, this area, as the name suggests, modulates the identification of faces as well as their recognition. Unlike the STS, which recognises facial expressions, the fusiform face area's unique function in the brain is that it identifies and reacts to faces, regardless of the emotion that the face may portray (Schultz, 2005). Lesions to this area result in prosopagnosia, which is the inability to recognize faces (Buzsáki, 2009). It has been theorized that the difficulties in processing faces could be the result of a missed signals from the amygdala that would also affect comprehension of social contexts (Schultz, 2005). In individuals with ASD it is consistently hypo-activated during face-processing tasks (Nickl-Jockschat et al., 2014). Schultz and Gauthier, et al., (2000) observed that in individuals with ASD, the activation in the right frontal gyrus (centre of activation of face perception) was significantly less activated

compared to neurotypical adults. The authors used fMRI to study brain region activation when viewing faces and objects. The differences in activation was only present when viewing faces and did not discriminate between different emotions on the faces.

Early reports of hypoactivation have not been replicated in all studies that investigated this phenomenon, and it is worth mentioning that in behavioural studies that focus on the identification of faces, qualitative differences are not conclusive between ASD and non-ASD population (Weigelt et al., 2012). Thus, more research into the role and mechanisms of the fusiform face gyrus both in ASD and non-ASD population is still needed.

1.1.4.2.3. The amygdala

The amygdala is a subcortical cluster of brain nuclei that are located deep within the temporal lobes, at the anterior end of the hippocampal formation and the inferior horn of the lateral ventricle of each hemisphere (Baron-Cohen et al., 2000). The main functions associated with the amygdala are related to emotions, biological based functions, attention, memory and learning (Binder et al., 2009). In humans, when processing stimuli of social relevance, such as recognition of facial expressions and gaze as well as body movements, the amygdala (specifically the left) has an enhanced activation (Baron-Cohen et al., 2000). It also has an influence in behaviour that involves motivation and emotion, most commonly, fear and its manifestation of autonomic signals. Because of its associated functions and anatomical location, the amygdala it is thought to play a connecting role between brain regions that process sensory information and those that elicit the corresponding emotional and motivational reactions (Binder et al., 2009). Howard et al. (2000) reports that when damage to amygdala is found, difficulties with memory regarding faces arises as well as detection

of the direction for eye gaze, resulting in impairments of socio-affective function. Not only do behavioural symptoms of ASD resemble those that have been described in patients with amygdala and temporal lobe damage, different types of structural neuroimaging studies have also found evidence to support a dysfunction of the amygdala in ASD (Sweeten et al., 2002). Using fMRI, Baron-Cohen et al. (1999) found that during a task designed to activate the amygdala (determining what a person is thinking/feeling based only on eye expression) the group with ASD lacked activation of their left amygdala but displayed increased activation in the STS compared to the control group. Another fMRI study found that participants with high functioning ASD had greater activity in the left superior temporal gyrus and peristriate visual cortex and less activity in the right fusiform cortex compared to the control group. The task consisted of assessing emotions while viewing photos of faces and ASD participants were matched to their controls in age and intelligence (Critchley et al., 2000).

From a neuroanatomical perspective, the development of the amygdala in individuals with ASD has a different course than typically developed individuals. In ASD, the size of the amygdala is larger during early years of life but its growth is diminished when reaching pre-adolescence (Schumann et al., 2004). What is curious is that this difference in size has not been found in older age groups (Haznedar et al., 2000). Two possible explanations for amygdala abnormalities in ASD is that there is either an overall lower number of neurons produced in the amygdala in the first years of life or that the amount of neurons produced is normal but is possibly eliminated through excessive pruning in adolescence Amaral et al. (2008).

1.1.4.3. The mirror neuron system

As this system is central to the thesis as a whole, it will be described in more detail in the following section. For the purpose of completeness, a short introduction is included here. Mirror neurons are a type of cell that fire both when a specific action is observed and when it is executed. Initial research in these neurons was conducted in monkeys as they were originally found in the F5 area of the premotor cortex of the macaque monkey (Rizzolatti & Craighero, 2004). The mirror neuron system is integrated by the right rostral inferior parietal lobule and inferior frontal cortex, these areas were found to form a cohesive system with physiological properties important in the incorporation of sensorimotor information and have also been associated with the neural basis of imitation (Iacoboni & Dapretto, 2006). The actions that were found to elicit mirror neurons response in monkeys corresponded to the mouth and hands (Ferrari et al., 2003, 2005). For example, behaviours associated with communication (lip smacking, lip/tongue protrusion) as well as those related to feeding (chewing, sucking) as well as grasping, placing, holding etc. Such behaviours are important in learning and imitation and ultimately in the survival of a species (Cook et al., 2014).

Research was later done on humans using non-invasive neuroimaging techniques such as EEG and fMRI to investigate the existence of a human mirror neuron system (hMNS) with similar reactivity and topographic correspondence (Braadbaart et al., 2013; Hamilton, 2013; Hobson & Bishop, 2016). This mirror neuron system described in humans allows not only the observation/execution of actions, but may also be involved in different cognitive functions such as imitation, theory of mind, language and empathy; as well as the perception of sensations and vicarious emotions (Keysers & Gazzola, 2009). As was reviewed above, these cognitive functions have been reported as impaired in individuals with ASD, which is why the study of mirror neurons has gained great

interest as the neural substrate for the ability to ability to understand and imitate the behaviour of others (Oberman et al., 2005). A more in-depth view of mirror neurons and their relation to ASD will be covered in the following section.

1.2. Mirror neurons

1.2.1. Overview and historical findings

In the 1990s, while several studies were involved in researching the F5 area of the premotor cortex in the macaque monkey, Rizzolati´s research team in the University of Parma found a specific type of neuron that fired not only when a monkey executed an action, but reacted in the same way when it observed the experimenter or another monkey perform that same action. This reactivity, mirroring the activity in others, earned them the name Mirror Neurons (MNs) (Rizzolatti, Fadiga, Gallese, & Fogassi, 1996).

The distinguishing trait of these neurons inspired further studies into revealing properties surrounding the conditions under which the mirror reaction was evoked. Classical studies continued using single cell neurophysiology in F5 and focusing on the firing specificity of mirror neurons in the monkey brain. The main findings of these studies were as follows:

- MNs respond to goal directed mouth and hand actions.

After recording the electrical activity from 532 neurons in area F5 of two macaque monkeys, Gallese, Fadiga, Fogassi, and Rizzolatti (1996) found that the visual stimuli that evoked the highest response in mirror neurons were those that involved object interactions with either the mouth or the hand of the experimenter. Furthermore, hand movements resulted in increased discharges in

the dorsal section of F5 while mouth movements had the same results with neuronal activity in the ventral section of this area. The authors also concluded that the distance of the object from the monkey did not affect MN reaction and intensity.

On the other hand, Ferrari, Gallese, Rizzolatti, and Fogassi (2003) found that a third of the mirror neurons, that were reactive to mouth actions, were particularly active during the observation and execution of actions associated to consuming food, for example: grasping, sucking or breaking food. These were referred to as ingestive MNs. They also found reactivity of mouth MNs with gestures that are associated with communication. This information is specifically relevant in supporting the extension of studies of MNs in monkeys to humans because area F5 of the monkey brain is considered to be the homologue Broca's area; an area that is also involved in communicative functions in humans.

- MNs respond ambidextrously and specifically to a type of hand movements

A same mirror neuron can fire for both right and left hand actions but is specific in the firing pattern depending on the type of hand action involved (Rizzolatti et al., 1988). For example, when picking up an object, the type of grasp the monkey uses causes different firing patterns in F5 (whole hand grip vs precision grip).

- Their activity "represents" the observed action.

When executing a movement, the individual usually predicts its consequences (the goal of the motor action). The meaning of the reactivity of MNs goes beyond a system limited to matching observation with execution. By recognizing that it is a another individual that is executing a goal

oriented action, and distinguishing it from other actions, an individual can use the information to act accordingly depending on the context (Rizzolatti et al., 1996). For example, in learning a skill that will aid in obtaining food. By matching an observed motor action with the corresponding activity that happens when the individual performs the same action, the individual could obtain the meaning of the observed action (di Pellegrino et al., 1992). The meaning of the movement is represented by a specific cortical pattern. When an external stimulus evokes a neural activity that represents a certain action similar to one generated internally, the similarity between the neural pattern of the internally represented action and the one evoked by an external stimulus results in the recognition of the meaning of the action (Rizzolatti et al., 1996).

- MNS are implicated in action recognition.

In Umiltá et al.´s (2001) study the researchers found evidence suggesting MN's role in action recognition. They recorded 220 individual neurons under two experimental conditions (A and B), at the beginning of both an object was presented on a table; however in one condition ("full vision") the monkey was presented with a goal directed action (an experimenter's hand reaching for the object). In the second condition this same action was presented but the final interaction between the hand and object (goal) was hidden behind a screen, that is, the monkey only had visibility of a hand reaching behind a screen. Of the neurons recorded, 103 were found to have mirror properties and 50 % responded in both conditions. This was is interesting because in the hidden condition, the information on the goal of the action could only be inferred. It was assumed that the MNs were able to code certain actions without having a full visibility of them, suggesting an understanding of the action that the observed individual is doing.

- MNs in monkeys are not responsive to pantomime actions.

In this same study, Umiltá et al., (2001) explored a further two further conditions with the same terms as before, but in these cases there was no initial presentation of an object. In both cases (with and without the presentation of a screen) the monkey only saw a hand reaching with a grasping action; that is, the action was merely a pantomime of the previous goal directed action. In these cases, they found that the MNs did not respond. The authors suggested that MNs use prior information to distinguish meaning of actions that are not explicit but that are usually identical in their final goals.

- MNs distinguish between same actions associated with different intentions

Fogassi et al., (2005) recorded neurons from the inferior parietal lobe from two monkeys during different conditions of observation and execution of grasping actions. The first two conditions were as follows: 1) the monkey reached for and grasped an inedible object and placed it in a container (which resulted in a reward for the monkey) 2) the monkeys reached for and grasped a piece of food that was subsequently eaten. Although both actions elicited a MN response, most neurons recorded had increased reactivity to the grasping of food (around 75%) suggesting that the intention of the action is relevant in the MN reactivity. In a third experimental condition, the monkey underwent training to grasp the piece of food and place it in a container. All neurons that discharged highest during the grasping-to-eat condition were less reactive in the grasping-to-place condition. Finally, the three previous experimental setups were replicated but, in this case, the monkey observed the experimenter performing the conditions. The neuronal firing during these three conditions resembled the execution conditions, supporting the relevance of the intention of the action for MN reactivity.

- MNs have the possibility of acquiring new properties.

Ferrari, Rozzi, and Fogassi, (2005) found a specific type of MNs that respond to actions performed with tools. After recording 209 neurons from the lateral section of the ventral premotor area F5 of two macaque monkeys, they observed that a group of neurons discharged when the monkey observed actions performed by an experimenter with a tool (a stick or a pair of pliers). They also found that the response was stronger than when the monkey observed a similar action made by the experimenter using their hand or mouth. The goal of the actions was the same, yet the monkeys were not previously trained for the manipulation of the tools used. The authors propose that a visual association was formed between the hand and the tool after the visual exposure to tool actions, which would suggest that these tool-responding mirror neurons would allow the observing monkey to extend action-understanding capacity beyond its initial motor representations in order to acquire new motor skills, in this case, the use of the tools used in the experiment.

- MN´s are involved in the neural basis for recognition of imitation of others as well as recognition of external imitation of the observer.

In another study by Paukner, Anderson, Borelli, Visalberghi, and Ferrari, (2005) two experimenters faced a monkey that was given a wooden cube with a hole on its side. The experimenters also had wooden cubes. One experimenter imitated the actions of the monkey while the other experimenter performed different actions involving a cube. In this experiment there was no recording of single cells, trials were recorded by video and analysed for the behaviour of the monkey. The monkeys exhibited a visual preference for the experimenter that imitated the manual object manipulations. The authors proposed their findings as support in the role of MNs as a neural

basis for recognizing imitation in the individual given that they have both visual and motor properties.

1.2.1.1. Classification

Classic studies in monkeys of field properties in MNs also resulted in different classifications, for example, based on how MNs reacted or to what stimuli they were sensitive to (hand or mouth actions, and within this last realm MNs dedicated to either eating or communicating). Gallese et al., (1996) proposed that MNs fall into three categories according to the visuo-motor congruence between the action that is observed, and the motor response coded by the neuron. These are: strictly congruent, broadly congruent and logically related.

1.2.1.1.1. Strictly congruent

"Strictly congruent" are the mirror neurons that have classic mirror properties: that is, they fire when the observed and executed action is identical (Gallese et al., 1996). Anatomically, these properties are associated to the dorsal part of pars opercularis in the caudal part of the inferior frontal gyrus (Molnar-Szakacs et al., 2005). The reactivity in MNs with correspondence in terms of the goal and the way it is executed, represents roughly one third of the mirror neurons in F5 (Iacoboni & Dapretto, 2006; Rizzolatti & Craighero, 2004). Rizzolati and Craighero (2004) also found that one third of ingestive MNs correspond to this category.

1.2.1.1.2. Broadly congruent

“Broadly congruent” mirror neurons can react during the observation/execution of an action even if the action observed is not identical to the one that they would execute (Rizzolatti & Craighero, 2004). They´ve been found in the ventral sector of pars opercularis in the caudal part of the inferior frontal gyrus (representing about two thirds of F5 mirror neurons), and could have an active role in predicting sensory outcomes of motor output as they generalize the outcome of the observed action (Iacoboni & Dapretto, 2006; Molnar-Szakacs et al., 2005;Rizzolatti & Craighero, 2004; Umiltá et al., 2001). Gallese et al., (1996) identified three additional groups within broadly congruent neurons that react to hand actions:

1. Highly specific for motor activity. Although they do not react to a single type of hand action (grasping vs holding), they do respond to specific type of grip.
2. Active during the execution of a specific hand action but also to the observation of two or more hand actions that are similar to the executed one.
3. Activated by the goal of an observed action but the way that the motor goal is reached does not affect the neuron´s reactivity.

Ingestive functions have been found to elicit reaction in broadly congruent MNs as well, as Rizzolatti and Craighero (2004) have described this property in two thirds of MNs associated to mouth functions.

1.2.1.1.3. Logically related

Initially described as 'non-congruent' by Gallese et al., (1996) because there was no specific relationship between the action that was observed and the movement that the monkey performed.

In observe and execute conditions, they may encode associations between successive components of an action sequence to get to an end goal or action (Cook et al., 2014). Therefore, the goal of the action in the observation condition will generally lead to the execution of a second action with a similar goal (Iacoboni & Dapretto, 2006). This type of mirror neuron is associated with the neural basis for the ascription of intentions. They are thought to code not only the action that is observed, but also, the motor acts that would be expected to follow in the context of the action: first recognizing the goal, and, consequently linking or associating the actions that would need to be followed in order to achieve an expected goal (Gallese, 2007).

1.2.2. The Human Mirror Neuron System (hMNS)

1.2.2.1. Overview

Humans are included within the great ape family (Cook et al., 2014). As was mentioned in the previous section, initial MNs studies and their properties were done on macaque monkeys, and MNs have been identified for mouth and hand movements that include both communicative and ingestive functions. It is the involvement of MNs in language and communication as well as the homology between area F5 in the monkey brain and Brocca´s region in humans, that encouraged Gallese et al., (1996) to propose a matching system of mirror neurons in humans that was involved not only in the recognition of action but also in phonetic gestures important for communication.

This proposal brought on a great deal of interest in studying the existence of MNs in humans not only in the role of language and speech perception (Rizzolatti & Arbib, 1998), but also as a path to understanding the behavioural correlates of the basic MN features (recognition, imitation and understanding goal oriented actions) (Arbib, 2005; Enticott et al., 2013; Molenberghs, Cunnington,

& Mattingley, 2009; Perry et al., 2017; Pineda, 2008; Williams et al., 2006). However, in human studies, an interest also arose in the implication of these features for human social cognition, specifically, in the development of key social skills (Gallese, 2007; Hamilton, 2013; Parsons & Mitchell, 2002; Pineda & Hecht, 2009; Rochat, Serra, Fadiga, & Gallese, 2008).

Understandably, the study of a mirror neuron system in humans (HMNs) presented a methodological challenge, as for ethical reasons, the single cell neurophysiological protocols used on macaques could not be replicated in humans. This matter has been addressed by using indirect methods of measuring MN activity such as: Transcranial Magnetic Stimulation (TMS) (Fadiga, Craighero, Buccino, & Rizzolatti, 2002; Fadiga, Fogassi, Pavesi, & Rizzolatti, 1995; Heiser, Iacoboni, Maeda, Marcus, & Mazziotta, 2003; Mark, 2000; Puzzo, Cooper, Cantarella, Fitzgerald, & Russo, 2013), magnetoencephalography (MEG) (Gaetz & Cheyne, 2006; Hari et al., 1998; Nishitani & Hari, 2000), functional magnetic resonance imaging (fMRI) (Buccino et al., 2007; Calvo-Merino, Glaser, Grèzes, Passingham, & Haggard, 2005; Calvo-Merino, Grèzes, Glaser, Passingham, & Haggard, 2006; Molenberghs et al., 2009) as well as Electroencephalography (EEG) (Bernier, Dawson, Webb, & Murias, 2007; Braadbaart, Williams, & Waiter, 2013; Hari & Salmelin, 1997; Hari, 2006; Muthukumaraswamy, Johnson, & McNair, 2004; Pfurtscheller, Neuper, & Krausz, 2000; Pineda, 2005; Thorpe, Cannon, & Fox, 2016). What has been found using these different neurophysiological techniques can be summarized as follows:

TMS

In studies that use transcranial magnetic stimulation (TMS), a pulsed magnetic field is generated with a magnetic coil over the scalp; this magnetic field can have a temporary effect that can be

excitatory or inhibitory on specific areas of the brain as a result of the induction of electrical currents in brain tissue (Mark, 2000).

Research with TMS on the MNS in humans have focused mainly on the responses in muscles as a result of the observation of assorted stimulus involving hand, arm or finger movements. These studies have provided valuable information about the role of MNs in goal and non-goal oriented actions, imitation as well as a link between MNs and speech and their anatomical correlates (Costantini et al., 2014; Fadiga et al., 2002; Fadiga et al., 1995; Galantucci et al., 2006; Gangitano et al., 2001; Heiser et al., 2003; Pobric & Hamilton, 2006; Strafella & Paus, 2000; Watkins et al., 2003).

Fadiga et al. (1995) used TMS to validate classic MN properties in humans. In research, participants observed scenarios that involved actions with different goals: passive observation of an object, the grasping of that object by an experimenter, movements traced in the air by the arm of the experimenter (non-goal directed or intransitive movements), as well as the dimming of a light while receiving TMS over the left motor cortex. The motor evoked potentials (MEPs) recorded from arm and hand muscles increased significantly when participants observed the experimenter performing the grasping action. The MEPs also increased during the intransitive arm movements performed by the experimenters, although to a lesser degree. The pattern of muscle activation generated by the TMS resembled that of muscle contraction when the participant executed the same action. Their results were among the first to support a corresponding action-observation-execution on humans as had been found in monkeys.

Additional aspects of MN properties in humans were sought after by other research groups. Gangitano et al. (2001), for example, aimed to analyse the temporal structure during the processing

of goal-oriented actions. Participants received TMS pulses to the left motor cortex at key moments during the observation of a reaching-grasping action dividing the movement into different phases. The amplitude of MEPs varied according the temporal phase of the movement, the MEP reached its maximum amplitude during the opening of the fingers in the grasping movement and decreased as the fingers closed. Their results suggest the importance of temporal coding in processing goal-oriented actions in the human mirror neuron system.

As for TMS studies focused on the imitative aspect of mirror systems in humans, Heiser et al. (2003) used repetitive TMS (rTMS) to generate a temporary neural disruption in Brodmann area 44 (BA44) while participants were asked to execute either imitative or control action tasks involving finger movements on a keyboard while receiving rTMS over this area. Their results showed a diminished performance during the imitative tasks as a result of the rTMS, the control group was unaffected in its performance. This suggests the importance of Brodmann area 44 (a premotor region associated to MNs) in imitation in addition to the more commonly known role in language.

rTMS has also been used on the mirror neuron system to test the role of MNs in action understanding. By temporarily altering activity in the inferior frontal gyrus during a perceptual weight judgement task, Pobric & Hamilton (2006) found that performance during the task was impaired when the judgement task involved a person. The three conditions were as follows: 1) observing a hand lifting a box and placing it on a shelf (participants had to judge the weight of the box); 2) observing a bouncing ball (participants had to judge the weight of the ball); and 3) a duration-judgement task as control condition, where participants observed a hand lifting a box and had to determine how long the box had been on screen. While viewing the stimulus videos rTMS was administered over the left pars opercularis. rTMS only affected participant´s performance in

the condition where they had to make judgements over an action being performed by a person (condition 1). These results support the role of the left pars opercularis in the inferior frontal gyrus in understanding the actions of others.

The use of TMS has also been employed to provide evidence on the role of mirror neurons in speech. Under the motor theory of speech perception, in order to produce language, muscles involved in the production of speech must activate to perform the necessary motor gestures for the articulation of speech, when listening, the individual´s own articulatory gestures are triggered and the message is understood (Galantucci et al., 2006). In this perspective mirror neurons play an important role in speech perception and understanding. To test if cortical centres associated to language and speech are triggered when listening to verbal cues Fadiga et al. (2002) used TMS to stimulate the left motor cortex while participant listened to spoken words. Their results showed increase in MEP in tongue muscles when listening to words that involved movement of those muscles when pronouncing the word.

Another study that supports the role of the motor system and mirror mechanisms involved in the production of speech is by Watkins et al. (2003) who tested not only auditory but visual perception of speech as well using TMS over the face area of the primary motor cortex. MEPs in lip and hand muscles were recorded during four experimental conditions: listening to speech, listening to non-verbal sounds (both while viewing visual noise), viewing speech related lip movement and viewing eye and brow movements (both while listening to white noise. MEPs were increased during conditions related to speech in both auditory and visual conditions during the stimulation of the left hemisphere.

Research interest in this area has also focused on how the hMNS can be influenced by sensorimotor experience (Catmur et al., 2007, 2009). By measuring MEPs on specific finger muscles during TMS before and after training participants in action-observation-execution of compatible and incompatible finger movements, Catmur et al., (2007) found that mirror properties can be modified with sensorimotor training. In their study, two training groups were formed randomly depending on the type of training that participants received: compatible and incompatible. In the training sessions participants were showed videos of index and little finger movements and were asked to perform a finger movement in correspondence. In the compatible training group, they performed the same finger movement observed in the video. In the incompatible training group, they were asked to perform the movement with an opposite finger (index movement observed- little finger movement performed and viceversa). As the authors predicted, in the compatible training group the pattern of muscle specific activation was preserved. However, in the incompatible training group the original mirror response, that is, MEP enhancement with corresponding finger movement was reversed. In ecological terms, this supports the notion that the human mirror system can be shaped as a result of day to day experiences and social interactions.

Functional magnetic resonance imaging (fMRI)

Brain imaging techniques that offer high spatial resolution such as fMRI allow us to study specific anatomical brain structures and their involvement in cognitive functions (Mark, 2000; Shafi et al., 2013). In the case of MNs, studies with fMRI have compared cortical areas with mirror mechanism properties of motor actions in humans with those where mirror neurons were found in monkeys and found a general correspondence of MN regions with shared reactivity of the cells (Buccino et al., 2007; Calvo-Merino et al., 2005; Calvo-Merino et al., 2006; Cross, Hamilton, & Grafton, 2006;

Fabbri-Destro & Rizzolatti, 2008; Heiser et al., 2003; Molenberghs et al., 2009; Molnar-Szakacs et al., 2005).

In terms of imitation the areas that have been found to be activated during tasks where participants performed hand and finger imitative motor actions are: the superior and inferior parietal lobule and the dorsal premotor cortex (Molenberghs et al., 2009).

As for passive action observation MN tasks, when participants observe an action, these are mapped onto the observers´ own cortical motor network (Fabbri-Destro & Rizzolatti, 2008). The type of action observed affects the strength of the activation; for example, when individuals have a specific motor skill, such as dancers or tennis players, the cortical activation is stronger in these individuals than in those without the specific motor expertise (Calvo-Merino, Glaser, Grèzes, Passingham, & Haggard, 2005; Calvo-Merino, Grèzes, Glaser, Passingham, & Haggard, 2006; Cross, Hamilton, & Grafton, 2006)

Finally, the involvement of the hMNs in understanding the intentionality of goal oriented action has also been supported by results of fMRI studies. Iacoboni et al. (2005), used three types of stimuli involving intentionality were used: hand grasping actions in two different contexts, hand grasping actions without a context and a scene with objects but no hand. They found that hand grasping actions in which a context was present resulted in an increase in the posterior part of the inferior frontal gyrus as well as the ventral premotor cortex; this suggests that these areas (associated with action recognition) are also playing a role in the understanding intentions of actions.

Understanding intentions also means distinguishing between intentional and non-intentional actions. This has also been supported by fMRI research; Buccino et al., (2007), found that the mirror mechanism is activated in both intentional and non-intentional actions. The areas that signal spatial and temporal information of unexpected events were found to be activated in addition to mirror neuron areas when individuals recognise if an action has a specific intention.

Electroencephalography (EEG)

Electroencephalography is a neuroimaging technique that reads electrical activity at the scalp that is the result of millions of synchronous, neuronal postsynaptic potentials (Angelakis et al., 2007; Teplan, 2002). The electrical activity recorded by the EEG comes mostly from pyramidal neurons in the cerebral cortex. Brain electrical currents are made up of sodium (Na+), potassium (K+), calcium (Ca++), and chloride (Cl-) ions which travel in and out of the neuron depending on the electromagnetic potential of the membrane (Teplan, 2002). Differences in the electrical fields between the soma (body of neuron) and apical dendrites (neural branches) create dipoles that generate electrical potentials, known as local field potentials. Neural oscillations express cyclical voltage changes in local field potentials from areas of brain tissue and, via volume conduction, are translated into amplitude of the EEG signal recorded from the scalp (Mathalon & Sohal, 2015; Ros et al., 2014).

Neurons are mutually connected into neural networks through synapses that resonate through the cortex when firing in a synchronous manner (Teplan, 2002). The cortex operates spatially in terms of three resonant loop-types: local, regional and global. Local resonances occur between macrocolumns, that is, a cortical unit of operation (DeFelipe et al., 2012); regional resonances occur between macrocolumns that are further apart from each other, and global resonances develop

between different areas (for example, between frontal and parietal networks). All of these can either occur spontaneously or as the result of thalamic pacemakers (Lubar, 1997).

In terms of how these loops can be observed in the EEG, the closer the occurrence of resonances, the higher the frequency that is observed. For example, local resonances are typically associated with gamma frequencies, regional resonances produce alpha and lower beta frequencies and global resonances are more often responsible for slower activity such as delta and theta (Klimesch, 1999; Lubar, 1997).

The number of neurons that are synchronised in time and space are what makes it possible to observe what are originally local field potentials in an EEG recorded from the scalp. Arbitrarily activated electrical fields are usually cancelled out in an EEG signal (Mathalon & Sohal, 2015). Oscillations allow communication between different areas of the brain, both at a micro and macro level, in an organised way that enables processing streams of endogenous and exogenous information (Ros et al., 2014). The origin of EEG network oscillation is illustrated in figure 1.2.

Like MEG, this neurophysiological technique offers a temporal advantage in the study of functional neuronal activity. The minimal invasiveness that it causes to participants is also an advantage as it only requires participants to wear a cap (in most cases) while performing tasks or watching stimulus, a feat much less intimidating than when it is performed in a MEG or fMRI machine. Although stillness is compulsory to get clean signals and usable data; the use of a cap is much more comfortable for participants and can be adjusted to many experimental designs in the study of MNs.

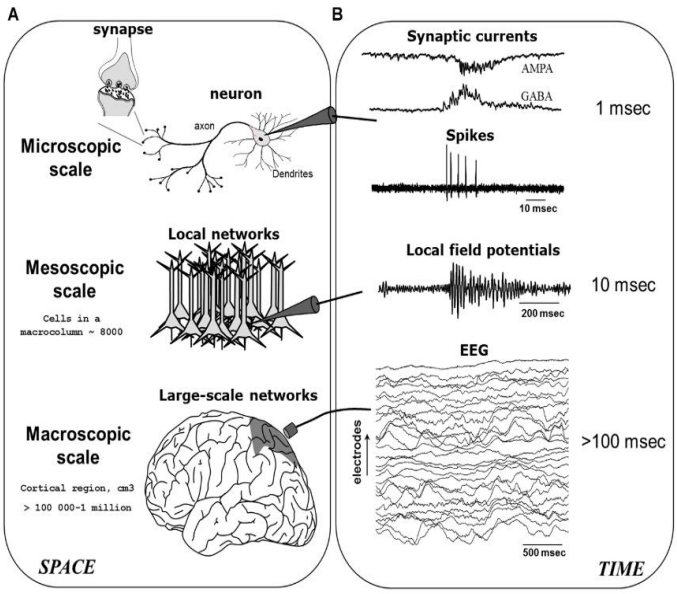

Fig. 1.2 The origin of EEG network oscillations. A) Spatial and B) Temporal scales of how neural activity produces the signals shown on the EEG (adapted from Ros et al., 2014).

In EEG studies that focus on the hMNs, typical protocols used in these studies also request that the participant undergo three conditions: passive observation of motor actions performed by another individual, execution of the motor action and a third condition which may involve the motor action without a biological component (Braadbaart et al., 2013; Hari, 2006; Muthukumaraswamy et al., 2004; Pfurtscheller et al., 2000; Thorpe et al., 2016). Depending on the purpose of the studies, these conditions may vary, however what they seek is to record and analyse electrical current variations in the brain as a result of the MN reactivity.

The EEG frequencies that typically exhibit variations when individuals are presented with stimuli intended to elicit MN response are the Alpha (8-13Hz) and Beta (13-30Hz) bands recorded over the sensorimotor cortex; these frequencies recorded over central areas are components of the mu rhythm (Denis et al., 2016; Hari & Salmelin, 1997). The mu rhythm band can be recorded at rest with eyes open and display an attenuation in response to the observation and execution of motor

actions (Bernier, Dawson, Webb, & Murias, 2007). Because of the reactivity and topography of the mu rhythm and the traits previously described of MNs, it is considered to reflect a downstream modulation of sensorimotor areas by mirror neuron activity (Pineda, 2005). The relation between mu rhythms and MNs will be covered specifically in the following section. Of the neurophysiological techniques described, there will be a further focus on the study of MNs through EEG studies, as this will be the technique employed in the collection of data in the future chapters.

MEG

Another non-invasive neurophysiological technique employed in the field of the hMNS is magnetoencephalography. Through this technique magnetic fields produced by electrical currents of neuronal activity are measured by a superconducting quantum interference device (SQUID) and can be anatomically localized. It has a high temporal resolution that allows for the study of temporal aspects in the functional connectivity of mirror neurons (Shafi et al., 2013).

In the field of MNs, studies that employ MEG generally record neuromagnetic signals of participants while they perform experimental tasks attuned with MN reactivity, i.e. passive observation of an action, execution of an action and imitation (Gaetz & Cheyne, 2006; Hari et al., 1998; Nishitani & Hari, 2000).

As in the case of TMS, early studies using this technique also focused on validating the existence of a corresponding MNs in humans with the properties found in monkeys. Hari et al. (1998) recorded rhythmic neuromagnetic oscillatory activity of approximately 20 Hz over the precentral cortex during tasks related to MNs. Participant´s neuronal activity was recorded in three conditions: 1) resting state 2) during the manipulation of an object with their hands and 3) in a

passive observation of another person manipulating the same object. In order to assess precentral motor cortex activity, the post stimulus rebound of the 20Hz frequency was measured after stimulating the left and right median nerves at the wrists in an alternate manner. Suppression of the rebound was taken as the reflection of increased activity in the motor cortex (MN activity); the greater the suppression the more MN activity reflected in the area. In addition to the experimental conditions, spontaneous cortical activity was also recorded without the stimulation of the median nerves. They found that the greatest suppression of post-stimulus activity occurred during the observation of another individual manipulating the object with their hand, the second condition also elicited a suppression of the 20 Hz frequency, although not as great as condition 2. The authors explain these findings as a result of the mirror mechanism in the precentral cortex and proposed the measurement of the 20Hz rebound effect as a method of studying disorders that involve alterations in the action-representation system.

Nishitani & Hari (2000) also used experimental conditions with MEG to measure MN reactivity; in this study, an imitation condition was also included. Neuromagnetic signals were recorded during task performance of all experimental conditions. Their result also confirmed correspondence between the mirror systems in monkeys and humans, and that in the case of humans, mirror systems are most reactive to the observation of another individual performing a hand action while imitating the action themselves. In this study, the left Brodmann´s area (BA44) was found to have initial activation during the execution of the action followed by activation in BA4. In imitation and observation conditions, activation in these areas was similar. Based on these findings, the authors also conclude that BA44 is relevant in the temporal dynamics of mirror mechanisms.

These different physiological measures have also been used in juxtaposition as a way to further examine MNs and their functional role. Arnstein et al. (2011) for example, compared the EEG mu rhythm to the BOLD fMRI signal during action execution and observation as a step towards understanding the origin of mu rhythm suppression. By recording collecting this simultaneous physiological information, the authors found evidence of mu rhythm suppression as an indicator of MN activity in primary somatosensory cortex, IPL, and dorsal premotor cortex. Nevertheless, this was not the case for mirror neurons in Brodmann area 44, which has been strongly linked to mu rhythm (Pineda, 2005). Similar findings by Perry & Bentin (2009) support the correspondence between EEG and fMRI for mu suppression and the human MN system. Overall, by comparing mu rhythm suppression to fMRI indexes of human cortical activity during tasks that elicit the hMNS, they found a parallel between the two physiological measures. The authors propose that future studies use simultaneous physiological measures in order to better characterise different traits of the MNS.

1.2.2.2. Mu Rhythms and mirror neurons

The mu rhythm is an EEG oscillation originally described within the alpha frequency bandwidth of 8-13 Hz (Niedermeyer, 1997). Its discovery dates back to the first half of the 20th century, initially in studies focused on the precentral and occipital areas by Jasper & Andrews (1938), who named it the precentral Alpha rhythm and later described it in studies as ‘alfoide activity’, or ‘rolandic alpha’. In the 1950s, more features regarding this rhythm were described by Gastaut and collaborators, who introduced the term "rhythme rolandique in arceau" which means ‘Rolandic wicket rhythm’ (Gastaut, 1952, 1954; Gastaut et al., 1952). It is currently referred to as the mu rhythm as a result of the Greek alphabet letter that identifies it (μ). The different ways in which this rhythm is referred to emphasizes its morphology as well as the topography. On the EEG trace,

the mu rhythm has an arch shape, composed of an acute negative component and a rounded positive component (Kuhlman, 1978); the spatial distribution is limited to the pre and post central area in the cortex (mainly over the sensorimotor cortex in the post-Rolandic area), and it is observed at its peak when a person is not moving (Arroyo et al., 1993; Oberman et al., 2008; Pfurtscheller & da Silva, 1999); They can therefore be located roughly over electrodes C3 and C4 according to the international system 10/20 and are limited to periods of 0.5 to 2 seconds (Niedermeyer, 1997; Niedermeyer & Silva, 2004).

Although the frequency and amplitude of mu is similar to the alpha rhythm, the topography and reactivity of mu is unique to the rhythm. For many years the mu rhythm was regarded in a similar way as alpha in terms of functional significance, that is, as a indicator of an idling state. While the alpha activity can be observed in the parieto-occipital cortex and reacts to the opening and closing of the eyes, The mu rhythm is modulated during the preparation of a movement by an individual as well as when the movement is observed and executed (Hauswald et al., 2013). The attenuation of the rhythm in observation-only conditions depends on the level and quality of attention given to action but can reach the same level of attenuation as performing the action itself (Brunsdon et al., 2019; Covello et al., 1975; Hari, 2006; Pfurtscheller & da Silva, 1999). After the initial desynchronization of mu when viewing or executing an action, there is generally a rebound in the synchronization of mu activity that can be observed as an increase in the amplitude of the rhythm. The event-related rebound is dominant over the contralateral primary sensorimotor area after the movement-offset (see figure 1.3).

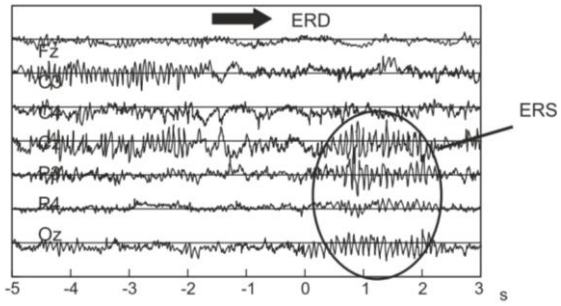

Fig 1.3 Event related desynchronization (ERD) and subsequent Event related synchronization (ERS) rebound over central areas recorded during a right finger movement. Adapted from Nicolas-Alonso and Gomez-Gil (2012).

The sensorimotor cortex shows a variety of mu rhythms with specific topographic and functional properties. Different frequency components of mu have been described where the reactive somatosensory rhythm can be found in the beta frequency (13-30Hz) as well as in Alpha (8-13Hz) (Arroyo et al., 1993; Brunsdon et al., 2019; Denis et al., 2016; Gaetz & Cheyne, 2006; Hari & Salmelin, 1997; Jasper & Penfield, 1949; Nam et al., 2011; Pfurtscheller & da Silva, 1999).

The particular arch-shape of mu rhythms in the EEG is the result of two frequency components that have been identified within the rhythm: 8-13 Hz and from 13-30 Hz, that is, an alpha and beta frequency component (Hari & Salmelin, 1997). The spectral peaks of these frequency component are 10z and 20Hz and have been associated to somatosensory and motor cortical functions respectively (Avanzini et al., 2012).

In pioneering studies of functional anatomy of the brain, Jasper and Penfield (1949) described a precentral beta rhythm in the motor hand area that was blocked briefly at the beginning of a simple motor task, (clenching a fist) and was blocked again briefly immediately after the movement was over. The precentral beta rhythm was also identified in Brodmann areas 44 and 6 using electrocorticography (a technique that measures brain activity directly from the exposed cerebral cortex) they identified alpha and beta frequencies as representative activity of the resting motor cortex (see figure 1.4). This was supported by studies done in the 1990s that suggested the source

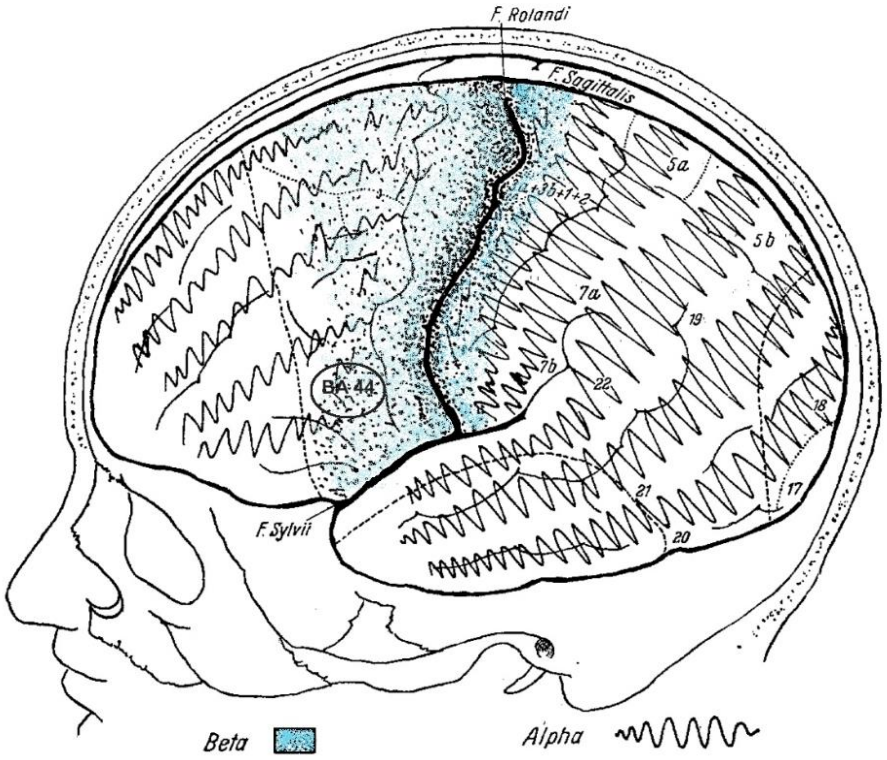

Fig. 1.4 Representation of cortical areas giving rise to the sensorimotor alpha rhythm and beta rhythms. BA represent Brodmann area 44. Adapted from Jasper and Penfield (1949).

of mu cortical location to be focused around the central sulcus and sensorimotor areas as well as some parietal cortical areas (Salenius et al., 1997; Salmelin et al., 1995).

The mu rhythm, shows a different cortical reactivity depending on the body part that is involved in a motor task: i.e. an area where mu is blocked upon moving arms and/or hands does not show the same reactivity when a facial movement is involved and vice versa (Arroyo et al., 1993). Such

movements can be voluntary, passive or reflexes and the effect is generally bilateral although higher in the contralateral rolandic region to the site where the limb movement was originated (Niedermeyer, 1997; Pineda, 2005). The correspondence between the area of movement of the body that blocks the mu rhythm at a specific site and the region of the body that is affected by the stimulation on the same site has been tested empirically in humans at a single

In a study by Arroyo et al., (1993), a correspondence was found between the area of movement of the body that blocks the mu rhythm at a specific site and the region of the body that is affected by the stimulation on the same site. Using subdural electrode implanted on patients with intractable epilepsy, they were able to detect a cortical mu rhythm of 7-11Hz by means of electrical stimulation on 5-16 electrodes placed over sensorimotor cortex. They concluded that both the presence and the attenuation of the rhythm were specific to the somatic representation of the cortex where it was registered.

Further research into the behaviour of this rhythm found that the scope of its reactivity went beyond action observation and execution and also included motor imagery. When instructing individuals to imagine movements of their hand in accordance to a visual stimulus showed on a screen, an ipsilateral attenuation of the mu rhythm was found in two-thirds of the participants tested. This pattern was similar to the one described before the execution of a movement (Pfurtscheller & Neuper, 1997).

A study by Puzzo et al., (2011) focused on methodological issues of the EEG as an index to study the hMNs in order to obtain a robust protocol for the study of sensorimotor reactivity. After testing trials of different lengths and repetitions (longer trials with less repetitions vs shorter trials with multiple repetitions), they found that multiple repetitions in stimuli presentation over longer

exposure to movement observation is better suited to the study of sensorimotor reactivity. Furthermore, they found that the low beta frequency component of the mu rhythm was a more sensitive index for the study of hMNs activation during the observation of hand movements. In the next chapter, we will include this protocol in order to test if it is also optimal for the assessment of a neurofeedback protocol that aims to uptrain sensorimotor activity.

Earlier in this section we reviewed the implication of mirror neurons in perception, action understanding as well as in the imitation of facial expressions and language. The existence of mirror systems in the human brain, and their role in the transformation of representations perceptual executable actions, coupled with new conceptualizations about mu, resulted in the hypothesis of mu rhythms as the EEG index of the hMNS (Pineda, 2005), that is, the mu rhythm is generated as the result of synchronous downstream modulations of neurons in the sensorimotor area of the brain in which only some of them are mirror neurons (Braadbaart et al., 2013; Pineda, 2005). This makes sense as the phenomenology of mirror neurons and mu rhythms is similar. Both are sensitive to movement and cognitive imagery as well as the observation of actions that are relevant to the subject, in addition to a common frontoparietal source localization. When studying the validity of MN activity with EEG mu rhythm through a meta-analysis Fox et al. (2016) also found significant effect sizes for mu during execution (Cohen's $d = 0.46$, $N = 701$) and observation (Cohen's $d = 0.31$, $N = 1{,}508$) of actions. Their results support the use of EEG, and specifically changes in mu activity in the study of mirror neurons in humans.

With the goal of deciphering the association of mirror-like cortical rhythm mechanism to action observation, Avanzini et al. (2012) characterized somatosensory rhythm topography and temporal course throughout the observation of different types of hand movements. After analysing the high-density EEG recordings of all movement observation conditions (target directed, non-target

directed, cyclic and non-cyclic) in epochs of 200s, they obtained a somatosensory activity time course of action observation. What they found is that all movement types generated desynchronization of alpha and beta rhythms in central and parietal regions. The desynchronization of both frequencies occurred the moment the hand movement started with their corresponding rebound of activity once the movement had ended. In terms of mu frequency component findings, there was a higher desynchronization and longer rebound synchronisation in the alpha band for target directed motor acts. The beta band modulation however, displayed a strong correlation with the speed of the action. Their findings suggest the mu rhythm's variation during action observation is comparable to the execution of the action. This study is of particular relevance because it strongly supports a mirror mechanism in humans for action observation and execution by providing evidence that the cortical motor system is temporally parallel to movements that we observe.

What can be concluded from the various neurophysiological methods employed in the research of hMNs, is that there is indeed evidence supporting a corresponding system in mirror neurons as has been described in monkeys. Although the properties and functions described by research thus far do not support an identical correspondence, the basic mirror mechanism in action, observation-execution and imitation appear to be present in humans.

Humans obtain ample experience observing and performing similar actions, and MNs can adapt to react in accordance (Heyes, 2010). MNs in humans fire in broader contexts that correlate action-observation-execution than MNs in monkeys. For example, monkey MNs do not fire in the presence of isolated hand actions, even if they are performing goal-oriented actions; unlike human MNs, in which many of the stimuli used in experiments show a single hand performing several goal and non-goal oriented actions (Turella et al., 2009).

As for the location of the hMNs, the main areas where mirror networks have been found and studied are the inferior parietal lobule (IPL) and the ventral premotor cortex (PMv) in addition to the caudal part of the inferior frontal gyrus (IFG) (Fabbri-Destro & Rizzolatti, 2008; Molenberghs et al., 2009; Molnar-Szakacs et al., 2005).

Mirror mechanisms in the human brain are not only limited to hand or finger motor actions. Depending on the anatomical location of the network that contains neurons with mirror properties, they have been associated to different cognitive functions (Fabbri-Destro & Rizzolatti, 2008). For example, MNs found in Brodmann area 44 have a role in motor gestures necessary for the production and understanding of speech (Watkins et al., 2003), MNs in sensorimotor areas are strongly linked to the processing of goal-oriented actions, etc (Cook et al., 2014).

A study by Costantini et al., (2014) found that the left PMv is also involved in driving an individual's gaze when observing a goal directed action (a hand reaching for a tomato). When participants underwent repetitive transcranial magnetic stimulation (rTMS) over the left PMv and the posterior part of the left superior temporal sulcus, it affected the eye behaviour when following the trajectory of the hand towards the goal. The authors concluded that eye movements may also be driven by motor processes comparable to a mirror response, that is, activating motor processes that they would use in order to execute the action themselves. Because of the association to anatomic areas where the hMNs has been described and the suggested association of a mirror response, this study is relevant to the study of mirror neurons and suggests that additional physiological measures such as eye movements should be included in the study of the hMNs. This premise will be studied in further experimental chapters (see chapter 2 and 4)

The role of MNs in mutual representation of motor actions between observer and performer of the action as well as the understanding of intentions has been suggested to be the base upon which individuals develop key social skills, such as understanding facial expressions, language and empathy (Hamilton, 2013; Iacoboni & Dapretto, 2006).

Hamilton, (2013) offers another interpretation of mirror neuron function, focused on response to social contexts rather than only action comprehension and prediction. As the author explains, in our day to day life we are rarely passive observers of the actions that happen around us; we spend most of our time responding and engaging in actions that involve social interaction. Hamilton therefore proposes that an important function of the mirror neuron system is to coordinate appropriate dynamic responses to real life social interactions and that the mirror neuron system's response favours situations involving social situations over predicting actions. The basis for this interpretation stems from studies done on automatic imitation, where participants that imitate hand positions (fist/shake/grasp) respond faster with their right hand when viewing images of a right handed shake hands image, suggesting that the automatic imitation can be facilitated by a social response preparation (Heyes, 2011). Further evidence for this can be found in as study by Sartori et al., (2013) where participants observed videos of action sequences with variations of hand postures (whole hand grip vs precision grips) while TMS-induced motor evoked potentials (MEP) were taken from their hand and finger muscles. The authors found that in addition to a mirror response reflected in large MEPs when observing whole hand grips, there were also large MEPs when the stimuli presented prompted a social response from the participant (watching a cup placed closer to the participant and the actor on screen reaching towards it). These studies support the function of mirror neurons beyond action observation and imitation towards a more dynamic role for social response and interaction. We will be testing this social response paradigm in future experiment chapters 3 and 4.

1.2.2.3. ASD and the hMNS

Given the properties of the hMNs discovered so far, it is not surprising to find an hypothesis that posits that a dysfunction in the mirror neuron system might be one of the main causes in disorders such as Autism, as imitation is key in the development of social skills (Bernier, Dawson, Webb, & Murias, 2007;Bernier et al., 2013; Braadbaart, Williams, & Waiter, 2013; Iacoboni et al., 1999; Meltzoff & Prinz, 2002; Williams, Whiten, Suddendorf, & Perrett, 2001; Williams et al., 2006).

Nonetheless, empirical findings from functional and structural imaging studies are not yet conclusive in elucidating the role of the hMNs in ASD. Hadjikhani et al. (2014) found that for participants with ASD, there was decreased grey matter in regions where the MNS was classically described, this included inferior parietal lobe, STS and Broca's area. Furthermore, this reduction in cortical thickness was positively correlated with communication and social difficulties. However, more recently, Fründt et al., (2018), focused on the white matter structure of the hMNS in ASD and control participants; the authors found a similar anatomical network of the hMNS between experimental and control groups. Both groups had strong connections in the areas that have been typically associated with the hMNS. Therefore, this study does not support an anomaly in this network in ASD from a structural perspective. They did, however, find that the connection of white matter microstructure between the right IFG and supramarginal gyrus (SMG) was significantly related to the severity of ASD symptoms.

Variability in findings could be accounted for by Hamilton et al.'s (2007) hypothesis that deficits in ASD cannot be accounted for solely by the hMNS. In their study, although the authors found that ASD individuals had impaired performance in ToM tasks compared to control participants, they discovered that this impairment did not translate to imitation ability. Both ASD and their

matched controls displayed similar ability to imitate grasping actions. The authors concluded that ASD symptoms should not be attributed to one system in the brain (hMNS), but that research should take into account multiple brain systems that can explain the multifaceted manifestations of action understanding and imitation.

Another factor that could be playing a role in the activation of the hMNS in ASD is the personal relationship of the stimuli to the individual. Using mu suppression over the sensorimotor cortex as a measure of the hMNs, Oberman et al. (2008) found that in contrast to neurotypical children, participants with ASD showed a higher mu suppression when the actions were performed by someone that they were familiar with. Although this reactivity was different to neurotypical participants whose levels of mu suppression was stable throughout the stimuli presentation, these results suggest a different functioning of the hMNS rather than an overall dysfunction of the system. Evidence against a global dysfunction of the hMNS can also be found in studies using motor-evoked potentials (MEP). Enticott et al. (2013) used MEPs in order to generate an index of interpersonal motor resonance (IMR) while watching others execute actions. They also tested the degree of social relevance of the stimuli presented and found that the lack of an overall dysfunction in the hMNS in ASD persisted regardless of the social relevance of the stimuli. Taken together, the research suggests that other factors such as visual processing and attentional influences should be investigated further in order to provide more insight in the role of the hMNs in ASD (see chapter 3).

1.2.2.4. Considerations and limitations

The mirror neuron theory of action understanding has received criticism largely in terms of the generalization of functional significance roles found in monkeys to humans and in their protagonist role in explaining action understanding.

As Hickok, (2009) frames it, mirror neurons were empirically discovered in a species that does not possess the same higher order cognitive processes as seen in humans. Subsequently, these findings were translated to describe functional significance of higher order cognitive processes in a separate species where the empirical data on mirror neurons is, as of yet, inconclusive. For example, in the case of imitation, the role of mirror neurons in humans has been questioned because, macaques do not have the ability to imitate. If this is not a function described in the studies where mirror neurons can be studied on a cellular level, it is questionable that this function could be attributed to mirror neurons in humans. A possible explanation that Hickok provides is that mirror neurons have functionally evolved in humans in order to serve behavioural adaptative needs that are specific to the species whilst maintaining their original properties described in macaques. However, the author emphasizes the need for more research into these alternative explanations of mirror neuron functionality.

In terms of the role of mirror neurons as the neural basis of action understanding, this has also been the target of disparagement as some researchers consider that it oversimplifies the concept of what understanding an action means. It could be argued that when a person imitates an action that they have observed in someone else, they have understood the action as a consequence of the motor representation and further execution orchestrated by mirror neurons. Furthermore, within the mirror neuron theory it is proposed that we understand actions by interpreting the end goal of

said action (Rizzolatti & Craighero, 2004). However, it is important to consider that an action rarely leads to a sole goal. Equally important, the final goal of an action can be ambiguous until its completion. One may be reaching for an object, to pick up, to throw, to eat it or simply signal towards it but not pick it up. It is not clear in the literature if each action for a same goal and vice versa, would have to be processed uniquely and independently by mirror neurons.

Another argument against mirror neurons as the neural basis for action understanding comes from the data obtained from patients with apraxia and focal brain lesions, as well as patients who have inferior frontal gyrus damage (Buxbaum et al., 2005). There seems to be a lack of association between the ability to execute an action and the ability to recognise it. In both of those cases one would expect that the ability to recognise or execute actions would (under the mirror neuron theory) also impair the ability to understand actions. If mirror neurons are the neural basis of action understanding, then there would need to be strong empirical data both associating recognition, understanding and execution. This is also true for the role of mirror neurons in speech, as damage to the motor speech area does not result in an absence of speech recognition. This would mean that although mirror neurons play a role in action understanding it may not be a central one as was originally proposed (Birch, 2017).

An alternative explanation to how actions are understood through mirror neurons is that they are part of a larger system that processes actions. Hickok, (2013a) proposes that actions are processed concurrently in the inferior parietal cortex which projects to F5 associative mechanisms which in turn receives input from the superior temporal sulcus. The result is a “mirror response” which would explain the high-level functions/attributes that have been placed upon mirror neurons in the F5 area. This would mean that what has been thought of as the human mirror neuron system is actually the facilitation of adaptive associations dependent on tasks that have been learned through

the motor system and a more concrete function of the mirror system proposed is that mirror-like activity is most likely reflecting sensory-motor associations but these associations lack a more abstract content (Hickok, 2013b).

Silas et al. (2010, 2012) propose the existence of two mirror neuron systems that process behavioural and neural correlates differently in action prediction. In one study participants were instructed to execute an action based on the visual stimuli that they were presented with. The video clips were movements that either had goal-related relevance or had a visuospatial overlap to the action the participant was meant to execute. Participants were assessed based on the reaction time when they executed the action according to the presented stimuli. EEG data was also recorded during this activity. The researchers found that the behavioural measures (reaction time) were consistent with a direct matching interpretation regardless of the relevance of the stimuli. However, the EEG findings displayed a preference for the relevance of observed actions supporting the role of action prediction in the hMNs (Silas et al., 2012).

Along these lines, other explanations proposed include Hickok and Hauser's (2010) action selection model where the function of mirror neurons is to code associations between actions perceived in the environment and the resulting observed response. Csibra (1993) proposes that mirror neurons are activated after an action has been processed rather than being responsible for its production. Kosonogov (2012) also subscribes to the theory that actions are coded outside of the mirror neuron system but adds that the relevance of the action to the individual will determine if it is reproduced by the mirror neurons in order to add it to their motor “collection” and will then be used in the future.

Given that the mirror neuron theory has been linked as a possible explanation of ASD, the plausibility of this theory has also been put into question in the realm of ASD. Southgate & Hamilton (2008) argue that imitation is a process that goes beyond mirroring behaviour. Although performance in imitation tasks is often impaired in individuals with ASD, this is not always the case (Bird et al., 2007) which questions the broken mirror theory of ASD. A second argument is that because imitation as a cognitive process is not yet fully understood, and it is not a process that relies on a sole brain system. Without fully understanding the neural underpinning of imitation levels/components, it would be difficult to generalise deficiencies in imitation and furthermore, social interaction in ASD as a result of a "broken" mirror neuron system. Furthermore, when looking at changes in the mu rhythm suppression (EEG index of mirror neurons in humans) while ASD and control participants executed or observed hand actions (as well as a non-biological visual stimuli as a control condition), Fan et al. (2010) found that although the suppression of mu was significantly different across conditions, it did not vary significantly between groups. While the ASD group did have difficulties imitating, the suppression of mu during the imitation task was not affected. This research also brings into question the broken mirror theory in ASD as it suggests that the dysfunction of the mirror neuron system is more complex than originally described.

What we can conclude from these different theories and perspectives on mirror neurons is that there is still much more research that is needed in this field in order to elucidate these theories and determine concrete mirror neuron functions and their association to the understanding of actions. Further experimental chapters will test different aspects of mirror neurons such the attentional bias (see chapter 3), individual differences with autistic traits (see chapter 2) and if it is possible to modulate them through neurofeedback (chapter 4). But before moving on to experimental chapters, we will cover a brief theoretical overview into the neuromodulation technique that will be used in chapter 4, neurofeedback.

1.3. Neurofeedback

1.3.1. Overview and basic concepts

As mentioned in the previous sections, EEG patterns are a reflection of pyramidal neuron activity (Kirschstein & Köhling, 2009). The brain is affected by both exogenous and endogenous factors that can range from the biochemical, metabolic and hormonal to the behavioural (Teplan, 2002). The cortex works in terms of resonant loops. Excitatory and inhibitory interactions of neuromodulators within the cortex as well as between cortex and thalamus are responsible for such loops, referred to as pacemakers (Lubar, 1997).The competition created between the synchrony of endogenous oscillations and the environmental factors that have an effect on them creates opportunity to modify brain patterns with voluntary practice in an attempt to find balance and adapt to the environment (Buzsáki,2009).

One way to do this is through the use of neurofeedback, an operant conditioning paradigm where a participant is trained to modulate aspects of their EEG (Vernon et al., 2003a). Through the use of NFT the pacing of cortical resonant loops can be modified, and in so doing, participants can also modify their neurophysiology (Lubar, 1997; Reiner, Gruzelier, et al., 2018). The temporal resolution and high sampling rate of the EEG makes it possible for the participant to receive real time feedback on their present brain activity and by bringing it into the realm of consciousness, to learn to modulate it (Congedo, 2013).

As sensory stimuli have been observed to induce time-locked changes in neuronal activity, the neurofeedback participant receives auditory, visual or tactile stimuli and feedback (Hammond, 2011). By using a stimuli to signal moments in time to the brain, that moment and the EEG activity associated to it can be categorized to a positive or negative situation and therefore enhanced or

inhibited (Collura, 2014). The use of NFT in multiple disorders is grounded on the casualty hypothesis that attributes the behavioural symptoms of neuro-psychological disorders to variations in how the brain of a given individual is functioning (Omejc et al., 2018)**.** With continued training, changes in brain activity can generate long-term improvements in behaviour (Coben & Evans, 2011; Gruzelier, 2014c; Hammond, 2011; Kouijzer et al., 2010). This is why its implementation has increased not only in the treatment of symptoms for disorders such as ADHD, epilepsy, depression, anxiety and obsessive compulsive disorder, but there are also studies that show its benefits on musical as well as cognitive performance such as increased concentration and memory (Gruzelier, Egner, & Vernon, 2006; Gruzelier, Foks, Steffert, Chen, & Ros, 2014; Vernon et al., 2003).

NFT takes advantage of the brain's autoregulation and plasticity to provide regulatory capacity to an otherwise dysregulated brain (in the case of specific disorders), or to enhance cognitive abilities from concentration to musical or even surgical skills (Brandmeyer & Delorme, 2013; Collura, 2014;. Gruzelier, 2009; Gruzelier et al., 2010; Jia et al., 2014; Omejc et al., 2018). Because the brain self-regulates, the process is always gradual and within individual biological parameters. The process always allows the brain to adapt to a new state following an individual pace of progress. This provides an element of safety to the technique resulting in minimum adverse side effects. Additionally, by using EEG, researchers and clinicians can pinpoint locations to train using standardized electrode systems (10-20 or 10-10 systems; (Collura, 2014). It is not without its disadvantages as it requires active motivation from the participant which may be dampened by the number of sessions that an average NFT requires. The number of NFT sessions required means that it can also be a costly treatment both in time and financially. However, depending on individual goals and circumstances, it is a neuromodulation technique with both high levels of specificity whilst maintaining a low risk to the participant (see table 1.3).

Table 1.3: Options for mental health interventions (collura, 2014)

Modality	Method	Invasive	Biological basis	Specificity	Directedness
Talk/ Behavioural therapy	Learning (various)	No	Moderate (when neuroscience driven)	Moderate	High (can focus on issue or problem)
Pharmaceutical	Altering (chemistry)	Yes	High (chemical change)	Moderate (neurotransmitters)	Low (widely distributed in brain, side effects and abreactions can occur)
Stimulation	Altering (electrical)	Yes	High (electrical conduction)	Moderate (location on head)	Moderate polarity, location)
Neurofeedback	Learning (operant)	No	High (eeg and learning process)	High (site specific or loreta)	High (wide range of protocols, settings, sites)

1.3.2. Historical background

The earliest reports of biofeedback and neurofeedback date back to the 1960s, where an interest flourished among different researchers to study whether having information about one's own physiological processes could result in a degree of control over specific internal processes (Wagner, 1975). Some of the first studies concerning neurofeedback as a form of intervention involved the modulation of 12-15Hz activity recorded over the primary sensorimotor cortex, otherwise known as the sensorimotor rhythm (Coben, Linden, & Myers, 2010). The benefits of training the SMR were discovered somewhat accidentally, in the 1960s, when Sterman and his colleagues were conducting studies on the SMR rhythm in cats. By training the cats to remain still, they found the rhythm increased. Later on, the same cats were included in a different study that was focused on the toxic effects of aviation fuel. It was during this study, where the researchers

observed that the cats that had been previously trained to enhance SMR were significantly more resistant to the epileptic seizures induced by the aviation fuel than non-trained cats (Collura, 2014). This finding sparked curiosity for further studies into the training of SMR and led to the its application as a non-invasive therapy for humans who experienced seizures. The success of those studies sprouted the application of NFT as a therapeutic option for alcoholism, anxiety, concentration and hyperactivity and set the foundation for NFT as a scientific and non-invasive technique to regulate brain activity (Angelakis et al., 2007; Collura, 2014)

While initial studies were often limited to the technology of their time, more recent studies take advantage of the technological advancements to produce increasingly creative NFT protocols whilst maintaining the same principles. For example, Pineda, Silverman, Vankov, and Hestenes (2003), trained participants to manipulate the SMR in a 3D first person shooter video game. Right- and left-hand movements in the game were controlled by high and low mu respectively. The authors concluded that the element of a brain computer interface in NFT accelerates the learning curve in NFT goals as it is more engaging.

Seo, Noh, and Jeong (2018) used a smartphone based NFT to increase their index of concentration. They used a portable EEG headband that recorded activity over FP1 and FP2. Feedback was given in the form of an archery game on a smartphone. When participants increased the concentration index (given by an algorithm that summed activity in SMR and beta, divided it by the amount of theta and then multiplied it by 100) the aim moved closer to the target on the screen. The researchers were able to successfully improve participant's concentration index and maintain the effects after the completion of training. The authors also concluded that the use of portable devices can be harnessed to produce NFT effects without the constraints of a lab.

Faller et al., (2019) used online NFT to adjust arousal levels through a brain–computer interface (BCI) that modified the feedback according to the participant's arousal level during a boundary-avoidance task (BAT), the scenario consisted of aerial navigation task in virtual reality that generated conditions to stimulate arousal (measured by pupil dilation and heart rate). Information from the spontaneous EEG was decoded into an index of inferred task-dependent arousal. When the participant was more engaged in the activity, they received feedback. The authors managed to improve performance in the BAT by shifting the participant's state of arousal.

The advantage of technology to provide a more engaging reward/feedback for participants in these studies, support the notion that the choice of the reward element creates the value of the NFT (Collura, 2014), as will be discussed further in the next section.

1.3.3. Protocols

1.3.3.1. General considerations

The type of protocol employed depends on the ultimate goal of NFT. Whether it is seeking peak performance training to improve a skill or upregulating a rhythm in a clinical setting, when choosing a protocol for NFT two things are central in order to achieve the goal of the training, these are: threshold and feedback.

Threshold

The threshold refers to the microvolt level that the EEG amplitude of the selected activity band (alpha, theta, beta etc) must reach in order to achieve feedback (Collura, 2014). Depending on the

protocol, the threshold can be used as a signal for the deployment of rewards, or as the threshold cut-off point for receiving said reward. An NFT protocol can have multiple thresholds depending on the end goal of the training. For example, Wang et al. (2016) sought to improve attention in individuals diagnosed with ASD by concurrently training to increase relative power in gamma while decreasing the power in the cortical theta/beta ratio. In terms of thresholds, this means that every time a participant's gamma power surpassed the thresholds set by the authors, they received a reward, however, the reward also depended on the participants staying below the threshold for the theta/beta ratio. The theta/beta ratio theta condition required participants to enhance the low frequency range (theta) in comparison to the amplitude of beta range. In this study one threshold had to be surpassed in conjunction with maintaining limits set for the second threshold.

Feedback

As in the classic operant conditioning paradigm, the types of feedback that a participant receives in NFT can be in the form of reinforcement/reward, aimed to increase a behaviour (in this case, a specific brain activity), or a punishment, aimed to decrease the brain activity of interest (Staddon & Cerutti, 2003). In order to inhibit the brain activity of interest, rewards can be withheld, or a participant can receive feedback to indicate that they have deviated from the session target so they can regulate back towards the goal.

The feedback used for NFT protocols are mainly classified into visual, auditory and tactile stimuli (Collura, 2014; Reiner, Gruzelier, et al., 2018). Examples of each classification and how they are implemented as a reward or stimuli to inhibit undesired brain activity can be found in table 1.4. Depending on the goal and set up of the protocol, multiple feedback modalities can be provided to

the participant. For example, the display of a video where the image gets brighter when the desired brain activity is achieved, and concurrently, the participant hears a tone when they fall out of the established threshold.

Table 1.4. Types of feedback

Classification	Feedback type	Reward indication /control
Visual	Video(s) Static image Text	Stop-start Brightness modulation Contrast Zoom
Auditory	Discrete (appears only when the reward is achieved) Continuous (throughout the whole session)	Volume control Stop-start
Tactile	Vibration	Start-stop Increase/decrease intensity

1.3.3.2. Low vs high frequency protocols

In general, NFT has two general directions depending on the oscillatory frequency that we wish to train, either low or high frequencies. Overall, the selection of the frequency we wish to train will determine the type of protocol. As mentioned in the previous section, a protocol can also train multiple frequencies, requiring the enhancement or inhibition of simultaneous frequencies in order to receive the reward.

Low Frequency Protocols

Low frequency protocols comprise delta, theta, alpha and slow cortical potential NFT. These protocols are mainly focused on strengthening relaxation and focus or attaining altered states of consciousness (Collura, 2014; Marzbani et al., 2016). The goal of these protocols is to achieve the

desired state through less effort by allowing their natural flow of brain activity and releasing of control over them. The sessions for these protocols can be longer and without breaks because the goal is more aimed towards total relaxion (Collura, 2014).

Delta (0.5 - 4Hz)

Delta waves are characteristically associated with stage 3 of Non-REM sleep. In sleep they are associated to physical restoration (Buzsáki, 2009; Le Bon et al., 2012). In individuals with migraines and impairment of consciousness an abnormal rhythmic delta activity has been described in frontal areas (Walser & Isler, 1982). Thus the training of this frequency is used to improve sleep and alleviate headaches (Marzbani et al., 2016).

Theta (4-8Hz)

Theta is associated with memory, emotion, creativity, meditation and the first phase of sleep (Buzsáki, 2009). The training of this frequency is generally employed to improve anxiety, emotional disorders and lack of attention and is often trained with other frequencies, such alpha and/or beta. (Gruzelier, Foks, et al., 2014; Gruzelier, Thompson, et al., 2014; Reiner et al., 2014; Reiner, Lev, et al., 2018; . Vernon, 2005).

Alpha (8-12 Hz)

Usually associated with a calm yet alert state, the alpha rhythm has often been referred to as the “idle state” of the brain (Buzsáki, 2009; Niedermeyer, 1997; Pfurtscheller et al., 1996; Pineda, 2005). The so-called alpha mood is associated with calm and pleasantness in the individual experiencing it (Binder et al., 2009; Niedermeyer, 1997), which is why alpha has been shown to increase during meditation and those who are experienced meditators have an increase in this band

compared to others (Fell et al., 2010; van Lutterveld et al., 2017). Consequently, depending on the frequency trained within the alpha band, the application of this band in NFT protocols is mainly used for: increasing states of relaxation (Omejc et al., 2018), reducing stress anxiety, and pain (7-10 Hz) (Marzbani et al., 2016), and improving meditation practice (Brandmeyer & Delorme, 2013; Chow, 2014; Ford et al., 2016; Gruzelier, 2014a).

Alpha training can also be combined with theta (increasing theta over alpha activity) to improve creativity in artistic activities or performance whilst favouring relaxation (Gruzelier, 2009; Gruzelier, Foks, et al., 2014; Gruzelier, 2014b). This protocol is usually done with eyes closed, using auditory feedback. Alpha/theta training can induce a hypnagogic state in the participants which can be applied to enhance creativity or gain deeper awareness (Collura, 2014).

High frequency protocols

The goals of high frequency protocols are usually the reinforcement of activation, organizing, concentration and inhibiting distractibility (Collura, 2014; Marzbani et al., 2016). It is best to set up the training in the form of trials and with a more interactive quality in the feedback in order to avoid fatigue or lack of motivation from the participants (Collura, 2014).

Beta

These brain waves are associated with concentration on a task, problem solving, a state of deliberate attentiveness as well as movement (Buzsáki, 2009; Herrmann et al., 2016).

Training beta is used to improve concentration and attention. This has resulted in an interest for scholastic applications of the protocol in order to improve performance (Hosťovecký & Babušiak,

2018; Jurewicz et al., 2018; Steiner et al., 2010). It has also been widely used in the treatment of ADHD, alcoholism and epilepsy (Ko & Park, 2018; Marzbani et al., 2016; Steiner et al., 2010; Thompson et al., 2010). The training of this band has also been seen to reduce anxiety and stress by achieving a state of relaxed focus (Hammond, 2011; Michael et al., 2005; Walker, 2009; Wang et al., 2019). In individuals who do not have clinical complaints it improves performance in tasks related to attention or mental acuity (Pimenta et al., 2018; Vernon et al., 2003b).

Sensorimotor Rhythm (SMR: 12-15Hz)

Measured over the sensorimotor cortex, this rhythm is associated with a state of rest of the motor system (Sabate et al., 2012) this why they are the most discernible when the person is inactive. Training this rhythm is related to body stability and resistance to stress (Collura, 2014). The training of SMR has shown positive results in patients with seizures, insomnia, poor concentration and ADHD (Collura, 2014; Janssen et al., 2017; Jeon & Choi, 2017; Ko & Park, 2018; Kober et al., 2015; Reddy & Sneha, 2019; Vernon et al., 2003b).

Gamma

These waves are related to cognitive processing and information exchange with the outside world (Marzbani et al., 2016). Therefore the training of this rhythm has been used to improve performance in cognitive, and problem solving tasks, as well as memory tasks (Chauvière & Singer, 2019; Gruzelier, 2014a; Ninaus et al., 2015; Salari et al., 2014).

1.3.4. Applications

As touched upon above, applications for NFT span far and wide. From improving attention and cognitive processing in healthy individuals to rehabilitating therapy for brain surgery and treating

depression, ADHD and ASD. Although these are not the only realms where NFT can be applied, some of the of the main areas are described below:

1.3.4.1. Performance enhancement (sports, music, cognition)

There is increasing interest in the use of NFT protocols in healthy individuals in order to improve their performance in a particular task or skill. This is particularly so in sectors where performing a skill successfully results in tangible consequences, whether it be financial (in the case of a professional athlete or an artist) or even result in life and death situations (in the case of a surgeon). NFT has become a viable addition for training high performance skills by enhancing the neuroplasticity. Many of the protocols take advantage of the SMR, beta, theta and alpha training protocols depending on the skill they wish to enhance. For example, NFT has yielded advantages on surgical skills of micro-surgeons who trained SMR ratio during 8 sessions compared to their peers who did not include it in their training (Ros et al., 2009).

In the use of NFT to enhance performance in athletes, the protocol used is usually focused on the regulation of SMR to improve performance and reaction times in motor tasks. Reviews in this field of study show that although many support the use of NFT for sports performance, the presence of placebo effects and inconsistency in results when replicating protocols across different studies suggest that the research in this application of NFT is still in its early stages (Mirifar et al., 2017, 2018; Xiang et al., 2018).

For example, Paul et al. (2012) trained archery players to improve accuracy during archery performances. The authors recruited university level archery players and subsequently divided them into control and experimental groups. After twelve sessions of NFT on SMR to theta ratio

the archers in the experimental group were able to improve their scoring accuracy score on a regular basis. Other sports where NFT has yielded positive results in performance enhancement is football (Wilson, Peper & Moss, 2006), gymnastics (Shaw et al., 2012), golfing (Sherlin et al., 2015) , tennis (Gracz et al., 2007), baseball (Sherlin et al., 2013), canoeing (Perry et al., 2011) and speedskating (Beauchamp et al., 2012).

The association of alpha and theta with creative process has also sparked the interest of implementing NFT to enhance creative/artistic skills and performance. The training of theta has been implicated in the induction of hypnagogic states that favour creative 'flow'. (Collura, 2014; Gruzelier, 2014a, 2014b). The term "flow" refers to a psychological construct that describes when a person experiences an optimal balance between skill, mastery and challenge while receiving feedback during a task/activity. This state involves an increased amount of concentration while maintaining a low amount of awareness and results in a feeling of satisfaction (Gruzelier et al., 2010).

Alpha/theta training has had positive results in music and dance students (Egner & Sterman, 2006; Raymond et al., 2005) and this protocol has been extended to dancers by combining it with heart rate variability feedback; in both cases participants have shown improvement in their performance (Gruzelier, 2014b; Raymond et al., 2005).

It is important to note that although there is evidence to the improvement of performance in sports and the arts through NFT, there is also a widespread heterogeneity in the methodological set up, and some controlled studies have not been successful. For example, Mirifar et al., (2018) found no improvement to reaction times and attention when training theta/beta ratio. This stresses the need

to outline consistent protocols in order to establish NFT as a valid and standardized method for improving cognition.

1.3.4.2. Cognition in elderly

Due to medical and technological advancements, the average life expectancy has increased over the last decades. A longer life expectancy also brings about ailments that are linked to ageing. Although it is normal for a degree of cognitive decline to occur with natural ageing (memory, language, attention, executive functions etc), pathological deterioration of cognition is also becoming more frequent and therefore, a need to counteract if not prevent or treat these pathological cognitive decline becomes ever more relevant.

Studies that use NFT as an intervention to improve cognition have so far, been scarce (Angelakis et al., 2007; Becerra et al., 2011). They focus on EEG biomarkers of ageing and train the activity to generate improvements on cognitive tasks. For example, Becerra et al. (2011) took an excess of slow theta absolute power in awake resting state as the EEG-based predictor of cognitive impairment in normal elderly participants. They designed an NFT protocol that consisted of 30 sessions, lasting 30 minutes each, where participants received auditory feedback as a reward when they decreased their theta absolute power. The participants were randomly divided into experimental and control groups and were assessed pre and post NFT sessions using cognitive tasks that evaluated attention, executive functioning and memory. As expected, their experimental group had a significant improvement in cognitive and EEG measures. However, the authors also found improvements in the control group, which they attributed to a placebo effect, not only of the sham NFT but also of taking part in the study and regularly interacting with the experimenters and therapists which in itself was a change from the participants' day to day life.

Another EEG-based parameter of ageing that has been used in NFT protocols is the alpha peak frequency, which correlates positively with cognitive performance and negatively with ageing (Angelakis et al., 2007). A pilot study done by these authors where they had a control group and two experimental groups, (one that trained alpha amplitude and one that trained alpha peak frequency), suggested that NFT of the peak alpha frequency resulted in improvement on cognitive processing speed and executive functions task. Neither training had an effect on memory.

Other types of feedback, such as tactile, have also been explored in this population. Basta et al. (2011) for example, implemented a vibro-tactile feedback protocol in order to rehabilitate symptoms of body sway in elderly adults with balance disorders. NFT was performed daily for 15 min over the course of two weeks in a control and experimental group. The improvements found in the experimental group were not observed in the control group, The authors concluded that vibro-tactile stimulation was a valid alternative for rehabilitation for these disorders, because it was easy to implement, individualized as well as cost effective in terms of time when compared to other rehabilitation alternatives.

The above studies suggest that the implementation of NFT in the elderly population could have potential significant applications on the improvement of quality of life and treatment of pathological cognitive decline. However, there is still a need for further research in this area.

1.3.4.3. NFT as a treatment for disorders (ADHD, Epilepsy, Depression)

The use of NFT in the treatment of disorders, has had success as a non-invasive therapeutic option, whether it be on its own or in combination with other treatments. Overall, this neuromodulation technique targets cortical activity and neuroanatomical locations that have been described as

abnormal in a specific disorder and aims to train the specific brain activity in order to increase the regulation of the area and/or frequency. For example, in the treatment of epilepsy, the protocols that have reported successful reductions in seizure frequency are those that train either the SMR or slow cortical potentials (Egner & Sterman, 2006; Marzbani et al., 2016; Reddy & Sneha, 2019; Sterman & Egner, 2006; Walker & Kozlowski, 2005). As epilepsy is the result of an overexcitation of cortical and/or thalamocortical structures, the training of SMR has been shown to help regulate the firing pattern of thalamic nuclei to a more rhythmic pattern (Reddy & Sneha, 2019). A second protocol that has yielded success in reducing seizures, is the training of Slow cortical potentials (SCPs).

SCPs refer to changes in cortical polarization of the EEG that serve as a mechanism of threshold regulation for local excitatory or inhibitory cortical networks (Birbaumer, 1999). They can be time locked to external events or self-regulated through training (Strehl, 2009). As they are within the range of 0-1Hz which is below spontaneous oscillatory activity like delta, theta, alpha, beta, and gamma, the benefits from NFT come from gaining awareness of the SCP themselves. Shifts in SCP have been observed before and during seizures; in NFT, patients are trained to identify these shifts and therefore increase control over the activity (Reddy & Sneha, 2019; Strehl, 2009).

In depression, the use of NFT as a therapeutic option has support from both asymmetry theory (Davidson, 1992) and social learning theory (Linden, 2014). In a broad sense, the symptoms of depression span emotion regulation, cognition, motivation, and homoeostasis (Hammond, 2005; Linden, 2014). This results in treatment options that include, psychological, pharmacological, or physical interventions, and the combination of these treatments have also yielded positive results (Khan et al., 2012). Medication side-effects, as well as a lack of response from some participants present significant limitations to the afore mentioned treatments. Based on the asymmetry theory

of depression which suggests that the symptoms are a result of a hypoactivity on the left frontal alpha power compared to the right, NFT aims to shift that asymmetry towards an increased activation in the left hemisphere. Although there are mixed results with this theory, metanalyses have supported this asymmetry model as the basis for NFT protocols to treat depression (Linden, 2014).

The social learning theory posits that depression is a result of a general low sense of agency and loss of experience of control of the environment (Bandura, 1999). Within this frame NFT improves depressive symptoms by improving participant's sense of agency when they successfully gain control/awareness over their own brain activity (Linden, 2014).

As for ADHD, NFT has consistently demonstrated positive results in improvement of ADHD symptoms (Angelakis et al., 2007; Baumeister et al., 2018; Cortese et al., 2016; Logemann et al., 2010; Van Doren et al., 2019). The basis of NFT as a viable therapeutic alternative for individuals with ADHD is that by modifying electrical brain frequencies that are correlated with attention, impulse control and adaptative behaviour, negative behavioural problems can be reduced (Riesco-Matías et al., 2019). The protocols mainly train sensorimotor rhythm (SMR), slow cortical potentials (SCP) and, more frequently, the ratio between the theta and beta band frequencies (Enriquez-Geppert et al., 2019). At a first glance, NFT has been demonstrated to be an effective alternative at reducing symptoms of ADHD. However, it is currently not as effective as pharmacological treatments, which are still the gold standard for ADHD (Van Doren et al., 2019). The advantage of NFT is the lack of negative side-effects whilst improving behavioural symptoms as well as having long-term effects (Enriquez-Geppert et al., 2019). Nevertheless, there has been a debate when analysing the methodological considerations of some of the studies, as the pre-, post- and long-term assessments are sometimes fragile, in the sense that they depend on

observational reports from parent or teachers, who can be biased by the experimental conditions (Van Doren et al., 2019). Another topic of debate is the placebo effect that some authors claim as the explanation for success in the efficacy of NFT in ADHD (Ghaziri & Thibault, 2019). This perspective has also been met with criticisms of exaggerating the effect of the placebo and committing type III statistical errors (Trullinger et al., 2019). This implies that more stringent assessments be made to determine the success/effectiveness of NFT protocols.

1.3.5. NFT and ASD

As with other disorders, protocols for NFT in ASD are mainly focused on improving the core symptoms either by improving indirect symptoms that have an effect on daily performance (such as attention and anxiety/impulsivity) or targeting brain activity that has been described as being abnormal in this population. Specific NFT protocols that are implemented vary depending on the particular characteristics of the participants, if they have concurrent diagnostics, and the symptom that is being targeted (Marzbani et al., 2016).

The frequencies most often trained are the inhibition of the theta-beta ratio whilst enhancing beta activity (Marzbani et al., 2016; Wang et al., 2016), for improvement of attention and concentration (school performance); and enhancement of the mu rhythm in order to affect some of the social symptoms of ASD. Studies in this line of research show positive outcomes manifested by a decrease in symptoms as well improvements in post-protocol assessments (Coben & Padolsky, 2016; Datko et al., 2017; Holtmann et al., 2011; Jarusiewicz, 2016; Kouijzer et al., 2009; Kouijzer et al., 2009, 2010; Pineda et al., 2008, 2008; Pineda et al., 2014).

However, the topic of assessment of NFT is somewhat controversial. As with ADHD, the definition of "success" in a training protocol is often based on parent or teacher perceptions reported on questionnaires before and after the intervention (Jarusiewicz, 2016; Kouijzer et al., 2010; Pineda et al., 2008; Pineda et al., 2014). Indeed, studies that include physiological measures such as EEG or even fMRI as a pre- and post-assessment are scarce (Datko et al., 2017) (EEG REF). Other methodological limitations that have been described are the size of the sample groups (Kouijzer et al., 2009, 2010; Pineda et al., 2014), unequal group sizes (Coben et al., 2014), and unclear assessment in the initial diagnosis (Pineda et al., 2014).

One of the few studies that has controlled participants for age, gender, race, handedness, other treatments, and severity of ASD was done by Coben & Padolsky (2016), where they aimed to reduce the EEG hypoconnectivity previously observed in ASD populations. They had a sample size of 37 participants that attended 20 NFT sessions. Although their pre- and post-assessments were largely based on parent or teacher observation-based questionnaires, they also included a pre- and post-NFT EEG as well as the monitoring of cerebral blood flow through research infrared cameras. To determine training band and threshold, the researchers took information from all pre-NFT assessments. Post-assessment revealed significant reduction in symptoms observed by parent/teachers and correlated to a general reduction in hypoconnectivity among the experimental group. This study was the continuation of a pilot study done by Jarusiewicz (2002) where they also found improvements in symptoms as reported by parent/teacher observation based questionnaires.

In high functioning ASD individuals, NFT has been used to train the mirror neuron system via modulation of the mu rhythm. Pineda et al. (2014) used an NFT protocol focused on increasing the mu rhythm within (8-12Hz) whilst reducing theta and beta. Participants were either high-

functioning ASD or neurotypical individuals. Thresholds were determined individually from a baseline period at the beginning of each session and calibrated according to each participant's performance throughout the sessions. Pre- and post-assessment included both an EEG based mu suppression index task as well as observational questionnaires completed by parents or guardians. The authors found improvements only in the ASD group in terms of pre- and post-assessment of behaviour and EEG. Other studies within this line of research state that the changes in behaviour and EEG following a NFT protocol for mu do not necessarily extend to imitation behaviour (Pineda et al., 2008). However further studies that include pre- and post-assessments by means of fMRI imitation and observation tasks provide support for implementing this protocol in ASD population (Datko et al., 2017). Although all these studies compare the effect of NFT between ASD and neurotypical population, it would be important to include comparisons between participant who are on the spectrum but either do not receive NFT or are subjected to sham NFT in order to elucidate more about the efficacy of NFT itself as a therapeutic option.

Until these methodological topics are addressed, it remains unclear to what extent and how NFT may be improving symptoms in ASD. This in turn creates obstacles for the use of NFT as a standardized treatment option. More studies are therefore needed in order to objectively assess the efficacy of this treatment as a non-invasive alternative for individuals with ASD.

2. Resting state EEG (rEEG) in autistic traits

2.1. Aims and overview

Autism spectrum disorders (ASD) encase a variety of developmental symptoms that affect how individuals interact and communicate socially and are also characterized by repetitive and stereotyped behaviours (American Psychiatric Association, 2013b). A single underlying cause has not yet been pinpointed but numerous neural system dysfunctions have been linked to this disorder (see chapter 1 for a detailed description).

The evolution in the characterization and diagnostic features of ASD is a result of the many variations in clinical presentation of those who are affected by it (Wang et al., 2013). The study of the heterogeneity in autistic traits and symptoms alongside the discovery of the Broader Autistic Phenotype (see chapter 1) has led to the characterization of autism as a spectrum. Autistic-like traits have been described within the neurotypical population with ASD as the extreme of a dimension that includes both social and functional connectivity traits (Barttfeld et al., 2013).

There has been a growing interest in studying the brain during resting state. The brain is a system that operates on an endogenous level, reacting to external sensory information as it arises (Wang et al., 2013). When the brain is in resting state, it reflects the brain's endogenous activity including data concerning communication between different brain areas. In this state one can also observe spontaneous variations in neural activity and these can be associated to individual differences in terms of cognitive decline as well as a variety of disorders (van Diessen et al., 2015).

Studies done on resting state in individuals with ASD show that compared to neurotypical individuals, there is typically higher power in the alpha bandwidth of the EEG and decreased power in lower frequency bands such as delta and theta (Wang et al., 2013). A meta-analysis on resting state functional brain activity done by Wang et al. (2018) also reported alterations in individuals with ASD, specifically in the cerebellum and language areas. Despite the information that the resting state could provide on endogenous brain activity, the literature on resting state brain activity in ASD is not extensive.

Many paradigms use magnetoencephalography (MEG) and functional magnetic resonance imaging (fMRI) to study functional connectivity of the resting state. However, these techniques often have high costs and can be difficult to use in specific populations (Subha et al., 2010). Electroencephalography (EEG) offers both practical and methodological advantages for the study of the resting EEG (rEEG); it is less expensive, has a higher tolerance for the participant's movement and can be used in various groups regardless of age as it is more clinically available and offers a higher temporal resolution (Wang et al., 2013).

Another approach to the resting brain is to study the functional connectivity between networks in the brain. Rojas et al., (2018) describe a model based on the combination and correlation of resting-state functional magnetic resonance (rs-fMRI) with EEG, in order to obtain the localization of functional networks in the brain (Default mode, Frontoparietal, Ventral attention, Dorsal Attention, Somatomotor and Visual Functional network). This model takes temporal and spatial resolution advantages of both neuroimaging techniques and can be replicated using the 10/20 system in order to identify abnormal function in these networks. This approach will enable us to increase the spatial resolution of our EEG data by comparing it to a model that is based of rs-fMRI

whilst maintaining the temporal resolution of the EEG and thereby maximise the information obtained in order to describe the rEEG of our sample in a more complete manner.

In terms of analysis that are often used with EEG data, most rEEG studies focus on the decomposition of the signal into the different oscillatory frequency bands that have been associated to physiological properties and cognitive processes (Wang et al., 2013). Different measures of the EEG such as frequency, amplitude and power can then be taken and analysed to gain insight into brain activity within and between regions. Traditional EEG analysis use the fast Fourier transform. This technique assumes that the EEG signal is both linear and stationary in order to decompose the signal in the traditional bands (alpha, delta, theta, beta and gamma) (Schwilden, 2006). However, the brain is not a linear system (Schwilden, 2006) and a less used and more recent approach takes into consideration these non-linear aspects of the EEG. The most widespread used nonlinear methods are focused on entropy and fractal concepts and they assume that the EEG signal frequency components have variations in amplitude and shape as time progresses and these fluxes provide information about the underlying intrinsic dynamics of the EEG (Ma et al., 2018). As physiological signals are generated by self-regulating biological systems, nonlinear approaches can reflect the characteristics of signal complexity of these systems (Klonowski et al., 2000). It is likely that the activity detected by each method is generated by different mechanisms, which means their combined use could provide distinct information regarding large scale networks (Wen & Liu, 2016).

In ASD, studies focusing on EEG complexity and nonlinear measures have been carried out in order to identify biomarkers of the disorder (Bosl et al., 2011; Bosl et al., 2017; Catarino et al., 2011; Kang et al., 2018, 2019). Including non-linear EEG signal analysis to existing classification

methods has been suggested as a valid complementary measures to the increase the information in order to improve classification and diagnosis of neurodevelopmental disorders (Bosl et al., 2017). Ahmadlou et al., (2010) for example, used fractal dimensions to identify complexity and dynamic changes in the rEEG of ASD children. These authors found that the fractal dimensions 'Higuchi' and 'Kantz' were significantly different between the samples and proposed the use of fractal dimensions as a possible tool for the diagnosis of ASD.

With that in mind, this chapter aims to explore both oscillatory and nonlinear measures of the rEEG in neurotypical individuals with high and low autistic traits. We hypothesize that although our sample is not from a clinical ASD population, there will be differences in brain activity between the groups, as would be expected within the spectrum approach to autistic traits (Barttfeld et al., 2013; Hurst et al., 2007; Tavassoli et al., 2014; Wakabayashi, Baron-Cohen, Wheelwright, et al., 2006). Within linear/oscillatory measures of absolute power and functional connectivity, we expect to see higher alpha power in the low AQ group. For nonlinear measures we would expect significant group differences in entropy and fractal measures in accordance with previous studies on ASD and entropy measures.

2.2. Methods

2.2.1. Participants

5000 participants were initially contacted via e-mail through the university mailing list for research participation, SONA and word of mouth to be screened for the study using the Autism-Spectrum Quotient (AQ) (Baron-Cohen et al., 2001). A total of 291 people answered and returned the AQ. High and low group scores were selected by taking one standard deviation above and below from the mean score among the sample (M=18, SD=6). This resulted in the high AQ group being

comprised of those scoring ≥24 and the low AQ group scoring ≤13. These scores also correspond to the extreme of the normal distribution described by Baron-Cohen et al., (2001); that is to say, although the high group was one standard deviation higher than the normal population average, these scores are not considered at high risk for diagnosis of ASD. Subsequently, 40 participants (20 high AQ and 20 low AQ) were invited take part in the study. Participants had a mean age of 23.57 ± 3.67 for the high AQ group and 21.67 ± 3.6 for the low AQ group. In terms of gender, the high group had 11 female and 9 males, and the low group had 14 female and 6 male

The University of Essex Ethics Committee approved this study. All participants read and signed and informed consent prior to their participation. The procedures used in this study were non-invasive and in accord with the Declaration of Helsinki; at no time were the participants or the researcher at risk for their safety.

2.2.2. Materials

2.2.2.1. Pre-screening

The Autism-Spectrum Quotient (AQ) is a self-administered short scale that measures the degree to which an adult of normal IQ exhibits traits associated with the autistic spectrum. The participant scores a point for each answer that resembles an autistic answer. Higher scores equal a higher level of autistic traits. It is comprised of 50 items that are divided into 5 different areas: social skills, attention switching, attention to detail, communication and imagination (Simon Baron-Cohen et al., 2001). The AQ has proven to be suitable for screening purposes in research and clinical settings due to the high internal consistency and reliability across different samples (Baron-Cohen et al., 2001; Hurst, Mitchell, Kimbrel, Kwapil, & Nelson-Gray, 2007; Wakabayashi, Baron-Cohen, & Wheelwright, 2006; Wakabayashi, Baron-Cohen, Wheelwright, et al., 2006).

2.2.2.2. Protocol

2.2.3. Experimental Design

This was an independent measures design. Dependent variables were absolute power, entropy and fractal measures as well as mu microstructure measures. Each variable and its subcategories will be described in detail in the results section. Independent variable was AQ group (high and low).

2.2.4. Procedure

At the beginning of the session the participant was asked to read and sign a consent form to take part of the experiment. After placement of the EEG cap, the resting state EEG was recorded for 2 minutes. Participants were instructed to sit still with their eyes closed during that time. An electrooculogram (EOG) calibration task was also done in order to form the basis for subsequent EOG artefact reduction for the EEG (see Croft & Barry, 1998).

All EEG data was simultaneously recorded with a Neuroscan 4.4 acquisition software and Synamps II amplifiers using a 64 channel Quick-Cap arranged according to the international 10–10 system (Compumedics, Melbourne, Australia). Eye movements were recorded additionally using four facial electrodes: 1 horizontal electrode for each eye (approximately 1cm on the outer cantus of each eye) and 2 vertical electrodes (above and below the left pupil). Impedances for all electrodes were reduced to below 10 kOhm before the start of each session. All EEG data were continuously sampled at 1000Hz with a bandpass filter of 0.15–200Hz and a 50Hz notch filter. Online, EEG data were referenced to an electrode between Cz & CPz, and grounded midway between Fz and FPz.

2.2.4.1. EEG data preparation

Bad electrodes were excluded on a subject by subject basis. Principal components analysis was performed on the acquired eye movement data to obtain components reflecting saccades and blinks. To carry out ocular artefact rejection, the acquired components were subsequently rejected from the task data traces (Vigario, 1997; Vigario et al., 2000).

Using EEGlab software toolbox from Matlab (Delorme & Makeig, 2004), the data were then re-referenced to the average of the scalp electrodes and filtered using a bandpass filter f 0.5- 50Hz using a finite impulse response (FIR) filter with automatic estimation of filter order. If the signal had any isoelectric sections of >5seconds it was removed from the recording. Artefact subspace reconstruction was performed using bad burst correction with a max accelerating window of .5 and standard deviation of 20 to correct bad periods.

Independent component analysis was done using a SOBI algorithm. Components were flagged and labelled for removal using IC label. Components that contained less than 50% of brain activity were removed from the signal.

2.2.5. Results

Linear analysis

Absolute power

Bandpower analysis was performed using the python toolbox YASA (https://raphaelvallat.com/yasa/build/html/index.html) which performed a fast fourier transformation (FFT). The FFT yields a number for each designated frequency band from which

the amplitude and phase of the signal can be extracted. This type of analysis assumes that the oscillations in the EEG signal are sine waves which can be fragmented into different frequency components (Ma et al., 2018). The bands that were selected for analysis were: Delta (0.5-4 Hz), Broadband Theta (4-8 Hz), Broadband Alpha (8-12 Hz), Low Alpha (8-10 Hz), High Alpha (10-12 Hz), Broadband Beta (12-30 Hz), Low Beta (12-20 Hz), High Beta (20-30 Hz), and Gamma (30-45 Hz). Electrodes were grouped into right and left brain regions: frontal(F), frontocentral (FC), central (C), centroparietal (CP), parieto-occipital (PO), parietal (P) and occipital(O). The electrodes that made formed part of each brain region group were as follows:

Frontal:

Right: F8, F6, F4, F2

Left: F7, F5, F3, F1

Frontocentral:

Right: FC6, FC4, FC2

Left: FC5, FC3, FC1

Central:

Right: C6, C4, C2

Left: C5, C3, C1

Centroparietal:

Right: CP6, CP4, CP2

Left: CP5, CP3, CP1

Parieto-occipital:

Right: PO8, PO6, PO4

Left: PO7, PO5, PO3

Parietal:

Right: P8, P6, P4, P2

Left: P7, P5, P3, P1

Occipital:

Right: O2

Left: O1

All variables were checked for normality to determine what type of independent samples statistic should be used. Normally distributed variables were analysed using t-test and non-normally distributed variables were analysed using the Mann-Whitney test.

Theta

For broadband theta and significant group differences were found over left frontocentral U=103, p=.024, central U=102, p=.023 and centroparietal areas U=110, p=.041. The low AQ group had a higher absolute power than the high AQ group: Md= 3.78 vs Md= 2.21; Md= 3.16 vs Md= 1.55 and Md= 3.16 vs Md= 2.03 respectively.

Over right central areas, group differences were also significant U=112, p=.048. Again, the low AQ group had a higher absolute power than the high AQ group Md=3.40 vs Md= 1.80.

Alpha

For broadband alpha, significant group differences were found over right frontal regions U= 106, p=.048; the low AQ group had a greater absolute alpha power than the high AQ group: Md=16.36 vs Md=6.45.

High Alpha

For the high Alpha band, significant group differences were found in over right frontal areas (U= 105, p= .045); left frontocentral areas (U= 97, p= .015) and right occipital areas (U=54, p=.018). In all three areas the low AQ group had a greater absolute power than the high AQ score group: Md= 5.66 vs Md= 3.01; Md= 6.83 vs Md= 2.55; Md=36.39 vs Md= 8.9 respectively.

Low Alpha

For the low Alpha band, significant group differences were found over left centroparietal U=110, p=.041 and parietal U=108, p=.035. The Low AQ group had a greater absolute power than the high group (Md=5.12 vs Md=2.40 and Md=9.97 vs Md= 5.82) respectively.

Beta band

Significant group differences for beta were found over left central areas U=112, p=.048. The Low AQ group had a greater absolute power than the high group (Md=3.9 vs Md=2.35).

No other bandwidths showed significant group differences between groups (ps > .05). A visual summary of results can be found in Fig 2.1.

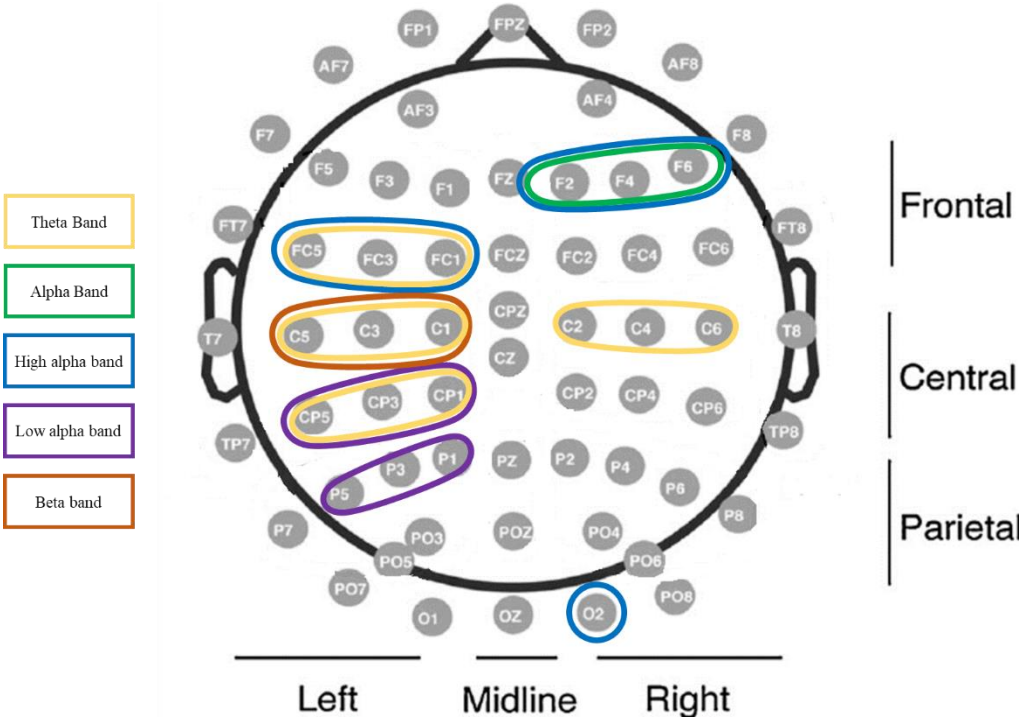

Fig.2.1. Areas with significant group differences in absolute power analysis. Specific bands are located on the left. Adapted from Kielar and Joanisse (2011).

Functional connectivity

Additionally electrodes were grouped according to functional connectivity networks as suggested by Rojas et al., (2018) into the frontoparietal (Fp1, Fp2, F3, F4, Fz), somatomotor functional network (Cz, C3, C4) and visual functional network (O1, O2, Oz) (see fig 2.2).

Group differences were found in the Somatomotor functional network for Theta U=100, p=.033. The low AQ group had a higher absolute power in this network compared to the high group (Md=3.14 vs Md= 1.70).

The frontoparietal functional network in low alpha was also significantly different between groups U=110, p=.041. Again, the low AQ group had a higher absolute power in this network compared to the high group Md= 7.20 vs Md=3.54.

A visual summary of functional connectivity findings can be found in fig. 2.2

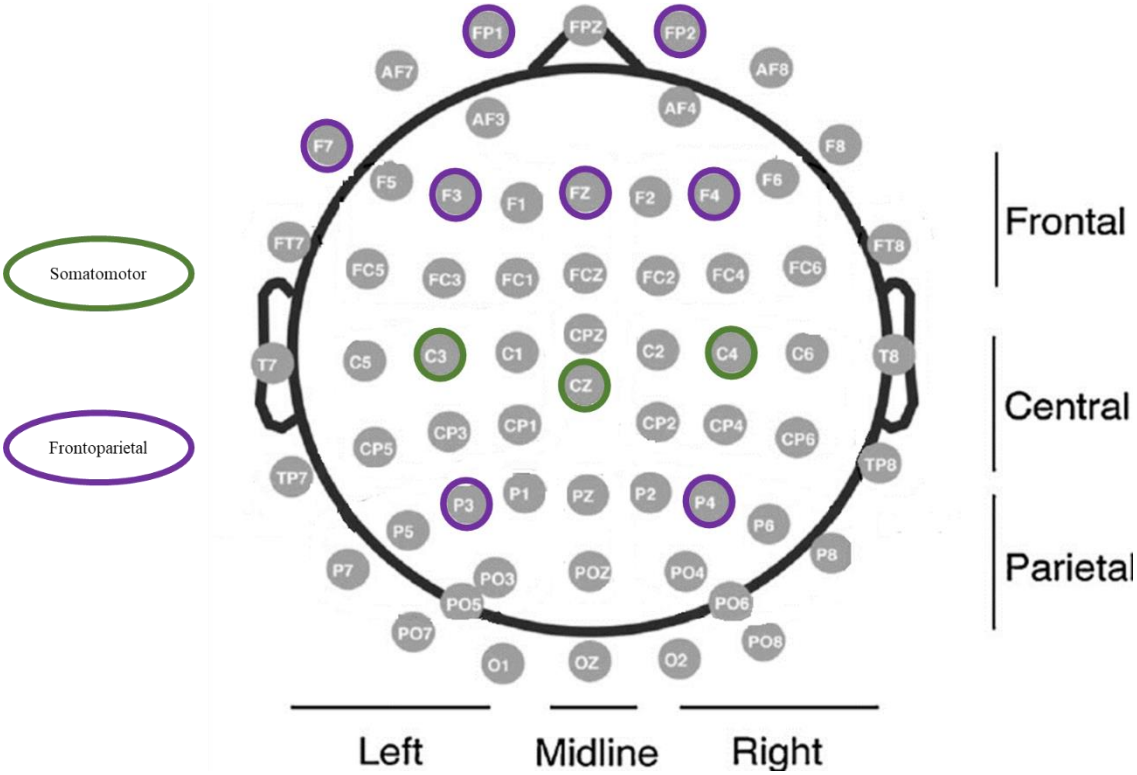

Fig. 2.2. Functional connectivity network findings, Significant group differences were found in the frontoparietal and somatomotor networks for theta and low alpha respectively, the low AQ group had a higher absolute power over the high AQ group. Adapted from Kielar and Joanisse (2011).

Mu Microstructure analysis

Differences in mu rhythm reactivity between neurotypical individuals and ASD populations were described in chapter 1; building on past research, it was of particular interest in this experiment to also focus on the mu rhythm as a microstructure event of the rEEG in order to identify if there were any group differences in the morphology of the mu rhythm associated to AQ scores on an endogenous level that could be affecting its reactivity. Microstates analysis of the mu rhythm were analysed using the python toolbox YASA

(https://raphaelvallat.com/yasa/build/html/index.html) to detect mu rhythm as an EEG microstructure event as well as obtaining absolute power for the central (C1, C2, C3, C4, C5, C6) and central parietal (CP2, CP3, CP4, CP5, CP6) channels. Parameters for detection of mu were as follow: frequency from 8-13Hz, duration of 0.5 second minimum and 10 seconds as a maximum. Duration, amplitude, peak frequency, oscillation and symmetry values for each of the mu rhythm events were obtained and averaged per participant. Absolute power (μV2) of the rEEG was retrieved for Alpha (8-13hz), Alpha Low (8-10Hz), Alpha High (10-13Hz).

All variables were checked for normality to determine what type of independent samples statistic would be used. Normally distributed variables were analysed using t-test and non-normally distributed variables were analysed using the Mann-Whitney test.

Variables with significant group differences were:

Duration

At electrode C5 there was a significant difference between AQ groups, U=45.5, p = .002. Mu events detected in the high AQ group had a higher mean rank duration in seconds than the low AQ group (Md=1.29 vs Md=1.03).

Absolute power

At electrodes CP4 there was a significant difference between AQ groups, U= 110, p= .041 and CP6 U= 103, p= 103, p= .039. Mu events detected in the low AQ group had a higher mean rank (the mean of all values ranked low to high) in absolute power (mV^2) than the high AQ group (Md= 1.875 vs Md=1.695 and Md=1.865 vs Md=1.620for CP4 and CP6 respectively).

Oscillatory Activity

The number of oscillations measures the balance between time and frequency resolution (Vallat, 2018). This is based on the number of peaks and troughs of the mu rhythm event identified (Purcell et al., 2017). The increase in the number of oscillations refers to the specificity of the wavelet to the central frequency determined (in this case 8-13Hz). Inversely, a lower number of oscillations indicates that the temporal resolution of the wavelet is enhanced.

For oscillatory activity, overall the high AQ group had a higher mean rank number of oscillations compared to the low AQ group. This effect was observed at the following electrodes:

C3 U=88.5, p=.021 (Md=12.4 vs 11.63)

C4 U=88.5, p=.034 (Md=13.1 vs 11.7)

C5 U= 43.5, p= .002 (Md=13.81vs 10.54)

CP4 U= 105, p= .028 (Md=13.24 vs 12.32)

Symmetry

In this context, symmetry is a measure based on an index that compares the longitudinal montage of electrodes between hemispheres in order to identify spectral features of time. Values range from zero to one, where figures closer to cero indicate higher levels of symmetry (Luccas et al., 2016).

At electrode CP2 there was a significant difference between AQ groups, U= 82, p= .007. Mu events detected in the high AQ group had a higher mean rank symmetry than the low AQ group (Md=.525 N=20 vs Md=.450 N=17).

Non-linear analysis.

Non-linear measures of the EEG are a measure of how complex the signal is, they are based on chaos theory and provide information regarding brain dynamics associated with different functional states. On a general level, they provide a measure of the amount of information that is in the signal, hence, the more information (components) in the signal, the more irregular and uncertain (complex) it will be (Ahmadlou et al., 2010; Kesić & Spasić, 2016; Ma et al., 2018). Contrary to linear analyses, such as FFTs, nonlinear analyses take into consideration that the dynamic nature of the EEG, and how it changes in amplitude or shape over time (resulting in a non-linear physiological signal).This allows us to extract information about the underlying neural mechanisms of the phenomenon of interest (Ma et al., 2018).

Non-linear variables of the rEEG were analysed using the python toolbox YASA (https://raphaelvallat.com/yasa/build/html/index.html). Measures of entropy and fractal dimension were calculated across all 64 channels. For the fractal dimensions Petrosian and Higuchi,

algorithms were calculated. For entropy, permutation entropy measures were taken (Gao et al., 2011; Klonowski et al., 2000; Schwilden, 2006).

All variables were checked for normality using the Shapiro-Wilk test to determine what type of independent samples statistic would be used. Normally distributed variables were analysed using t-test and non-normally distributed variables were analysed using the Mann-Whitney test.

Entropy

Entropy is a measure of complexity in the EEG signal, the higher this value is, indicates a measure of increased complexity in the signal (Schwilden, 2006). Permutation entropy describes the comparative occurrence of the sequential patterns that make up the EEG waveform. When it is high, it is indicating that the signal is made up of mainly higher irregular frequencies and when it is low then it is the low more regular frequencies that predominate (Olofsen et al., 2008).

Significant findings concentrated over the left frontal, frontotemporal and parietal occipital areas as well as right frontocentral and parieto-occipital areas. For the purpose of clarity, all significant findings are displayed in table 2.1. The high AQ group had a higher permutation entropy than the low AQ group in all the electrodes with significant differences.

Table 2.1. Summary of results for Permutation entropy.		
Electrode	**Normally distributed**	**Significant group difference**
AF4	Yes	[t(33)=2.086, p= .045]
CP5	Yes	[t(35)=2.221, p=.033]
F7	Yes	[t(33)=2.498, p=.018]
FC6	Yes	[t(31)=2.179, p=.037]
FT7	Yes	[t(32)=2.506, p=.017]
FT8	Yes	[t(35)=2.307, p=.027]
02	Yes	[t(28)=2.740, p=.011]
P7	Yes	[t(31)=2.482, p=.019]
PO4	Yes	[t(35)=2.187, p=.035]
PO5	Yes	[t(36)=2.088, p=.044]
PO6	Yes	[t(36)=2.114, p=.042]
PO7	Yes	[t(36)=2.081, p=.045]
T7	Yes	[t(23)=2.223, p=.036]
TP7	Yes	[t(27)=2.886, p=.008]

Fractal dimensions

Fractal dimensions of the EEG are also a measure of the complexity of the signal. A fractal refers to variations in time or figures in space that display similar patterns at progressively small scales (self-similarity) and cannot be defined by an integer value (Goh et al., 2009). The EEG is a fractal-like signal that also measures the complexity of the underlying mechanisms that make up the signal

(Cusenza, 2012). The fractal dimension of the EEG reveals the level of self-similarity and complexity of the signal (Ahmadlou et al., 2010). This type of analysis is useful for depicting electrical brain dynamics in pathological and physiological conditions because it is very sensitive in identifying hidden information embedded in physiological signals (Acharya U. et al., 2005; Gao et al., 2011; Kesić & Spasić, 2016).

Petrosian

The Petrosian algorithm is a fractal dimension algorithm of the EEG that compares the total number of units that make up a curve with the minimum number of units needed to reproduce the pattern of the curve. The Petrosian algorithm decodes the time series into a binary classification of one or zero. The value given depends on whether the difference between the consecutive waveforms exceeds or not a standard deviation of the waveform.

Significant findings concentrated over the left frontal, frontotemporal and parietal occipital areas as well as right frontocentral and parieto-occipital areas. For the purpose of clarity, all significant findings are displayed in Table 2.2. The high AQ group had a higher Petrosian fractal dimension values than the low AQ in all findings.

Table 2.2. Summary of results for Petrosian fractal dimension.		
Electrode	**Normally distributed**	**Significant group difference**
AF4	Yes	[t(33)=2.107, p=.043]
CP5	Yes	[t(35)=2.210, p=.034]
F7	Yes	[t(33)=2.516, p=.017]
FC6	Yes	[t(31)=2.197, p=.036]
FT7	Yes	[t(32)=2.521, p=.017]
FT8	Yes	[t(35)=2.293, p=.028]
O2	Yes	[t(28)=2.740, p=.011]
P7	Yes	[t(31)=2.467, p=.019]
PO4	Yes	[t(35)=2.211, p=.034]
PO5	Yes	[t(36)=2.096, p=.043]
PO6	Yes	[t(36)=2.121, p=.041]
PO7	Yes	[t(36)=2.87, p=.044]
T7	Yes	[t(23)=2.240, p=.035]
TP7	Yes	[t(27)=2.882, p=.008]

Higuchi

This algorithm measures the complexity of a signal in the time domain.

All variables that were not normally distributed were analysed using a Mann Whitney test and correlated using Spearman's Rho. Significant findings concentrated over the left frontal, frontotemporal and parietal occipital areas as well as right frontocentral and parieto-occipital areas.

The high AQ group had higher Higuchi fractal dimension values over the low AQ group. Again, all significant findings are displayed in table 2.3

Table 2.3. Summary of results for Higuchi fractal dimension.

Electrode	**Normally distributed**	**Significant group difference**
P7	No	U= 68, p=.014 Ranks: High: 21.25 Low: 13.00
PO4	No	U= 104, p=.042 Ranks: High: 22.53 Low: 15.28
PO5	No	U= 109, p=.038 Ranks: High: 23.05 Low: 15.56
PO6	No	U= 108, p=.035 Ranks: High: 23.10 Low: 15.50
PO7	No	U= 106, p=.031 Ranks: High: 23.20 Low:15.39
POz	No	U= 105, p=.045 Ranks: High: 22.47 Low:15.33
TP7	No	U= 50, p=.016 Ranks: High: 18.93 Low:11.33
AF4	Yes	[t(33)=2.123, p=.041]
CP5	Yes	[t(35)=2.171, p=.037]
F7	Yes	[t(33)=2.573, p=.015]
F8	Yes	[t(34)=2.042, p=.049]
FC6	Yes	[t(31)=2.321, p=.027]
FT7	Yes	[t(32)=2.533, p=.016]
FT8	Yes	[t(35)=2.623, p=.013]
O2	Yes	[t(28)=2.830, p=.009]
T7	Yes	[t(23)=2.222, p=.036]

A visual summary of the nonlinear findings according to the entropy /fractal measure used can be found in fig 2.3.

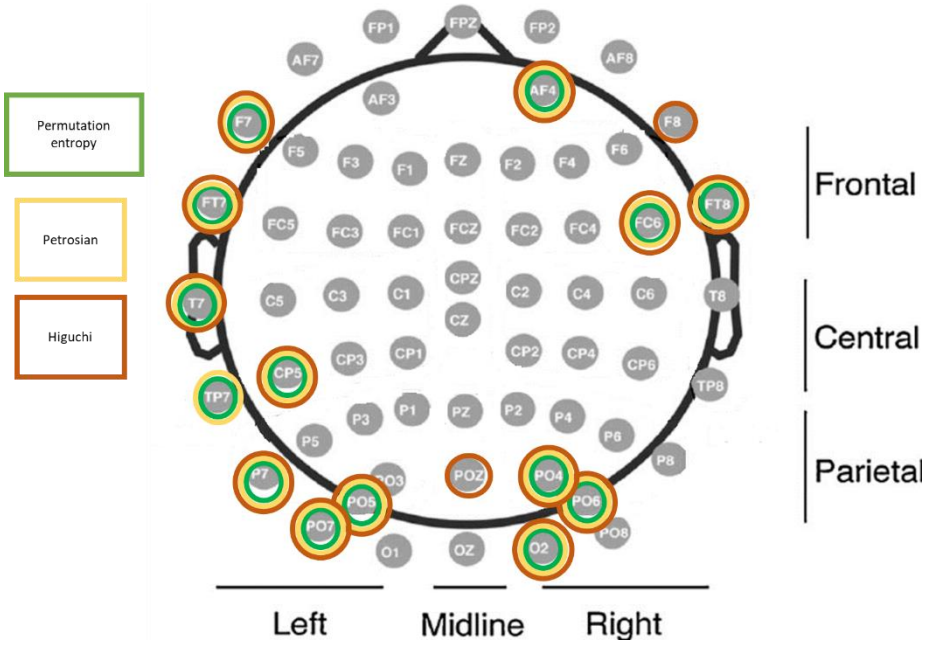

Fig 2.3. Non-linear analysis findings, colour and name of the algorithm used are located on the left. All the algorithms displayed significant group differences. Adapted from Kielar and Joanisse (2011).

The final step in the analysis was to run a correlational analysis between significant findings in the microstructure of mu and complexity measures. There were significant positive correlations between oscillation in mu for electrodes C5 and CP4 in both entropy and fractal dimension values (see Table 2.4 for a detailed report of correlation values). This means that as the number in oscillations in mu increases so do the values of the complexity measures. There were also significant negative correlations for absolute power in electrodes CP4 and CP6 with both entropy and fractal dimension values (see Table 2.5, 2.6 and 2.7 for a detailed report of correlation values). This means that as absolute power in these electrodes increases, complexity measures decrease. A visual summary of the correlation between these findings can be found in Fig. 2.4a & b.

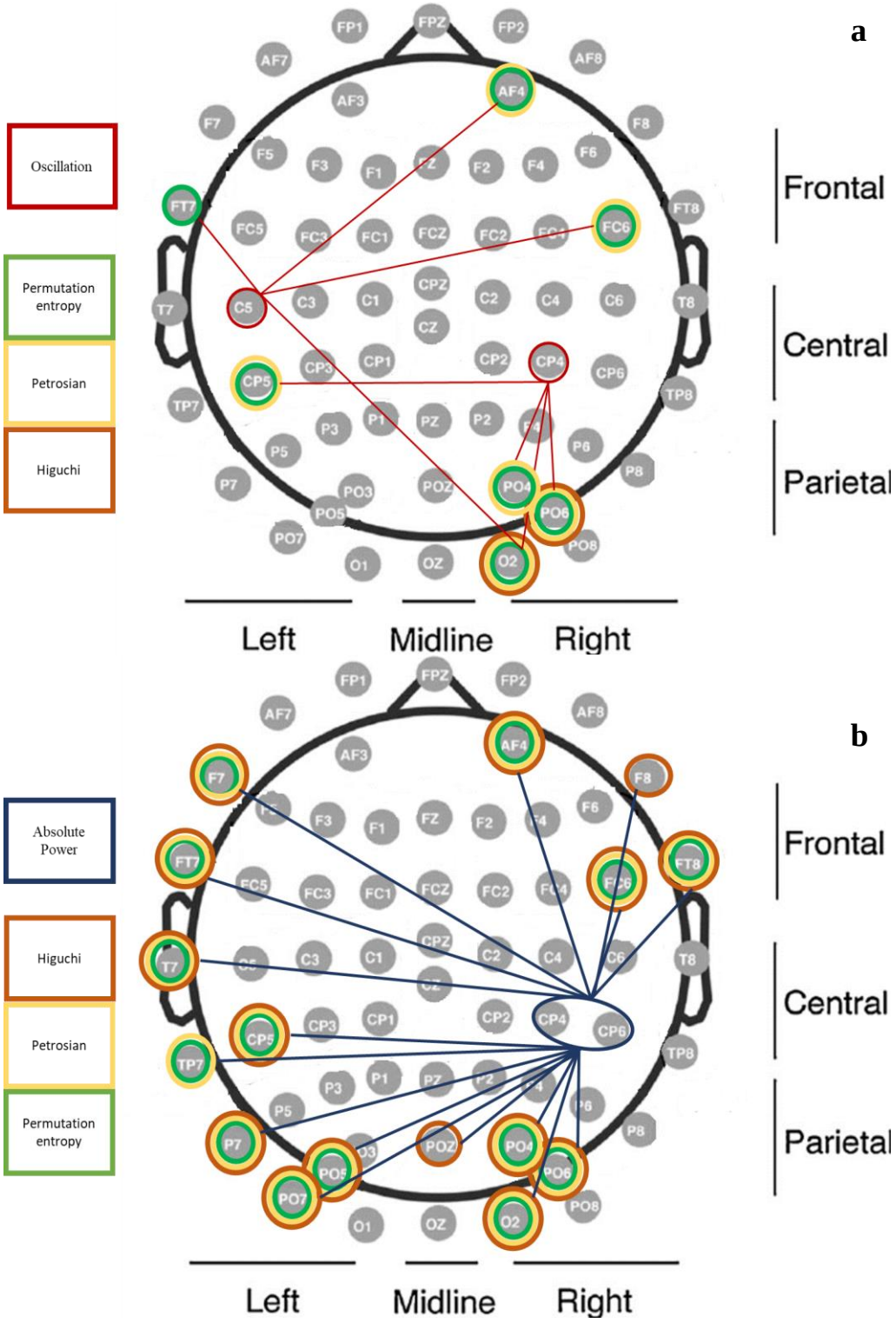

Fig.2.4. Significant correlations between microstructure of mu variables and complexity measures. **a)** Significant positive correlations between oscillations in Mu rhythm and measures of complexity.
b) Significant negative correlations between absolute power in Mu rhythm and measures of complexity.

Table 2.4. Summary of correlation results between oscillation and complexity measures.

Oscillation electrode	Complexity measure	Complexity Electrode	Correlation
C5	Permutation Entropy	AF4	r = .388, p=.037 N= 29
		FC6	r = .404, p=.033 N=28
		FT7	r =.378, p=.047 N=28
		O2	r =.386, p=.047 N=27
	Petrosian	AF4	r =.390, p=.036 N=29
		FC6	r =.406, p=.032 N=28
		O2	r =.389, p=.045 N=27
CP4	Permutation Entropy	CP5	r =.372, p=.023 N=37
		O2	r =.448, p=.013 N=30
		PO4	r =.381, p=.020 N=37
		PO6	r =.397, p=.014 N=38
	Petrosian	CP5	r =.372, p=.023 N=37
		O2	r =.452, p=.012 N=30
		PO4	r =.380, p=.020 N=37
		PO6	r =.397, p=.014 N=37
	Higuchi	PO6	r = .322 p=.049 N=38
		O2	r = .418, p=.022 N=30

Table 2.5. Summary of correlation results between significant absolute power and permutation entropy

Permutation Entropy	**Absolute Power electrode**	**Correlation**
AF4	CP4	r =-.699, p <.000 N=35
	CP6	r =-.705, p <.000 N=34
CP5	CP4	r =-.743, p <.000 N=37
	CP6	r =-.770, p <.000 N=36
F7	CP4	r =-.680, p <.000 N=35
	CP6	r =-.663, p <.000 N=35
FC6	CP4	r =-.677, p <.000 N=33
	CP6	r =-.644, p <.000 N=33
FT7	CP4	r =-.685, p <.000 N=34
	CP6	r =-.658, p <.000 N=33
FT8	CP4	r =-.645, p <.000 N=37
	CP6	r =-.616, p <.000 N=36
O2	CP4	r =-.571, p =.001 N=30
	CP6	r =-.560, p =.002 N=29
P7	CP4	r =-.692, p <.000 N=33
	CP6	r =-.705, p <.000 N=32
PO4	CP4	r =-.676, p <.000 N=37
	CP6	r =-.668, p <.000 N=36
PO5	CP4	r =-.620, p <.000 N=38
	CP6	r =-.600, p <.000 N=37
PO6	CP4	r =-.724, p <.000 N=38

	CP6	r =-.713, p <.000 N=37
PO7	CP4	r =-.603, p <.000 N=38
	CP6	r =-.574, p <.000 N=37
T7	CP4	r =-.684, p <.000 N=25
	CP6	r =-.698, p <.000 N=24
TP7	CP4	r =-.714, p <.000 N=29
	CP6	r =-.695, p <.000 N=28

Table 2.6. Summary of correlation results between significant absolute power and Petrosian fractal dimension

Petrosian	**Absolute Power electrode**	**Correlation**
AF4	CP4	r =-.707, p <.000 N=35
	CP6	r =-.714, p <.000 N=34
CP5	CP4	r =-.749, p <.000 N=37
	CP6	r =-.776, p <.000 N=36
F7	CP4	r =-.684, p <.000 N=35
	CP6	r =-.667, p <.000 N=34
FC6	CP4	r =-.688, p <.000 N=33
	CP6	r =-.655, p <.000 N=33
FT7	CP4	r =-.689, p <.000 N=34
	CP6	r =-.663, p <.000 N=33
FT8	CP4	r =-.649, p <.000 N=37
	CP6	r =-.619, p <.000 N=36
O2	CP4	r =-.578, p =.001 N=30

	CP6	r =-.566, p=.001 N=29
P7	CP4	r =-.698, p <.000 N=33
	CP6	r =-.712, p <.000 N=32
PO4	CP4	r =-.687, p <.000 N=37
	CP6	r =-.679, p <.000 N=39
PO5	CP4	r =-.630, p <.000 N=38
	CP6	r =-.612, p <.000 N=37
PO6	CP4	r =-.738, p <.000 N=38
	CP6	r =-.727, p <.000 N=37
PO7	CP4	r =-.613, p <.000 N=38
	CP6	r =-.586, p <.000 N=37
T7	CP4	r =-.691, p <.000 N=25
	CP6	r =-.706, p <.000 N=24
TP7	CP4	r =-.720, p <.000 N=29
	CP6	r =-.701, p <.000 N=28

Table 2.7. Summary of correlation results between significant absolute power and Higuchi fractal dimension.

Higuchi	**Absolute Power electrode**	**Correlation**
P7	CP4	r =-.713, p <.000 N=33
	CP6	r =-.735, p <.000 N=32
PO4	CP4	r =-.749, p <.000 N=37
	CP6	r =-742, p <.000 N=36
PO5	CP4	r =-.664, p <.000 N=38
	CP6	r =-.653, p <.000 N=37
PO6	CP4	r =-.800, p <.000 N=38
	CP6	r =-.788, p <.000 N=37
PO7	CP4	r =-.651, p <.000 N=38
	CP6	r =-.632, p <.000 N=37
POz	CP4	r =-.812, p <.000 N=37
	CP6	r =-.813, p <.000 N=36
TP7	CP4	r =-.728, p <.000 N=29
	CP6	r =-.710, p <.000 N=28
AF4	CP4	r =-.737, p <.000 N=35
	CP6	r =-.754, p <.000 N=34
CP5	CP4	r =-.754, p <.000 N=37
	CP6	r =-.778, p <.000 N=36
F7	CP4	r =-.658, p <.000 N=35
	CP6	r =-.637, p <.000 N=34
F8	CP4	r =-.669, p <.000 N=36

	CP6	r =-.626, p <.000 N=35
FC6	CP4	r =-.735, p <.000 N=33
	CP6	r =-.703, p <.000 N=33
FT7	CP4	r =-.661, p <.000 N=34
	CP6	r =-.632, p <.000 N=33
FT8	CP4	r =-.666, p <.000 N=37
	CP6	r =-.641, p <.000 N=36
O2	CP4	r =-.599, p <.000 N=30
	CP6	r =-.577, p =.001 N=29
T7	CP4	r =-.716, p <.000 N=25
	CP6	r =-.736, p <.000 N=24

2.2.6. Discussion

The object of this study was to explore oscillatory and nonlinear features of the resting state EEG in participants with high and low AQ scores in order to identify group differences that were specifically associated to their degree of autistic traits. Significant group differences were identified in both oscillatory and non-linear measures.

For absolute power, the differences associated with AQ traits were identified within the broadband alpha (8-12Hz), and low alpha (8-10Hz), beta (12-30 Hz), and theta (4-8 Hz). For broadband alpha and high alpha differences were located in the right frontal, left frontocentral and right occipital areas. In right frontal areas, this corresponds to Brodmann area 08 which encases the premotor cortex and the pre supplementary motor area (Strotzer, 2009). This is also the case for findings in the left frontocentral areas, which corresponds to the supplementary motor area as well as Broca's language area and the inferior frontal gyrus (Heiser et al., 2003; Nishitani & Hari, 2000; L. M. Parsons & Osherson, 2001; Strotzer, 2009). Findings for low alpha were located over left centroparietal and parietal areas which overlap with the angular and supramarginal gyrus, which have been considered as part of Wernicke's area (Strotzer, 2009). For broadband beta, group differences were located over left central areas, this matches the primary somatosensory cortex (Thies et al., 2018). The findings in theta are also concentrated over left frontocentral and centroparietal areas as well as central areas on both hemispheres. The negative association of absolute power in these bands to AQ traits is in line with multiple studies that have reported abnormalities in these areas in individuals with ASD (Bastiaansen et al., 2011; Brambilla et al., 2004; Haznedar et al., 2000; Heiser et al., 2003; Iacoboni & Dapretto, 2006; Nickl-Jockschat et al., 2014; Perkins et al., 2015; Puzzo et al., 2010; Wang et al., 2018). It is noteworthy that regions such as the supplementary motor area and inferior frontal gyrus have also been described as part

of the human mirror neuron system, and the dysfunction of this system has also been associated to difficulties in social and action understanding in ASD (Denis et al., 2016; Falck-Ytter, 2010; Fründt et al., 2018; A. F. de C. Hamilton, 2013; Riitta Hari, 2006; Nishitani & Hari, 2000; Jaime A. Pineda, 2005; Puzzo et al., 2010). Equally important, the alpha and beta bands over central areas point to the mu rhythm, which as was described in chapter 1, has been widely linked to both the human mirror neuron system and ASD.

These findings are further supported by the significant group differences in the somatomotor functional network for low alpha and the frontoparietal functional network for broadband theta. Both of these networks showed significantly less absolute power for the high AQ group compared to the low AQ group and overlap with the areas previously described that have been associated with abnormalities in ASD. Taken together with the findings in absolute power, we can deduce that there was a general trend of higher absolute power in lower AQ traits throughout all linear measures, group differences were only observed both on specific brain regions and at a functional network level.

Although our sample is drawn from neurotypical individuals, the findings support the concept of autistic traits as a spectrum and thus would suggest that as AQ traits increase into clinical diagnosis of autism the trend of under activation in these areas may follow into levels of clinical abnormality. In terms of nonlinear measures, the trend of significant differences associated to AQ scores moved in the opposite direction. Permutation entropy and fractal dimension measures correlated positively with the degree of autistic traits that participants had. These measures both indicate a level of randomness and irregularity in the EEG signal as their values increase (Olofsen et al., 2008; Schwilden, 2006). In our findings, the high AQ group had higher levels on both permutation entropy and fractal dimension measures. The higher the level of AQ traits, the faster and more

predominant higher frequencies were found in the signal of frontal, frontotemporal, and parieto-occipital areas on the left hemisphere and the right frontocentral and parieto-occipital areas. Regions of increased complexity in high AQ traits included electrode sites thought to lay over the fusiform face area (Brodmann area 21,21), the opercular area (Brodmann area 44) which is involved in motor functions, orbitofrontal (Brodmann area 47) and part of Wernicke's area (Brodmann area 42) and the supramarginal gyrus (Brodmann area 40). These regions have also been associated with the symptomatology of ASD (Bird et al., 2006; Brambilla et al., 2004; Dalton et al., 2005; Hoffmann et al., 2016; Iacoboni et al., 1999; Mundy, 2018; Nickl-Jockschat et al., 2014; Pelphrey et al., 2007; Pereira et al., 2019; Salmi et al., 2013; Schultz, 2005). Based on the findings it could be suggested that these areas display a higher level of randomness, irregularity and higher frequencies as AQ traits increase and could at some point influence proper functioning of the underlying neural networks. However, as this study did not include specific source localisation, it would be necessary to conduct further studies utilising this approach to confirm these findings.

It is also worth noting that the findings in complexity measures are different from other studies using rEEG, where entropy values in ASD are lower than neurotypical individuals. (Bosl et al., 2011; Kang et al., 2019). We attribute this to 1) the age of our sample and 2) the degree of AQ that our sample had, which did not fall into a clinical diagnosis classification. Previous studies have been done on children that had a clinical diagnosis of ASD. Further studies would also need to be done in clinically diagnosed adults with ASD in order to assess if the differences in complexity described in childhood remain lower than neurotypical individuals or if there are changes in EEG complexity during adolescence.

As for the microstructure of mu, there were interesting groups differences over central areas (C3, C4, C5) and right centroparietal areas (CP4 and CP6). Our results found that overall in individuals with high AQ traits, the mu rhythm has more oscillations and lower absolute power. This was the case in the right centroparietal area, but it was in C5 where in addition to lower absolute power and more oscillations the mu rhythm events also had a longer duration. These findings support widespread research on the mu rhythm and ASD where mu rhythm reactivity has been described as abnormal (Bernier et al., 2007; García Vite et al., 2018; Lepage & Théoret, 2006; Oberman et al., 2005; Ruysschaert et al., 2014). Additionally, the significant positive correlation of oscillation for electrodes C5 and CP4 and negative correlation of absolute power for electrodes CP4 and CP6 with measures of both entropy and fractal dimension values complexity also supports the findings of higher levels of randomness and irregularity with high autistic traits. In other words, our results suggest that a higher degree of AQ traits, would reflect a mu rhythm with more oscillations, lower absolute power, and a higher degree of complexity in left central areas and right centroparietal areas. More studies that replicate the analysis of mu microstructure and complexity measures in clinically diagnosed studies are needed but these results suggest this characterization of the mu rhythm is specifically associated to AQ traits and could be used as a possible, future biomarker for these traits.

Limitations and future directions

One of the main limitations to this study is that it was not focused on the age group that typically, studies of rEEG complexity have focused upon. Therefore, the differences in our findings for complexity measures to other studies may be due to factors other than those we sought to explore. For example, Bosl et al., (2011) found that complexity measures from the rEEG in infants could be used as a biomarker for those at risk. Given the results of this study, it would be interesting to

replicate it in samples that include different age groups as well as clinical populations of ASD in order to identify if the differences in the rEEG according to AQ traits change/evolve across the life span or if it depends on the severity of ASD. Another aspect worth mentioning is that as this study was exploratory, the different analyses were done on a sole database retrieved from participants rEEG and there were multiple hypotheses of interest, as a result of this, the occurrence of multiple comparisons in the linear analysis of band power could have influenced our findings. When performing multiple comparisons, it is possible that the likelihood of encountering significant findings will increase, and erroneous inferences could arise (Dunn, 1961). Although the findings from the linear analysis were consistent with the nonlinear analysis, it is important to acknowledge that multiple comparisons were performed and could have had an effect on the results.

Additionally, it is worth keeping in mind that he EEG as a neuroimaging technique, although high in temporal resolution is not as consistent in terms of spatial resolution. Therefore, the findings regarding brain regions should be interpreted with caution, as a formal source localization analysis was not conducted. Future studies could replicate this study using neuroimaging techniques with higher spatial in order to confirm differences in the brain areas identified in this study.

2.2.7. Conclusion

In this study, we aimed to explore linear oscillatory and nonlinear features of the rEEG in participants with high and low AQ traits in order to identify group differences that were specifically associated to their degree of AQ traits. The negative and positive associations found in oscillatory and nonlinear measures (respectively) found in individuals with high AQ traits

suggests that there is less activation of frontal and fronto-central regions combined with higher levels of complexity and irregular activity in fronto-temporal, temporal, parietal and parieto-occipital areas. The overlap with brain regions that have been associated to ASD abnormalities suggests that the individual differences in the spectrum of autistic traits go beyond personality traits and are also represented on a physiological level in neurotypical individuals.

3. EEG protocols on hMNS activation (mu suppression) and its difference according to self-reported traits of autism in neurotypical individuals.

3.1. Aims and overview

As discussed in earlier chapters, people who are diagnosed with Autism Spectrum disorder (ASD) experience persistent difficulties with social interaction and communication in an array of contexts (American Psychiatric Association, 2013a). The severity of symptoms varies and people with this disorder can require different levels of support to fulfill daily activities and interactions. Autistic traits have been identified in family members of individuals (broader autistic phenotype) and also distributed normally among the general adult population (Baron-Cohen et al., 2001; Constantino & Todd, 2003; Wakabayashi, Baron-Cohen, & Wheelwright, 2006)

Among the neural systems explored in the study of ASD is the mirror neuron system (Schultz, Romanski, et al., 2000). Mirror neurons fire both when a specific action is observed and executed and have been linked to action processing, motor cognition, goal understanding, speech perception and language development, as well as the development of key social skills (Hickok, 2013; Jeannerod, 2001; Neuper et al., 2009; Ruysschaert et al., 2014; Sinigaglia, 2013). Because mirror neurons were originally described in macaque monkeys though the use of subdural electrodes (Rizzolatti & Craighero, 2004), more indirect methods had to be employed in order to study this system in humans. The EEG has been widely used in the study of the human mirror neuron system (hMNS) because of its high temporal resolution and non-invasive method (Bernier et al., 2014). The EEG records electrical oscillations of pyramidal cells that reflect the activity of the cerebral

cortex (Bosl et al., 2011). One such oscillation, that has been associated to the hMNS, is the so-called mu rhythm, a cortical oscillation generally measured at 8–13hz and 15–25 Hz EEG over sensorimotor areas, at rest with eyes open (Hari & Salmelin, 1997). Because of the topographic and physiologic reactivity resemblance between mu rhythms and mirror neurons, this rhythm has been considered as an EEG marker for mirror neuron activity (Hari, 2006; Pineda & Hecht, 2009; Virji-Babul et al., 2008; Yin, Liu, & Ding, 2016). In individuals with ASD, abnormal mu modulation has been identified and associated with abnormalities in the motor system as well as social function difficulties (Hauswald et al., 2013). For example, when observing a grasping action executed by strangers, individuals with ASD exhibit reduced desynchronization of mu compared to control participants (Oberman et al., 2008). Further examples involving ASD and the hMNS can be found in section 2.2.2.3 of this thesis.

Another technique that has been used in the study of ASD and the hMNs is eye-tracking. Eye movements provide an objective measure of where a participant's interest and attention is when observing a visual display (Duchowski, 2017; Hamilton, 2013; Poole & Ball, 2011). Most eye-trackers work by directing infrared light from an LED embedded in the infrared camera of the eye-tracker into the eye to generate reflection in the pupil and cornea. The eye-tracker software measures these two reflections as a vector to identify a reference of where the eye is located and hence, where the person is looking on the screen (Poole & Ball, 2011). In terms of the mirror neuron system, Rosander and von Hofsten (2011) described a predictive eye movement in neurotypical infants and adults when performing and observing goal-directed actions. Both adults and infants had to either move a ball between 2 locations on the visual field or observe the same movement performed by an experimenter. Although adults and infants executed the movements with great resemblance, adults had a better performance during observation. The authors also observed that when executing the movements, the speed of the participant's saccades was faster

than the peak velocity of the hand. This is particularly relevant in the development of prediction of other people´s actions as the ability to interpret actions (in oneself or others) and how it relates to the goal is considered as the basis for constructing a repertoire of actions that are used by the MNS. In this study the authors also concluded that in order to predict other´s actions the development of one´s own motor actions must first be learned.

Under the broken mirror theory of autism, one would expect a significant difference in predictive eye movements during goal directed action observation compared to neurotypical individuals. In ASD, this predictive eye movement is not manifested differently except when there is a social cue involved in the gaze (Falck-Ytter, 2010; Falck-Ytter et al., 2012). Wild, Poliakoff, Jerrison, and Gowen (2012) found that when observing and imitating hand directed movements, participants with ASD spend more time on the end goal rather than tracking the observed movement during the action leading to differences in imitation when compared to controls.

Other studies involving eye -tracking and ASD report differences in fixation patterns when observing social stimuli, in particular when focusing on the eye or face region (Boraston & Blakemore, 2007; Dalton et al., 2005; Papagiannopoulou et al., 2014). The importance of fixations when studying eye movements through eye-tracking is its implication in the processing of visual information; the premise being that when the gaze is focused on a specific point, the time spent there allows for processing visual information from that point (Boraston & Blakemore, 2007). Differences in fixations and gaze patterns have also been associated to brain activation. Using eye-tracking with fMRI, Dalton et al. (2005) found an under-activation of the fusiform gyrus that correlated with a decreased number of fixations in individuals with ASD. Furthermore, recent studies involving eye-tracking in the study of ASD have looked into the development and validation of measures based on eye-tracking to estimate ASD risk and severity (Frazier et al.,

2018). Although it is not the focus of this thesis, it is worth mentioning that a recent study found that an oxytocin intervention increased the number of fixations to eyes in a real time, naturalistic social interaction (Auyeung et al., 2015). Evidence has shown that oxytocin and arginine vasopressin play a pivotal role in regulating social and affiliative behaviours (Kosfeld et al., 2005; Shamay-Tsoory & Abu-Akel, 2016). In both humans and animal models that study ASD, abnormalities in the oxytocin receptor gene (OXTR) have been previously described (Liu et al., 2010; Loparo & Waldman, 2015b). Specifically, plasma oxytocin levels in individuals with autism display abnormalities in their expression in comparison with neurotypical individuals (Green et al., 2001; Modahl et al., 1998; Zhang et al., 2016).

A parallel aspect that this experiment aims to address is the attention bias debate that has been associated with mirror neurons, mu rhythm and autism. A recent study by Hobson & Bishop, (2016) questions the validity of mu suppression as a measure of the hMNs by suggesting that the overlap of frequency between alpha and mu could cause confounds in alpha activity and attentional engagement, due to the sensitivity of alpha to attention. They conclude that the effect of mu is weak and unreliable and strict controls for attention should be included in studies involving the mu rhythm. They further highlight that the role of beta must be explored as one of the spectral peaks that comprise mu rhythm. Fox et al. (2016) on the other hand, conclude the opposite. In their meta-analysis, they review 85 studies of mu that infer human mirror neuron system activity and although they suggest experimental and methodological improvements in the approaches to study mu, they agree that the changes in the EEG are a valid measure of human neural mirroring. In both studies however, attention was controlled through behavioural tasks that could in themselves, be creating a confound in attention on a cortical level. In terms of ASD, within the spectrum of autism, there is a heterogeneity of sensitivity to types of cues as well as different capacities in attention that could be interacting with the clinical features of the disorder (Ames & Fletcher-Watson, 2010).

Different lines of research have explored alternative explanations to the differences in mu suppression over the sensorimotor cortex in individuals with ASD such as variations in the processing of early attention and biological motion as well as a decreased attention to social cues (see chapter 1). What has been theorized is that it is not the activation of mirror neurons that explains difference in mu suppression among neurotypical individuals and those with ASD but rather differences in the attention that each population pays to the stimuli themselves. As a tool for assessing attention, eye-tracking also provides a measurement of interface-evaluation through eye movements and fixations that are captured objectively during the presentation of stimuli (Poole & Ball, 2011). By simultaneously recording eye movement variables(such as dwell time, fixation time, saccade amplitude etc.) with the EEG during the presentation of a stimuli, we will also have information as to the attention that each individual and therefore each group of interests, pays to the stimuli. For example, if the fixations were nowhere near the stimuli, but rather at the edge of the screen, it would tell us that the EEG data from that participant would not be reliable as they were not paying attention to the stimuli, therefore, any brain activity elicited would have to be accounted for something other than the stimuli presented. This makes eye-tracking quite useful not only for evaluating the participant, but in this case, the EEG stimuli as well, by controlling for any attention bias.

For these reasons, to further understand the role that mirror neurons may have in goal-oriented movements and in neurotypical adults with high and low autistic traits, the aim of this experiment was to compare 3 different protocols that had been previously associated with the hMNS in terms of anatomy as well as mu-suppression (in sensorimotor alpha and low beta bands) and observe if individual levels in autistic traits within neurotypical individuals causes differences in EEG and eye-movement behaviour in order to further explore HMN reactivity in the spectrum of autistic traits in less studied populations (within the neurotypical adults) from different theoretical

perspectives as well as address the potential attentional bias that has sparked some debate when talking about mirror neurons and ASD.

Each protocol selected assessed a different aspect that has been associated with the mirror neuron system: 1) The mu multiple repetitions protocol (MMR) - a classic MN reactivity protocol using hand movements (Puzzo et al., 2011); 2) The proactive gaze protocol – based on Costantini, Ambrosini, Cardellicchio, & Sinigaglia's (2014) protocol where they found that proactive gaze behaviour had an anatomical association to motor areas that would be involved in the execution of the action observed and 3) The Social Response Protocol - observation of movement involving a goal-directed hand action, but this time, adding a social response component (Clausen et al., 2015). This third protocol takes into consideration another suggested function of the mirror system, (mentioned in chapter 1), which is to respond to the actions of others in a manner that is socially acceptable rather than only understand or predict actions (Hamilton, 2013).

As was briefly mentioned above, the goal of these experiments is three-fold. Firstly, it increases the literature on individual differences in autistic traits regarding reactivity of the HMNs. Secondly, it addresses a topic that has been the focus of some debate in the study of mirror neurons and ASD: the putative attentional bias in ASD population, which has been suggested as a determinant factor in the impairments described when comparing to a neurotypical population that inherently focuses on people and faces (Ames & Fletcher-Watson, 2010). These experiments address this topic by including EEG and the simultaneous measure of eye movements with the use of an eye-tracker that records where the participant is looking at the screen throughout the experiment, and therefore providing an objective measure of where the individuals are placing visual attention. Thirdly, in the context of the overall PhD project, the three protocols were selected

with the aim of obtaining the best psychophysiological protocol for the pre-post assessment in the following experiment: a neurofeedback study on neurotypical individuals with high autistic traits.

3.2. Methods

Participants, materials and scales were the same for all three protocols:

3.2.1. Participants

5000 participants were initially contacted via e-mail through the university mailing list for research participation, SONA and word of mouth to be screened for the study using the Autism-Spectrum Quotient (AQ) (Baron-Cohen et al., 2001). A total of 291 people answered and returned the AQ. High and low group scores were selected by taking one standard deviation above and below from the mean score among the sample (M=18, SD=6). This resulted in the high AQ group being comprised of those scoring ≥24 and the low AQ group scoring ≤13. These scores also correspond to the extreme of the normal distribution described by Baron-Cohen et al., (2001); that is to say, although the high group was one standard deviation higher than the normal population average, these scores are not considered at high risk for diagnosis of ASD. Participants had a mean age of 23.57 ± 3.67 for the high AQ group and 21.67 ± 3.6 for the low AQ group. In terms of gender, the high group had 11 female and 9 males, and the low group had 14 female and 6 male

Subsequently, 40 participants (20 high AQ and 20 low AQ) were invited take part in the EEG and eye-tracking study.

The University of Essex Ethics Committee approved this study. All participants read and signed and informed consent prior to their participation. The procedures used in this study were non-

invasive and in accord with the Declaration of Helsinki; at no time were the participants or the researcher at risk for their safety.

3.2.2. Materials

3.2.2.1. Scales

The Autism-Spectrum Quotient (AQ) is a self-administered short scale that measures the degree to which an adult of normal IQ exhibits traits associated with the autistic spectrum. The participant scores a point for each answer that resembles an autistic answer. Higher scores equal a higher level of autistic traits. It is comprised of 50 items that are divided into 5 different areas: social skills, attention switching, attention to detail, communication and imagination (Simon Baron-Cohen et al., 2001). The AQ has proven to be suitable for screening purposes in research and clinical settings due to the high internal consistency and reliability across different samples (Baron-Cohen et al., 2001; Hurst, Mitchell, Kimbrel, Kwapil, & Nelson-Gray, 2007; Wakabayashi, Baron-Cohen, & Wheelwright, 2006; Wakabayashi, Baron-Cohen, Wheelwright, et al., 2006).

3.3.Mu multiple repetitions protocol (MMR)

For a detailed view at participants, materials and scales see section 3.2.

3.3.1. Stimuli

The mu multiple repetitions protocol (MMR) used by Puzzo, Cooper, Cantarella and Russo (2011) in order to elicit the EEG indexed hMNS during movement observation. In this set there are 3 videos, each lasting 3000ms. A fixation cross was shown on a black background before each video for 1000ms. The video clips depict either a moving hand, a static hand or two balls moving at

approximately 1Hz vertically toward each other in the centre of the screen. In the moving hand condition, the fingertips and thumb of the hand touch in the centre of the screen, again moving at 1Hz. In the static hand condition, the fingers and thumb do not touch. In all three videos the objects viewed are light pink viewed against a black background. The videos were showed in random order, repeated 30 times each (90 trials in total). In order to avoid expectancy effects, between each trial a fixation cross appeared in the starting position on a black background for a randomized period of 1000-2000ms (Fig 3.1).

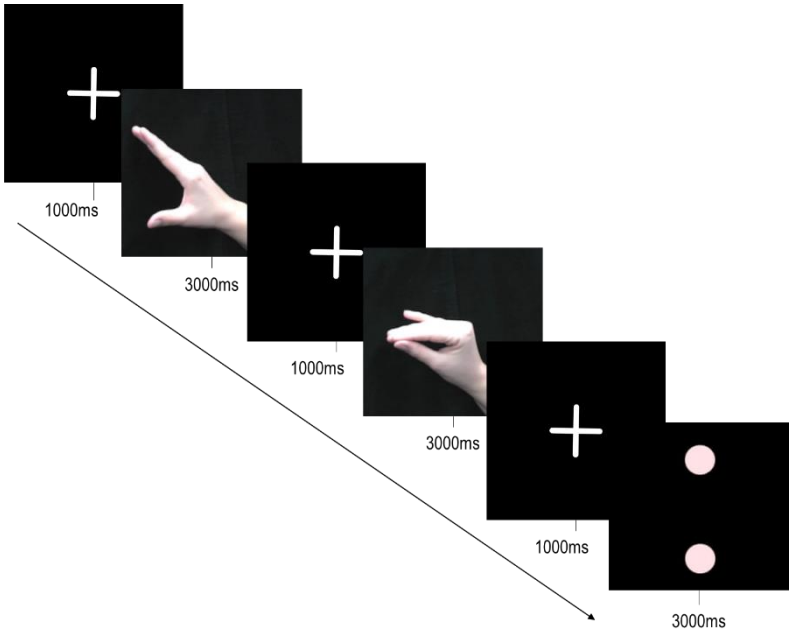

Fig 3.1. Experimental procedure for the mu multiple repetitions protocol (MMR)

In addition to the stimuli protocols, an electrooculogram (EOG) calibration task was used to form the basis for subsequent EOG artefact reduction for the EEG (Croft & Barry, 1998).

All stimuli were constructed on the SR Research Experiment Builder (Version 1.6.12) and presented on the Eyelink 1000 Eye-tracker (Mississauga, Ontario, Canada) an infrared video-based eye-tracking device. Stimuli were presented on a 1027 x 768 pixel screen 60cm away from the

participant, this equals to a visual angle of 25° 31' 0.06" (measure of the size of the object's image on the retina (Swearer, 2011)).

The triggers were sent to the EEG Neuroscan 4.4 acquisition software and Synamps II amplifiers through a parallel port while the protocols were displayed. Data for the EEG and eye-tracker were recorded simultaneously.

3.3.2. Experimental design

3.3.2.1. EEG

This was a mixed measures design. Independent variables (IV) were stimuli and hemisphere and dependent variables (DV) were sensorimotor alpha and beta event related desynchronization (ERD) values. Within-subjects variables were stimuli and hemisphere and between-subjects variable was group. The specific design and levels for each factor will be explained further in the results section. To control for attention, trial fixation count was recorded with the eye-tracker according to the interest areas as described in the following section.

3.3.2.2. Eye-tracking

This was also a mixed measures design. IV was the stimuli (within subjects variable) and AQ group was the between subjects variable. There was only one interest area across all stimuli which served as a control for attention between groups (see fig 3.2). DVs were fixation count, average fixation duration, pupil size and average saccade amplitude.

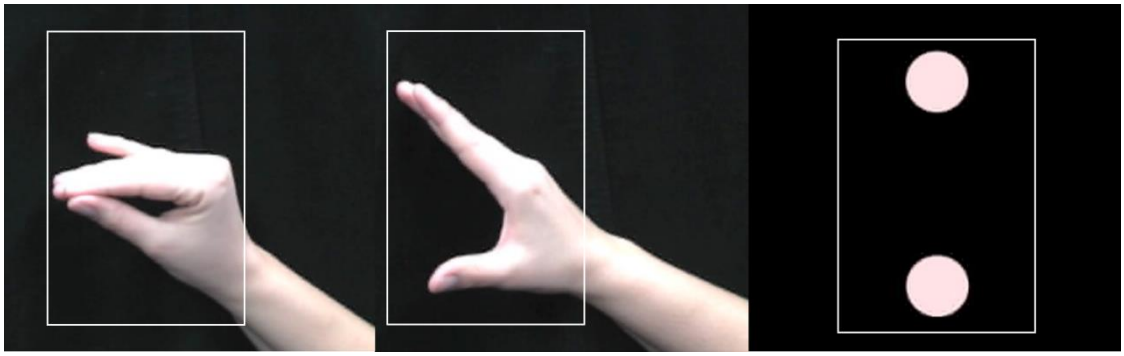

Fig 3.2. Interest area across all stimuli. From left to right:" moving", "still" and "balls".

3.3.3. Research Hypotheses

Research hypotheses for this protocol were focused on how mirror neuron activation during action observation is moderated by high and low autistic traits within a neurotypical population. For EEG variables, we predict that mu ERD will be significantly higher in the low AQ score group. Furthermore, we predict that EEG differences between groups will not be found in the eye-tracking variables fixation count. As for the remaining eye-tracking variables: pupil size, average fixation duration and average saccade amplitude, we predict differences between groups.

3.3.4. Procedure

At the beginning of the session the participant was asked to read and sign a consent form to take part of the experiment. After placement of the EEG cap they were then instructed to place their chin on the chin rest that was located at a fixed parallel position to the monitor of the EyeLink 1000 (Mississauga, Ontario, Canada).

Before the presentation of the stimuli, each participant's resting state EEG as well as a short calibration for the ocular artefact reduction was recorded (Croft & Barry, 1998). Another short calibration with the eye-tracker was performed before the start of the experiment.

Sampling rate for the eye-tracker was set at 1000Hz. All EEG data was simultaneously recorded with a Neuroscan 4.4 acquisition software and Synamps II amplifiers using a 64 channel Quick-Cap arranged according to the international 10–10 system (Compumedics, Melbourne, Australia). Eye movements were recorded additionally using four facial electrodes: 1 horizontal electrode for each eye (approximately 1cm on the outer cantus of each eye) and 2 vertical electrodes (above and below the left pupil). Impedances for all electrodes were reduced to below 10 kOhm before the start of each session. All EEG data were continuously sampled at 1000Hz with a bandpass filter of 0.15–200Hz and a 50Hz notch filter. Online, EEG data were referenced to an electrode between Cz & CPz, and grounded midway between Fz and FPz.

3.3.4.1. EEG data preparation

Following visual inspection of the data, noisy data blocks were rejected. Bad electrodes were excluded on a subject by subject basis. Principal components analysis was performed on the acquired eye movement data to obtain components reflecting saccades and blinks. To carry out ocular artefact rejection, the acquired components were subsequently rejected from the task data traces (Vigario, 1997; Vigario et al., 2000).

The data were then re-referenced to the average of the scalp electrodes, epoched from -2000 to 4000ms around the start of each video and then an automatic artefact rejection algorithm was applied (+/- 100mV). For the calculation of ERD/S the following steps were carried out: the data

underwent complex demodulation and concurrent filtering (zero phase-shift, 24dB roll-off, envelope computed) into the bandwidths alpha (7.812-12.695 Hz), low alpha (7.812-9.765Hz), high alpha (9.765-12.695Hz) and low beta (12-20 Hz): the data was trimmed (1000ms from each side) to remove filter artefacts and averaged. A reference interval of -1000 to 0ms was used to calculate the percentage change between the active period (0 to 3000ms) and the reference using the classic method adapted from Pfurtscheller and colleagues (e.g. 1977, 1999): ERD% = (R - A)/R*100, where R = the reference interval and A = the active or task phase. Thus, desynchronization and synchronisation are expressed as a percentage of activity relative to the reference interval (as a result, this formula ERD produces positive scores and ERS negative). ERD/S data contain both phase-locked and non-phase-locked activity.

3.3.5. Results

3.3.5.1. EEG

Trials were divided into three time frames for separate analyses (early, mid and late). Time frames were as follows:

- Early: 0-1000ms
- Mid: 1000-2000ms
- Late: 2000-3000ms

For the factorial design the electrodes were collapsed across hemispheres resulting in a 3 x 2 x 2 (stimulus x hemispheres x group) design with AQ score as a between subjects factor (high vs low score), and two within subjects factors: stimuli (Still, moving and balls) and hemisphere (left and right). Event related desynchronization for bands alpha (7.812-12.695 Hz), low alpha (7.812-9.765Hz), high alpha (9.765-12.695) and low beta (12.695-19.531Hz) as a measure of mu rhythm desynchronization were the dependent variables. This analysis was done for electrodes over Frontocentral (FC), Central (C) and Centroparietal (CP).

Early time period (0-1000ms)

Frontocentral electrodes

A significant interaction of "stimuli x hemispheres" was observed in high alpha [F (2, 76) = 4.491, p =.014] over FC electrodes. Post-hoc t-tests revealed a significant difference between FC Left and FC Right in the still condition [t (39) = 2.732, p =.009]. The right hemisphere had a significantly higher ERD (M= - 14.61, SD= 48.18) over the left hemisphere (M= - 2.51, SD=38.79).

There were no significant findings over central (maximum F= 2.849, p=.100) and centroparietal electrodes (maximum F= 1.749, p=.234).

Mid time period (1000-2000ms)

Central electrodes

A significant main effect of stimuli was found in alpha [F (2, 76) = 4.081, p =.021] and low alpha [F (2, 76) = 7.471, p=.001]. Post hoc t-tests revealed a significant difference between the move and balls stimuli [t (39) = - 2.779, p=.008] in the alpha band. The differences between these two stimuli were also found to be significant in low alpha [t (39) =-3.588, p=.001]. In both bands the move condition had a greater ERD than the balls condition; alpha (M=30.83, SD= 22.76 vs M=22.39, SD=22.01) low alpha (M =31.74, SD =18.89 vs M = 20.12, SD =18.89) (Fig. 3.3).

Main effect of hemisphere was found in high alpha [F (1, 38) = 5.355, p =.026] over central electrodes. The left hemisphere had a significantly higher ERD than the right (M=23.62, SD=25.15 vs M=15.27, SD=32.89).

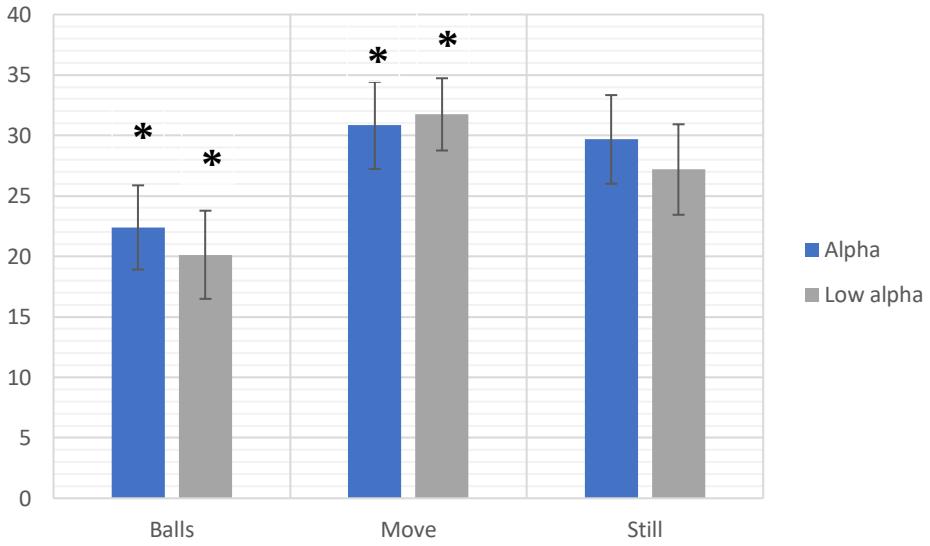

Fig. 3.3 Main effect of stimuli in central electrodes for alpha and low alpha in mid time period.

Centroparietal electrodes

A significant main effect of stimuli was found in low alpha [F (2, 76) = 4.531, p < .05]. Post hoc t-tests revealed a significant difference between the balls and move stimuli [t (39) = -2.666, p=.011] in low alpha. The move stimulus had a greater ERD (M=37.67, SD=20.33) than balls (M=27.56, SD= 24.14). In beta a significant main effect of stimuli was also found [F (1.55, 58.9) = 5.033, p < .05]. Post hoc t-tests revealed significant differences were found between balls and move [t (39) =-3.007, p=.005] and between still and balls [t (39) =2.694, p=.010]. The move stimulus had a higher ERD than balls (M=32.10, SD=12.51 vs M=24.14, SD=15.19) and still had a higher ERD than balls (M=29.11, SD=17.95 vs M=24.14, SD=15.19) (Fig 3.4).

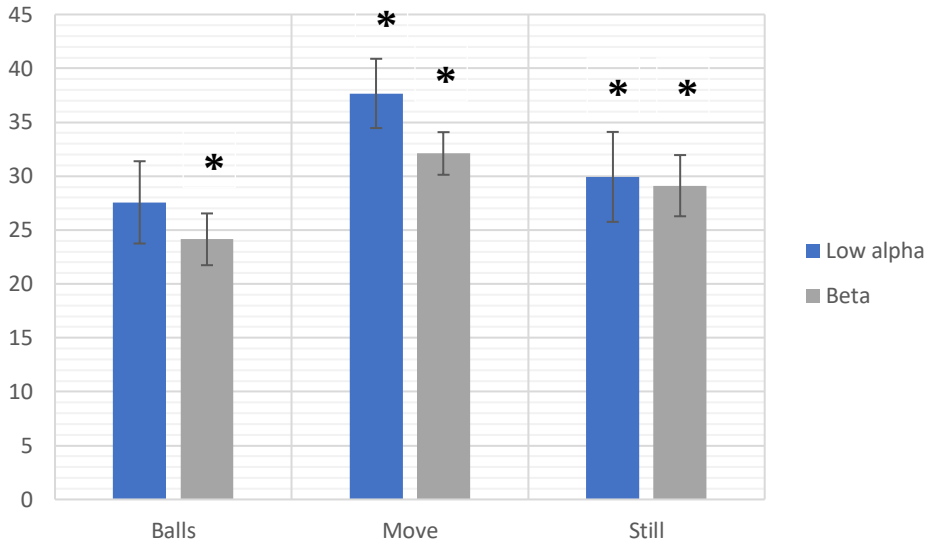

Fig. 3.4 Main effect of stimuli in centroparietal electrodes during the mid-period in low alpha and beta bands.

Late time period (2000-3000ms)

Frontocentral electrodes

A significant main effect of stimuli was found in alpha [F (2, 76) = 3.474, p = 0.36], low alpha [F (2, 76) = 7.241, p = .001] and beta [F (2, 76) = 4.782, p= .011]. Post hocs did not reveal significant differences in alpha. In low alpha, there were significant differences between the balls and move stimuli [t (39) = -3.524, p=.001] as well as between the move and still stimuli [t (39) =3.156, p=.003]. This was also true for beta, between balls and move stimuli [t (39) = -2.589, p=.013] as well as between the move and still stimuli [t (39) =2.630, p=.012]. In both bands, the move stimulus had a higher ERD than both still and balls stimuli: low alpha (move: 41.26, SD=21.89; still: M=28.47, SD= 30.74 and balls: M=24, SD= 33.132); and beta (move: M=31.16, SD=15.62; still: M=22.62, SD= 17.89 and balls: M=22.05, SD= 18.79) (fig 3.5).

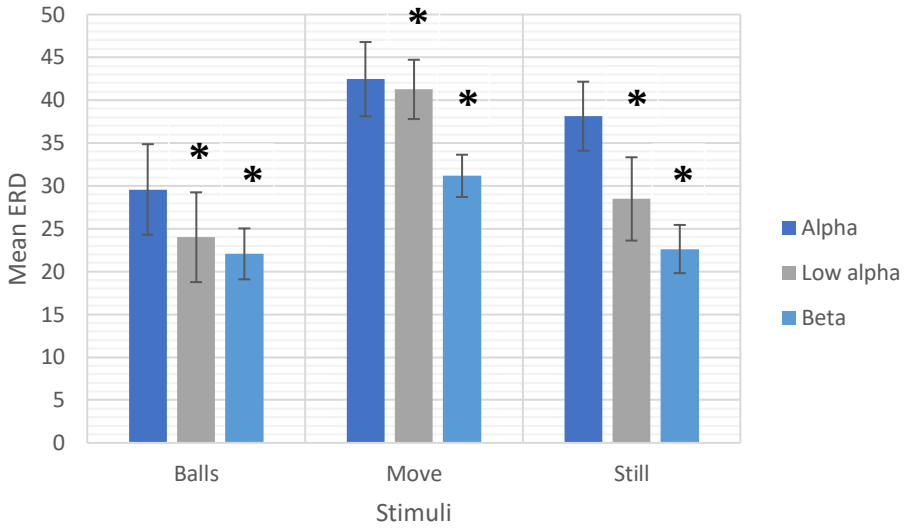

Fig.3.5 Main effect of stimuli in frontocentral electrodes during the late period in alpha, low alpha and beta bands.

Additionally, a main effect of hemisphere was observed for low alpha [F (1, 38) = 4.392, p=.043]; in this case the right hemisphere (M=27.26, SD= 24.41) had a higher ERD over the left (M=23.89, SD=26.88).

Central electrodes

A significant main effect of stimuli was found in alpha [F (2, 76) = 5.007, p =.009], low alpha [F (2, 76) = 7.830, p= .001] and beta [F (2, 76) = 7.765, p = .001] displaying the same trend as in the other time periods. For alpha there was a significant difference between balls and move stimuli [t (39) = -3.389, p=.002]; the move stimulus (M=36.62, SD=27.47) had a higher ERD over balls stimulus (M=24.03, SD=25.24). For low alpha, was there was a significant difference between balls and move stimuli [t (39) = -3.879, p<.000]. Move and still stimuli also showed a significant difference [t (39) =3.276, p=.002]. The move stimulus (M=40.69, SD=21.72) had a higher ERD than still (M=27.05, SD=30.12) and balls stimulus (M=23.61, SD=28.52). For the beta band there was a significant difference between balls and move condition [t (39) = -4.011, p<.000] as well as

between move and still stimuli [t (39) = 2.707, p=.010]. In this band, the move (M=31.09, SD=16.02) stimulus had greater ERD than both the still (M=21.76, SD=17.27) and balls stimuli (M=19.5794, SD= 13.87) (Fig 3.6).

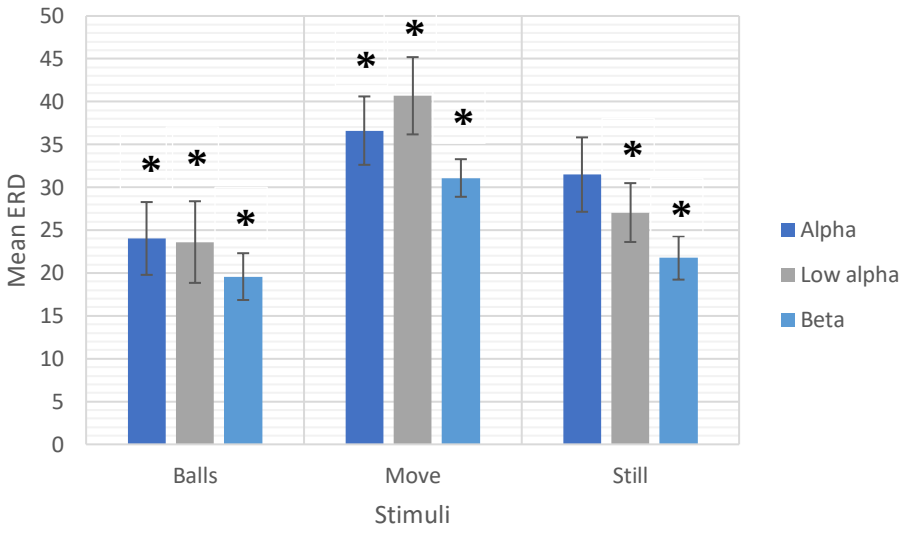

Fig. 3.6 Main effect of stimuli in central electrodes during the late period in alpha, low alpha and beta bands.

A significant main effect in hemisphere for alpha 2 [F (1, 38) = 4.129, p = .049] showed a higher ERD in left (M=26.69, SD=30.63) hemisphere over right (M=19.88, SD=35.13).

Centroparietal Electrodes

A significant main effect of stimuli was found in low alpha [F (2, 76) = 4.456, p < .015] and beta [F (1.662, 63.159) = 8.567, p = .001]. In low alpha, move and still stimuli showed a significant difference [t (39) = 2.806, p=.008]. Move stimulus (M=43.40, SD=26.77) had a higher ERD than the still condition (M=29.76, SD=31.62). In beta, the move and still stimuli were significantly different [t (39) = 2.233, p=.031] as were the balls and move stimuli [t (39) = -4.384, p<.000]. Move (M= 36.44, SD=13.97) had a higher ERD than still (M=29.52, SD=16.94) and balls stimuli (M=26.57, SD=13.47) (Fig 3.7).

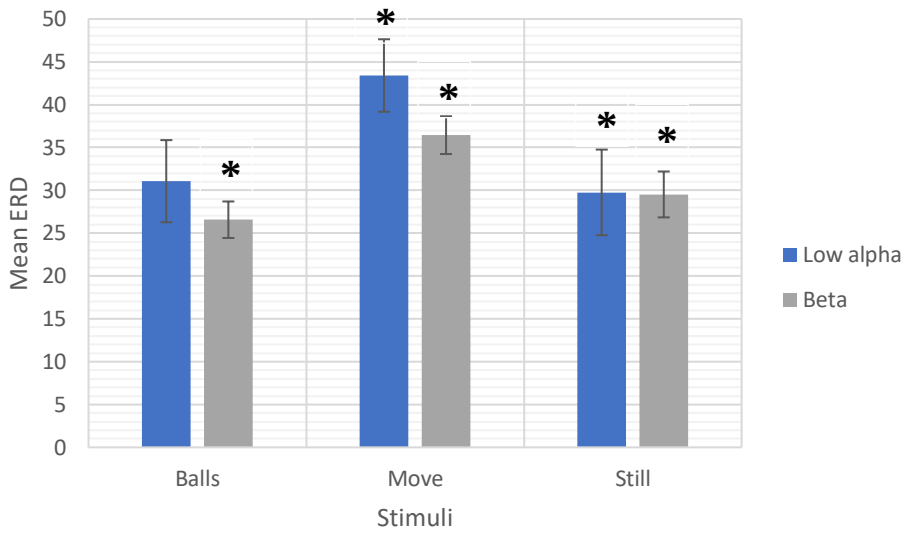

Fig. 3.7 Main effect of stimuli in centroparietal electrodes during the late period in low alpha and beta bands.

Further to the factorial analysis a Person correlation was performed between significant ERD and AQ score in each time period in order to determine if this effect was specifically associated to AQ score. Two findings revealed positive low to moderate correlations. In the mid time period over centroparietal areas in ball stimuli for beta ERD (r=.337, n=40, p=.033), and in the late time period over centroparietal areas in ball stimuli for beta ERD (r=.320, n=40, p=.044). These results suggest that the higher the AQ score, the greater ERD in beta over central and centroparietal areas for the ball stimuli (see fig 3.8 a and b).

A summary of EEG findings can be found in table 3.1, section 3.8.

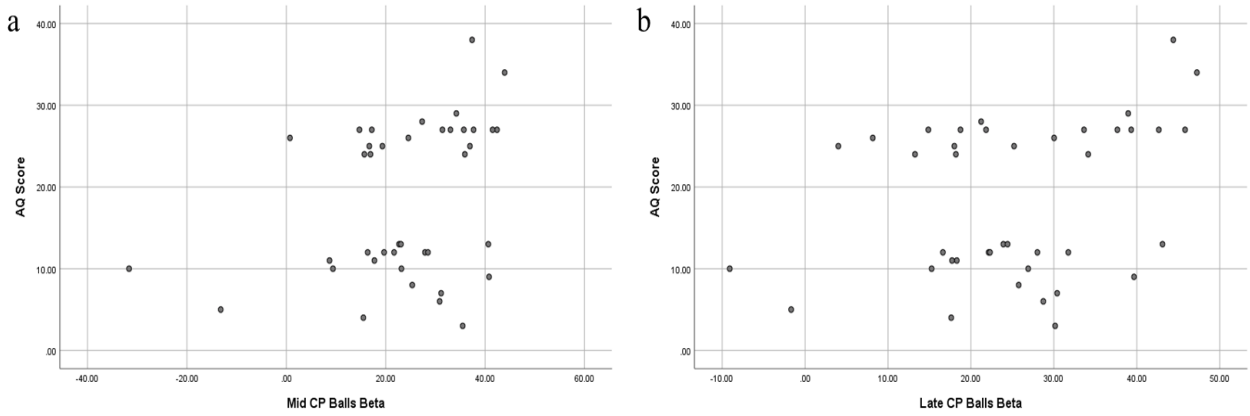

Figure 3.8 .Significant correlations for a) The mid time period over centroparietal areas in ball stimuli for beta ERD (r=.337, n=40, p=.033), and b)The late time period over centroparietal areas in ball stimuli for beta ERD (r=.320, n=40, p=.044).

3.3.5.2. Eye-tracking

A 2 x 2 (stimuli x group) mixed factorial design was conducted for DV pupil size. Within-subject factors was pupil size difference between baseline (still) and the moving/balls stimuli. Between subjects factor was group (high and low AQ). For DVs: trial fixation count, average fixation duration, and average saccade amplitude a 3 x 2 (stimuli x group) mixed factorial design was conducted. Within-subject factors were stimuli (balls, moving, still) and group was the between-subjects factor (high and low AQ).

Pupil size difference

Refers to changes in the diameter of the pupil as a possible indication of cognitive load (Krejtz et al., 2018; Kret & Sjak-shie, 2018). A significant main effect of stimuli was found in this variable [F (1, 38) = 96.832, p= .000] there was a larger pupil size difference in the balls stimuli (M= 287.082 SE= 25.35) than the moving stimuli (M= 51.28 SE= 15.06).

Average saccade amplitude

Saccade amplitude refers to the angular distance that the eye covers during a movement (in degrees of visual angle) (Bahill et al., 1975). It is has been associated to saccade accuracy and has been described as abnormal in individuals with high functioning autism (Johnson et al., 2012). A significant main effect for average saccade amplitude was found for stimuli [F (2, 76) = 10.86, p <.001]. The moving stimuli reflected the lowest average saccade amplitude (M=1.95, SD=.88) followed by balls (M=2.11, SD=.92), and still (M=2.38, SD=1). Post hoc tests indicated that this group difference was only significant in stimuli balls and moving [t (38) = -2.751, p =.009] and [t (38) = 2.47, p=.018] respectively. A between subjects effect for group [F (1, 38) = 5.75, p =.002] was also found. For all stimuli, these results indicate that the high AQ score group had a higher average saccade amplitude than the Low AQ score group (fig 3.9).

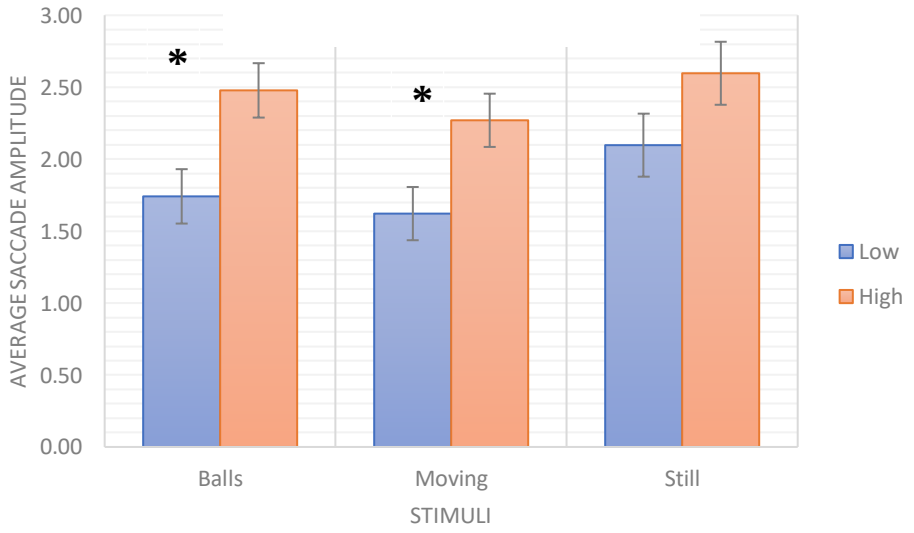

Figure 3.9 Differences in average saccade amplitude across groups and stimuli. * indicates significant differences “Balls” [t (38) = -2.751, p = .009] and “Moving” [t(38)= 2.47, p=.018].

In order to determine if this effect was specifically associated to AQ score, a Pearson correlation was performed between average saccade amplitude and AQ score for each stimulus. Results indicated a significant moderate to strong positive correlation in all stimuli: balls (r= 0.461, n=40,

p= .003), moving (r= 0.365, n=40, p= .021) and still (r= .354, n=40, p=.025). These results suggest that the higher the AQ score, the larger the saccade amplitude across all stimuli.

No significant results were found for fixation count (maximum F= 1.865 , p=.180) and average fixation duration (maximum F=1.275 , p=.266).

Average saccade amplitude was also correlated with ERD but no significant results were found.

3.3.6. Discussion

For this protocol, we expected to find differences in mu desynchronization between the high and low AQ group. However, no group differences were found in our EEG variables. The main effect that was found in stimuli (mainly between the moving and the ball) was correlated with AQ traits and revealed moderate positive correlations in the beta band with the balls stimuli over central and centroparietal areas in mid and late time periods. This means that the higher the AQ traits, the greater the desynchronization of mu (in the beta band frequency component) when presented with the balls stimuli. Beta event related desynchronization as a frequency component of the mu rhythm is more associated to motor imagery in the primary motor cortex and may influence preparation for movement as well as the understanding of more intricate actions (Hari & Salmelin, 1997; Ritter, Moosmann, & Villringer, 2009); it therefore makes sense that this aspect of mu would have more presence given that the stimuli involved observation of hand movements. Beta event-related desynchronization has also been previously described an index of individual differences in AQ traits associated to the hMNS (Cooper et al., 2013) as well as during the observation of hand actions in a typically developed sample (Puzzo et al., 2011).

In terms of the eye-tracking variables, for saccade amplitude, we had hypothesized differences between groups; our findings revealed that the high AQ group had a statistically significant higher

saccade amplitude than the low AQ traits group. This was further positively correlated with their AQ score. It has been suggested that as the amplitude of a saccade becomes higher, the saccade becomes less accurate because the velocity of the saccade tends to increase as well (Bahill et al., 1975). The circuits that play an important role in saccade metrics (saccade amplitude included) are within the cerebellum and brainstem (Soetedjo et al., 2019), both of which have been associated to certain "mirroring" tasks such as action observation and understanding due to the role of the cerebellum in the sequencing of executed movements (Cattaneo et al., 2012). Cerebellar abnormalities have also been described in individuals with ASD (Amaral et al., 2008; Casartelli et al., 2018; Johnson et al., 2012; Schmitt et al., 2014) (for a more detailed view of neuroanatomy abnormalities in ASD see chapter 1). This suggests that the saccade amplitude found in the high AQ group indicates less saccade accuracy and possibly differences in cerebellum activity. However, this will need to be confirmed with more studies that test this

Absence of group differences was also true for pupil size. Contrary to our initial hypotheses, we were not able to find differences between groups, only between stimuli and this is to be expected due to a luminescence effect between the different conditions. This was unexpected, as alterations in pupil size have been described and proposed as possible indicators of an autonomic dysfunction in autism spectrum disorder (Anderson et al., 2013; Anderson & Colombo, 2009; Bast et al., 2019; Krejtz et al., 2018; Martineau et al., 2011a). We provide 2 possible explanations for this:

- Variability in stimuli type. Martineau et al. (2011) used still colour photographs of faces (neutral and virtual) and found an 89% match of accuracy in classifying individuals with ASD based on their pupil responses. Other studies involving faces or gaze dependent social cues have found similar associations (Boraston & Blakemore, 2007; Falck-Ytter, 2010; Falck-Ytter et al., 2012). In this protocol although the stimuli involved were a human hand

(moving and still), the stimuli did not include faces or a social component (see social response protocol).

- Degree of autism. Although our sample had individuals with high autistic traits, they did not meet the criteria for clinical ASD as in previous studies (Anderson et al., 2013; Martineau et al., 2011b). This questions the use of pupil dilation as an early biomarker of ASD or at the very least raises questions as to the sensitivity of this measure as a diagnostic tool, as in this study it was not possible to distinguish individuals from the high or low autistic traits group using this eye-tracking variable alone. It would be interesting to see at what point of the autism spectrum this physiological measure becomes a diagnostic measure. Thus, further studies as well as replications of current studies supporting this are suggested.

Of equal importance, no group differences were found in fixation count within the interest area defined as the perimeter of the stimuli, which suggests a lack of attentional bias over the findings (see section 4.6 in this chapter).

3.4. Proactive gaze protocol

For a detailed view at participants, materials and scales see section 3.2.

3.4.1. Stimuli

The proactive gaze protocol by Costantini, Ambrosini, Cardellicchio and Sinigaglia (2014). The video clips show a side-on view of an actor performing an unpredictable reach movement toward either a small or a large tomato (targets), in half the videos the actor performs the hand movement with a closed no pre-shape in order to touch the top of the target, while in the other half the actor

reaches toward the target with a pre-shaped hand depending on the target to be grasped. A fixation cross was shown in the starting position on a black background before each video for 1000ms. Each video lasts 2500ms. Specific timeline for the video was: 1000ms of a white fixation cross over the actor's hand at the movement onset location, the following 1000ms showed the hand movement and the last 500ms was a still of the last frame of the stimulus video. At the beginning of each video, there is a white fixation cross over the actor's hand to signal the starting position, followed by the arm movement of the actor toward the target. Four different layouts counterbalanced the hand trajectories, which meant 16 different stimulus videos (2 targets x 2 movement types x 4 layouts). Video clips were presented in the middle of the screen against a black background. The videos were showed in random order, repeated 8 times each (128 trials in total). Between each trial a fixation cross appeared in the starting position on a black background for a randomized period of 1000-2000ms in order to avoid expectancy effects (see Fig.3.10).

In addition to the stimuli protocols, resting state EEG was recorded for 2 minutes while the participant was at rest with eyes closed; as well as an electrooculogram (EOG) calibration task used to form the basis for subsequent EOG artefact reduction for the EEG (Croft & Barry, 1998).

All stimuli were constructed on the SR Research Experiment Builder (Version 1.6.12) and presented on the Eyelink 1000 Eye-tracker (Mississauga, Ontario, Canada) an infrared video-based eye-tracking device. Stimuli were presented on a 1027 x 768 pixel screen 60cm away from the participant, this equals to a visual angle of 25° 31' 0.06" (measure of the size of the object's image on the retina(Swearer, 2011)). The triggers were sent to the EEG Neuroscan 4.4 acquisition software and Synamps II amplifiers through a parallel port while the protocols were displayed. Data for the EEG and eye-tracker were recorded simultaneously.

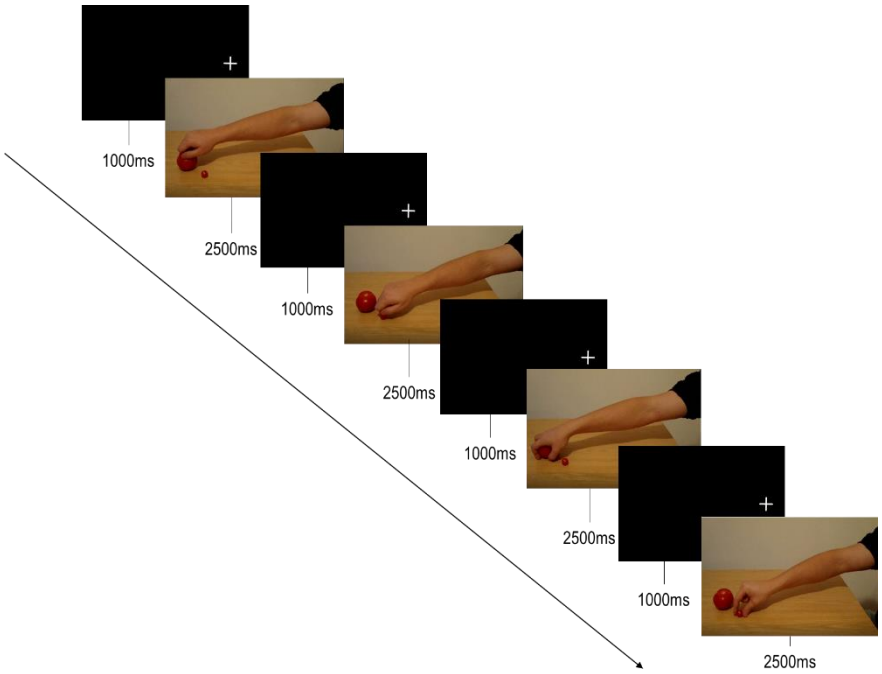

Fig 3.10. Experimental procedure for the proactive gaze protocol.

3.4.2. Experimental design

3.4.2.1. EEG

This was a mixed measures design. Independent variables (IV) were stimuli and hemisphere and dependent variables (DV) were sensorimotor alpha and beta event related desynchronization (ERD) values. Within-subjects variables were stimuli and hemisphere and between-subjects variable was group. The specific design and levels for each factor will be explained further in the results section. To control for attention, trial fixation count was recorded according to each protocol's different interest areas as described in the following section.

3.4.2.2. Eye-tracking

This was also a mixed measures design. IVs were stimuli and interest area (IA). DVs and IA were as follows:

DVs were trial fixation count, average fixation duration per trial, average saccade amplitude and proactive gaze behaviour, this last measure was defined as the time from the first fixation in the onset hand IA to the first fixation in the target interest area. However, this behaviour was not observed in all trials so the frequency of successful trials as well as the total percentage of successful trials per participant were also measured.

Given that in this protocol, the participant observes an unpredictable reach movement toward either a small or a large tomato (targets); two IA were determined based on the target of the trial (large or small tomato) as well as the onset hand location at the beginning of the video. These interest areas match those in Costantini et al.´s, (2014) original paper (see figure 3.11).

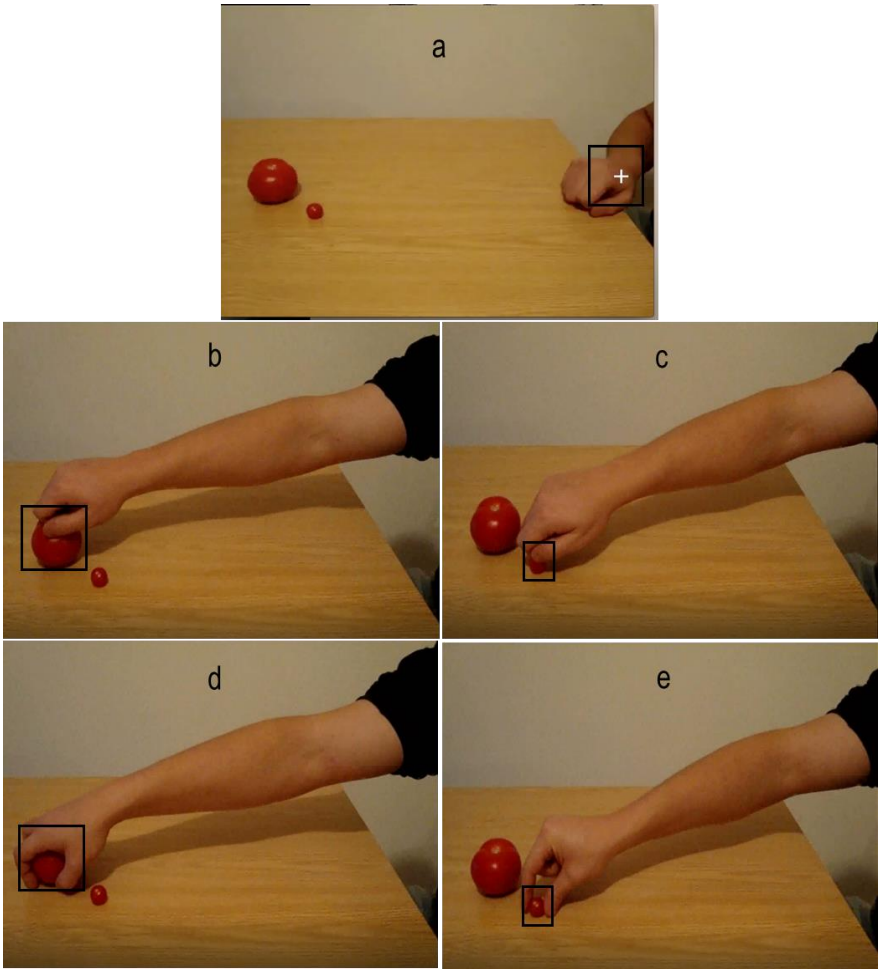

Fig 3.11. Interest areas for the proactive gaze protocol a) Hand onset (same for every condition) b) No Power /Target large c) No precision/Target small d) Power/Target large e) Precision/Target small.

3.4.3. Research Hypotheses

Research hypotheses for this protocol were focused on high and low autistic traits within a neurotypical population and how this reflects on mirror neuron activation during action observation. For EEG variables, we predict that mu desynchronization will be significantly higher overall in the low AQ score group. Based on the original article, we predict that the pre-shape grasp will have a higher ERD than the non pre-shape grasp, regardless of the size of the goal the grasp goes towards. We expect to find differences in proactive gaze behaviour, average saccade amplitude and average fixation duration, However, we predict that differences between groups will not be found in fixation count.

3.4.4. Procedure

At the beginning of the session the participant was asked to read and sign a consent form to take part of the experiment. After placement of the EEG cap they were then instructed to place their chin on the chin rest that was located at a fixed parallel position to the monitor of the EyeLink 1000 (Mississauga, Ontario, Canada)

Before the presentation of the stimuli each participant's resting state EEG as well as a short calibration for the ocular artefact reduction was recorded (Croft & Barry, 1998). Another short calibration with the eye-tracker was performed before the start of the experiment.

Sampling rate for the eyetracker was set at 1000Hz. All EEG data was simultaneously recorded with a Neuroscan 4.4 acquisition software and Synamps II amplifiers using a 64 channel Quick-Cap arranged according to the international 10–10 system (Compumedics, Melbourne, Australia). Eye movements were recorded additionally using four facial electrodes: 1 horizontal electrode for

each eye (approximately 1cm on the outer cantus of each eye) and 2 vertical electrodes (above and below the left pupil). Impedances for all electrodes were reduced to below 10 kOhm before the start of each session. All EEG data were continuously sampled at 1000Hz with a bandpass filter of 0.15–200Hz and a 50Hz notch filter. Online, EEG data were referenced to an electrode between Cz & CPz, and grounded midway between Fz and FPz.

3.4.4.1. EEG data preparation

Following visual inspection of the data, noisy data blocks were rejected. Bad electrodes were excluded on a subject by subject basis. Principal components analysis was performed on the acquired eye movement data to obtain components reflecting saccades and blinks. To carry out ocular artefact rejection, the acquired components were subsequently rejected from the task data traces (Vigario, 1997; Vigario et al., 2000).

The data were then re-referenced to the average of the scalp electrodes, epoched from -2000 to 3500ms and around the start of each video and then an automatic artefact rejection algorithm was applied (+/- 100mV). For the calculation of ERD/S the following steps were carried out: the data underwent complex demodulation and concurrent filtering (zero phase-shift, 24dB roll-off, envelope computed) into the bandwidths: alpha (7.812-12.695 Hz) low alpha (7.812-9.765Hz), high alpha (9.765-12.695), and beta (19.531-29.296) : the data was trimmed (1000ms from each side) to remove filter artefacts and averaged. A reference interval of -1000 to 0ms was used to calculate the percentage change between the active period (0-2500ms) and the reference using the classic method adapted from Pfurtscheller and colleagues (e.g. 1977, 1999): ERD% = (R - A)/R*100, where R = the reference interval and A = the active or task phase. Thus, desynchronization and synchronisation are expressed as a percentage of activity relative to the

reference interval (as a result, this formula ERD produces positive scores and ERS negative). ERD/S data contain both phase-locked and non-phase-locked activity.

3.4.5. Results

3.4.5.1. EEG

Trials were divided into two time frames for separate analyses (early and late). Time frames were as follows:

- Early: 500-1500ms
- Late: 1500-2500ms

A mixed factorial design was conducted on each time frame. For the factorial design the electrodes were collapsed within hemispheres resulting in a 2 x 2 x 2 x 2 (target size x grasp type x hemisphere x group) design. Within subject factors were target size (large vs small), grasp (non-pre-shape vs pre-shaped) and hemispheres (left vs right). AQ group was the between subjects factor (high vs low score). Event-related desynchronization for bands alpha (7.812-12.695 Hz), low alpha (7.812-9.765Hz), high alpha (9.765-12.695) and beta (12.695-19.531Hz) were the dependent variables. This analysis was carried out for electrodes over frontocentral (FC), central areas (C) and centroparietal (CP).

<u>Early time period (0-1000ms)</u>

Frontocentral electrodes

Significant main effect of target size was found in high alpha [F (1, 37) = 6.286, p =.017]. The small target had a greater ERD than the large (M= 24.02 SE=3.51 vs M=16.27 SE=4.06).

Significant main effect of target size was also found in beta [F (1, 37) = 4.318, p =.045]. The small target had a greater ERD than the large (M=16.134, SE=1.824 vs M=12.902, SE=1.902). Significant interactions found in this band were target size x grasp type [F (1, 37) = 4.266, p =.046]. To correct for a Type I error Bonferroni corrections were conducted for this interaction and provided a new p value of p<.025. Post-hoc tests revealed a significant difference between grasp type in the small target size [t (38) = 2.357, p = .024]. Pre-shaped condition had a greater ERD over non pre-shape condition (M=18.71 SD=13.24 vs 14.08 SD=12.40). A second significant interaction was found in beta for target size x grasp type x hemisphere [F (1, 37) = 8.062, p =.007]. Bonferroni correction for this interaction provided p<.012. Post hoc tests indicated a significant difference between grasp type in the small target over left hemisphere [t (38) = -2.809, p=.008]. The pre-shape had a greater ERD over non pre-shape grasp type (M=18.94 SD=12.42 vs M=12.43 SD=13.80) (Fig 3.12).

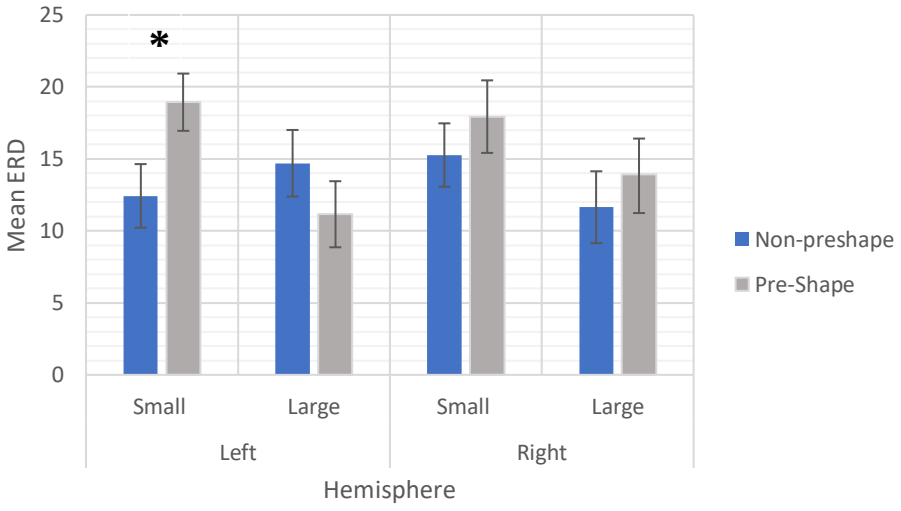

Fig. 3.12 Significant interaction for target size x grasp type x hemisphere in beta during the early time period.

Late time period (1000-2500ms)

Central electrodes

Significant main effect of hemisphere was found in Beta [F (1, 37) = 4.147, p=.049]. The left hemisphere had a greater ERD over the right (M= 24.777 SE=1.844 vs M= 22.505 SE=1.805).

Further to the factorial analysis a Pearson correlation was performed between significant ERDs and AQ score in each time period in order to determine if this effect was specifically associated to AQ score. No correlations were found.

A summary of EEG findings can be found in table 3.2, section 3.8.

3.4.5.2. Eye-tracking

A 2 x 2 x 2 (target size x grasp type x group) mixed ANOVA was conducted for trial fixation count, average fixation duration per trial and average saccade amplitude. Within subject factors were target size (large vs small) and grasp type (No pre-shape vs pre-shaped). Between-subjects factor for all variables was group.

Average fixation duration per trial

Average fixation duration refers to the average duration (in milliseconds) of all fixations in the trial (SR Reasearch, 2007) A significant main effect was found for target size in average fixation duration (F (1, 37) = 8.66, p= .006). Participants focused on the larger target for a longer period (M= 510.58, SD=27.46) in milliseconds compared to the small target (M= 488.47, SD=24.26).

Average saccade amplitude

Saccade amplitude refers to the angular distance that the eye covers during a movement (in degrees of visual angle) (Bahill et al., 1975). It has been associated to saccade accuracy and has been described as abnormal in individuals with high functioning autism (Johnson et al., 2012). For average saccade amplitude a significant main effect of grasp type was found [F (1, 37) = 13.660, p=.001] as well as a significant interaction of grasp x target type [F (1, 37) =10.01, p=.003]. Post-hoc tests revealed that regardless of the target size, in the stimuli where the actor approached the target with a pre-shaped grasp participants showed a larger saccade magnitude (in degrees of visual angle) (M= 4.224, SE=.150) in contrast to the no pre-shape grasp (M= 4.030, SE= .155). No significant difference was found between groups.

As for the proactive gaze behaviour, t-tests were conducted on the percentage of trials where proactive gaze behaviour was successfully completed. No significant differences were found between groups (p>.05). This was also true of the variable trial fixation count (minimum p value = .215, F= 1.592) and average fixation duration (minimum p value = .530, F= .401).

3.4.6. Discussion

The current protocol investigated mu desynchronization in a high and low AQ traits sample during the observation of a goal directed action. Research hypotheses for this protocol were mainly focused on group differences among high and low autistic traits. Contrary to this, no significant group differences were found. We attribute this outcome to the level of autistic traits in our sample (see section 2.3.6). Equally important, no group differences were found in fixation count within

the interest area defined as the perimeter of the stimuli, which suggests a lack of attentional bias over the findings (see section 4.6 in this chapter).

Notwithstanding, there were some interesting findings in the rest of the variables. Average fixation duration was longer on the larger target. Although this could be a saliency effect due to the size of the target (larger target equals longer time/attention spent observing it), an alternative explanation is that fixation time acts as a measure of visual exploration (Unema et al., 2005). If the object is larger, than the fixation time on it is longer because it there is more information to be processed.

As for the mu desynchronization, we did find an increased ERD in the left frontocentral Beta band for the pre-shape condition, specifically, when the target was small. Given the location of this effect, this finding coincides with the original article where the authors provided evidence supporting the contribution of the left ventral premotor cortex (PMv) in proactive gaze behaviour (Costantini et al., 2014). The authors found that proactive gaze was influenced when specific motor cues (i.e. a pre-shaped grip) were present, the participant's gaze arrived at the target faster when the actor's hand was in a pre-shape grip; this eye-tracking behaviour was altered when they applied repetitive transcranial magnetic stimulation over the PMv cortex. Our eye-tracking findings in average saccade amplitude were also in line with this. When there was a motor cue provided by the actor's hand in the shape of a pre-grasp, participants showed a larger saccade magnitude. Furthermore, proactive gaze and saccades have been previously described both in neurotypical infants and adults when performing and observing goal-directed actions (Rosander & von Hofsten, 2011). In their study, both infants and adults had to either move an object between two places in their visual field or observe someone else perform the task. The authors found that the proactiveness of gaze shifts was associated with saccades that preceded the movement observed. Findings from this protocol add to this literature.

3.5. The Social Response Protocol

For a detailed view at participants, materials and scales see section 4.2.

3.5.1. Stimuli

The Social Response Protocol - involving goal directed observation with a social component protocol (Clausen et al., 2015) intended to elicit the EEG indexed hMNS during movement observation but adding a social response component. This protocol was designed to test a theory by Hamilton (2013) where it was proposed that an important purpose of the MNS is to respond, in real-time and in a socially appropriate fashion, to the actions of others. A fixation cross was shown in the starting position on a black background before each video for 1000ms. The videos last 3000ms, in each one a female actress wearing black clothing in front of a black background is holding a white mug. There are three stimuli, one condition shows the actress moving the mug towards the viewer, in the second condition the mug is moved backwards away from the viewer, and in the third condition the video has no movement. In all three stimuli the actress' facial expression is neutral and the video is shown against a black background. The videos were showed in random order, repeated 30 times each (90 trials in total in 10 blocks of 9 trials each). Between each trial a fixation cross appeared in the starting position on a black background for a randomized period of 1000-2000ms in order to avoid expectancy effects (Fig 3.13).

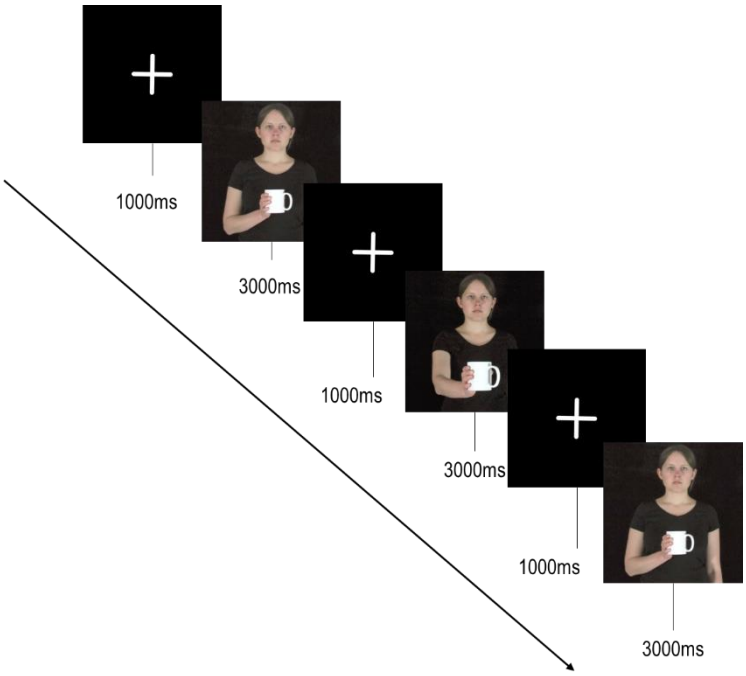

Fig 3.13. Experimental procedure for the Social Response Protocol

In addition to the stimuli protocols, resting state EEG was recorded for 2 minutes while the participant was at rest with eyes closed; as well as an electrooculogram (EOG) calibration task was used to form the basis for subsequent EOG artefact reduction for the EEG (Croft & Barry, 1998).

All stimuli were constructed on the SR Research Experiment Builder (Version 1.6.12) and presented on the Eyelink 1000 Eye-tracker (Mississauga, Ontario, Canada) an infrared video-based eye-tracking device. Stimuli were presented on a 1027 x 768 pixel screen 60cm away from the participant, this equals to a visual angle of 25° 31' 0.06" (measure of the size of the object's image on the retina (Swearer, 2011)). The triggers were sent to the EEG Neuroscan 4.4 acquisition software and Synamps II amplifiers through a parallel port while the protocols were displayed. Data for the EEG and eye-tracker were recorded simultaneously.

3.5.2. Experimental design

3.5.2.1. EEG

This was a mixed measures design. Independent variables (IV) were stimuli and hemisphere and dependent variables (DV) were sensorimotor alpha and beta event related desynchronization (ERD) values. Within-subjects variables were stimuli and hemisphere and between-subjects variable was group. The specific design and levels for each factor will be explained further in the results section. To control for attention, trial fixation count was recorded according to the interest areas described in the following section.

3.5.2.2. Eye-tracking

This was also a mixed measures design. Within subjects variables were stimuli and interest area (IA) and between subjects variable was group. IVs were stimuli and IA. DVs and IA were as follows:

The interest areas were eyes, face, mouth and cup (see figure 3.14). Interest areas mouth and eyes overlapped with face. DVs were trial and interest area fixation count, dwell time, average fixation pupil size, and average saccade amplitude.

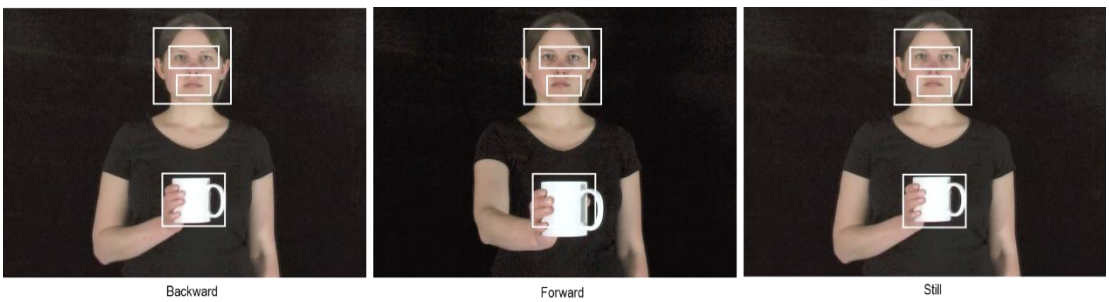

Fig 3.14. Interest areas for social response protocol across all stimuli. From left to right: “backward”, “forward” and “still”.

3.5.3. Research Hypotheses

Research hypotheses for this protocol were focused on high and low autistic traits within a neurotypical population and how this reflects on mirror neuron activation during action observation with a social component. For EEG variables, we predict that mu ERD will be significantly higher overall in the low AQ score group. Additionally, within the frame of Hamilton's (2013) theory of MN function, we expect the forward stimuli to show increased ERD reflecting an anticipatory response to a social cue. Furthermore, we predict that EEG differences between groups will not be found in eye-tracking variables fixation count. As for the remaining eye-tracking variables, pupil size, interest area fixation count, dwell time, average pupil size and average saccade amplitude, we predict differences between groups.

3.5.4. Procedure

At the beginning of the session the participant was asked to read and sign a consent form to take part of the experiment. After placement of the EEG cap they were then instructed to place their chin on the chin rest that was located at a fixed parallel position to the monitor of the EyeLink 1000 (Mississauga, Ontario, Canada)

Before the presentation of the stimuli each participants' completed a short calibration for the ocular artefact reduction (Croft & Barry, 1998). Another short calibration with the eye-tracker was performed at the beginning of each set of videos.

Sampling rate for the eye-tracker was set at 1000Hz. All EEG data was simultaneously recorded with a Neuroscan 4.4 acquisition software and Synamps II amplifiers using a 64 channel Quick-Cap arranged according to the international 10–10 system (Compumedics, Melbourne, Australia).

Eye movements were recorded additionally using four facial electrodes: 1 horizontal electrode for each eye (approximately 1cm on the outer cantus of each eye) and 2 vertical electrodes (above and below the left pupil). Impedances for all electrodes were reduced to below 10 kOhm before the start of each session. All EEG data were continuously sampled at 1000Hz with a bandpass filter of 0.15–200Hz and a 50Hz notch filter. Online, EEG data were referenced to an electrode between Cz & CPz, and grounded midway between Fz and FPz.

3.5.4.1. EEG data preparation

Following visual inspection of the data, noisy data blocks were rejected. Bad electrodes were excluded on a subject by subject basis. Principal components analysis was performed on the acquired eye movement data to obtain components reflecting saccades and blinks. To carry out ocular artefact rejection, the acquired components were subsequently rejected from the task data traces (Vigario, 1997; Vigario et al., 2000).

The data were then re-referenced to the average of the two mastoid electrodes, epoched from -2000 to 4000ms and around the start of each video and then an automatic artefact rejection algorithm was applied (+/- 100mV). For the calculation of ERD/S the following steps were carried out: the data underwent complex demodulation and concurrent filtering (zero phase-shift, 24dB roll-off, envelope computed) into the bandwidths low (8-10 Hz) and higher alpha (10-12 Hz) as well as beta (12-20 Hz): the data was trimmed (1000ms from each side) to remove filter artefacts and averaged. A reference interval of -1000 to 0ms was used to calculate the percentage change between the active period (0 to 3000ms) and the reference using the classic method adapted from Pfurtscheller and colleagues (1977, 1999): ERD% = (R - A)/R*100, where R = the reference interval and A = the active or task phase. Thus, desynchronization and synchronisation are

expressed as a percentage of activity relative to the reference interval (as a result, this formula ERD produces positive scores and ERS negative). ERD/S data contain both phase-locked and non-phase-locked activity.

3.5.5. Results

3.5.5.1. EEG

Trials were divided into three time frames for separate analyses (early, mid and late). Time frames were as follows:

- Early: 0-1000ms
- Mid: 1000-2000ms
- Late: 2000-3000ms

A mixed factorial design was conducted on each time frame. For the factorial design the electrodes were collapsed across hemispheres resulting in a 3 x 2 x 2 (stimulus x hemispheres x group) design with AQ score as a between subjects factor (high vs low score), and two within subjects factors: stimuli (backwards, forward and still) and hemisphere (left and right). Event related desynchronization for bands: alpha (7.812-12.695 Hz), low alpha (7.812-9.765Hz), alpha 2 (9.765-12.695), beta (12.695-19.531Hz) were the dependent variables. This analysis was done for electrodes over frontocentral (FC), central areas (C) and centroparietal (CP). To correct for a Type I error, a Bonferroni correction was conducted for all post-hoc t-tests and provided a new p value of $p<.016$.

Early time period (0-1000ms)

No significant interactions or main effects were found in this time period ($p > .080$).

Mid time period (1000-2000ms)

Frontocentral electrodes

Over FC areas, a significant main effect of stimuli was found in low alpha [$F(2, 74) = 5.001$, $p = .009$]. Follow up t-tests revealed significant differences between forwards and backward condition [$t(38) = 3.082$, $p = .004$]. Forward ($M = 27.14$, $SD = 21.96$) condition had a higher ERD than the backward condition ($M = 14.68$, $SD = 28.37$). Fig (3.15).

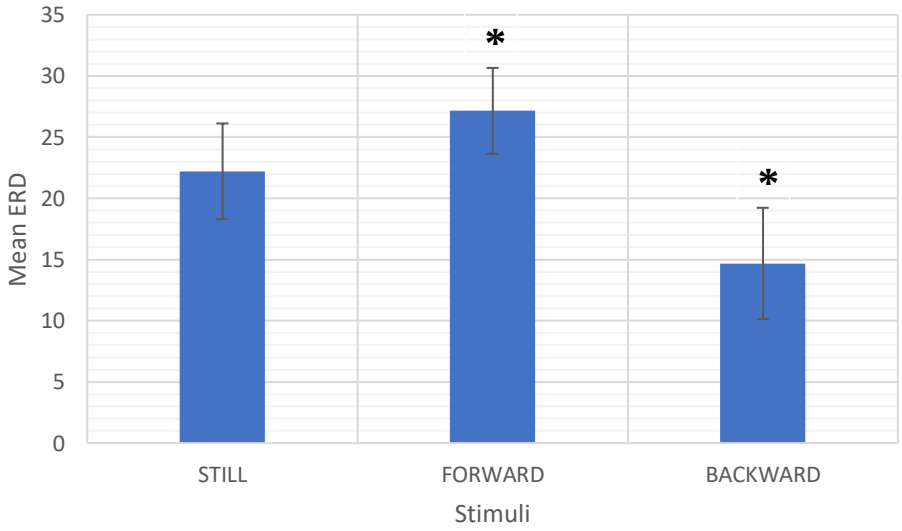

Fig. 3.15 Significant main effect of stimuli in low alpha over frontocentral electrodes in mid time period.

Central electrodes

In the C area, a significant main effect of hemisphere in alpha 2 was found [F (1,37) = 4.601, p < 05] where the left hemisphere (M= 27. 58 SD=26.19) showed a higher ERD than the right (M= 22.69, SD= 30.62).

No significant interactions or main effects were found in centroparietal electrodes (maximum F=.925, p=.342).

Late time period (2000-3000ms)

No significant interactions or main effects were found in Frontocentral electrodes (maximum F=1.544, p=.222).

Central electrodes

In central areas, a main effect of hemisphere was found in low alpha [F (1,37) = 4.42, p < .05] the right hemisphere showed a higher ERD (M=30.63, SD=25.96) than the left (M=26.46, SD=25.09).

Centroparietal electrodes

In centroparietal areas a significant main effect of hemisphere was found in low alpha [F (1, 37) =8.71, p < .05] the right hemisphere showed a higher ERD (M=37.42, SD=25.02) over the left (M=32.22, SD=24.14).

Further to the factorial analysis a Person correlation was performed between significant ERDs and AQ score in each time period in order to determine if this effect was specifically associated to AQ score. A low negative correlation was found in the right centroparietal hemisphere for the late time period (r= -.290, n=38, p=.077). This would suggest that the lower the AQ score the higher ERD in low alpha over right centroparietal areas 2000ms after the presentation of the stimuli (late period).

A summary of the main findings can be found in table 4.3, section 4.8.

3.5.5.2. Eye-tracking

Eye-tracking variables were analysed as follows:

A 3 x 4 x 2 (stimuli x interest area x group) mixed factorial design was conducted for fixation count, dwell time and pupil size. AQ score was the between subjects factor (high vs low score), and two within subjects factors: interest area (mouth, eyes, face, cup) and stimuli (Backwards, Forwards, Still). A 3 x 2 (stimuli x group) mixed factorial design was conducted for average saccade amplitude.

Significant findings were found in interest area fixation count, dwell time and average saccade amplitude.

Fixation count

Fixation count refers to the number of times a fixation appears within a certain interest area (SR Research, 2007)and was of interest in this protocol in order to determine where participants

focused their attention and if there were differences between groups in attention paid to the stimuli that could cause a bias in the EEG results. A significant main effect of stimuli was found [F (2, 76) = 10.877, p=.000]. To correct for a Type I error, a Bonferroni correction was conducted for post-hoc t-tests in stimuli and provided a new p value of p<.016. Post hoc tests revealed significant differences between forward and still stimuli [t (39) = -4.190, p= .000] and still and backward stimuli [t (39) = 3.162, p= .003]. No group differences were found.

Dwell time

Dwell time refers to the total time (in milliseconds) that the participant spent on a specific interest area (SR Research, 2007). This variable is of interest given that the stimuli includes a social component, group differences were of interest as well as specific interest areas in order to determine weight of attention given to the different parts of the stimuli. A significant main effect of stimuli was found [F (2, 76) = 4.742, p=.011]. To correct for a Type I error, a Bonferroni correction was conducted for post-hoc t-tests in stimuli and provided a new p value of p<.016. Significant differences were found between still and backward condition [t (39) = 2.85, p= .007]. Means across interest areas were as follows: backward (M= 500.40 SE= 31.11) forward (M= 505 SE= 31.45) and still and (M= 529.61 SE= 35.05).

A significant main effect of interest area was also revealed, [F (2, 76) = 39.307, p=.000]. Bonferroni correction provided a new p value of p<.008. Significant differences were found among the following interest area comparisons: mouth and cup [t (39) = 7.712, p= .000], mouth and face [t (39) = -6.956, p= .000], cup and face [t (39) =3.494, p= .001], cup and eyes [t (39) = -8.056, p= .000] and face and eyes [t (39) = -6.921, p= .000]. Means across interest areas were as follows:

mouth (M= 184.11, SE= 32.02) cup (M= 1112.94 SE=105), face (M=577.19, SE=76.86) and eyes (M= 172.45, SE =35.13).

A significant interaction between stimuli and interest areas was also found [F (2,76) = 24.776, p=.000]. Bonferroni correction provided a new p value of p<.004. Significant differences were found in interest area eyes between forward and still stimuli [t (39) = -3.746, p= .001] and still and backward stimuli [t (39) = 5.285, p< .000].

In cup interest area between backward and forward stimuli [t (39) = -3.375, p= .002] and forward and still stimuli [t (39) = 5.861, p< .000].

In face interest area between forward and still [t (39) = -5.892, p< .000] and still and backwards [t (39) = 5.734, p< .000] (fig 3.16).

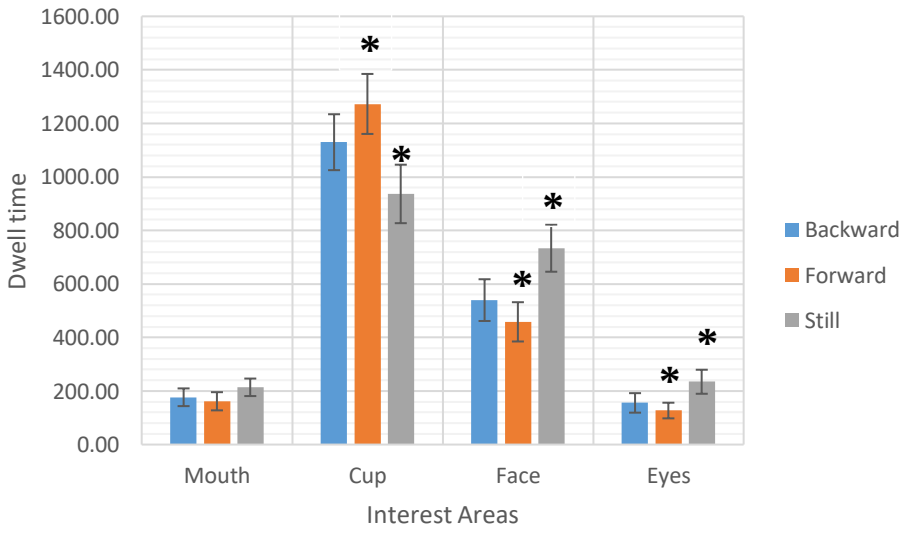

Fig. 3.16. Significant interaction between stimuli and interest area in dwell time. Significant differences in specific interest areas between conditions are signalled with a black cross.

Average saccade amplitude

Saccade amplitude refers to the angular distance that the eye covers during a movement (in degrees of visual angle) (Bahill et al., 1975). For average saccade amplitude a significant main effect between groups was found F (1, 38) = 7.354, p= .01 (Figure 3.17). No significant effects were found within subjects. This result reflects that the average size (in degrees of visual angle) of saccades in the trial were larger in the high AQ score group. Upon further t-tests, the significant difference was confirmed across all stimuli: back [t (38) =-2.21, p < .05], forward [t (38) =-2.56, p< .05], and still [t= (38) =-3.03, p<.05]. In order to determine if this effect was specifically associated to AQ score, a Pearson correlation was performed between average saccade amplitude and AQ score for each condition. Results indicated a significant moderate to strong positive correlation in all stimuli: backward (r= 0.421, n=40, p= .007), forward (r= 0.383, n=40, p= .015) and still (r= .451, n=40, p=.003). These results suggest that the higher the AQ score, the larger the saccade amplitude in all stimuli.

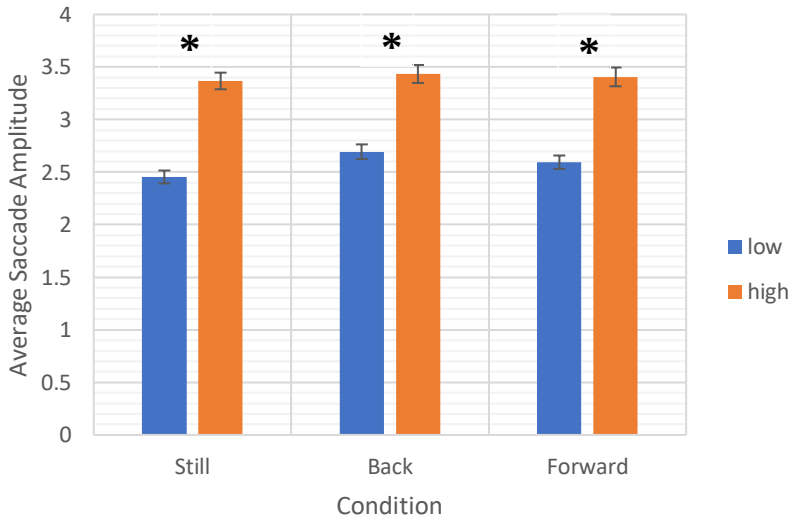

Fig 3.17. Differences in average saccade amplitude across groups and stimuli. Back [t(38)=-2.21, p < .05], forward [t(38)=-2.56, p< .05], and still [t=(38)=-3.03, p<.05].

We further correlated ERD with eye-tracking findings but no correlations were significant ($p>.087$).

3.5.6. Discussion

The current protocol investigated mu ERD in a high and low AQ traits sample with stimuli that included action observation with a social component. We hypothesised an overall higher ERD in the Low AQ traits group. Despite this, we did not find group differences. Much like the other protocols a possible explanation could lie in the degree of autistic traits in our sample (see section 4.3.6). Although part of our sample had high AQ traits they were not clinically diagnosed with ASD.

Interestingly, most of the mu ERD effects were identified in the Low Alpha band as opposed to the multiple repetitions protocol where they were in the Beta band. A possible explanation lies in the type of stimuli. This protocol involved social components, in that it contained faces and the action of offering and taking away an object from the participant. It is thus viable that the ERD would occur in the low alpha band as this band has been strongly associated to the primary somatosensory function, possibly reflecting an internal representation of movement observation (Hari & Salmelin, 1997). Signalling from the somatosensory cortex to the premotor cortex has been also been suggested to play an important role in the understanding of action and imitation (Gazzola & Keysers, 2009; Keysers & Gazzola, 2009).

It is worth noting that the faces included in this protocol lacked emotion which could have caused the main effect of mu desynchronization in low alpha despite no group differences. Karakale, Moore, and Kirk, (2019) suggest that higher desynchronization of mu during the observation of neutral faces plays a role in recognizing action and emotion because a “blank” canvas of a neutral

face would need increased sensorimotor engagement in light of the lack of information presented by the environment in this scenario. Thus, in this protocol, the stimuli involved a neutral face, also suggesting a recruitment of sensorimotor engagement by a higher ERD of low alpha.

By adding a social component into the stimuli, this protocol also intended to test an alternative mirror neuron purpose proposed by Hamilton (2013) which refers to the function on MN as a facilitation of a social response. In this protocol, the stimuli had a clear social cue: the person in the video had a mug with the handle towards the participant and the mug was moving either towards or away from the participant. It would be expected that the forward stimuli have a higher ERD as the person in the stimuli is "offering" the mug to the participant. In terms of our results, an effect of this nature was observed in the frontocentral electrodes, where there was a significantly higher ERD in low alpha for the forward stimuli. Within the frame of Hamilton's (2013) theory of social response, this suggests an anticipatory response from MNs (reflected by mu desynchronization) to the mug being directed at them. Liepelt, Prinz, and Brass (2010) found similar results when measuring a motor priming effect. They asked participants to produce an action matching an image that presented end positions of different actions. In images with a right-handed shake-hands image, participants had a higher response with their own right hand. This points to a response to the social cue of an offered handshake.

Another finding in the protocol that supports this social response function of the mirror neuron system is the eye-tracking variable dwell time. Cup as an interest area had the highest dwell time in the forward stimuli. As mentioned in the results section, dwell time refers to the total time (in milliseconds) that the participant spent on a specific interest area (SR Research, 2007). In terms of attention, longer dwell time would mean longer time paid to the cup when it was approaching the participant, also suggesting a motor anticipatory response to a social cue (receiving the cup).

It was interesting that there was an absence of group differences for pupil size, as alterations in pupil size have been described and proposed as possible indicators of an autonomic dysfunction in autism spectrum disorder (Anderson et al., 2013; Anderson & Colombo, 2009; Bast et al., 2019; Krejtz et al., 2018; Martineau et al., 2011a). And studies involving faces show similar findings (Boraston & Blakemore, 2007; Falck-Ytter, 2010; Falck-Ytter et al., 2012; Martineau et al., 2011a). In this protocol, the lack of pupil size differences among groups cannot be attributed to the type of stimuli, as it also included faces. Further studies with this protocol adding emotion to the stimuli would provide more insight on stimuli that involves faces with and without emotional content, pupil size and degree of AQ traits.

For trial fixation count, as predicted, no group differences were found. This supports a lack of attention bias among different levels of AQ traits that could have interfered with EEG results. For average saccade amplitude, in the same way as the mu multiple repetitions protocol, the high AQ group had a statistically significant higher saccade amplitude over the low AQ. This finding was further positively correlated to the AQ score. These results could be linked to differences in cerebellum activity that have been previously described in ASD (see 4.3.6); further studies will be needed to confirm this.

Another variable that correlated with participant's AQ traits despite the absence of group differences, was low alpha ERD in the right centroparietal electrodes for the late time period. The low negative correlation found suggests that as the AQ traits become more pronounced in a neurotypical population, the ERD in low alpha declines. If this effect were to perpetuate throughout the AQ spectrum, the aforementioned would be in line with what has been described as an altered desynchronization of mu and the somatosensory cortex in individuals with diagnosed autism (Bastiaansen et al., 2011; Bernier et al., 2014; Cook et al., 2014; Lepage & Théoret, 2006;

Oberman et al., 2005, 2013). This finding strongly supports the use of this protocol in the following experiment as the pre- and post-neurofeedback assessment as it will be interesting to see if this effect as well as average saccade amplitude, is modified through a neurofeedback protocol.

3.6. Attentional Control

As mentioned in previous results sections, fixation count did not prove to be significant between groups ($p>.05$ in the t-tests for fixation count across all protocols). However, given that the interest in fixation count was to measure attentional bias between the high and low AQ groups, in addition to the statistical analysis of fixation counts per interest areas, the distribution of all fixations counted according to condition and group were plotted in the form of a heat map for each protocol in order to better illustrate the attention paid on each stimuli (fig 3.18 a, b and c).

It is clear that not only are all participants paying attention to the stimuli displayed, but there are also no significant differences in attention between groups. This was confirmed by the non-significant statistical differences between groups in fixation count per trial. This suggests that the differences found in EEG and eye-tracking DV cannot be attributed to an attention bias among groups as was proposed in the research hypothesis. Furthermore, the correlations between eye-tracking variables (average saccade amplitude), ERDs and the AQ traits, support the lack of attentional bias across all protocols.

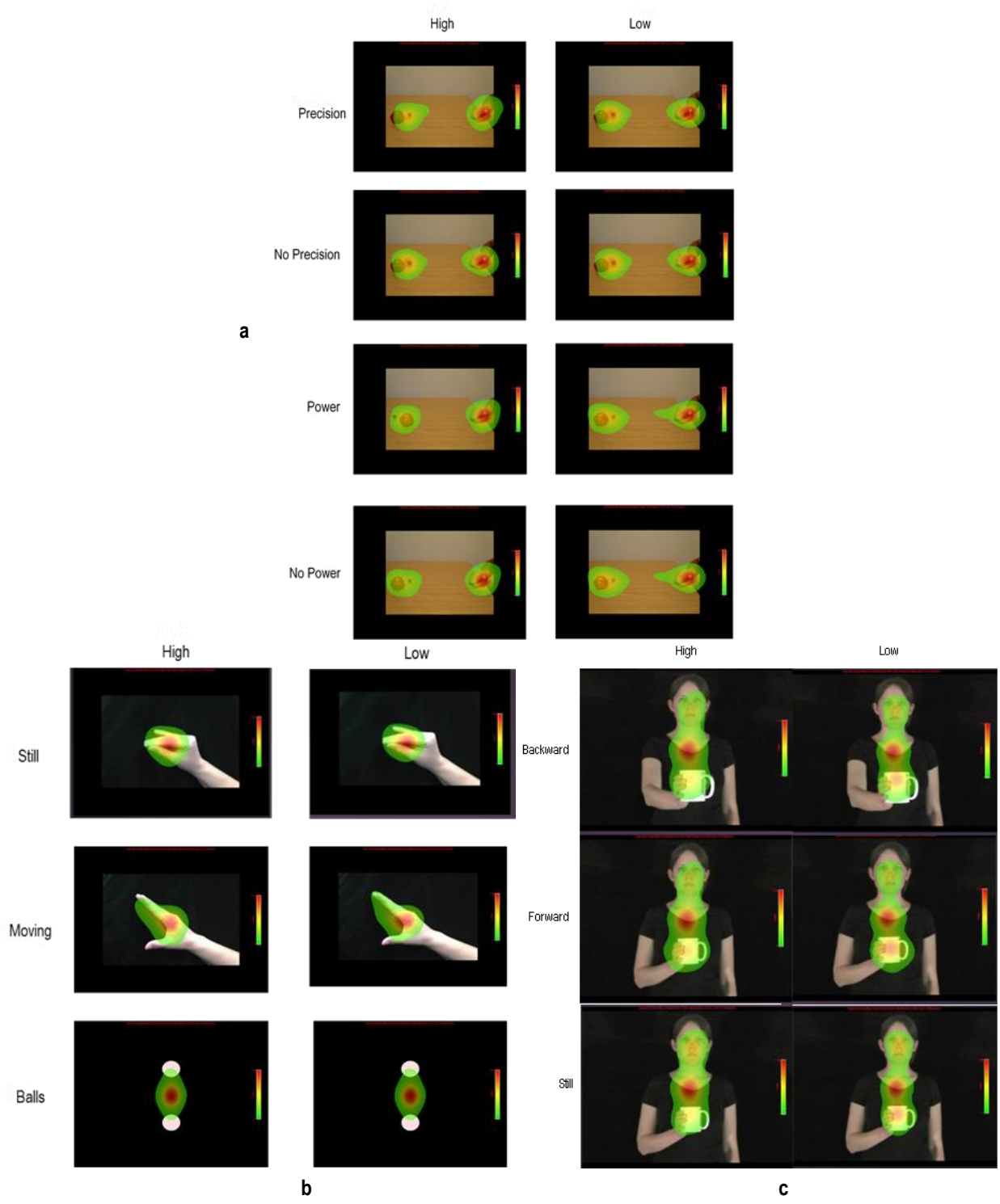

Figure 3.18 Heat map representing the distribution of all fixations across all trials divided by group and condition and condition. a) Proactive gaze b) Mu multiple repetitions c) Social Response Protocol. Colours indicate the density of fixations in a specific area green represents a lower frequency of fixations, whilst red represents the highest number of fixations in an area.

3.7. Conclusion

Contrary to our hypotheses in all protocols, there were no significant group differences among ERD variables. Notwithstanding, across all protocols average saccade amplitude did present differences between high and low AQ and was further correlated to AQ traits. The higher the AQ score, the larger the saccade amplitude across all stimuli. As explained above, this suggests that the saccade amplitude found in the high AQ group could be signalling to differences in cerebellum activity. Further studies will be needed to confirm this. Equally important, in the social response protocol, a negative correlation with low alpha ERD found in the right centroparietal areas suggests that as the AQ traits become more pronounced in neurotypical population, the ERD in low alpha declines. We conclude that the social response protocol is the most suitable as a pre and post physiological neurofeedback assessment.

3.8. Results tables

Table 3.1. Mu multiple repetitions protocol results summary

Time period	Electrode group	Effect/Interaction	Band	Post hoc	Significant Correlation with AQ
Early	Frontocentral	Stimuli x hemispheres	High alpha	Right>Left in Still	
Mid	Central	Stimuli	Alpha	Move>Balls	
			Low alpha	Move>Balls	
		Hemisphere	High alpha	Left>Right	
	Centroparietal	Stimuli	Low alpha	Move>Balls	
			Beta	Move>Balls	r=.337 with balls condition
Late	Frontocentral	Stimuli	Low alpha	Move>Balls Move>Still	
			Beta	Move>Still	
		Hemisphere	Low alpha	Right>Left	
	Central	Stimuli	Alpha	Move>Balls	
			Low alpha	Move>Still Move>Balls	
			Beta	Move>Still Move>Balls	
		Hemisphere	High alpha	Left>Right	
	Centroparietal	Stimuli	Low alpha	Move>Still	
			Beta	Move>Balls	r =.320 in balls condition

Table 3.2. Proactive gaze protocol results summary.

Time period	Electrode group	Effect /interaction	Band	Post hoc
Early	Frontocentral	Target Size	High Alpha	Small target >Large target
		Target size x Grasp type	Beta	Pre-shape> Non Pre-shape
		Target size x Grasp type x Hemisphere		Small target: Pre-shape> Non Pre-shape in Left hemisphere
Late	Central	Hemisphere		Left>Right

Table 3.3. Social Response Protocol results summary

Time period	Electrode group	Effect /Interaction	Band	Post hoc	Significant Correlation with AQ Score
Mid	Frontocentral	Stimuli	Low alpha	Forward>Backward	
	Central	Hemisphere	High alpha	Left> Right	
Late	Central	Hemisphere	Low alpha	Right>Left	
	Centroparietal	Hemisphere	Low Alpha	Right>Left	r= -.283 for the right hemisphere

4. Neurofeedback training on individuals with high autistic traits.

4.1. Aims and overview

There are several lines of research that are currently underway in trying to decipher the underlying neural substrates involved in the symptoms that characterize Autism spectrum disorder (ASD). Although no evidence-based cure exists for ASD, psychosocial and pharmacologic interventions can improve the quality of life of individuals with ASD and their families. These include behavioural therapy, social skills training, parental interventions and medication. Different intervention modalities have variation in degrees of invasiveness, biological basis, specificity and directedness(Collura, 2014).

Neurofeedback is a form of operant conditioning where a participant is taught to modulate brainwave patterns by monitoring and receiving feedback of physiological parameters in real time so they may learn to adjust certain frequencies (Coben & Evans, 2011). It is a biologically based intervention with a high level of specificity and directedness (location of brain areas to train as well as selection of frequencies can be adapted to individuals) but at the same time, it is completely non-invasive. The sensors are topical, and there are no negative side effects that could compare to the case of pharmaceutical treatments (Collura, 2014). Due to the demands on attention to the training sessions, some participants could report fatigue or headaches. These reports however, are scarce (Holtmann et al., 2011b).

The use of neurofeedback as a clinical tool in the study and treatment of several pathologies has slowly grown, due to the positive results individuals show when trained to control specific brain frequencies to enhance certain functions or reduce undesirable activity. Some of the first studies concerning neurofeedback as a form of intervention involved the modulation of sensorimotor rhythm brainwaves (Coben, Linden, & Myers, 2010). Vernon et al, (2003) investigated the possibility that training healthy individuals to enhance theta activity (4–7 Hz), and sensorimotor rhythm (SMR) activity (12–15 Hz) would specifically influence a particular aspect of cognitive performance, in contrast to a non-neurofeedback control-group. Their results suggested that normal healthy individuals can learn to increase a specific component of their EEG activity, and as a result, semantic processing in working memory tasks could be facilitated. The non-invasive approach and positive results in the treatment of individuals with ADHD (Arns, de Ridder, Strehl, Breteler, & Coenen, 2009), Epilepsy (Monderer, Harrison, & Haut, 2002), as well as optimising functions such as attention, creativity and creative music performance in non-clinical populations (Gruzelier, Foks, Steffert, Chen, & Ros, 2014), have grown the interest in neurofeedback both in clinical and research fields. The field of ASD research is not an exception (for further information see chapter 1).

A review of the effects of neurofeedback in autistic samples by Coben, Linden, and Myers (2010) mentions that the rate of abnormal EEGs reported in ASD individuals range from 10 to 83%. Such a broad range of abnormality in brain activity calls for an equally broad range of therapeutic options in order to minimise side-effects across the autistic spectrum. The authors suggest that as neural abnormalities in ASD activity are proposed to be a result of reduced connectivity between specialized local neural networks and overconnectivity within isolated neural assembles, neurofeedback as a non-invasive therapeutic alternative, could be used to enhance neuroregulation

and metabolic function with minimal side effects and adapt to the needs of the ASD individual depending on their particular brain patterns.

As for the different NFT protocols that are used in ASD, they can broadly be classified into 2 groups depending on the strategy: inducing changes in the pattern of EEG frequency bands (mainly theta, theta/beta ratio or gamma); and increasing mu suppression (Holtmann et al., 2011).
Kouijzer, de Moor, Gerrits, Buitelaar, and van Schie (2009) reported positive effects of neurofeedback in ASD individuals when learning how to mediate theta power in order to promote and improve executive functions. The authors found that sixty percent of the participants in the treatment group successfully reduced excessive theta power during neurofeedback. Parents reported an improvement in social interaction as assessed by the questionnaires Children's Communication Checklist (CCC-2) and the Auti-R. In addition to this, the authors report a maintenance of the effects 12 months after initial assessments post NFT. Participants underwent the same assessment of executive functions and social behaviour through the parent report-based questionnaires and improvement from the scores taken before the NFT was maintained.

Wang et al., (2016) used a NFT protocol to increase attentional abilities in participants with ASD. In their study, they focused on gradually decreasing theta/low beta and theta/high beta EEG ratios, while increasing the relative power of gamma (30–45Hz) band. Behavioural changes were measured pre- and post-neurofeedback data using the parent reported questionnaire: Aberrant Behaviour Checklist (ABC). After 18 weekly NFT sessions the authors reported a linear decrease of theta/beta ratio parallel to a linear increase of the relative power of gamma activity. Additionally, authors, reported correlations of the EEG findings to the behavioural measures. The subscale of the ABC Lethargy/Social Withdrawal correlated positively with relative gamma power changes whilst correlating negatively with changes in the theta/low beta and theta/high beta ratios.

Case studies with NFT and ASD are more variable in the selection of frequencies to be trained as they are usually tailored to the patient's individual EEG findings. For example, Karimi, Haghshenas, and Rostami, (2011) combined NFT aimed to increase the 4-7Hz band with speech therapy and reported improvement in signs and symptoms of the patient as well as changes to their EEG. Coben and Padolsky (2016) delivered NFT to a group of 37 participants over the course of 20 sessions, providing an unique NFT protocol according to individual EEG findings in the pre-assessment. Training bands ranged from 5-16 Hz combined with delta and beta inhibition based on a case by case assessment. Although both of these studies report improvement of symptoms and changes in EEG activity as a result of the NFT, their results bring to focus one of the challenges in NFT methodology, namely, the generalization of protocols that can serve to prove the efficacy and objectiveness of this neuromodulation method.

As mentioned by Holtmann et al. (2011) mu rhythms have also been among the focal frequencies trained in NFT protocols involving autism. This is due to multiple reports of differences in mu reactivity between neurotypical individuals and individuals with ASD (Bernier et al., 2014; Cooper et al., 2013; Fan et al., 2014; García Vite et al., 2018; Oberman et al., 2005; Ruysschaert et al., 2014). As was covered in chapter 1, strong associations between action observation, mu activity and social abilities have been described in neurotypical individuals and seem to be reduced in individuals with ASD who have social difficulties (Hauswald et al., 2013; Oberman et al., 2005, 2008). Mu rhythms have been proposed to reflect a downstream modulation of motor neurons in the premotor cortex, of which some have been identified as mirror neurons (Bernier, Dawson, Webb, & Murias, 2007). However, pyramidal tract neurons with mirror-like properties responding to action observation have also been observed in the primary motor cortex as well as the ventral premotor area (Vigneswaran et al., 2013). Given that mirror neuron studies done in monkeys cannot be replicated non-invasively in humans, this rhythm is taken as an index of mirror neuron

activity and is therefore instrumental in the study of ASD as part of the dysfunction in the mirror neuron system theory that has been proposed as a possible explanation for some of the abnormalities in social skills in those with ASD (Angelini et al., 2018; Bernier et al., 2014; Fründt et al., 2018; H. Jeon & Lee, 2018; Lepage & Théoret, 2006).

Two electrophysiological studies conducted by Pineda et al. (2008) tested the hypothesis that operant conditioning of mu rhythms via neurofeedback training can renormalize (increase) mu suppression and improve behaviour in children diagnosed with ASD. Their results showed decreases in amplitude but increases in phase coherence in mu rhythms and normalization of mu rhythm suppression when comparing experimental and placebo groups. The authors also report improvement in behaviours involving attention as well as behaviours observed by parents as assessed by different questionnaires. They conclude that training the mu rhythm can be effective in producing changes in EEG and behaviour in high-functioning ASD children, but that the effects do not necessarily impact imitation behaviour. Although these studies support the use of NFT as an effective method for inducing functional changes to neural networks, it is of notice that the behavioural assessments include questionnaires based on parental observations.

Further research involving the modulation of frequencies associated with autistics traits and differences in low beta during event related desynchronization (ERD) reactivity during hand action versus static hand observation, found that the EEG markers of mirror neuron system activation may differ between the different groups. Repetitive transcranial magnetic stimulation (rTMS) was applied to the inferior parietal lobe in order to modulate EEG sensorimotor reactivity induced by hand movement observation, where the results indicated that the modulation differed according to the degree of self-reported traits of autism (Puzzo, Cooper, Cantarella, Fitzgerald, & Russo, 2013), in this study however, no significant difference was found in participants with high autistic traits

in low beta sensorimotor reactivity between active and sham rTMS during static hand or hand movement observation.

According to Budzynski, Evans, & Abarbanel (2009), the broad range of applicability of modes of neuromodulation suggest that good brain function depends on tight constraints in the timing of neuronal information exchange, and that the failure of precision in the domain of timing and frequency represents the dominant failure mode of the central nervous system, accounting for much of mental dysfunction. This outlook supports NFT as a therapeutic alternative for regulating neural networks. However, there is also much work to be done in terms of scientific assessment and validation of the technique with ASD as there is great heterogeneity in methodologies used both in clinical and research trial. Due to the longitudinal nature of neurofeedback, most studies work with small samples that, in some cases do not include control groups. Additionally, pre-post assessments are often based on third party reports of symptoms (mainly parent/tutors). There is a need for further investigation within this field with control groups, physiological assessment of neurofeedback training (NFT) success and homogeneity in NFT protocol across all participants in order to elucidate the effectivity of this method in the modulation of autistic traits that cause difficulties in social interaction as well as the functioning of the neural substrates that underlie it.

As was covered in chapter 1, when talking about NFT, the designation of frequency band to be trained as well as the threshold for feedback is crucial. Literature has described interindividual differences when referring to frequency bands such as alpha and theta (Bazanova & Aftanas, 2006; Bazanova & Vernon, 2014; Klimesch, 1999). In the case of alpha, the peak alpha frequency (PAF) has also been shown to reflect individual differences that have been correlated to different cognitive abilities and disorders (Angelakis et al., 2004), among which ASD is included (Dickinson et al., 2018). This makes sense given that the alpha band has been associated to the

cognition of typical development (Klimesch et al., 2007). The use of alpha peak frequency for neurofeedback has also been associated to improvement in neurofeedback efficiency as it would affect the frequency limit of the bands to be trained (Bazanova & Aftanas, 2006; Mierau et al., 2017).

With this in mind, we proposed the use of 3 physiological measures in order to assess the success of the NFT: Pre/post EEG and eye-tracking using a protocol associated to the EEG index of the mirror neuron system (see chapter 1) as well as a measure of haemodynamic changes throughout the NFT using near-infrared spectroscopy (NIRS). In addition to the psychophysiological assessment, our sample was divided into subgroups of neurofeedback training and a sham training condition. All of these measures are non-invasive and provide objective information of changes (if any) produced by the NFT.

The EEG protocol selected for pre and post assessment was a result of the findings in chapter 3, in which the Social Response protocol yielded a negative correlation between autistic traits and ERD in low alpha on the right hemisphere. As this was measured over the sensorimotor cortex we chose to train mu over C4 for this study with the rationale that by increasing the power of mu through NFT this would also translate to an increase in the suppression of mu by increasing the neuronal population firing synchronously under this rhythm (Mierau et al., 2017). Training the motor cortex on the right hemisphere will improve the encoding of physical and cognitive tasks (Moore et al., 2012; Ozonoff & Miller, 1996). As far adding a measure of cortical haemodynamic changes, we chose NIRS as a non-invasive and reliable method of monitoring brain oxygenation from the cortical microcirculation blood vessels (Quaresima & Ferrari, 2019). The premise of this method is that the demand for oxygen in the brain is altered by neuronal activation. With the use of infrared light that is transmitted into the cortical area of interest, NIRS can provide a measure

of brain activation in the difference of light detected between a transmitter and receptor (optodes). The measures provided by NIRS are oxy- (O2Hb) and deoxy-haemoglobin (HHb) concentrations. Because human tissue is semi-transparent to light, allowing protons to spread, haemoglobin has unique absorbing characteristics that affect how light is absorbed in the tissue when it is projected through it. Less light detected by the receiver optode will translate to higher cortical activity as indicated by higher levels of oxy-haemoglobin (Tempest et al., 2014). Another measure to ensure objectivity in methodology and results was to include a sham group in addition to the training group that underwent the same number of training sessions.

The study aims to investigate the possibility that training healthy individuals with a high degree of autistic traits to enhance their mu rhythm (based on their individual alpha peak over right central areas) in their EEG via a neurofeedback protocol could influence their sensorimotor reactivity. As the mu rhythm spans both the alpha and low beta frequency bands (R Hari & Salmelin, 1997), we expected to find significant changes in the relative and absolute power of alpha and low beta over central areas in the resting state EEG between pre and post assessment time in the training group . We also expected that the training group would have an increase in relative and absolute power of mu over central areas between pre- and post-assessment, whilst the sham group would not show significant changes. Overall suppression of mu would also be expected to have an increase in the training group over central areas between pre and post assessment whilst the sham group would not show significant changes. Furthermore, based on the findings of Klimesch et al., (2007)if participants in the training group could increase mu power post-training, we expected them to also significantly increase mu event-related desynchronization (ERD) to the EEG mu ERD protocol in the low sensorimotor alpha and low beta band from the pre to the post assessment time whilst the sham group would not show significant changes to their mu ERD. When exploring changes during NFT sessions, we expected changes in the relative power of mu across sessions of the training

group as well as an increase in the suppression of mu whilst the sham group would not show significant changes.

In terms of the pre and post eye-tracking measures, we expected the training group to have significant differences in trial and interest area fixation count, dwell time, average fixation pupil size, and average saccade amplitude from the pre to the post assessment time whilst the sham group will not show significant changes. For NIRS measures taken throughout the NFT sessions, we expected to find an increase in oxy-haemoglobin between first and last sessions over C4 in the training group but not in the sham group. The results of this study could shed light on further understanding the neural substrates of the human mirror neuron system as well as offer further evidence on the effects of neurofeedback on autistic traits.

4.2. Methods

4.2.1. Pre-Screening

The Autism-Spectrum Quotient (AQ) is a self-administered short scale that measures the degree to which an adult of normal IQ exhibits traits associated with the autistic spectrum. The participant scores a point for each answer that resembles an autistic answer. Higher scores equal a higher level of autistic traits. It is comprised of 50 items that are divided into 5 different areas: social skills, attention switching, and attention to detail, communication, and imagination (Simon Baron-Cohen et al., 2001). The AQ has proven to be suitable for screening purposes in research and clinical settings due to the high internal consistency and reliability across different samples (Baron-Cohen et al., 2001; Hurst, Mitchell, Kimbrel, Kwapil, & Nelson-Gray, 2007; Wakabayashi, Baron-Cohen, & Wheelwright, 2006; Wakabayashi, Baron-Cohen, Wheelwright, et al., 2006). The purpose of

administering these scales over a large sample of the general population between 18 and 56 years old was to identify the sample to undergo NFT.

4.2.2. Participants

5000 participants were initially contacted via e-mail, through the university's online research participation system (SONA) and word of mouth to be screened through an online Qualtrics survey (REF for Qualtrics) which contained Autism-Spectrum Quotient (AQ). The survey was answered over a period of 5 months (October 2018-Feb 2019) by a total of 414 people. From this sample 20 participants with high AQ scores were invited to take part in the study. The high group scores were selected based on experiment 1 (AQ ≥24). As mentioned in the previous chapter, these scores also correspond to the extreme of the normal distribution described by Baron-Cohen, Wheelwright, Skinner, Martin, & Clubley, (2001), that is to say, although higher than the normal population, these scores are not considered as reflecting a diagnosis of ASD. Over the course of the study, 3 participants decided to withdraw from the study, which resulted in a final sample of 17 participants (9 female, 8 male) which were randomly allocated to either a sham or training group (9 training vs 8 sham). The overall sample had a mean age of 21.7 years (SD 2.9) Training group was comprised of 5 females and 4 males (mean age 22.4 ± 3.9) vs 4 females and 4 males in the sham group (mean age 21 ± 1.6).

Participants were naïve to the group allocation.

The University of Essex Ethics Committee approved this study. All participants read, signed and informed consent prior to their participation. The procedures used in this study were non-invasive, at no time were the participants or the researcher at risk for their safety.

4.2.3. Stimuli

4.2.3.1. Pre-Post physiological assessments

The Social Response Protocol - involving goal directed observation with a social component protocol (Clausen et al., 2015) intended to elicit the EEG indexed hMNS during movement observation whilst adding a social response component. This protocol was designed to test a theory by Hamilton (2013) where it was proposed that an important purpose of the MNS is to respond, in real-time and in a socially appropriate fashion, to the actions of others. This protocol was chosen based on the results of chapter 3). A fixation cross was shown in the starting position on a black background before each video for 1000ms. The videos last 3000ms, in each one a female actress wearing black clothing in front of a black background is holding a white mug. There are three stimuli, one shows the actress moving the mug towards the viewer, in the second the mug is moved backwards away from the viewer, and in the third the video has no movement. In all three stimuli the actress' facial expression is neutral and the video is shown against a black background. The videos were showed in random order, repeated 30 times each (90 trials in total in 10 blocks of 9

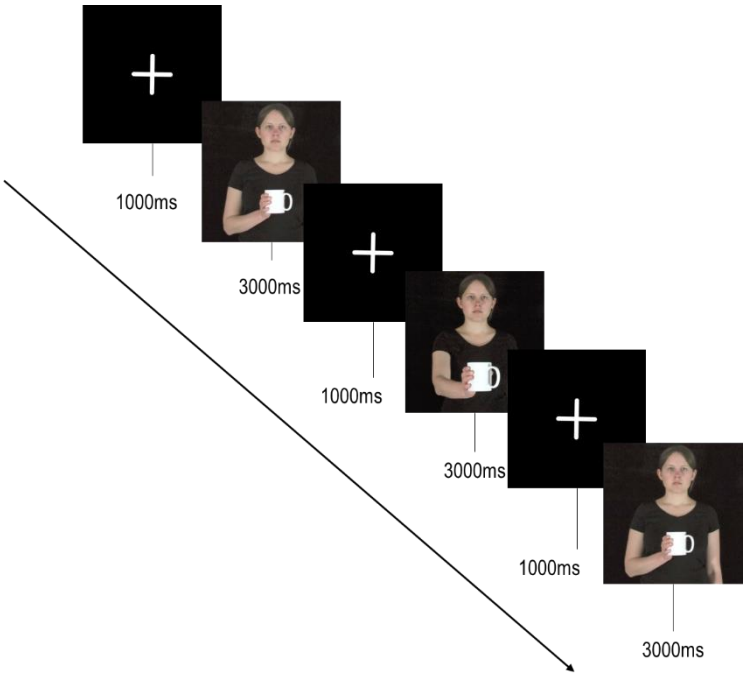

Fig 4.1. Experimental procedure for the Social Response Protocol

trials each). Between each trial a fixation cross appeared in the starting position on a black background for a randomized period of 1000-2000ms in order to avoid expectancy effects (see figure 4.1).

In addition to the stimuli protocols, resting state EEG was recorded for 2 minutes while the participant was at rest with eyes closed; as well as an electrooculogram (EOG) calibration task was used to form the basis for subsequent EOG artefact reduction for the EEG (Croft & Barry, 1998).

All stimuli were constructed on the SR Research Experiment Builder (Version 1.6.12) and presented on the Eyelink 1000 Eye-tracker (Mississauga, Ontario, Canada) an infrared video-based eye-tracking device. Stimuli were presented on a 1027 x 768 pixel screen 60cm away from the participant, this equals to a visual angle of 25° 31' 0.06" (measure of the size of the object's image on the retina (Swearer, 2011)). The triggers were sent to the EEG Neuroscan 4.4 acquisition software and Synamps II amplifiers through a parallel port while the protocols were displayed. Data for the EEG and eye-tracker were recorded simultaneously.

4.2.4. Experimental design

Blind factorial experimental design with between and within factors.

4.2.4.1. EEG

This was a mixed measures design. Independent variables (IV) were assessment time (pre and post), stimuli (backward, forward, and still) hemisphere (right and left) and group (sham and training). Dependent variables (DV) were sensorimotor alpha and low beta event related desynchronization (ERD) values. Within-subjects factors were assessment time, stimuli and hemisphere and between-subjects variable was NFT group. The specific design and levels for each factor will be explained further in the results section. To control for attention, trial fixation count was recorded.

4.2.4.2. Eye-tracking

This was also a mixed measures design. Within-subjects factors were assessment time, stimuli and interest area (IA). Between subjects factors was group (Sham and Training). DVs and IA were as follows:

The interest areas were eyes, face, mouth, and cup (see figure 4.2). Interest areas mouth and eyes overlapped with face. DVs were trial and interest area fixation count, dwell time, average fixation pupil size, and average saccade amplitude.

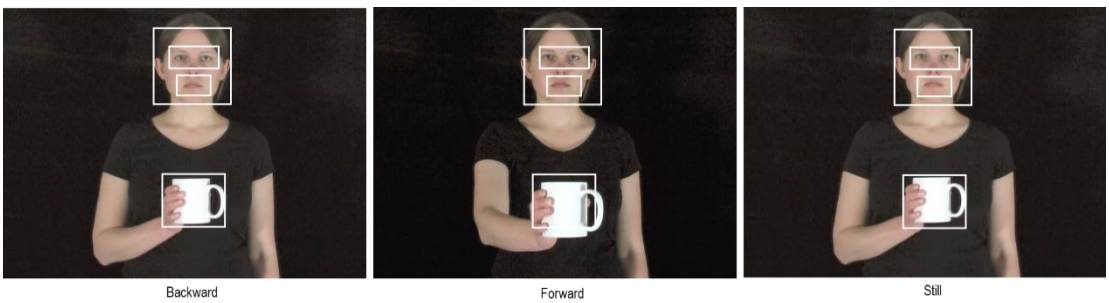

Fig 4.2. Interest areas for social response protocol across all stimuli. From left to right: “backward”, “forward” and “still”.

4.2.4.3. Neurofeedback sessions

Neurofeedback and sham sessions were done using a the 10-channel system Nexus-10 MKII (Mind-Media BV, Herten, The Netherlands) as well as the BioTrace+ software (MindMedia BV). In both groups the channel set-up was as follows: feedback EEG activity was taken from C4; this signal was referenced to the right earlobe. The ground electrode was placed at FPz. In order to control for movement activity that could affect the feedback electrode, an additional electrode was placed over the zygomaticus muscle and referenced to the left mastoid (M1) to record the electromyogram (EMG).

In addition to the EEG and EMG, cerebral haemodynamic was measured for all participants using a multi-channel NIRS (Oxymon III, Artinis Medical Systems, Zetten, the Netherlands). Optodes (one transmitter with its corresponding receiver) were secured into an EEG cap placed at 15mm on either side of C4 (30mm between optodes) in order to monitor concentration fluctuations of changes (Δ) in oxygenation (ΔO_2Hb) and blood volume (total haemoglobin Δ[tHb] defined as Δ[O2Hb] + Δ[HHb]. Sampling rate was 10Hz and recording lasted the whole of the NFT session (30 minutes).

During the NFT sessions all participants were placed in a soundproof booth where they were connected to both the NFT and the NIRS equipment. Light was turned off in the booth at the beginning of the session in order to avoid external light that could affect optode measurement.
Once all sensors had been calibrated, the NFT session began. For the training condition, feedback was delivered in the form of a video game similar to the classic “Space invaders” format. The threshold was determined according to each participant’s individual alpha peak frequency (as determined by the pre-EEG assessment). Feedback was delivered from the electrode placed over

C4 within the alpha frequency band (8-12Hz) approximately 2µv above their alpha peak frequency amplitude (M=9.7µv). When the participant reached and maintained their threshold for a minimum period of 250ms participants received 1 reward point, making the game move and the spaceship shoot towards the invading spaceships. For every 250ms maintained on or over the threshold, participants would get an additional point. When participants' activity over C4 went below the threshold all feedback would cease and the game would freeze. Feedback was also determined by the EMG activity being recorded over C4 and the zygomaticus muscle. When the voltage over these sensors was equal or higher than that of the EEG activity over C4, all feedback would cease. This ensured that muscle artefact or voluntary muscular activity by the participant would not mask progress in the feedback (see figure 4.3). Threshold was fixed for each participant throughout the 8 sessions,

Fig. 4.3. NFT session screens. a) screens viewed by the participant b) experimenter's screen with thresholds for EEG-blue, EMG (C4-red and on the zygomaticus muscle-orange).

In the sham condition, participants were presented with a video of a feedback session from another participant and received no feedback from their own brain activity. Nevertheless, their EEG was recorded from the same channels as the NFT group. At the end of each session participants were instructed to sit still for 5 minutes in order to obtain a resting period reading for the NIRS signal.

4.2.5. Procedure

20 participants were selected from the sample of 414 people that answered the Qualtrics survey online and invited take part in the study (see above). All participants read and signed a consent form in order to take part of the experiment.

Participants underwent 8 sessions that each lasted for 45 minutes (including the placement of sensors). In addition to this, all participants underwent pre and post psychophysiological assessments during a protocol intended to produce the EEG indexed hMNS during movement observation. Thus we used the Social Response protocol (Clausen et al., 2015) that was used in previous experiments (see chapter 3) while taking simultaneous EEG and eye-tracking measures.

Immediately after initial EEG/eye-tracking measures, each participant's alpha peak was taken by performing an average of their baseline resting state EEG. The target frequency broad band for NFT was alpha.

Participants were divided randomly into two groups to undergo either sham training or NFT. All participants were naïve as to which group they were placed in.

For an overview of the procedure can be found in figure 4.4.

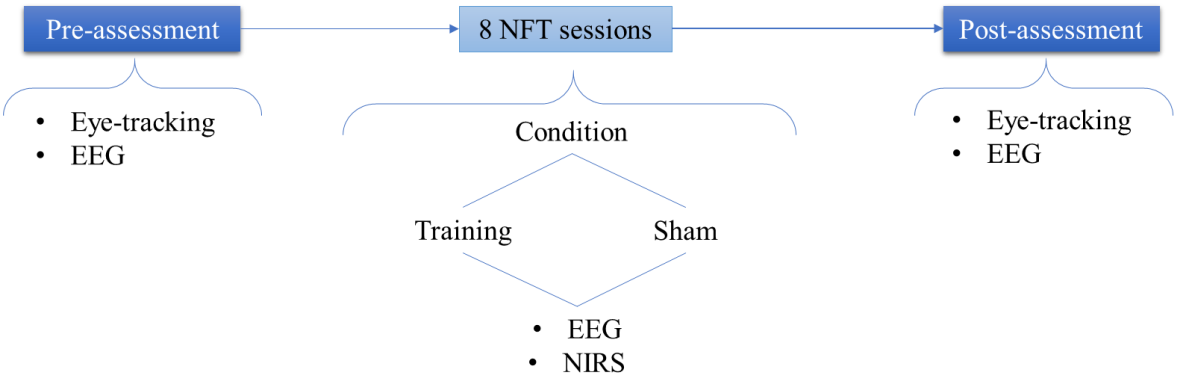

Fig. 4.4 Overview of the procedure.

4.2.5.1. EEG data preparation

For the pre and post physiological assessment, and REEG, following visual inspection of the data, noisy data blocks were rejected. Pre-processing was carried out using Neuroscan edit (Compumedics, Australia). Bad electrodes were excluded on a subject by subject basis. Principal components analysis was performed on the acquired eye movement data to obtain components reflecting saccades and blinks. To carry out ocular artefact rejection, the acquired components were subsequently rejected from the task data traces (Vigario, 1997; Vigario et al., 2000).

The data were then re-referenced to the average of the scalp electrodes, epoched from -2000 to 4000ms and around the start of each video and then an automatic artefact rejection algorithm was applied (+/- 100mV). For the calculation of ERD/S the following steps were carried out: the data underwent complex demodulation and concurrent filtering (zero phase-shift, 24dB roll-off, envelope computed) into the bandwidths low (8-10 Hz) and higher alpha (10-12 Hz) as well as low beta (12-20 Hz): the data was trimmed (1000ms from each side) to remove filter artefacts and averaged. A reference interval of -1000 to 0ms was used to calculate the percentage change between the active period (0 to 3000ms) and the reference using the classic method adapted from

Pfurtscheller and colleagues (e.g. 1977, 1999): ERD% = (R - A)/R*100, where R = the reference interval and A = the active or task phase. Thus, desynchronization and synchronisation are expressed as a percentage of activity relative to the reference interval (as a result, this formula ERD produces positive scores and ERS negative). ERD/S data contain both phase-locked and non-phase-locked activity.

For the NFT sessions, each session was epoched into six five-minute segments. Each segment was first bandpass filtered (0.5 – 45) in EEGlab using digital FIR order 2000. Continuous artefact detection was specified at a moving window width of 500ms and a window step parameter of 250ms checking for activity that exceeded the voltage threshold of -50:50. Segments separated by less than 500ms were joined and artefacts shorter than 100ms were not considered as artefacts.

4.2.5.2. NIRS Data preparation

For the NIRS data, files were pre-processed using the Fieldtrip software toolbox (Oostenveld et al., 2011) on MATLAB. Motion artefacts were identified and removed by means of thresholding the z-transformed value of the raw data. Threshold z-value for motion artefacts was 3.5. Once they were removed, the raw data from the optodes that reflected optical density of light was transformed to oxygenated and deoxygenated haemoglobin concentrations. Differential path factor was set at 5.9 in accordance to Scholkmann and Wolf's (2013) general equation based on wavelength and age of the participants. Bandpass filter (0.01 to 0.2) was applied in order to filter out other systemic responses as well as slow drifts. Remaining data was epoched into segments of 300 seconds that resulted in 3 epochs of 5 minutes each.

4.3. Results

4.3.1. Pre and post EEG analysis

4.3.1.1. Social response protocol

Trials were divided into three time frames for separate analyses (early, mid and late). Time frames were as follows:

- Early: 0-1000ms
- Mid: 1000-2000ms
- Late: 2000-3000ms

For the factorial design the electrodes were collapsed across hemispheres resulting in a 2 x 3 x 2 x 2 (assessment time x stimuli x hemispheres x group) design with three within subjects factors: assessment time (pre NFT vs post NFT), stimuli (still, moving and balls) and hemisphere (left and right) and one between subjects factor: NFT group (Active vs Sham). Event-related desynchronization for bands alpha (7.812-12.695 Hz), low alpha (7.812-9.765Hz), high alpha (9.765-12.695) and low beta (12.695-19.531Hz) as a measure of mu rhythm desynchronization were the dependent variables. This analysis was done for electrodes over Frontocentral (FC), Central (C) and Centroparietal (CP) areas.

Although there were some significant interactions and main effects, there were no significant findings in line with the hypotheses of this experiment in any of the time periods.

With this in mind, a 2 x 3 x 2 (assessment time x stimuli x group) mixed factorial design was performed on just electrode C4 in all time frames (early, mid and late) in order to assess the specific region where participants received feedback during the sessions. For this design there were two

within subjects' factors: assessment time (pre NFT vs post NFT), and stimuli (still, moving and balls) and one between subjects' factor: NFT group (Active vs Sham).

Significant results were found only in the early time frame. A significant between groups effect [F (1) = 7.167, p= .032)] revealed that overall, the active NFT group had a higher ERD than the Sham group in high alpha. Furthermore, a significant interaction between condition, stimuli and group was observed [F (1.51) = 4.462, p = .047]. To correct for a Type I error, Bonferroni corrections were conducted for this interaction and provided a new p value of p<.008. Subsequent post-hoc tests revealed a significant difference in the post condition, backward stimuli [t (10) = 3.656, p = .004] the training condition had greater high alpha ERD than the sham condition after the NFT sessions had concluded (see figure 4.5). A further paired-samples t-test for condition in the backwards stimuli of the training group showed a trend towards significance [t(15)=1.966, p=.068].

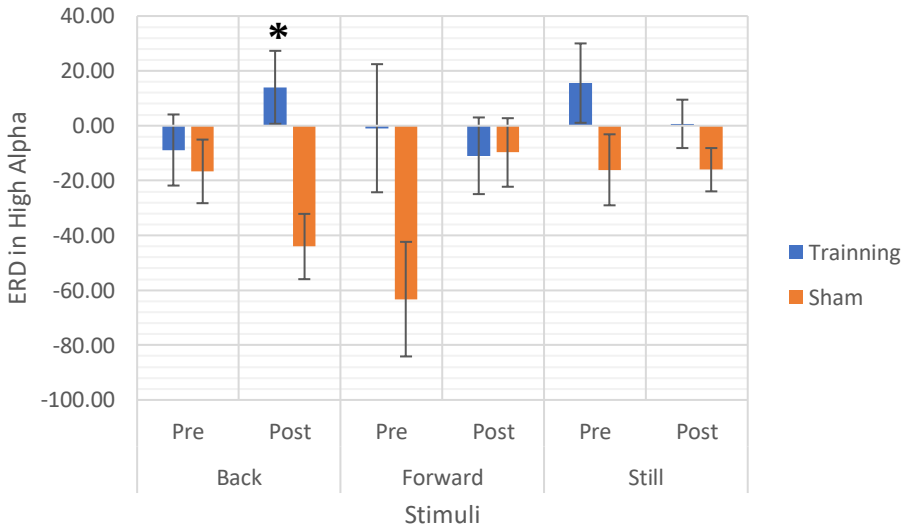

Figure 4.5 Significant interaction between condition, stimuli and group for ERD in High Alpha [F (1.51) = 4.462, p = .047]. Post-hoc test revealed significant differences in Post condition, Backward stimuli where the training condition had greater high alpha ERD than the sham.

4.3.2. Resting state EEG

Microstates analysis of the mu rhythm during pre- and post-NFT resting state EEG was performed using the python toolbox YASA (https://raphaelvallat.com/yasa/build/html/index.html) . Parameters for detection of mu were as follow: frequency from 8-13Hz, duration of 0.5 second minimum and 10 seconds as a maximum. We obtained values of mean amplitude, mean absolute power, mean relative power and mean frequency of the identified mu rhythm events. Electrodes were collapsed across central and centroparietal areas and 2 x 2 x 2 (assessment time x hemisphere x group) mixed factorial design analyses were performed for each of the variables. None of the variables yielded significant results (maximum F= 2.612, p=.130).

A 2 x 2 x 2 (assessment time x hemisphere x group) mixed factorial design was also performed for the relative power in a frequency band in relation to a percentage of the total power of the signal and the absolute power (µV2) of each of the bands: delta (.05- 4 hz), theta (4-8 hz) alpha (8-13 hz), low beta (13-30 hz) and gamma (30-45 hz). For this design there were two within subjects' factors: assessment time (pre NFT vs post NFT), and hemisphere (right vs left) and one between subjects' factor: NFT group (active vs Sham). Significant interactions were found for assessment time x hemisphere x group for relative power in gamma over central areas [F (1) =7.468, = .015]. The same significant interaction was found for absolute power in theta also over central areas [F (1) =5.113, p = .039]. However, post-hoc tests did not reveal any specific further findings for either case.

Furthermore, a 2 x 2 x2 (assessment time x stimuli x group) mixed factorial design was performed on just electrode C4 for the relative power in a frequency band as a percentage of the total power

of the signal and the absolute power (μV^2) of each of the EEG bands in order to assess the specific scalp area where participants received feedback during the sessions . For this design there were two within subjects' factors: assessment time (pre NFT vs post NFT), and stimuli (still, moving and balls) and one between subjects' factor: NFT group (active vs Sham). No significant findings were revealed (maximum F= 2.173, p = .151).

4.3.3. Neurofeedback sessions

4.3.3.1. EEG data

As mentioned in the EEG data section for the analysis of the neurofeedback sessions, NFT EEG data were obtained from electrode C4. In order to assess the effect of NFT, the first and last sessions were analysed. Each session was divided into six five-minute epochs. Epochs were analysed using the python toolbox YASA (https://raphaelvallat.com/yasa/build/html/index.html) to detect mu rhythm as an EEG microstructure event as well as obtaining absolute power for the channel. Parameters for detection of mu were as follow: frequency from 8-13Hz, duration of 0.5 second minimum and 10 seconds as a maximum. Event count, duration, amplitude and frequency for each of the mu rhythm events were obtained. Absolute power (µV2) of the epoch was also retrieved for Alpha (8-13hz), Alpha Low (8-10Hz), Alpha High (10-13Hz).

For the microstructure analysis, a 2 x 6 x 2 (session x epoch x group) mixed factorial design where session (first vs last) and epoch (1-6) were within subjects' factors and group (active training vs sham) was the between subjects' factor. Although there were some significant interactions and main effects, there were no significant findings pertaining to the hypotheses of this experiment (maximum F=2.064, p=.185).

Absolute power for alpha, alpha low, alpha high was analysed with Mann-Whitney tests given that these variables did not have a normal distribution. There were significant findings in high alpha for session 8: epoch 1 and 2 revealed greater absolute power for training (11.56 and 11.44) over sham group (6.13 and 6.25). U= 13, p= .027 and U=14, p = .036 for epoch 1 and 2 respectively (Fig 4.6).

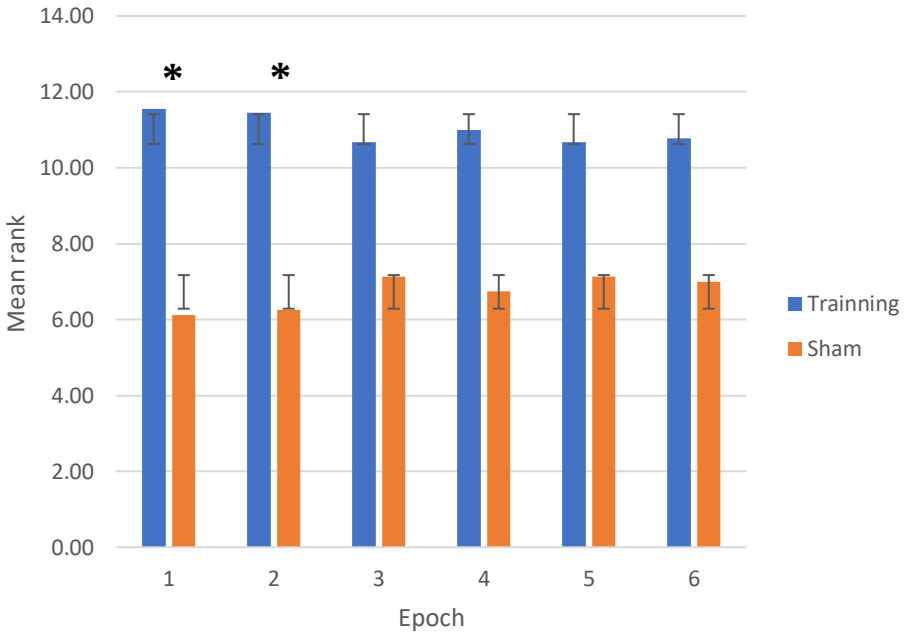

Fig 4.6 Mean ranks for absolute power in high alpha between groups during session 8.

4.3.3.2. NIRS

As there was no specific timing of stimuli during the NFT sessions but rather active NFT /sham presentation during the whole session, after the data was cleaned of artefacts and filtered, three 5-minute epochs were created for each NFT session. We did not analyse and compare effects across 8 sessions because the resulting model had insufficient residual degrees of freedom, meaning that more participants would be needed to make those comparisons. This was addressed by analysing the first and last sessions only, in order to assess the effect of NFT training. This was done by using a 2 x 3 x 2 (session x epoch x group) mixed factorial design where session (first vs last) and epoch (1, 2, or 3) were within subjects factors and group (active training vs sham) was the between

subjects factor. We opted for this approach, used extensively in previous fNIRS analyses (Tak & Ye, 2014), because our main interest was to reflect differences in activation between sham and active training groups at the beginning and the end of the NFT protocol. The dependent variables of interest were absolute values of oxygenated O_2Hb haemoglobin and blood volume (total haemoglobin Δ[tHb] defined as Δ[O2Hb] + Δ[HHb]. Independent variables were NFT group (training vs sham).

Although there were some significant interactions and main effects, there were no significant findings pertaining to the hypotheses of this experiment in any of the time periods (maximum F=.102, p= 3.101).

Further to group difference analysis, absolute values of oxygenated O_2Hb haemoglobin and blood volume (total haemoglobin [tHb]) per session were correlated to EEG activity per session across all participants. Significant correlations for O_2Hb during session 8 showed a positively moderate to strong correlation with alpha (8-12hz) [r (17)=.545, p= .024]; low alpha (7-10Hz) [r (17)= .546, p=.023]; high alpha (10-12Hz) [r (17)= .546, p=.023]; low beta (13-21 Hz) [r (17)= .496, p=.043]. These results indicate that higher levels of O_2Hb indicate higher activation in these bands over C4; particularly in the low alpha band, where the correlation was the strongest.

4.3.4. Eye-tracking

Eye-tracking variables were analysed as follows:

A 2 x 3 x 4 x 2 (assessment time x stimuli x interest area x group) mixed factorial design was conducted for fixation count and dwell time. For average saccade amplitude and trial fixation count a 2 x 3 x 2 (assessment time x stimuli x group) mixed factorial design was completed. For pupil

size, a 2 x 2 x 2 (assessment time x stimuli x group) mixed factorial design was performed. Within subjects' factors across analyses were assessment time (pre vs post), stimuli (Backwards, Forwards, Still) and interest area (mouth, eyes, face, cup); the between subjects' factor was group (training vs sham). For pupil size, within subject factor stimuli only had 2 levels (forward vs backward) as this refers to change in the diameter of the pupil from a baseline (in this case the still condition) to the stimuli (forward vs backward movement).

Interest area fixation count

Trial fixation count refers to the number of times a fixation appears within a certain interest area (SR Research, 2007) and was of interest in this protocol in order to determine where participants focused their attention and if there were differences between groups in attention paid to the different interest areas. A significant main effect of assessment time was found [$F(1)= 7.996$, $p=.013$] and stimuli [$F(1)= 8.494$, $p=.011$]. Significant interaction between assessment time and stimuli [$F(1)= 8.660$, $p=.010$]; assessment time x interest area [$F(1.41)= 5.556$, $p=.019$]; stimuli x interest area [$F(1.39)= 5.30$, $p=.022$] and assessment time x stimuli x interest area [$F(1)= 7.996$, $p=.013$]. However, there were no significant findings between groups, so no further analysis was taken on this variable.

Dwell time

Dwell time refers to the total time (in milliseconds) that the participant spent on a specific interest area (SR Research, 2007). This variable is of interest given that the stimuli includes a social component, group differences were of interest as well as specific interest areas in order to determine weight of attention given to the different parts of the stimuli. A significant main effect

of stimuli [F (1.04) = 6.379, p=.022] and interest area [F (1.433) = 17.201, p< .000] was observed. Furthermore, a significant interaction between stimuli and interest area [F (1.284) = 4.119, p=.048] and assessment time x stimuli x interest area [F (1.656) = 8.364, p=.003] was found. However, there were no significant findings between groups, so no further analysis was taken on this variable.

Average saccade amplitude

Saccade amplitude refers to the angular distance that the eye covers during a movement (in degrees of visual angle) (Bahill et al., 1975). There were no significant findings.

Trial fixation count

Trial fixation count refers to the number of times a fixation appears within a certain interest area (SR Research, 2007) and was of interest in this protocol in order to determine where participants focused their attention and if there were differences between groups in attention paid to the stimuli that could cause a bias in the EEG results. No significant differences were found.

Pupil size difference

Refers to changes in the diameter of the pupil as a possible indication of cognitive load (Krejtz et al., 2018; Kret & Sjak-shie, 2018). No significant changes were found.

As a consequence of the lack of group differences in any of the eye-tracking measures, we can be reassured that any differences found in oscillatory activity or blood oxygen levels between groups are not due to the visual behaviour of the participants.

4.4. Discussion

The study aimed to investigate if a neurofeedback protocol training individual alpha peaks over C4 could lead to an increase in mu rhythm suppression in healthy individuals with a high degree of autistic traits. Physiological measures assessed in this study were:
EEG sensorimotor activity and eye-tracking variables before and after the NFT, as well as their EEG activity and blood oxygen level dependent response throughout the NFT sessions.

Overall, we had three significant findings:

1) A significant group difference in absolute band power for high alpha (10-13Hz) during the start of the last NFT session. The active NFT group had a greater high alpha band over the sham group. This is in line with the goal of the NFT protocol as the participants were training to increase their amplitude within the alpha band over the right central area. Nevertheless, the purpose of increasing the synchronicity of neural populations within the alpha band was in the hope that this could also drive an increase of mu suppression the post-training EEG assessment (i.e. the Social Response protocol). This was found to be the case for the backward condition of the post Social response protocol (see below). This result should be taken carefully as the differences found were only during the last session and not between the first and last session, which would undoubtedly have provided greater evidence for the effect of NFT on the training group.

2) A positive moderate to strong correlation between the absolute band power in the EEG and NIRS in session 8 for oxygenated haemoglobin O_2Hb in low alpha (7-10Hz), high alpha (10-12Hz), broadband alpha (8-12hz), and low beta (13-21 Hz) indicating that the increase in absolute band power in these bands over C4 is associated with higher levels of O_2Hb in that same area.

3) A significant difference between groups in the pre/post-NFT Social response protocol. Specifically, greater ERD in the high alpha band (9-12Hz) during the backward stimuli was observed in the post-NFT assessment time for the active NFT group compared to the sham group. As the groups did not have any statistical differences in ERD before the NFT protocol, this result suggests that the increase in ERD in the high alpha band over C4 may result from the NFT protocol. However, when comparing the pre and post conditions in the backwards stimuli for the training group, the p value did not reach a significant value ($p=.068$). This could be attributed to the size of the sample, but further studies will need to be done with larger sample sizes in order to ascertain that the significant difference found between groups is due to the effect of NFT. Nonetheless, this should be taken into account and the effect of NFT as an outcome, should be also taken with caution.

The significant group differences for absolute high alpha power and its positive correlation to O_2Hb during the last NFT session suggest a cortical activation of the area that was trained.

This would in turn advocate that the NFT did indeed influence the mu rhythm as there were changes to the absolute power of high alpha (which is one of the frequency components of the mu rhythm) and that this is associated to a higher metabolic activity in the area as reflected by higher levels of O_2Hb. However, these changes were not manifested in changes of suppression of mu within social contexts. At least, not in the manner that we had hypothesised. We expected to find

differences in the forward condition of the post NFT assessment as it is this condition that is associated to a social response (the person on screen moves a mug with the handle towards the participant, seemingly offering them the mug). Nonetheless it was in the backward condition where the group difference was identified (i.e. where the mug is being withdrawn from the observer). One possible explanation for this, is that there was an effect of the NFT protocol on the pyramidal tract neurons with mirror-like properties. These neurons respond to action observation and have also been observed in the primary motor cortex (Vigneswaran et al., 2013). And so, in this case perhaps the choice of the pre- and post-NFT assessment stimuli (Social Responding protocol) was a limitation and a more specific action-observation stimuli (such as the multiple repetitions mu protocol described in chapter 3) would have been more tailored to evaluating a change (if any) in the suppression of mu between assessment times and among groups. In previous research with NFT, mu activity and ASD, the evaluation of social symptoms is usually done through parent/guardian observations and the EEG is analysed using protocols more attuned to the multiple repetitions protocol including action observation and, in some cases, action execution (LaMarca et al., 2018; Pineda et al., 2008; Pineda et al., 2014).

A second explanation, could be found in Kosonogov's (2012) view on the role of mirror neurons. The author proposes that the system first evaluates actions outside of the mirror neuron system in order to determine if the action observed is relevant to the observer and if this is the case, it is stored to the individual's archives of actions for future use. The action will then be reproduced implicitly through the mirror neurons. From this perspective and based on our findings, we could hypothesize that from an evolutionary standpoint something taken away from us (cup moving backward) is more relevant to the individual than something that is given to us (cup moving forward). When something is given to us, we can choose if we want to accept it, but it does not affect what we already have. On the contrary, something taken away from us could be interpreted

as more of a threat if it is something that could be critical to our survival (food or water). Giving the high level of AQ traits in the sample and the decreased interest in social activities typically associated to them, it could be proposed that in terms of relevance to actions there was a hierarchy to those more associated to biological needs (cup moving backward) before social ones (cup moving forward) and that although there was a change in mu activity after the NFT, this hierarchy was not modified regardless of the increase in mu response. This is also in line with Hamilton's (2013) social response theory of mirror neurons, where she proposes that the main function of mirror neurons is to respond by representing one's own action in a social situation rather than only mirror the action. What each person represents as their own action however, would invariably differ from person to person and could depend on the perceived relevance of said action observed to the individual as Kosonogov (2012) proposes. In the context of this study it is possible that the increase in mu rhythm activity as a result of the NFT reflected the representation of participants' own action, but the response was elicited according to the action (condition) that represented most relevance to them. Contrary to our hypothesis, no significant findings were found between groups in the resting EEG for pre- versus post-NFT. And this was also reflected in the eye-tracker variables where there were no findings in line with the hypothesis of the study. It is noteworthy that in the case of the eye-tracking variables, the lack of significant statistical differences between groups within each assessment time (before or after NFT), also suggests that there was no difference in the visual attention paid by either group to the stimuli that they were presented with on each occasion. Therefore, any EEG and NIRS differences that were observed between groups cannot be ascribed to simple differences in attention.

Significant findings were also missing from the NIRS and EEG analysis conducted on the first and last session in order to assess the effect of NFT training. However, these results could be explained by methodological limitations of the study which we will touch upon below.

Limitations and future directions.

Methodological limitations

Duration of active training during each session.

Due to the nature of the NFT stimuli (a videogame) participants were instructed to train continuously for the duration of the session, that is, 30 minutes. In retrospect, having a continuous session of NFT could have had an adverse effect on the attention and motivation of the participants and therefore affected their performance in the training itself. The significant group differences found only during the start of the last session (and lack thereafter) may be signalling a drop of motivation throughout the session due to the length of active NFT that the participants were expected to do. This aspect could also explain the absence of significant findings in the NIRS variables. Future studies should address this aspect by dividing the NFT effective training time into smaller training trials throughout the session (Collura, 2014).

Threshold

It is said that a truly successful NFT protocol is a science but also an art (Collura, 2014). What this means is that the level of individuality provided in a NFT protocol ensures success to the brain activity that is being trained. Some NFT protocols modify the threshold throughout the session in order to maintain a predefined percentage of successful brain activity (De Zambotti et al., 2012). However, the manner in which a threshold is modified and set for different participants can also depend on the experience of the NFT practitioner. Ioannides (2018) considers this aspect of NFT as a key clash with controlled neuroscience experiments. In NFT, the conditions can vary greatly from one session to the other or even within the same session. On the contrary, in neuroscience

experiments everything must be controlled and any deviation from the fixed protocol makes the experiment invalid.

Would this mean that in order to structure a valid NFT protocol one must deviate from controlled measures? We do not consider that to be the case, one aspect that this study offers to the existing literature on NFT are the measures of control that were included to enhance the methodological validity of the study. These were: the inclusion of a sham NFT group and relying on multiple physiological assessments pre/post NFT as well as throughout the training. Thresholds were set according to each participant's alpha peak and were fixed throughout the NFT sessions for all participants in the training group. However, the level of control could have played a part in the lack of additional findings across all physiological variables.

Thresholding in the field of NFT is still largely arbitrary, even when it is based on physiological/anatomical parameters Collura (2014). How much should a threshold be manipulated and what are the parameters that determine an optimal window of learning in an individual? If a threshold is adjusted excessively throughout the session it begs the question - is the neurofeedback training the participant or vice versa? In this study the threshold that participants were expected to reach and improve on was based on their alpha peak. As a group, the participants in active NFT had a mean threshold value of 9.7 µV. This threshold was fixed throughout the whole NFT protocol. However, many participants had difficulty achieving their thresholds and this, coupled with the duration of the NFT sessions could have explained the drop-in motivation and therefore the lack of large-scale changes in brain activity from pre to post assessment time in EEG, eye-tracking as well as the NIRS findings across sessions.

Further studies should focus on methodological aspects of NFT, in order to obtain guidelines for: the threshold of feedback and its modification throughout the protocol; the number of sessions required for the training to have lasting effects; the duration of each session. It is in these aspects where we find the most heterogeneity in the literature of NFT (Enriquez-Geppert et al., 2017; Friedrich et al., 2014; Holtmann et al., 2011b; Pineda et al., 2014; Rogala et al., 2016).

More research into this topic would also provide further insight into the psychophysiological basis of learning. NFT is in its essence teaching the brain about its own state at a given time/circumstance and how to modify it towards a goal that is beneficial to the individual. Deciphering and setting guidelines to strengthen the methodological basis of NFT protocols would also give rise to applications of NFT in predicting and enhancing the ability of those who undertake NFT to learn. By identifying individual physiological, anatomical and psychological factors that can prove to be an advantage or obstacle in NFT it could lead a path into the standardization of the construction of NFT protocols based on individual learnability (Alkoby et al., 2018; Reiner, Gruzelier, et al., 2018; Rogala et al., 2016).

As for limitations in the use of NIRS and eye-tracking, the lack of more significant findings is attributed to the limitations in the threshold and session duration described previously. It is important to note, that contrary to being a limitation, from our perspective the inclusion of NIRS and eye-tracking to the methodology enriched the data obtained and allowed for a broader physiological picture, they did not interfere or overlap within each other or the EEG and neurofeedback sensors. Furthermore, in future studies it would be interesting to include eye-tracking throughout the NFT sessions as well in order to have a fuller picture of the learning process of training brain activity and if/how eye movements change throughout.

4.5. Conclusions

After undergoing a NFT of 8 sessions, we found an increase in ERD in high alpha for participants who underwent active training as opposed to a sham group. The EEG and NIRS data of the first and last NFT sessions revealed a greater high alpha absolute power in active training group which was correlated to the higher levels of oxygenated haemoglobin in the last session.

The findings in the microstructure of mu throughout the NFT and the corresponding correlation with the blood oxygen levels suggest that the NFT protocol had an effect both in the amplitude and frequency of mu in the group that underwent training. This was also reflected in the increase in mu activity during the post backward condition of the social response protocol. The significance of these results could be more associated to a mirror neuron response to the action that had the most relevance to the individual given the context of the study. Nonetheless, the NFT protocol was successful in increasing the reactivity of mu in an action observation context.

Furthermore, research on thresholds in NFT are needed in order to provide a standardized method that will allow us not only to improve neurofeedback training but also to make the protocols more scientifically rigorous and help to elucidate the anatomical/neurochemical substrates and neuronal mechanisms behind this non-invasive neuromodulation technique that will provide insights into learnability aspects as well as expanding its applications.

5. General Discussion

The goal of this thesis was to investigate cortical oscillatory activity in a neurotypical population with individual differences in autistic traits and explore the effect of non-invasive neuromodulatory training using multiple psychophysiological methods. For this reason, this project revolved around 3 main topics:

- The autism spectrum within neurotypical individuals.
- The mu rhythm as an EEG index of the human mirror neuron system (hMNs).
- The use of neurofeedback training (NFT) as a non-invasive neuromodulatory method.

Given that the association of these three topics has been studied in the past, whether it be individually or combined, this thesis sought to address methodological limitations that have been raised against each of these topics through the implementation of a multi-methods approach in order to provide novel insights and a deeper understanding of these topics.

5.1. Resting state EEG (rEEG) in autistic traits.

The first experimental step taken was to study the resting state of individuals with different degrees of autistic traits. Given that the brain is a system that reacts to both internal and external information (Wang et al., 2013), we considered it instrumental to study resting EEG (rEEG) in order to gain an understanding of the spontaneous variations in neural activity that could be associated to AQ traits before examining protocols meant to elicit cortical activity related to an event.

Although this first study did not employ a multi-methods approach, the analyses that were performed provided a novel and more in-depth perspective to the study of rEEG in neurotypical individuals with various levels of AQ. This experiment focused on both linear and nonlinear features of the rEEG, recorded while participants sat still with their eyes closed for two minutes.

Absolute power analyses revealed significant group differences in the theta, alpha and beta bands, mainly over the left hemisphere across frontocentral, central, central parietal and parietal areas. Higher AQ scores were associated with lower absolute power in these areas. A further study of the absolute power when looking at functional networks also showed differences in the frontoparietal and somatomotor network between high and low AQ scores. Low AQ score group had a higher absolute power in low alpha for frontoparietal networks. In the somatomotor functional network, low AQ score group also had a higher absolute power, this time in the theta band power. Again, high AQ scores tended to have a lower absolute power in these networks. These results replicate previous findings in ASD literature that identify these areas and bands as displaying abnormal activity and provide further information of this activity in neurotypical individuals with autistic traits (Barttfeld et al., 2013; Coben et al., 2008; Sperdin et al., 2018).

As this thesis is also focused on sensorimotor activity, an analysis of microstructure of the mu rhythm was also done on the rEEG. This analysis revealed that the mu rhythm in individuals with high AQ has lower absolute power but a higher number of oscillations per second (faster frequency) and longer duration of mu rhythm events, especially over the left central areas.

The nonlinear analysis of the EEG focused on obtaining the complexity of the signal. In this study, entropy and fractal dimension were measured in the rEEG, both of these measures indicate a level of randomness and irregularity in the EEG signal as their values increase (Olofsen et al., 2008;

Schwilden, 2006). In the study, these values were higher in individuals with high AQ. This would suggest that as the degree of AQ traits increases, the level of complexity and degree of randomness in brain activity increases; this was evident over left central and right centroparietal areas.

Taken together, this chapter provides a tentative characterization of rEEG (particularly, the resting mu rhythm) in autistic traits, and suggests that as the severity of AQ increases into clinically diagnosed populations, these markers would also become more pronounced and could be used as a possible biomarker in the future. However, more research in clinically diagnosed populations is needed in order to provide more insight on this matter.

5.2. EEG protocols on mu suppression and autistic traits

Once individual differences in endogenous sensorimotor brain activity had been ascertained, the next step in the project aimed to study this activity when it was associated with the observation of motor actions in different contexts. Although sensorimotor reactivity has been described as abnormal in individuals with ASD (see chapter 1 and 3) it has been suggested that differences in EEG sensorimotor activation during observation of motor actions that have been observed may be due to an attentional bias to the stimuli (Hobson & Bishop, 2016).

In chapter 3, this aspect was addressed through the simultaneous use of eye-tracking measures during the EEG task. Eye-tracking is a technique that has been used in the past to study ASD and the hMNs as it provides an objective measure of where a participant's interest and attention is when observing a visual display (Duchowski, 2017; Hamilton, 2013; Poole & Ball, 2011). Differences in eye-tracking variables such as fixation and gaze patterns has been reported in ASD populations when compared to neurotypical individuals (Boraston & Blakemore, 2007; Dalton et

al., 2005; Papagiannopoulou et al., 2014). Thus, given that this was a physiological measure that had been used in the study of ASD it was included in this experiment as a measure for controlling attention as well as to explore if significant differences in eye-tracking variables that had been reported in clinically diagnosed populations of ASD would be present in our sample.

In this experiment, the goal was threefold: 1) to gain more knowledge regarding the reactivity of the hMNs in neurotypical individuals as a function of individual differences in the degree of autistic traits; 2) to address the concerns of attentional bias that had been previously described as responsible for differences in sensorimotor activation in ASD; 3) to compare three protocols associated with sensorimotor/mirror neuron activity in order to identify the one that was more sensitive in eliciting mu rhythm reactivity in individuals with different levels of autistic traits. The result of goal 3 served to identify the best protocol to use as the pre/post-neurofeedback training assessment for chapter 4.

The three protocols used in this experiment were:

1) The mu multiple repetitions protocol (MMR) - a classic MN reactivity protocol using hand movements (Puzzo et al., 2011).

2) The proactive gaze protocol – based on Costantini, Ambrosini, Cardellicchio, & Sinigaglia's (2014) work, where they found that proactive gaze behaviour had an anatomical association to motor areas that would be involved in the execution of the action observed. It involves the goal-directed action of a hand reaching towards two targets of different sizes.

3) The Social Response Protocol - observation of human movement involving a goal-directed hand action, but this time, incorporating a social response component (Clausen et al., 2015).

The detailed description of each protocol can be found in chapter 3, however, the instructions across all protocols were the same, the participant was asked to observe a series of videos while having EEG and eye-tracking measures recorded.

In terms of the specific findings per protocol, the MMR protocol showed an overall difference between stimuli in the beta band over centroparietal areas in the non-biological stimuli (balls) over the still or moving hand stimuli in the mid and late time period of the trial. Although there were no significant differences between high and low AQ groups, this effect did have a significant positive correlation with AQ score. Meaning that even though there were no differences among our groups, this suggests that the higher the AQ score, the higher desynchronization in sensorimotor activity towards non-biological actions. Given that autistic traits are a spectrum it would be expected that individuals that rank high on AQ would have less interest in biological stimuli (hands) and more to a non-biological signal moving in a stereotypical manner (2 balls moving up and down towards the centre of the screen).

The proactive gaze protocol did not reveal group differences in mu desynchronization, or eye-tracking variables. This protocol was based on Costantini et al.'s (2014) paper describing the contribution of the left ventral premotor cortex (PMv) in proactive gaze behaviour. Notwithstanding, the eye-tracking findings in regard to average saccade amplitude for this protocol are in line with the findings from the original paper where the authors found that proactive gaze

was influenced when specific motor cues (i.e. a pre-shaped grip) were present. In our findings, average saccade amplitude was also larger when the actor's hand was in the shape of a pre-grasp. The social response protocol also had an absence of group differences for mu desynchronization. There was, however, an effect of hemisphere between left and right centroparietal areas with a negative correlation to AQ score over the right hemisphere, which suggests that as the AQ score increases the event-related desynchronization (ERD) in low alpha over these areas diminishes. This effect was attributed to the type of stimuli involved in this protocol. The faces included in this protocol were neutral, and studies suggest that the observation of neutral faces elicits a higher desynchronization of mu power because one requires more sensorimotor engagement in order to recognize action and emotion from a face that does not provide an emotional cue (Karakale et al., 2019). Another important aspect of this protocol is the use of a clear social cue: the person in the video had a mug with the handle towards the participant and the mug was moving either towards or away from the participant. Within the context of Hamilton's (2013) theory of social response (for MNs) we expected the forward condition to elicit a higher ERD. This was the case for low alpha over frontocentral areas and the effect suggests an anticipatory response from MNs (reflected by mu desynchronization) to the mug being directed at them. Other researchers have described a similar type of motor priming effect when asking participants to produce an action matching an image which had end positions corresponding to different actions (Liepelt et al., 2010). The findings in dwell time in the eye-tracking support the notion of increased attention on the social cue. As an interest area, the mug had a highest dwell time over the other interest areas (face, mouth, eyes) in the forward condition. Longer dwell time indicates more time spent on that area of interest.

One finding that was consistent across all protocols was the significant group differences in average saccade amplitude, which was greater in the high AQ group. Due to the role of the cerebellum in saccade metrics and the link in cerebellar abnormalities to ASD (Critchley et al.,

2000; Johnson et al., 2012; Schmitt et al., 2014), these results may suggest that high ASD traits could be also be associated to differences in the cerebellum function and these in turn could affect saccade metrics. However, the results from this experiment should be considered with caution as more research would have to be conducted between average saccade amplitude and cerebellar function and ASD in order to confirm this association.

By combining eye-tracking with EEG measures, this study was able to provide a physiological control for attention in the form of fixation counts over the image and in designated areas of interest. The results did not show significant differences in fixation count between those with high and low autistic traits in any of the interest areas in all protocols. This therefore indicates that the findings cannot be attributed to an attention bias among groups.

Taken together, this chapter provided 2 main outcomes: 1) although there were no significant group differences in ERD of the mu rhythm in the protocols, there were correlations between AQ score and ERD in low alpha and beta bands over frontocentral and centroparietal areas; 2) average saccade amplitude revealed significant differences among AQ groups. Based on results from all three protocols and given that the social response protocol had low beta ERD associations with AQ score, as well as eye-tracking group differences for dwell time, the social response protocol was selected as a pre- and post-NFT training assessment for the final, longitudinal study presented in chapter 4.

5.3. Neurofeedback training on individuals with high autistic traits.

Continuing with the goal of applying multi-method approaches to address previous methodological limitations, chapter 4 focused on NFT as a non-invasive neuromodulatory

technique in order to influence sensorimotor reactivity in high AQ trait individuals. In this instance, a multi-method approach was pursued using three physiological measures in order to assess the effect of NFT: Pre/post EEG and eye-tracking using a protocol associated to the EEG index of the mirror neuron system (see chapter 3) as well as the monitoring of haemodynamic changes throughout the NFT sessions using near-infrared spectroscopy (NIRS). In addition to the psychophysiological assessment, the sample was divided into subgroups: neurofeedback training (NFT) versus a sham training condition, in order to increase methodological strength.

Based on findings from chapter 3, the social response protocol was selected as the pre/post-NFT assessment measure, and neurofeedback training was done using each participant's peak alpha frequency amplitude over C4 as the threshold (see chapter 4 for a detailed description). Feedback was delivered in the form of a videogame that advanced when the participant's activity reached or crossed the threshold for at least 250ms. Each participant underwent 8 sessions where depending on the group that they were assigned to (participants were unaware as to which group they belonged to) they either did NFT or were presented with the video of a feedback session from another participant. All participants had EEG and NIRS sensors placed throughout each session.

This experiment had three main findings: 1) an increase in absolute band power for the high alpha band during the start of the final NFT session in the active NFT group; 2) a positive correlation between the absolute band power in the EEG and oxygenated haemoglobin (O_2Hb) in low alpha (7-10Hz), high alpha (10-12Hz), broadband alpha (8-12hz), and low beta (13-21 Hz) in session 8; 3) a significant group difference in the post-NFT assessment, specifically, in the ERD for the high alpha band (9-12Hz) during the backward stimuli. On an equally important note, eye-tracking results from this study revealed that there were no significant differences in the attention paid to the stimuli in the pre and post assessments.

These findings suggest that the active NFT achieved a modest increase in cortical activation of the trained area. Interestingly, this activation was identified in the stimuli that were not associated to a social response. The social response protocol was designed to test a theory by Hamilton (2013) where it was proposed that an important purpose of the MNS is to respond, in real-time and in a socially appropriate fashion, to the actions of others. In this protocol there are three stimuli, in each one a female actress wearing black clothing in front of a black background is holding a white mug. In one scenario, the actress moves the mug towards the viewer; in the second, the mug is moved backwards, away from the viewer, and in the third, the video has no movement. In all three stimulus conditions, the actress' facial expression is neutral, and the video is shown against a black background. Our hypothesis was that following active NFT, the increase in cortical activation would occur in the forward condition as this is thought to prime a social response in the observer. However, it was in the backward condition where the differences were identified. A possible explanation is that this reflects a mirror neuron response to the action that had the most relevance to the individual given the context of the study (Kosonogov, 2012). It is suggested that individuals with higher AQ traits would have preference and therefore a higher reactivity to the stimuli that had less association with social interaction. This preference was also portrayed in the previous experiment during the MMR protocol, where individuals with high AQ traits had a higher activation for the non-biological stimuli (the balls moving to and from the centre of the screen as opposed to the moving and static human hand).

Overall, by employing a multi-method approach, the results of each chapter provide a broader physiological picture of the topic under investigation. By gaining more physiological information, more questions are also raised in terms methodological improvements as well as future research paths. These will be addressed in the following section.

5.4. Limitations

The use of multiple methods for the study of neuro-cognitive phenomenon requires an increased control of factors that could affect the data obtained. In this project, the physiological methods employed were compatible with each other, and necessary measures were put in place so that the collection of data from different equipment would not interfere with one another.

However, one aspect that could have affected the results in the eye-tracking data for chapter 3, especially for pupil dilation, was the luminescence in the stimuli. The protocols used in this study were originally designed for EEG, and although all participants observed the stimuli from the same screen, in a room with under equal conditions of light, the luminescence value of each stimuli video was not taken. As we do not have this value, luminescence confounds that could have had an effect on pupil dilation cannot be discarded. As pupil dilation has been suggested as a potential early biomarker of ASD (Anderson & Colombo, 2009; Bast et al., 2019; Martineau et al., 2011b) and luminescence affects pupil dilation, it is important for future studies to control for this variable when using multi-method psychophysiological approaches. The results from chapter 3 raise questions as to the sensitivity of this measure as a diagnostic tool, as in this study it was not possible to distinguish individuals from the high or low autistic traits group using this eye-tracking variable alone but this cannot be confirmed until the appropriate controls for luminescence are made.

Another factor that could have affected the results was the degree of autism in the sample. Most studies done on ASD are focused on children that have been clinically diagnosed. In this project the focus was on neurotypical adults with varying degrees of autistic traits. The rationale behind choosing this population lay in the context of autism a spectrum as well as the description of the broader autistic phenotype that has been previously described in the literature (Grove et al., 2019;

Losh et al., 2009). Nonetheless, the difference in age and autistic severity could have been an explanation as to the lack of significant group differences where it was expected according to our hypothesis. It would be useful to replicate these studies in younger sample as well as those that have been clinically diagnosed to obtain further information regarding the spectrum and its traits in both clinically and non-clinically diagnosed population.

As for the use of NFT, 2 main limitations were identified: the duration of active training and the threshold. Upon closer consideration, the combination of these factors could have had a detrimental effect to the attention and motivation of the participants and may therefore have hindered their performance in the training. As was mentioned in chapter 4, the active NFT time that participants underwent was 30 minutes. It is noteworthy that although significant group differences were identified, these were limited to the start of the last session and noticeably dropped thereafter. This aspect would have also influenced the blood oxygen response, explaining the absence of significant differences in the NIRS variables. Based on this information, future studies should be constructed in terms of trials of active training with resting intervals in order to gain maximum effectivity of the NFT. Similarly, a future line of research could focus specifically on identifying the most effective parameters for the relationship between active training and resting times in order to maintain motivation and maximise the benefits of NFT.

Secondly, within the field of neurofeedback there is an ongoing debate as to the method of determining threshold parameters. In this study the threshold was set at the beginning and fixed for the whole of the protocol. This method could have also affected the motivation of participants if they had difficulty reaching their threshold. Since the threshold was fixed in order to have methodological control over this variable across participants, the participants that found it difficult did not receive the necessary feedback to maintain motivation in the protocol. Alongside the length

of the sessions it is most likely that these factors minimised the chances of finding large-scale changes in brain activity from pre to post assessment. Notwithstanding, in this context, the findings from this study suggest that NFT holds promise as an effective non-invasive neuromodulation technique.

5.6. Future directions

The limitations identified in this study sparked some interesting questions as to future lines of research. Results from chapter 3 showed significant differences in the average saccade amplitude. Saccade metrics have been associated to cerebellar activity which in turn has also been described as having abnormal functioning in ASD individuals (Schmitt et al., 2014; Soetedjo et al., 2019). Results from this work would suggest evidence to support the link between these topics but it would be relevant to conduct further studies using simultaneous EEG and eye-tracking that focused on assessing the role of the cerebellum in saccade metrics in order to gain more insight about this association.

Chapter 4 revealed the need for in-depth studies focused on methodological aspects of threshold, trial duration and number of sessions for NFT. By studying these aspects further, it would be possible to obtain methodological guidelines that would increase the efficacy and objectivity of this method as a non-invasive neuromodulatory technique. The goal of NFT is to condition the brain to modify its activity by gaining awareness about a specific desired state and learning how to access said desired state/activity. By increasing what is known about the individual differences of the psychophysiological factors that affect the learning process in NFT, standardized protocols based on individual learnability can be created and reliably applied in a variety of scenarios; for example, to boost education or home-based therapies.

5.7. Final Summary

The main aims of this thesis were to explore the relationship between cortical oscillatory activity and individual differences in autistic traits in a neurotypical population and also, to investigate how these differences moderate the effect of non-invasive neuromodulatory training on brain activity using multiple psychophysiological methods. The main findings can be summarised as follows:

When exploring how the rEEG of people varied according to individual differences in autistic traits, those who had a high level of AQ traits displayed less activation in frontal and fronto-central regions combined with higher levels of complexity and irregular activity in fronto-temporal, temporal, parietal and parieto-occipital areas (see chapter 2). Although no source localization was performed, the overall overlap of brain areas coincided with what has previously been described and associated with ASD abnormalities (Bastiaansen et al., 2011; Brambilla et al., 2004; Haznedar et al., 2000; Heiser et al., 2003; Iacoboni & Dapretto, 2006; Nickl-Jockschat et al., 2014; Perkins et al., 2015; Puzzo et al., 2010; Wang et al., 2018) (see chapter 1). Within the analysis of the microstructure of the mu rhythm (which has been associated to abnormal reactivity in ASD) it was of particular interest that individuals with high AQ traits displayed a mu rhythm with a higher number of oscillations per second, lower absolute power and higher degrees of complexity with entropy and fractal measures than those with low AQ traits. This finding is important as it could be a possible biomarker for autistic traits and it would be interesting conduct further studies in both diagnosed populations as well as samples of different ages in order to explore applications.

In chapter 3, different protocols that elicit sensorimotor reactivity were compared through a multi-method approach of eye-tracking and simultaneous EEG. This approach provided an interesting

insight into the putative attentional bias in ASD populations; something which has in the past been suggested as responsible for observed differences between ASD and neurotypical populations when stimuli involve people and/or faces.

In addition to identifying a pre-post intervention assessment tool for chapter 4, as well as individual differences in mu reactivity and average saccade amplitude (as measured by the eye tracker) the lack of significant differences in the number of fixations paid to the stimuli presented suggests that any variations found in brain activity cannot be attributed to differences in attention. In short, the amount of attention paid to the stimuli as well as the areas of interest did not differ according to AQ traits.

Additionally, the findings associated with lower alpha band ERD in high AQ individuals during the social response protocol led to our third and final study. The aim of this study was to train individuals with high AQ to enhance the mu rhythm using NFT. Using the social response protocol as the pre- and post-assessment of an 8 session NFT protocol, a multi-methods approach was carried out once more. On this occasion, the pre- and post-assessments included simultaneous EEG and eye-tracking and the NFT protocol also included simultaneous EEG and fNIRS recording of every session. This approach was valuable once more, as it provided parallel physiological evidence for the effects of NFT in sensorimotor reactivity, namely, an increase in ERD in high alpha, higher levels of oxygenated haemoglobin and changes to the amplitude and frequency in the microstructure of mu for participants who underwent active training as opposed to a sham group. Equally important, this study revealed the need for methodological improvements and standardization in the practice of NFT, specifically in the realm of thresholding.

In conclusion, a multi-method approach, such as the one employed in this thesis, is greatly recommended for future studies in this area; it proved to be advantageous in the study of cortical dynamics and provided a broader physiological picture into sensorimotor reactivity, autistic traits and neurofeedback.

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