An exploration of SSVEPs across development and autism spectrum conditions

By

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Abstract

This thesis contains an experimental investigation of networks dynamics across development and autism spectrum disorders (ASD). The interplay between functional segregation and integration within functional cortical networks was investigated based on the hypothesis that it plays a key role in development and ASD. Functional segregation refers to the synchronization between adjacent brain areas and functional integration indicates the synchronization between distributed brain regions. Steady-state visual evoked potentials (SSVEPs) to high contrast (90%) luminance and isoluminant chromatic (red-green) vertical gratings with two spatial frequencies (2.8 and 6 cpd) at 7.5 Hz (luminance) and 3.3 Hz (chromatic) were recorded in individuals with and without ASD. SSVEPs were analysed in the frequency and time domains to carrying out a detailed analysis of the dynamic functional connectivity elicited by perception of simple and complex visual stimuli.

The first research study explored aged-related changes in networks dynamics. Participants were 30 children aged 7 to 17 and 11 adults from the typical population. Our results suggest functional reorganization from local to distributed networks across development, and that networks underpinning medium spatial frequency change would be a useful biomarker of typical brain function. The second research study explored potential changes in networks dynamics between children with and without ASD. Participants were 20 children aged 7 to 17 (10 with ASD and 10 age-matched typically developing). The result of this study is a potential EEG biomarker to characterize atypical brain function in autism. Our results suggest a direct relationship between functional segregation and functional integration during visual perception; atypical functional connectivity in lower processing mechanisms might contribute to the disruption in long-range functional integration reported in ASD, because both abnormalities occur in concert in the autistic brain.

Overall this exploratory research shows that SSVEPs can elicit different functional networks involving local and distributed cortical brain systems, and can also show segregated and overlapping functional networks underlying neural mechanisms at early stages of visual processing during development and ASD. Therefore, SSVEPs would be a potentially useful technique to identify differences in the brains of people with and without autism.

Dedicated to my loving and caring daughter, Yaretzi.

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Glossary

2F, 3F, 4F	Second, third and fourth harmonic
ADHD	Attention Deficit Hyperactivity Disorder
ANOVA	Analysis of variance
ApEn	Approximate Entropy
ASD	Autism Spectrum Conditions
C1	The largest ERP component located around 100 ms after onset
CAMHS	Child and Adolescent Mental Health Services
cd/m2	Candela per square metre
CIE	International Commission on Illumination (in French)
CMS	Common Mode Sense active electrode
cpd	Cycles per degree
CRT	Cathode ray tube
DRL	Driven Right Leg passive electrode
DSM	Diagnostic and Statistical Manual
EEG	Electroencephalogram
ERP	Event Related Potentials
FFT	Fast Fourier Transform
fMRI	Functional Magnetic Resonance Imaging
GABA	Gamma-Aminobutyric acid
GE	Global Efficiency
HSF	High Spatial Frequency
Hz	Hertz or cycles per second
ICA	Independent Component Analysis
ICD	International Classification of Diseases
LE	Local Efficiency
LGN	Lateral Geniculate Nucleus
M	Cell of Magnocellular System
M-pathway	Magnocellular pathway
MEG	Magnetoencephalography
MRI	Magnetic Resonance Imaging
ms	milliseconds
MSF	Mid Spatial Frequency
MT	Medial Temporal Cortex
N1	Negative ERP component located between 150-200 ms
Oz	The central occipital EEG channel based on 10-20 system
P	Cell of Parvocellular System
P100	First positive ERP component around 100ms
P-pathway	Parvocellular pathway
PC	Personal computer
PET	Positron Emission Tomography
PLI	Phase Locking Index
PSD	Power Spectral Density
SSVEP	Steady-State Visual Evoked Potentials
TD	Typically developed subjects
TF	Time-frequency analysis
V1	Primary Visual Cortex
V2	Secondary Visual Cortex
VEP	Visual Evoked Potential

Chapter 1

Introduction

1.1 Current issues in neurodevelopmental diagnosis

Neurodevelopmental disorders have been considered a manifestation of atypical brain development. According to the Department for Work and Pensions (2012) Family Resources Survey 2010/11, neurodevelopmental disorders affect approximately 0.8 million children and young people, aged 0–18 in the UK. This group of conditions may include attention deficit hyperactivity disorder (ADHD), fragile X, autism spectrum conditions (ASD), etc. (DSM V, American Psychiatric Association, 2013). Regardless of diagnosis, all of these disorders involve cognitive, emotional, behavioural and interpersonal impairments. In many cases, behavioural phenotypes and symptoms of neurodevelopmental conditions changes with increasing age. In some other cases, individuals diagnosed with developmental disorders in childhood may face lifelong disabilities. Among these lifelong neurodevelopmental disorders, autism is considered one of the most severe conditions.

Despite advancements in the knowledge of the neuropathological underpinnings of autism, diagnosis and treatment of this condition can be difficult. There are two main frameworks for autism diagnosis, the Diagnostic and Statistical Manual (DSM-V) of the American Psychiatric Association (2013) and the International Classification of Diseases (ICD) of the World Health Organization (2010). Autism is currently diagnosed through subjective means such as observing behaviour and communication abilities, typically during preschool years at the earliest. DSM-V and ICD-10 consider three domains of behaviour: deficit in social

reciprocity, deficit in communication and presence of restricted and repetitive behaviours. Efforts are underway to identify biological quantitative methods that can assist behavioural methods to diagnose autism. For instance, a recent study (Wang et. al., 2009) aimed to determine a genetic biomarker based on the analysis in the effect of chromosome 5p14 in neural connectivity leading to ASD. On the other hand, studies on metabolic markers have demonstrated that the level of antioxidants excreted in urine is correlated with the degree of severity in ASD (Demoran et. al., 2011). Neuroimaging techniques have demonstrated altered density and distribution of the GABA receptor in subjects with ASD (Blatt and Fatemi, 2011).

In the quest for reliable biological markers for autism, the heterogeneity of this condition (Lenroot and Yeung, 2013), in terms of both phenotypes and outcome at any one age and over the course of development, has been the major challenge for researchers and clinicians. Biological evidence has shown a major difference in brain size and structure matter between individuals with autism and typically developing individuals at early stages of child's development (Courchesne et al., 2007; Damasio et al., 1980; Balottin et al., 1989). This difference, however, becomes less apparent with age, particularly from later childhood to adulthood (Lainhart et al., 2006; Silverman et al., 1998). Then, it has been suggested that atypical trajectory of autistic brain development underlies the evolution of its symptoms. Due the brain changes over time, the symptoms may also vary affecting the diagnosis. Nonetheless, the specific part of the brain that is affected is still under debate. Biomarkers must be useful at all points in childhood, thus, it is critical to determine how potential biomarkers vary over the course of development. Analysing the development of brain function over time may be key to successfully identify autism.

1.2 EEG based analysis

In recent years, the study of temporal changes in brain activity of children and adults has gained interest in developmental neuroscience. The study of normal brain development has been a focus of interest over the last decade, for better understanding of neurodevelopmental disorders. This view was encouraged by the advance of powerful non-invasive techniques for evaluating gross neuroanatomical changes and functional brain differences related to development. Among these neuroimaging techniques, the electroencephalogram (EEG) has several advantages for the study of brain function across development and neurodevelopmental conditions. First, the EEG equipment is portable and its cost is significantly lower than other neuroimaging methods. Second, its high temporal resolution (~ 1 ms) makes it suitable for the study of fast temporal changes in brain activity. Since the EEG is a measure of the electrical activity generated by millions of firing neurons in the brain, different rhythms shape its own dynamics in the recorded signal. EEG research (Nunez and Srinivasan, 2006) has suggested that rhythms of the brain change during the course of development, and can be modulated by neurodevelopmental disorders and other factors. For instance, EEG measurements in resting state have demonstrate that the amplitude of the activity in lower frequency bands tends to decrease with increasing age, whilst the amplitude of higher frequencies increases (Barriga-Paulino et al., 2011; Anokhin et al., 1996). Measures of synchronization among different parts of the brain have an increasing trend with age in resting state (Czigler et al., 2007), and when performing auditory tasks (Ho et al., 2012). A more detailed investigation of brain oscillations has been required in developmental studies, in order to determine whether neural measurements may be potential biomarker of atypical brain development.

Developmental disorders such as autism spectrum disorder (ASD), and other psychiatric and neurological disorders including schizophrenia and Alzheimer's disease involve a wide range of serious impairment in different areas. Although their symptoms and causes may be numerous and diverse, these conditions

share important characteristics related to neural connections (Anagnostou and Taylor, 2011; Kubicki et al., 2007; Daianu et al., 2013). Important progress has been made in identifying atypical brain activity in these conditions using electroencephalography (EEG). Relevant to the current study, individuals with ASD have shown a stronger and more rapid activation of early visual areas along with reduced interhemispheric synchrony during visual perception (Isler et al., 2008). As reported by these authors, the brain responses to long latency flash visual evoked potentials were compared between two groups of children (5.5–8.5 years), with and without autism spectrum disorders. Isler and colleagues (2008) interpreted their findings as evidence for local sensory hypersensitivity and reduced long-distance functional connectivity between early visual areas in ASD. Earlier and later stages of information processing have also been suggested impaired in autism based on results of EEG studies. Fujita et al. (2011) studied changes in chromatic and achromatic visual processing in subjects aged 17-38 years using event related potentials (ERPs). They found that the first component (N1) of ERPs to chromatic gratings was significantly prolonged in participants with autism compared to controls, whereas ERP responses to achromatic gratings did not differ significantly between the two groups. Given that chromatic stimuli preferentially stimulate colour sentitive cells in the parvocellular pathway, these results were interpreted as evidence for parvocellular pathway impairment with preserved magnocellular pathway function in the early visual system in ASD. Other EEG studies have investigated the integrity of early visual channels in ASD by means of ERP responses to gratings of different spatial frequencies, although results are diverse. Whereas Jemel et al. (2010) suggested reduced functional segregation of mid and high spatial filters in ASD participants aged 18-33 years, Vlamings et al. (2010) suggested enhanced visual processing of high spatial frequency in children with autism. Altered spatial frequency processing in ASD has been considered to contribute, at least in part, to the atypical ERP responses observed in ASD during face processing (Vlamings et al., 2010; Dawson et al., 2005). Some other EEG studies employed steady-state visual evoked potentials (SSVEPs) to study low-level visual processing (Pei et al., 2014) and visual spatial attention in autism

(Belmonte, 2000). Pei et al. (2014) found reduced activation of SSVEP responses to high spatial frequency gratings at twice the frequency of stimulus presentation (second harmonic) in ASD participants (aged 5-17 years) compared to controls, suggesting deficits at the earliest stages of sensory processing in autism. Belmonte (2000) reported an abnormally large activation of steady-state visual evoked potentials (SSVEPs) in both hemispheres regardless of visual stimuli in adults with autism (aged 19-32 years) compared to controls, which was interpreted by the author as an overconnected functional network for sensory inputs and a lack of perceptual specificity within sensory regions in autism. A more recent EEG study (Catarino et al., 2013) investigated perceptual specificity in ASD. The researchers found a lack of significant difference in the activation of areas involved in the processing of visual stimuli (faces or chairs) in participants with ASD (aged 21-37 years) compared to controls. In addition, they reported reduced interhemispheric synchrony in the ASD group regardless of visual stimuli. Their results provided support to previous studies reporting an impairment in the stimulus-specificity of the functional networks involved in visual perception in ASD (Belmonte, 2000), and to a diminished functional connectivity between brain regions during visual perception in individuals with autism (Milne et al., 2009; Isler et al., 2008). Their results, however, contrary to suggestion in the literature (Belmonte, 2000; Isler et al., 2008), provided no evidence for hyper-activity in early visual areas in individuals with ASD.

The previously mentioned findings have provided insight regarding the integrity of functional networks of individuals with autism during perceptual processing, and have shown that EEG tests can determine changes in brain activity in adults and children with and without autism. EEG offers several strategies for measuring brain activity changes, including event-related potentials (ERPs) and steady-state visual-evoked potentials (SSVEPs). ERPs and SSVEPs indicate responses elicited upon sensory stimulation at slow and fast presentation rate, respectively (Regan, 2009; Luck, 2005; Vialatte et al., 2010). EEG/ERPs/SSVEPs are noninvasive, requiring only that the participant tolerate an electrode hat for relatively short periods of time, and they do not necessarily require the

participant to perform cognitive tasks, making them well suited to the investigation of brain activity in younger children and participants who have limited cognitive abilities. Thus, the methodology can be used to investigate early stages of functional brain development in young children at risk of autism. An additional advantage of the technique is that EEG enable the measurement of brain activity with high temporal resolution. This is of particular interest for investigating the temporal dynamics of brain networks. The EEG/ERP/SSVEP signal consists of the summed electrical activities of a large population of neurons firing synchronously across a wide range of frequencies that can be recorded by means of electrodes from the scalp (Srinivasan et al., 2006; Niedermeyer and Lopes da Silva, 1999). The activation of neurons generates time-varying electrical currents. By correctly analysing the electric fields, EEG may provide reliable information about the neuronal activity in the brain and the temporal dynamics of this activity in the millisecond range. In addition, EEG equipment is relatively inexpensive compared with other brain imaging devices, less susceptible to motion artefacts that would confound connectivity results and simple to operate. Although the main disadvantage of EEG measures is poor spatial resolution, recent advances in high-density EEG equipment and signal analysis allow for more accurate estimates of the signal source (Makeig et al., 2002; Jung et al., 2001; Milne et al., 2008). Furthermore, the use of more advanced complex network approaches to EEG connectivity analysis have engendered EEG with the status of a true brain imaging technique, capable of providing a detailed description of functional brain dynamics for those who are interested in the temporal dynamics of local- and large-scale brain networks in neurodevelopmental and psychiatric conditions (Michel and Murray, 2012; Vissers et al., 2011; Boersma et al., 2011; Dimitriadis et al., 2010).

The examination of temporal dynamics is fundamental for all aspects of mental activity, including perception and cognition, because describes how the brain coordinates its different regions to process brain functions. The main feature of brain activity is the transient change of coordinated activity within brain networks even in a constant environment (Nenadovic, et al 2011; Karahanonoglu and Van De Ville, 2015). The next section describes perspectives on brain

dynamics that draw on recent advances in functional brain studies, computational approaches to transient brain dynamics are described in detail in chapter 2.

1.3 Brain dynamics as system responses

The human brain is considered the most complex and powerful information-processing system. The information is transmitted across brain systems at high-speed transmission times (Kent, 1981). Thus, a better understanding of the mechanisms of brain function would be possible by applying strategies to explore the organization of its systems that may be coded in the temporal relation of this interaction. Brain cells are organized in large groups over spatially distinct regions of the cerebral cortex. Each group may be considered as a functionally specialized system of the brain (Tononi et al., 1994; Dobkins, 2009). Visual and motor cortex, the fusiform face area (Kanwisher et al., 1997) and the parahippocampal place area (Epstein et al., 1999), are some of the anatomical systems that have been identified in the brain since the early nineteenth century (Gazzaniaga, 1989). The structural and functional organization of the brain systems follows a hierarchical organization. From a bottom-up perspective, simple processing of visual input is performed at the lower levels, and progressively the complexity of the processing increases, so that the highest levels process the general information about the environment rather than details (Riesenhuber and Poggio, 1999). The notion of different brain areas interacting to perform any perceptual or cognitive process is now widely accepted. The development of brain function, from the integrative point of view, has been a focus of interest in recent years (Braddick and Atkinson, 2011).

Functional interactions between distributed brain areas are modulated by the transient formation and dissolution of coordinated activity within segregated neuronal populations. These two properties of functional brain organization, local functional segregation and long-range functional integration co-exist within networks underpinning perceptual and cognitive tasks (Tononi et al., 1994;

Friston, 2002). The dynamic organization of functional interactions occurs at the millisecond time scale and shape the electrical activity measured from the surface of the skull by means of the EEG. Currently, electrophysiological studies on age-related synchronized activity between brain regions have been made predominantly during resting state (Nenadovic et al., 2011). However, the functionality of a complex system, as the human brain is, can be better understood if analysed as a hierarchical system where lower processing levels of the cortex are stimulated to elicit brain responses that are correlated to the external input. Thus, the use of visual stimuli allows studying changes in the response of brain systems to different physical properties. This is important because, it is suggested that the earliest processing levels of the cortex are the result of evolutionary selection (Hofman, 2014) and are functionally developed soon after birth, such as the early visual mechanisms that extract the spatial information of visual images (Dobkins, 2009). Meanwhile, the higher processing levels are likely to develop as the person grows up.

Research into the development of brain function is thus an important approach to develop effective tools for identifying the changes in brain activity that might reveal neurodevelopmental abnormalities.

1.4 Aims of the research

In this context, this work explores the development of brain function at the lower processing levels of the cortex. In particular, the visual system is a hierarchical system, with functionally segregated modules processing different aspects of vision (Dobkins, 2009). Early mechanisms of spatial frequency processing are at the lower levels, whereas at higher levels colour, motion and form are processed (Zeki et al., 1991). We aim to investigate functional segregation of early visual channels and integrative processing within functional networks involving higher visual areas over the course of development and its alteration in ASD.

The main purpose of the thesis is to identify potential changes in networks dynamics across development and in autism spectrum conditions with EEG. For this, we explore the use of steady-state visual evoked potentials (SSVEPs) to study the dynamics of functional networks over time. These are potentials elicited by a train of rapidly repetitive stimuli, which are traditionally analysed in the frequency domain (Reagan, 1989). We also explore the use of complex networks measures (Dimitriadis et al., 2010) that might reveal the interplay between functional segregation and integration within the functional networks underpinning vision processing.
In particular, the objectives of this thesis are listed below:

- To identify age-related changes in networks dynamics during low-level visual perception. Our rationale for this study is that different visual stimuli will generate different functional networks within the visual system (see Chapter 2). Luminance- and chromatic-defined gratings at two different spatial frequencies were used to test this hypothesis (see Chapter 3). The research question, what EEG indices can reflect age-related changes in the networks dynamics during visual perception? was investigated by performing a cross-sectional study where subjects included 30 children aged 7-17 and 11 adults aged 23-37.

- To identify potential changes in networks dynamics between children with and without ASD. We will investigate whether the EEG indices identified in typical development (chapter 4) are atypical in ASD.

1.5 Structure of this research

This dissertation is structured as follows:

- Chapter 2 provides an overview on the basic principles of structural and functional organization of the brain. The neurophysiological rationale for the experiments carried out in this thesis is also described.

- Chapter 3 describes the methods employed throughout the thesis to analyse the EEG data. Stimuli and analysis are widely explained.
- Chapter 4 describes our first study investigating age-related changes in networks dynamics during visual perception. EEG data from adults and children are analysed and compared to identify potential EEG biomarkers of typical neurodevelopment. The results were presented in the 2013 International Conference on Basic and Clinical Multimodal Imaging, Geneva, Switzerland.
- Chapter 5 describes a second study investigating potential changes in networks dynamics between children with and without ASD.
- In the last chapter, we summarize our contributions, providing general conclusions and pointing out to possible future directions.

Chapter 2

Brain dynamics

The human brain is known to be a complex anatomical and functional network of interconnected elements, at different scales, interacting to generate perceptual, cognitive and behavioural functions. Each one of our cognitive capabilities, our perceptions and emotions are processed in the human brain at high speeds with an optimal architectural design. Its structure and processing capacity was developed in an evolutionary manner to handle different problems and environmental challenges. Firstly, fast information processing was a significant characteristic to rise when avoiding dangerous species, and recognizing and hunting small preys to increase the chances of survival (Hofman, 2014). The ability to perform high-level cognitive tasks, such as planning and executing goal-directed movement (Sarlegna and Sainburg, 2009; Dinstein et al., 2007), was later developed in response to increases in the complexity within more sophisticated environments. Thus, perception and manipulation of large amount of sensory data to execute intelligent behaviour in a minimum of time became the kind of problems that the human brain had to solve with efficient processing and organization.

Not only size and function of brain structure has improved during the course of evolution, but also our understanding of its organizational principles. For instance, Camillo Golgi, Nobel Prize winner in the early nineteenth century, believed that information processing, whether it is simple or complex, was carried out by a unique big piece of continuously connected brain tissue (Gazzaniga, 1989). Instead, the processing capacity of the brain is determined by interacting clusters of discrete units physically interconnected and functionally

organized. This notion was first introduced by Santiago Ramón y Cajal in his Neuron Theory work (Llinas, 2003), which disproved Golgi's believes about brain functioning. Despite their contrasting theories, Golgi and Ramón y Cajal shared the Nobel Prize in 1906 due to their contribution to functional neuroscience. The advancement of imaging technology has given support to the Neuron Theory and improved our understanding of the functional organization of the brain.

In this context, this chapter is aimed at describing the principles of brain function since a system-based perspective, leading to the rationale of the studies presented in the thesis. This chapter is divided in two sections. The section 2.1 provides a comprehensive discussion on the functional organization of the brain, bringing attention to the modules of the visual system. Posteriorly, the section 2.2 presents a brief review of the connectivity analysis methods, emphasizing in measures of brain synchronization.

2.1. Functional organization and brain connectivity

Neurons maintain anatomical and functional connections with thousands of other neurons, forming neural networks of multiple levels of scale and sophistication. At the micro scale, neurons tend to connect with other neurons within a small and localized brain area, forming local networks. At the macro scale, functional connections transcend the boundaries of a single cortical region, forming large-scale networks.

Despite their size differences, neuronal networks exhibit two fundamental properties of structural and functional organization: functional segregation and integration. Functional segregation occurs when neurons with a dedicated processing function group together to form different neuronal populations or cortical areas. Functional integration refers to the coordinated interaction of neuronal populations across distributed regions of the brain (Tononi et al., 1994; Tononi et al., 1998; Friston, 2011). Both, functional segregation and integration in the brain have been extensively studied through the history of neuroscience,

with most theoretical accounts on brain function focusing on either aspects of localization or integration properties. These viewpoints of brain function, localizationist and holistic, are briefly described in the following sections.

2.1.1. Functional segregation

The localizationist theory has been concerned with the identification of different brain regions that execute specific functions. Early attempts to prove the existence of particular brain regions with specific function include neuroanatomical studies from Franz Josef Gal (1758 -1828). Examination of the skull shape of patients with speech problems, allowed him to suggest that the region responsible for language processing was localized in the frontal area of the brain. Although the examination of cranial bumps was considered not enough evidence to support Gall's theory, it encouraged other scientists to carry out neurological examinations to prove his theory. In 1851, Paul Broca presented a post-mortem study of pathological brain tissue. He showed that a lesion on the frontal region of the brain impaired the speech capacity of his patient, providing the first widely accepted evidence of cortical localization in the brain. Soon after, Carl Wernicke supported the theory of specialized cortical areas by associating several difficulties in speaking to damage to the frontal and temporal lobes. Since then, the location and organization of other cortical areas such as the primary visual, auditory and somatosensory areas have been identified as shown in Figure 2.1.

A more detailed map of the cerebral cortex was provided by Korbinian Brodmann in the early 20th century. On the basis of regional variations in the cytoarchitectonic structure of the cortex, Brodmann identified 52 brain areas, numbered according to its location and function, as shown in Table 2.1.

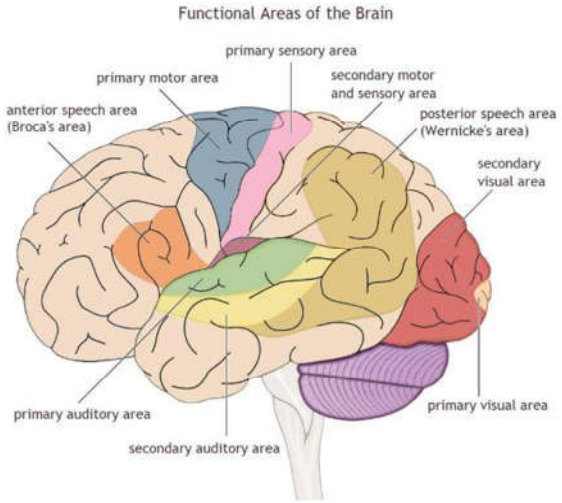


Figure 2.1. Main functional areas of the brain. Extracted from http://www.my-ms.org/anatomy_brain_part1.htm

Area number	**Area name**	**Location**
1, 2, and 3	Primary somatosensory cortex	Parietal lobe
4	Primary motor cortex	Frontal lobe
5 and 7	Somatosensory association cortex	Parietal lobe
6	Supplementary motor area and premotor cortex	Frontal lobe
8	Frontal eye field	Frontal lobe
9–12 and 46–47	Prefrontal cortex	Frontal lobe
17	Primary visual cortex	Occipital lobe
18 and 19	Visual association cortex	Occipital lobe
22	Wernicke's speech area	Temporal lobe
41 and 42	Primary auditory cortex	Temporal lobe
44 and 45	Broca's speech area (dominant	Frontal lobe

Table 2.1. Brodmann's areas

Although many more areas have been identified since then, and their delimitation and function remain under debate, Brodmann's original classification is still widely used. It has provided strong evidence of a functionally segregated organization of neurons in the cerebral cortex. However,

characterising brain function in terms of functional specialization does not reveal anything about how different brain regions exchange with each other.

2.1.2. Functional Integration

Contrary to the localizationist theory, which assigned a specific perceptual or cognitive function to a particular brain region, a holistic approach suggests that these processes cannot be performed by a brain region solely. Instead, perceptual and cognitive functions require the coordinated activation of many neuronal populations at distinct levels of scale across the cortex (Tononi et al., 1994). Functional neuroimaging studies of human participants have shown that a given task co-activates more than one brain region simultaneously. For instance, fMRI examination of human brain has shown a co-activation of prefrontal and parietal cortex during a working memory task (Cohen et al., 1997). Object recognition, attentional processing and motor planning are also considered the result of coordinated activity within large-scale functional brain networks.

Electrophysiological studies have shown that the interactions between different neuronal populations change over time, generating dynamic patterns of neural connectivity (Barriga-Paulino et al., 2011). The temporal correlation between different neuronal populations or regions of the cortex is referred to as functional connectivity. Thus, anatomical and functional connectivity are closely related in the study of brain function as they capture important aspects of brain networks. The neural connectivity in a typical developing brain determines the conditions for adequate segregation and integration, when any stimulus is present. As the brain evolves, it is expected that functional connectivity also varies over time. Thus, it is important for this work to analyse the neural measurements that reflect variations on neural conditions, when segregation and integration is degraded during atypical development. In order to determine the scope of this document, this analysis is addressed over a specific part of the cortex whose functional organization is briefly described in the rest of the section.

2.1.3. Hierarchical and parallel processing

The basic organization of the brain's architecture corresponds in a general way to the evolutionary sequence of increasingly more advanced levels of processing, from most detailed and specific to most general and abstract. At the lowest levels of the hierarchical organization, relatively simple processing is carried out, whereas more complex and powerful processing take place at higher levels. The interactions of the different parts of the hierarchical architecture have been classified according to the main routes of flow of information in the system: bottom-up and top-down (Riesenhuber and Poggio, 1999; Van Essen et al., 1992). When information processing is carried out moving forward from lower-level regions in the occipital lobe of the brain to higher-level regions in the prefrontal cortex, bottom-up processing is considered to take place. On the other hand, in top-down processing, the direction of information processing is considered to flow from higher-level regions to lower-level regions.

The distinction between bottom-up and top-down processing, establishes that in bottom-up processing, local features of a visual input are first processed by the primary visual area V1, whose functionality provides basic information about contours and orientation of the object to ascending modules along the ventral pathway. Neuroimaging studies have shown that a complete representation of a visual input, involves the activation of specialized groups of neurons in lower and higher-levels of visual areas. For example, face recognition activates specialized face-processing regions in the prefrontal cortex, but also the functionality of V1 and V2 are considered to play an important role in this task. A nose contour might suggest that the observed visual stimulus is a face (Galuske et al., 2002; Hochstein and Ahissar, 2002; Lamme and Roelfsema, 2000; Pascual-Leone and Walsh, 2001). It is still unclear whether object recognition is the result of a bottom-up processing or, the result of information feeding back from higher-level cortical regions in the parietal and prefrontal cortex to early processing stations.

Hubel and Wiesel's work (Hubel and Wiesel, 1959) led to the notion that the visual brain is organised in a hierarchical manner, with one area of simpler physiological properties feeding another in succession with more complex ones to produce visual perception. Recent studies however have also provided physiological evidence to suggest that, in addition to the hierarchical strategy, the brain may also use a parallel model to provide a unified and coherent perceptual experience. Shigihara and Zeki (2014) suggest that a parallel strategy in the brain's visual system would activate different areas of the visual cortex with similar latencies and strength during the processing of forms. In two separate studies, the authors used magnetoencephalography (MEG) and functional magnetic resonance imaging (fMRI) to investigate the activity produced in early visual areas (V1–V3) when twenty subjects viewed stimuli of increasing perceptual complexity. Three geometric stimuli (lines, angles and rhombuses) were used to test the parallel model of form processing in the brain. In the first study, a time-based analysis of the first component of event-related magnetic fields (ERFs) and an estimation of the sources of the responses showed that all visual stimuli activated all early visual areas with similar peak latencies. This finding does not reflect the temporal dynamics of brain activation that one might expect from a hierarchical strategy: shorter latencies for simple stimuli and longer ones for perceptually more complex stimuli (Shigihara and Zeki, 2013). In a second study, the research group found that all three stimuli activated all three visual areas, each stimulus activating each visual area with the same strength, which is the opposite of a hierarchically organized model dedicated to form processing, in which later visual areas (e.g., V3) are strongly activated by complex forms, while the simplest form would produce stronger activation of earlier visual area V1 (Shigihara and Zeki, 2014). Stimuli of faces and houses composed of straight lines have also been used to test the parallel strategy used by the visual brain (Shigihara and Zeki, 2014). Similar latencies were found in the MEG responses to both categories of stimuli in V1 and other specialized areas of the visual cortex outside it, in addition, face stimuli produced a larger amplitude than that elicited by house stimuli. These results have been supported by other psychophysical study, in which 26 subjects participated in

three different masking experiments to test the parallel strategy used by the visual brain (Lo and Zeki, 2014). The authors reported that, simple and mid-complex forms were well masked by each other, whereas the more complex forms were strongly masked only by the more complex forms but weakly masked by simple or mid-complex forms, which was interpreted by the authors as evidence of parallel processing of forms in the visual brain. According to the authors, the resistance of more complex forms to be masked by low-level forms suggests that complex forms may be processed in a separate parallel system, whereas simpler forms are processed in the same one. As conclusion, these results suggest that low-level and high-level information processing may be carried out in parallel by V1 and successive specialized visual areas outside it during visual perception.

Some other anatomical studies suggest parallel processing in the extrastriate cortex, the parietal, temporal, and frontal brain regions to deal with distinct aspects of stimulus information (Binfoski et al., 2013). After recombination of different subdivisions, two streams emerge: the dorsal and ventral streams (Figure 2.2), to perform integrative processing (see next section for a detailed description of these streams). It is suggested that parallel processing involves three connection types: feedforward, feedback and horizontal connections (Lamme and Roelfsema, 2000). Feedforward connections are those that provide input from cells at lower levels of brain organization, whereas feedback connections provide input from higher levels. Horizontal connections provide input from cells at the same level.

In summary, findings reviewed here suggest that the organization of the visual system has hierarchical as well as parallel properties. Although it is difficult to identify hierarchical o parallel processing on the basis of a sigle criterion, such as anatomical connections or latencies, they are useful concepts to increase our understanding of brain function. Hierarchical processing is mainly associated with feedforward connections involved in perception, whereas parallel

processing is thought to be required for visual attention and higher cognitive processes (Lamme and Roelfsema, 2000; Sigman and Dehaene, 2008).

2.1.4. The lower-levels of organization in visual system

At the lower-levels of brain organization, the visual cortex exhibit intra-areal connections, forming anatomical and functional networks at the local level. Overall, five relatively independent processing areas have been identified in the human visual cortex: V1 (also known as striate cortex), V2, V3, V4 and V5 (also known as medial temporal MT). Each of these visual areas contains neurons with a dedicated function. Hubel and Wiesel (Hubel & Wiesel, 1968) found that neurons in V1 fired strongly to the presence of bars and edges, behaving as spatial filters that selectively respond to oriented bars. Colour processing and vision motion have been attributed to V4 and V5 respectively (Zeki et al., 1991; Zeki, 2004; Watson et al., 1993). In the first place, patterns of light entering the eyes produce neural impulses, which go through the optic nerves, lateral geniculate nucleus (LGN) and then activate neurons in multiple brain regions starting from the primary visual cortex (V1). From there, the signals are passed on to a number of functionally distinct areas of the visual cortex, through two visual channels known as the dorsal and ventral streams, where further visual processing takes place.

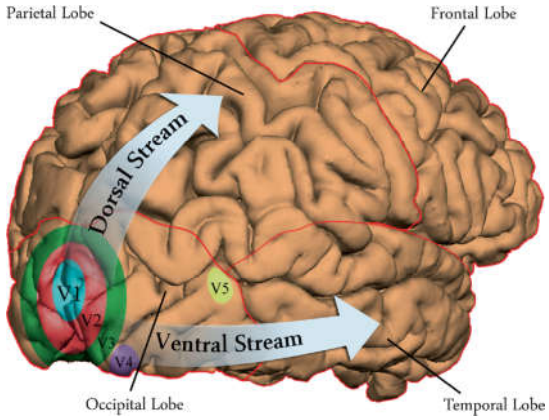


Figure 2.2. Visual streams. Form and recognition is processed in the visual ventral pathway. Spatial information is processed in dorsal stream. Extracted from (Possin, 2010).

The ventral pathway, also known as the "what" stream (Goodale and Milner, 1992) deals with the identification and recognition of objects. This channel connects primary and secondary cortical visual areas (V1 and V2) with visual area 4 (V4). Then, visual information from the occipital lobe travels to the temporal lobe and projects to prefrontal regions. The dorsal pathway, also known as the "where" stream (Goodale and Milner, 1992), is crucial for spatial and motion detection of objects in space. It connects primary and secondary visual areas (V1 and V2) with visual area 3 (V3) and middle temporal area (V5/MT), and further parietal and prefrontal regions (Cloutman, 2013).

The dorsal and ventral streams are considered to be broadly mapped onto sub-cortical streams originating in the LGN and termed the magnocellular (M) and parvocellular (P) streams. Functional studies have shown that neurons contained in the M and P pathways have distinct properties. Neurons in the M pathway are large cells considered to be unresponsive to colour under equiluminant conditions (Merigan, 1989), sensitive to higher contrast, responsive to lower spatial frequencies and higher temporal frequencies (Derrington & Lennie, 1984; Fujita et al., 2011) with transient responses. Neural properties in the P pathway include colour sensitiveness, lower contrast sensitivity, with preference to higher spatial frequencies and lower temporal frequencies, and sustained responses.

The projections of the M and P cells to higher-order visual channels appear to be distinct. The M pathway projects to the dorsal stream through the middle temporal area (MT) probably accounting for motion perception. Because of the different physiological properties of the M and P pathways, it is a common strategy to use stimuli that preferentially activate the individual pathways in order to study conditions, such as autism and dyslexia where one or other of the pathways has been suggested to be impaired (Milne et. al. 2002; Talcott et. al. 2000). Gratings with different visual attributes such as luminance contrast, chromaticity, and spatial and temporal frequencies are widely used to preferentially activate the two pathways. In particular, temporal characteristics

of vision perception at early levels of processing have been measured using steady-state paradigms, which require the presentation of a long-lasting train of stimulus (Burr and Morrone, 1996; Fujita et al., 2011; Fernandes Costa, 2011). The information processing carried out within these streams haw been considered evidence of anatomical and functional networks interacting to accomplish a common goal in the human brain (Binkofski and Buxbaum, 2013). This work aims to investigate functional integration between visual cortical areas by identifying age-related changes in functional networks during visual perception and networks dynamics in autism using neuronal synchronization techniques such as the steady-state visual evoked (SSVEP) paradigm, which is described in the following section.

2.2. Neuronal synchronization

Neurons in the cerebral cortex fire electrical pulses that travel down their axons in order to transmit the results of information processing to other neurons. The firing rates of single neurons form an electric field that can be recorded from the scalp using the EEG. The oscillatory activity showed in the EEG signal represents the mean summation of the firing signalling in the time domain. In this thesis, we are interested on analysing the EEG response of synchronized brain networks, when a specific stimulus is present, through the paradigm described in this section.

2.2.1 Steady-State Visual Evoked Potentials

Neurons dedicated to specific visual processing fire stronger than others non-related to stimulus processing, elicited by a functional network. Numerous studies have used transient stimuli to assess the integrity of networks within early visual pathways, visual cortex, and higher cognitive processing (Silberstein, 1990). The waveforms elicited by this kind of stimuli are widely known as Event Related Potentials (ERPs). Hundreds of transient ERPs are needed to obtain a reliable average ERP waveform. This related-stimulus approach has demonstrated that neurodevelopmental conditions affect EEG response, due to

atypical functional connectivity (Milne et. al., 2009). Therefore, this approach has had immense practical value in segregating stimulus related functional neural networks from background brain activity in clinical and cognitive studies.

In contrast to these transient ERPs, when a visual stimulus is presented to the participant in a rapidly repetitive fashion, the brain generates steady-state visual evoked potentials (SSVEPs). Electrophysiological experiments have shown synchronous activity of large populations of neurons in response to temporally modulated flickering stimuli (Okamoto and Nakagawa, 2011). The oscillatory activity recorded in the electrophysiological signal correlates with the neural circuits that support such an activity. A range of temporal frequencies have been used to assess the capacity of different neural circuits and the underlying mechanisms associated with them (Srinivasan, 2006; Herrmann, 2001).

SSVEPs are best analysed in the frequency domain, which require only a short time of recorded signal. This kind of brain response can be obtained noninvasively with minimal task demands. The recorded data present an excellent signal-to noise-ratio, and are relatively immune to participant movement or artefacts, which make this technique suitable for studying brain activity in young children and cognitive affected participants (Brenner, 2009). Because of all these features, recent electrophysiological studies on brain function have suggested that the integrity of functional networks would be best assessed using SSVEPs. In particular, functional brain networks that process visual sensory information can be investigated using SSVEPs paradigms, as the functional and physical properties of neurons in this region are well characterized. Experiments with humans have shown that steady-state responses elicited by flashing visual stimuli in a range of temporal frequencies generated different peaks in the magnitude of the brain activity (Herrmann, 2001; Lin et. al., 2006). Figure 2.3 illustrates how brain response varies when temporal frequency of the SSVEP is modulated.

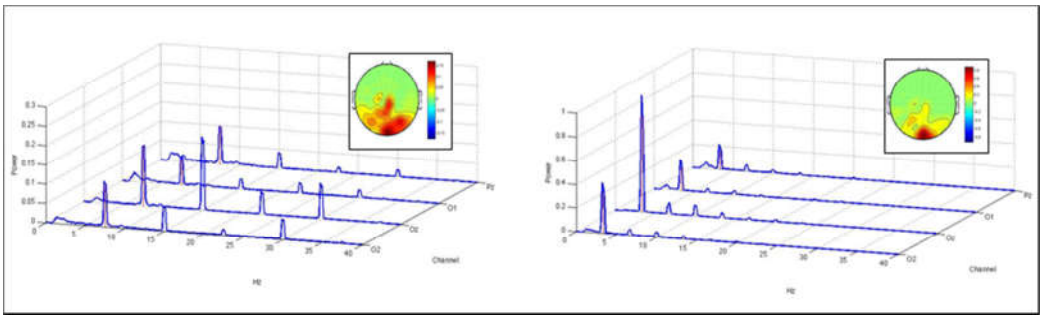


Figure 2.3. Spectral response of SSVEP. EEG responses with stimulus at high and low temporal frequencies, respectively (see Chapter 4).

SSVEP responses have also shown different sensitivities to physical stimulus parameters such as spatial frequency and colour (Song and Keil, 2013; Muller et al., 2006). This difference in stimulus-related brain responses has suggested that SSVEPs can entrain functionally distinct although spatially overlapping cortical networks that can be recorded using the EEG.

Current neuroimaging research suggests that SSVEP paradigm may provide promising results in neurodevelopmental analysis. SSVEP-based studies, driven on children and adults, have shown that adults present superior response with decreased stimulation frequency (Ehlers et al., 2012). This distinction may be caused by the developmental changes of the underlying neuronal structures. Furthermore, when ASD is present, atypical EEG measures by SSVEP elucidate compromised specific neural substrate in the early visual system (Pei el al., 2014).

2.2.2. Measuring brain dynamics

The brain is a complex mechanism made up of individual elements: from cells to neural ensembles. These elements are connected, so the brain can combine their individual actions to do everything it does, through their interactions. The combined responses of discrete populations of neurons allow interacting brain areas an extensive response repertoire, ranging from perception to cognition. When neuroimaging data are examined in terms of brain interactions, it is observed that many regions cooperate in perceptual and mental processes.

Emerging neurobiological theories emphasize the combined activity of interacting brain elements at multiple scales (cells to ensembles to regions) as the basis for brain function (Tononi et al., 1994; Friston, 2002; McIntosh, 2000). Thus, over the last several years, there has been a growing interest in studying both normal and pathological brain function by identifying variations in activation of brain areas and the functional interactions among them. However, studying the functional interactions among the neural assemblies distributed across different brain regions is one of the most challenging aspects of brain function due to the brain's adaptive capabilities. The brain can reorganize in response to external stimuli and after brain lesions and disease (Zola-Morgan, 1995; Gazanniga, 1989; Friston, 2005). However, the dynamics of this organization seem overlooked by traditional research on brain activity. Instead, a modern network perspective focuses on the structure and dynamics of neuronal networks underlying perception and cognition (Weiss and Mueller, 2003; Bassett et al., 2006). The key point here is that a more complex representation of the brain as a dynamic interconnected network, capable of plasticity and adaptation, inherently emerge from this network perspective.

The complex network-based time series analysis can be described as the transformation of a time series into a network using methods derived from the theory of graphs (Chavez and Valencia, 2010; Dimitriadis, 2010), and on the subsequent extraction of information about the time series through the analysis of the obtained network. The configuration of the network can be captured by its adjacency matrix, that contain the interdependencies between every possible pair of nodes of a graph (the nodes correspond to distinct brain regions or recording sites) as the strength of the functional connection between a particular pair, yielding so called functional networks, that have been used in brain-imaging techniques, such as Electroencephalography (EEG), Magnetoencephalography (MEG), Functional Magnetic Resonance imaging (fMRI) and Positron Emission Tomography (PET), to study the brain's functional connectivity (Yang et al., 2015; Valencia et al., 2008; van Straaten and Stam, 2013). Sendina (2011) applied a network approach to time series analysis of MEG data to evaluate the

balance between segregation and integration in functional brain networks during a memory task in healthy individuals. By means of EEG recordings, Sargolzaei (2014) applied a network approach to explore on the functional connectivity networks of paediatric subjects with epilepsy. For a review of functional networks examined in developmental disorders and other psychiatric conditions such as autism, schizophrenia and Alzheimer's disease using different neuroimaging techniques see (Takahashi, 2012; van Straatenn and Stam, 2013). Overall, the interpretation of brain connectivity measures in terms of functional networks has allowed the identification of basic patterns of brain dynamics: high functional coordination and fewer fluctuations in brain activity within the neuronal networks in the mature brain (Damoiseaux et al., 2006), transient coordinated activity within the developing brain's functional networks (Nenadovic et al., 2011), whereas both hyper- and hypo-synchronous activity of local and distributed neuronal networks are associated with impaired information processing (Schindler et al., 2007; Isler et al., 2010).

It is interesting to note that increasing evidence suggest that the brain is a network of interacting elements at multiple spatial scales ranging from the neuron to inter-areal interactions that, when functioning properly, produces an extensive range of dynamic, adaptable behaviour. However, when a specific element of this network fail or its integrity is affected by anything, then it will necessarily influence the function of the entire network or networks in which this element participates, contributing to brain disorders, including autism, epilepsy, and Alzheimer's disease. Thus, much could be learned about normal brain functioning and its alterations in neurological or psychiatric conditions, by examining functional networks in subjects where behavioural deficits are present due to damage or disease.

Tononi and colleagues (1994) have shown that functional segregation of specialized brain areas and their functional integration to produce perception and behaviour occurs at multiple levels of organization, from local to global, ranging from primary visual area to remote brain regions. Also, they suggested

that the interplay between these two aspects of brain organization is crucial to understanding brain function. Recent experimental work has shown that the interplay between segregation and integration in functional brain networks can be captured by a complex-network approach, allowing the study of the brain as a dynamical system (Sendina et al., 2011; Rudie et al., 2011). Using signal-processing techniques for quantifying the brain networks, functional connectivity can identify brain regions or recording sites that have similar frequency, phase and/or amplitude of correlated activity, revealing the topologies of their network representations. For a review of connectivity measures used in neuroimaging techniques see (Sakkalis, 2011; van Straatenn and Stam, 2013). The interactions between elements of the networks however are oscillating over short and long periods of time, thus, the EEG technique with its excellent temporal resolution, is optimal for examining the dynamic patterns of functional connectivity. A more recent perspective deals with tracking brain dynamics of the networks via time-dependent network analysis, with the aim of describing the overall structure of functional networks, in time series terms, which may reveal the dynamic properties of the brain's functional connectivity. This time-dependent network approach has made possible to investigate connectivity changes accompanying reorganization and pattern formation in the functional networks elicited during mental calculations (Dimitriadis et al., 2010), what remains unexplored is the applicability of this approach to lower-levels of information processing. In the current study, we examined the network-metric time series during visual perception.

Temporal synchronization of neuronal systems is essential for perceptual and cognitive processes (Dimitriadis, 2010; Nenadovic, 2011; Michel and Murray, 2012; Burr and Morrone, 1996). The electroencephalogram (EEG) provides a temporal resolution in the order of milliseconds for studying temporal evolution of the brain activity associated with brain processes in health and disease (Banaschewski and Brandeis, 2007). However, temporal changes in brain activity, as reflected in EEG, are rarely exploited due to lack of analytical tools and methodology. Special techniques are required for the detection of such

dynamics. That is why this project aims to explore a neurophysiological concept of functional connectivity that utilizes, within a unified framework, both local and remote neuronal interactions at the millisecond scale of EEG. Local neuronal assemblies underlie particular brain operations such as perception and low-level processing (local functional connectivity); however, cognitive operations arise from combined synchronous actions/operations of many neural assemblies (long-scale functional connectivity).

Traditionally, coherence and correlation have been the main methods to assess the degree of functional connectivity between brain areas (Shaw, 1984; Weiss and Mueller, 2003). However, mathematically, the coherence value indicates only the linear statistical link between time-series curves in a frequency band (Lachaux et al., 2002). Meanwhile, in general, the absence of some types of statistical interrelation between two processes does not mean the absence of any interaction between them at all. Therefore, one must be careful when interpreting coherence as an indicator of functional connectivity (Sakkalis, 2011).

Recently, several new methods for detecting functional connectivity between cortical areas have been published: partial directed coherence (Baccalá and, Sameshima, 2001), dynamic imaging of coherent sources (Gross et al., 2001), and phase synchrony based on wavelet or Hilbert transforms (Stam, 2005). However, all these methods have one or several of the following limitations. They do not take into consideration the nonstationary nature of the signal, they require a large number of trials of analysis (Luck et al., 2000), they use averaging and smoothing procedures, and they use linear models which for the brain is not typically the case (Dimitriadis et al., 2010). For the phase concept to be successfully used the frequencies of the signal should be locked, otherwise multiple harmonics of these frequencies may overlap and lead to ambiguous phase information (Tognoli and Kelso, 2008). Another drawback is that all these approaches do not consider that brain dynamics is a phenomenon variant over the time and space, which in general, require a spatiotemporal approach. In

conclusion, none of the methods above mentioned alone allows the direct characterization of brain dynamics at the EEG level.
This work aims to explore a new multidisciplinary approach of complex-network analysis that has emerged with the scope of characterizing these datasets and understanding the underlying mechanisms.

In summary, the study of cortical network organization may increase our understanding of normal brain function as well as functional brain disorders associated with abnormal connectivity. The complex network approach has proven to be a valuable tool to capture the dynamics of network activity during several mental processes, focusing primarily on the topological organization of the functional networks involved in specific tasks. A more recent complex network approach proposes a time-dependent characterization of brain connectivity by investigating functional segregation and integration, the two aspects of brain organization that coexist in the human brain. This approach has successfully been applied to EEG recordings of healthy subjects during the execution of mental calculations of increasing complexity (Dimitriadis et al., 2010). The incorporation of a network-metric time series allowed following the time evolution of the interplay between the integrated and segregated activity in functional brain networks, suggesting a time-dependent organization of the cortical structure of the functional networks during the mental calculations. Although this kind of analysis has only been applied to high-level mental calculations, we believe that it could be applied to low-level visual processing to explore differences in the dynamics of functional connectivity between children and adults, and between individuals with and without autism.

Chapter 3

General Methods

This chapter describes the materials and methods common to the experimental studies contained within this thesis. A detailed description of the actual electrophysiological procedure, from setup and recording to EEG analysis, is provided.

3.1 Participants

Due this thesis aims to investigate the effect of development in early visual and how ASD affect those observations, we decide to recruit three groups of subjects for the experiments: typical developing children, healthy adults and diagnosed ADS children. The total sample comprised 10 children diagnosed with ASD (mean age: 12.1 years, range 8.2–17.8 years, 2 female), 30 typically developing children (mean age: 11.09 years, range: 7.3–17.5 years, 7 female), and 11 healthy adults (mean age: 30.08 years, range: 23.7–37.9 years, 7 female).

Typical developing children were recruited by distributing the Parent Information Sheet, provided in Appendix A, at mainstream schools. In this document the aims of the test and the process is clearly described. In addition, the document explains the advantages and the risks for participating in the study, provides contact information in case of any complaint, and stablishes the usage of the data acquired during the test session. The interested families in participating were provided with the Letter to parents presented in Appendix B, where they provided general data of their child and contact information. On the other hand, children with ASD were recruited by approaching autism-specialist schools and NHS-units supporting patient on the autistic spectrum. Interested families with ASD diagnosed children were provided for the Parent Information

Sheet and the Letter to parents from Child and Adolescent Mental Health Services (CAMHS), in Appendix C.

At the beginning of the session, adults and children, along with their parents, were informed of methods and purposes of the examination and they provided written consent by the form in Appendix D. Parent had to fill out the Developmental History Questionnaire, presented in Appendix E. All the participants answered a Brief Medical History Questionnaire, such as the form presented in Appendix F, reporting normal or corrected to normal vision. As well, participants who required corrective eyewear for reading were encouraged to wear eyeglasses as opposed to contacts to reduce eyestrain and irritation. None of them reported a known history of epileptic seizure or head injury. All participants were right-handed. However, although it has been suggested that handedness may affect high-level functions involving both cognition and perception such as object recognition (Ferneyhough et al., 2010) and language processing (Newman et al., 2014), we did not include handedness in the exclusion criteria because our experimental paradigm involves no instructions or tasks, and is relatively unaffected by attention or other cognitive processes (Pei et al., 2014), which makes it well suited to the investigation of visual perception in infants and individuals with impaired cognitive and speaking capabilities.

The experimental procedure of this research was reviewed and approved by the Ethics Committee of Sheffield University for the study on adult participants. NHS ethics approval was given for the studies on children.

3.2. Apparatus

The EEG signals for all subjects were acquired following the EEG Data Acquisition Protocol used in the Sheffield Autism Research Lab (ShARL) at The University of Sheffield (http://autismresearchlab.group.shef.ac.uk/). The EEGs were recorded from the scalp using a 128 -channel Biosemi ActiveTwo system

(Biosemi, Amsterdam, Netherlands). The Biosemi configuration considers two separate electrodes located just posterior to the vertex, which are labelled as CMS and DRL (http://www.biosemi.com/faq/cms&drl.htm) to replace the reference and ground electrodes used in traditional amplifiers. See Figure 3.1 for electrode layout.

All subjects had two electrodes placed 1.5 cm above and below both eyes to record blinks and vertical eye movement. Horizontal eye movement was recorded by placing one electrode 1 cm lateral to the outer canthi of both eyes. EEGs were recorded with a sampling rate of 2048 Hz, processed online using a 0.16-100 Hz bandpass filter and stored on a hard drive for off-line analysis.

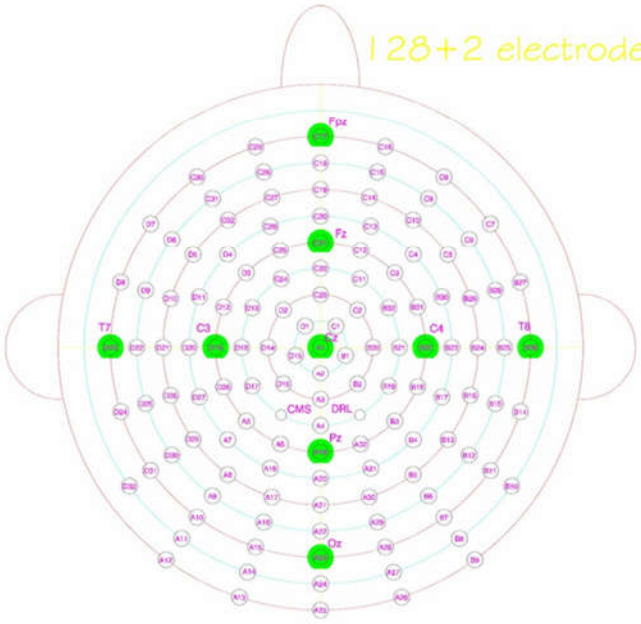


Figure 3.1: Sensor layout of the Biosemi 128-channel system. Green sites approximately correspond to sites of the international 10-20 EEG arrangement.

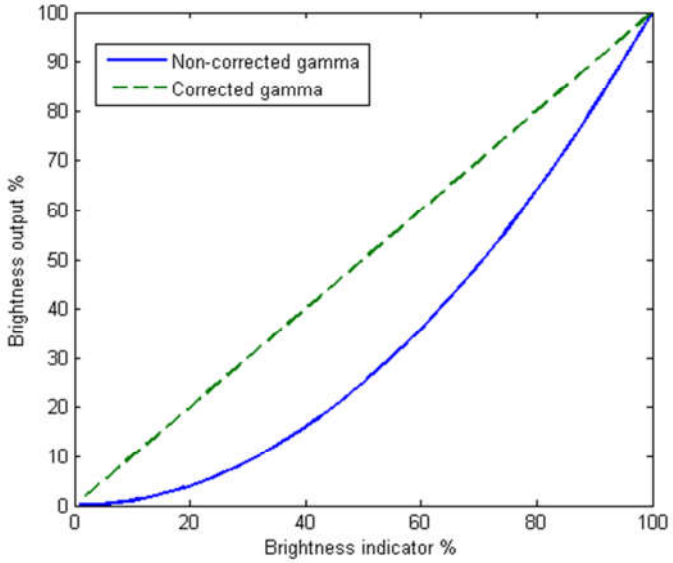


Figure 3.2: Gamma monitor response. The non-corrected signal will produce an inappropriate visual stimuli caused by the non-linearity. When gamma is corrected, the monitor will generate proportional luminance.

Stimuli were generated using the software packages Matlab (The Mathworks, Inc) and Psychtoolbox (Brainard, 1997). Stimuli were displayed on a 17-inch colour CRT monitor with a spatial resolution of 1280 x 1024 pixels and a frame rate of 60 Hz. Gamma correction was performed prior the experiments, which is required due that PC monitors do not produce light intensity in a proportional manner than the input voltage. Gamma is the non-linear effect in the monitor with respect to the input voltage regulated by the processor (Kubinger et al., 1998). Figure 3.2 illustrates the gamma response before correction. First, monitor luminance was measured with a ColorCAL MKII colorimeter (Cambridge Research Systems, Rochester, UK), in a light-controlled room. The measurements were used to approximate an exponential function using the Matlab Curve Fitting toolbox. Posteriorly, an intensity 8-bit lookup table was generated to be used to calibrate the monitor prior each experimental session and ensuring that the monitor was linear over the entire luminance range used in the experiments. The study took place in a dark room and the screen mean background luminance was set to 21 cd/m2. Stimuli were centrally presented in a square, which subtended

14.4° x 10.5° of visual angle, and observed from a distance of 70 cm., see Figure 3.3.

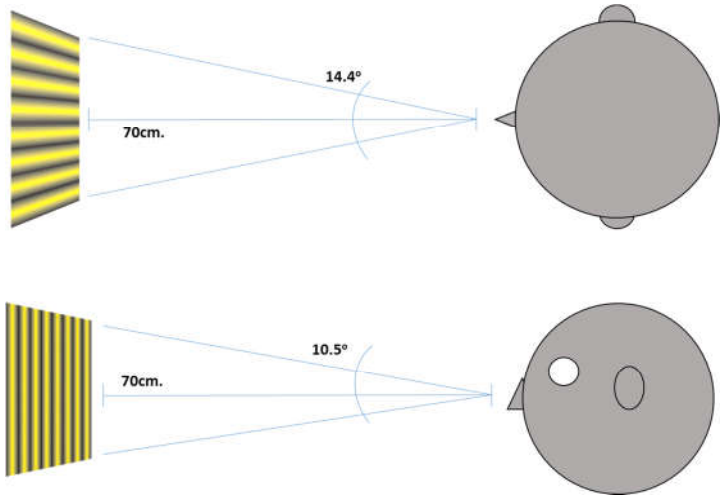


Figure 3.3: Experimental setup.

3.3. Stimuli

The EEG variable of interest for the main aim of this study was the dynamics of brain connectivity in local and distributed regions of the brain involved in lower-level visual processing. Stimuli consisted of vertically oriented sinusoidal gratings of two different spatial frequencies, defined by either colour or luminance contrast presented in a flickering way, in order to synchronize the response of specific cortical networks, using the Steady-State Visual Evoked Potential paradigm described in Chapter 2.

In order to reduce effects related to stimulus display artefacts (McCleery et al., 2007), the stimuli were presented in a yellow background where its chromaticity was defined, based on the International Commission on Illumination (CIE) space, with x=0.45123 and y=0.47826, as a mixture of the chromatic and luminance gratings. This condition ensured that the mean luminance and chromaticity of the background stayed constant across trials, allowing the extraction of the impact of SSVEP on the brain activity related to stimulus specificity.

3.3.1. EEG paradigm

During the experiment, participants underwent a Steady-State Visual Evoked Potential (SSVEP) paradigm, where each stimulus was presented in a rapid flickering manner. Four different visual stimuli were presented in random order, each one in trials of 8 sec of duration to elicit steady state brain responses, such as it is showed by the Figure 3.4. At the end of each trial, a cartoon character appeared for 1000 ms. to indicate a break to the participant, but also to keep child subjects alert. In case of eye tiredness, the subject was able to stop the current trial by pressing any key of the computer.

The rate of inter-trial interval was self-paced: participants were asked to initiate the upcoming trial by pressing the space bar key on a computer keyboard (ranged from 5 s to 10 s). The selection of a self-paced inter-trial interval was made in an attempt to minimize boredom and maximize alertness. ERP studies suggest that the duration of inter-trial interval may alter the cognitive processes involved in visual attention, affecting the amplitude of the N200 and P3 ERP components, while keeping the amplitude of the early perceptual ERP component (C1) relatively unaffected (Van der Borght et al., 2016). According to this, because the current study focuses on low-level perceptual processing, the amplitude of C1 would only be affected by the stimulus properties but not for the inter-trial interval, which may be useful in our studies with children and individuals with autism.

A white fixation cross subtending a visual angle of 0.2° remained in the centre of the screen during the experiment. In total, an approximated of 40 trials (10 times each different stimulus condition) were executed for five minutes plus breaks minutes, the total time of the experiment, including pauses, did not exceed 20 minutes.

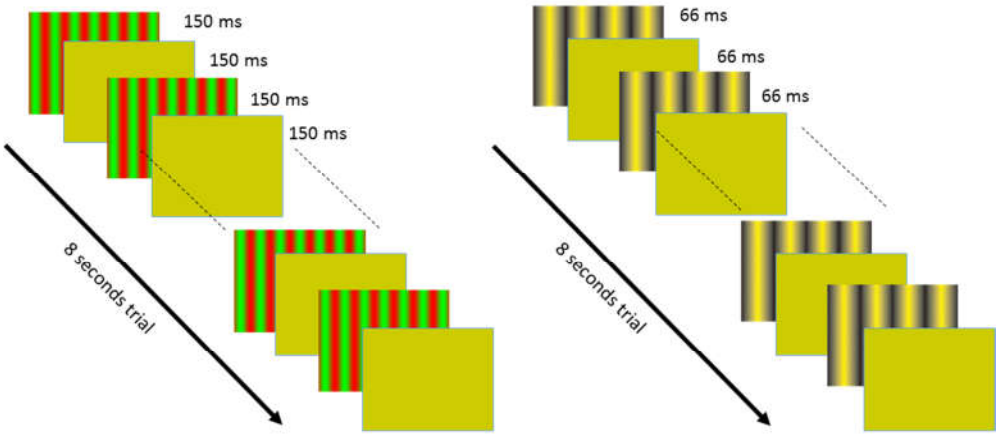
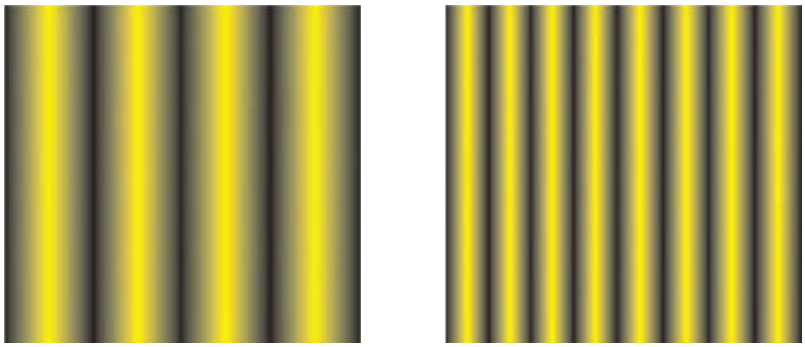


Figure 3.4: SSVEP paradigm. Each stimulus was presented in 8 seconds trials, in a flickering manner with a yellow-homogenous background. Each image in chromatic stimuli was displayed for 150ms. Meanwhile, each image in luminance stimuli was presented by 66ms.

The entire procedure lasted approximately one hour including equipment setup and data collection. Each participant was properly briefed regarding the procedure for ERP experiments and the importance of staying focused and still during the experiment. Once participants were comfortable with the EEG setup, they were instructed to remain in a relaxed position for the duration of the experiment and to keep at minimum eye blinks during trials.

3.3.2. Luminance stimuli

Luminance stimuli based on gratings was selected to elicit Magnocellular characteristics in visual cortical networks. The Magnocellular system is originated from the retina and projected to V1, with connectivity in MT. Magnocellular neurons are characterized by high luminance contrast sensitivity and response to high temporal frequencies (McCleery et al., 2007, Fujita, et.al.,2011). In order to compare the brain activity produced by high luminance contrast, sinusoidal gratings with two different spatial frequencies were defined, based on ASD studies (Jemel et. al., 2010), where mid spatial frequency (MSF) was 2.8 cpd (cycles per degree) and high spatial frequency (HSF) was established as 6 cpd, such it is illustrated in Figure 3.5.



a) 2.8 cpd **b) 6 cpd**

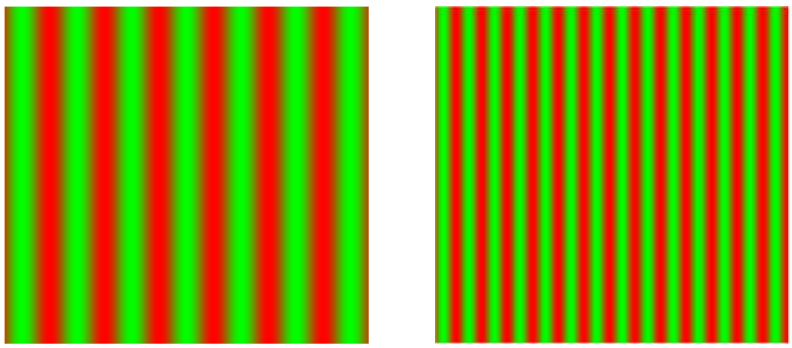
Figure 3.5: Yellow-black luminance stimuli. Based on gratings with different spatial frequencies. The MSF sinusoidal grating showed in a) presents 2.8 cpd and the HSF grating in b) is 6 cpd.

The foreground held a maximum luminance L_{max} =21cd/m2 and a minimum luminance L_{min}=0.5cd/m2. Due this combination of luminance, the Michelson contrast, defined by C=100 $(L_{max} \quad L_{min})/(L_{max} + L_{min})$, was 95%. The stimulus was presented against the yellow background for 4 refresh cycles (66 ms, 'on') and disappeared for 4 refresh cycles (66 ms, 'off') resulting in a stimulation frequency of 7.5 Hz. This frequency was established based on studies that demonstrated brain responses positively as temporary frequency of luminance gratings increases (Henning and Derrington, 1994). However, we had to consider that high temporal frequencies (>13Hz) may cause undesirable seizures to the subjects (Fisher et. al., 2005). This issue was reported to the Ethical Committee of the University of Sheffield and informed to all of the subjects before any test. Subjects with seizure antecedents were rejected of the study.

3.3.3 Chromatic stimuli

Grating-based chromatic stimuli were utilized for elicit Parvocellular characteristics of networks in visual cortex. Parvocellular system arises from retina to VI, with connectivity in V4 which plays an important role in colour perception (Livingstone and Hubel, 1988). Parvocellular neurons present low contrast sensitivity but high colour sensitivity when stimulus is presented at low temporal frequencies (Henning and Derrington, 1994). Vertical sinusoidal

gratings with two spatial frequencies (MSF=2.8 cpd, HSF=6 cpd) were used to compare the brain activity produced by colour contrast at medium- versus high-spatial frequency in visual cortex, such it is illustrated in Figure 3.6. The selection of colours was motivated by the red-green opponent processes in the midget retinal ganglion cells (80% of total retinal cells), which have on-off receptive field with those colours and because there is good evidence for human psychophysical and electrophysiological measures of spatial contrast response (Mullen, 1985).



a) 2.8 cpd **b) 6 cpd**

Figure 3.6: Red-green isoluminance stimuli. Based on gratings with different spatial frequencies. The MSF sinusoidal grating showed in a) presents 2.8 cpd and the HSF grating in b) is 6 cpd.

Red-green chromatic gratings varied only in chromaticity with equal luminance of red and green. The chromaticity was defined, based on the International Commission on Illumination (CIE) space, were x=0.6234, y=0.343 for red, and x=0.27906, y=0.61352 for green. Stimuli were surrounded by the yellow background with chromaticity x=0.45123 and y=0.47826. The luminance of red and green, as well as the homogeneous background, was 21 cd/m2. The contrast level was 0% as defined by the Michelson contrast. The chromatic stimulus remained on screen for 9 refresh cycles (150ms, 'on') and replaced by a homogeneous background for 9 (150ms, 'off') refresh cycles. This stimulus was presented in SSVEP with temporal frequency of 3.3 Hz, because visual system has been found to be more sensitive to the colour at this temporal frequency (McKeefry et al., 1996). The duration of one trial was 8000 ms of continuous flickering stimuli.

3.4. EEG Data Analysis

3.4.1 Pre-processing

After the raw EEG data were recorded, a digital pre-processing stage took place in order to make the signals suitable for algorithms of pattern analysis. The EEG signals were down sampled to 512 Hz to reduce file size and computing time. As illustrated in Figure 3.7, raw signals are vulnerable to low frequency drifting and high frequency noise caused by power supply. Hence, a bandpass filter of 1 and 40 Hz was applied to all EEGs. This frequency range would allow us to analyse the neural responses elicited by our visual stimuli, avoiding undesirable signals generated by electrical sources whose frequency spectrum falls between 50 and 60 Hz. This step was always executed in all EEG signals.

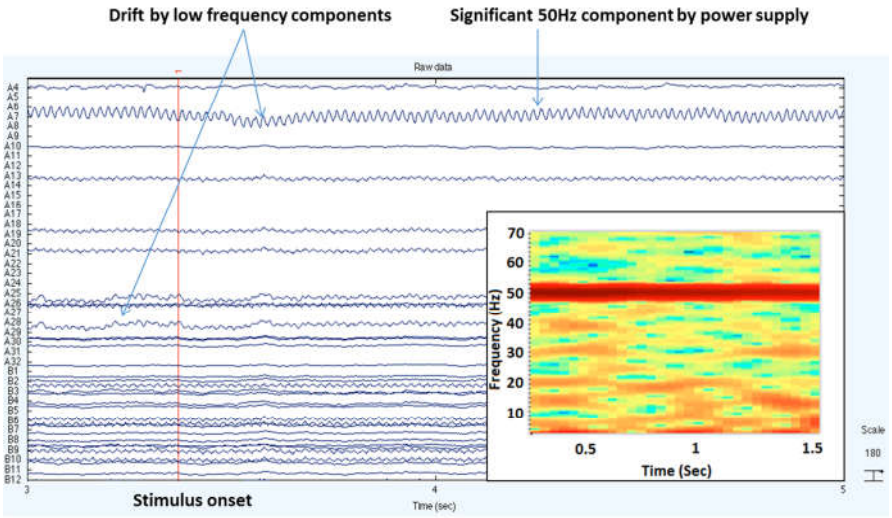


Figure 3.7. Drifting and high frequency noise in EEG signals. The spectrogram shows the 50Hz dominance in channel A7 by the power supply.

Bad channels with poor EEG signal resulting from insufficient contact with the scalp were identified by visual examination of the time series data and their spectra, and replaced by an interpolated channel obtained from neighbouring electrodes by means of linear interpolation (Kahaner et al., 1989). Figure 3.8 shows how this function computes the interpolated channel: the value of the bad channel is replaced, in each point of the time series, by the median value among

the value of the surrounding electrodes (Delorme and Makeig, 2004). Any channel where the maximum amplitude exceeded 100μV was also replaced. This was necessary in less than 40% of subjects for fewer than 10% affected channels, which were mainly located on the peripheral ring of the electrode arrangement.

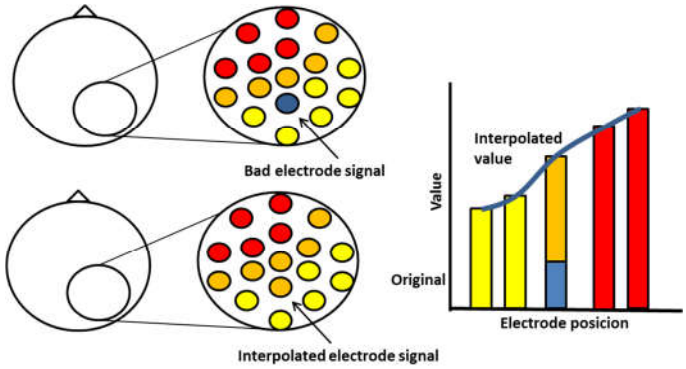
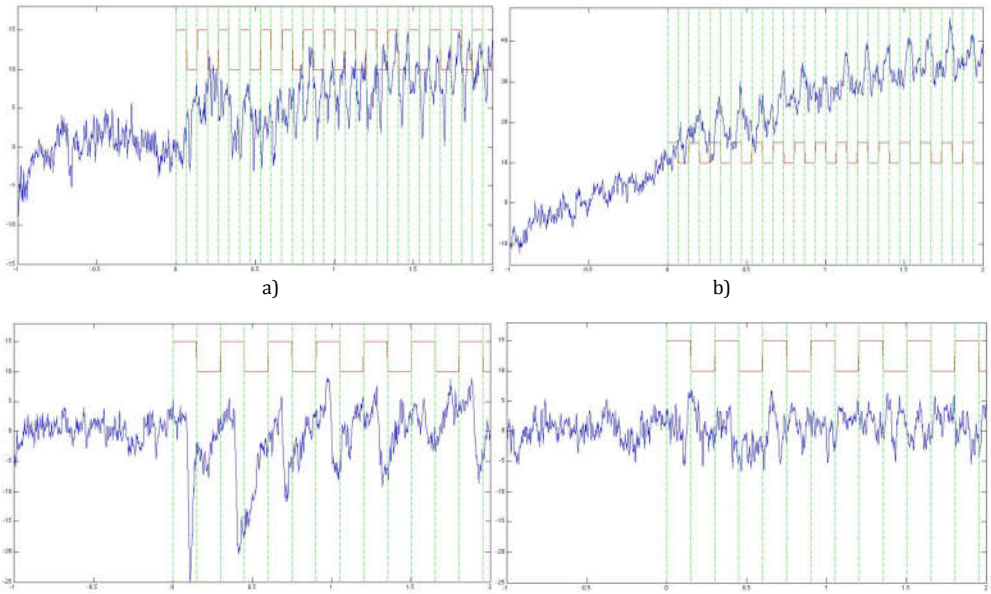


Figure 3.8. Interpolating a bad electrode. The value of a bad electrode is obtained by computing the median value of the surrounding electrodes.

After bad channels correction, EEG data were segmented into epochs based on stimulus type. The experiment was designed to obtain EEG datasets containing data in each visual stimulus over subjects of different chronological age. The extraction of individual epochs was performed time-locked with respect to the onset of each type of visual stimuli for each subject at all scalp sites. The epoch limits were defined from 500 ms before to 7500 ms after stimulus onset. Figure 3.9 shows the channel signal located in the centre of the occipital area (Oz) from an adult subject, in each stimulus, and SSVEP is illustrated as a square signal. In some cases, the presence of the synchronized response is evident from the stimulus onset, such as the responds to the chromatic MSF stimulus (Figure 3.9-c). However, in other cases, the signal presents a drift caused by varying temperature in the skin of the subject (Figure 3.9-b).

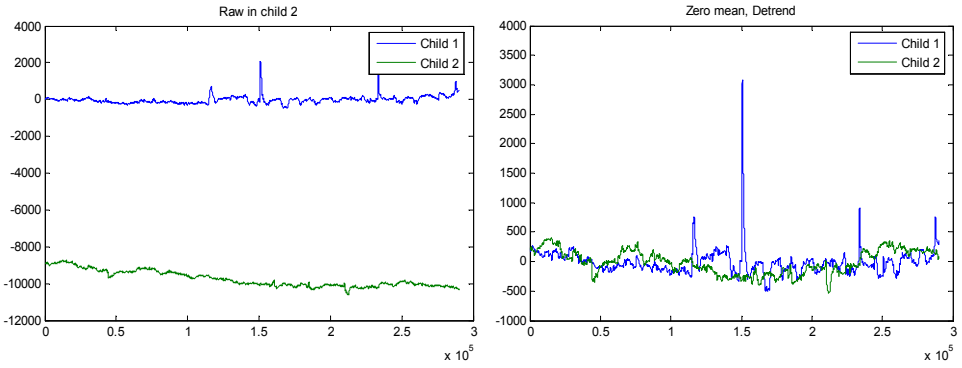


a) b)

c) d)

Figure 3.9: Response at the occipital channel (Oz). Signal of an adult subject, when stimulus is a) luminance LSF, b) luminance HSF, c) chromatic LSF and d) chromatic HSF.

For baseline correction, the mean value computed in the pre-stimulus period was removed from each single epoch. Also, data detreding was executed over drifted signals, using EEGLab toolbox functions. This allowed computing statistics over signal from different subject, such as it is illustrated in Figure 3.10.



a) b)

Figure 3.10: Response at the occipital channel (Oz) of two TD child subjects. The two raw signals differ significantly because of physical factor in acquisition a). After baseline removing and detrending, signals can be compared.

An Independent Component Analysis (ICA) (Jung et al., 2000) using the algorithm Sobi was applied to the SSVEP epochs in order to remove blinks and

other artefacts, such as the ones illustrated in Figure 3.11. This algorithm has previously been described as being effective for high-density EEG data artefact removal (Akaysha C. Tang et al., 2004). For every participant, a total of 134 (128 + 6) channels entered into the ICA analysis, resulting in an equal number of independent components. Each component was visually examined using the topography, power spectrum, and time course of its activity. Those independent components corresponding to blinks and eye movement artefacts were removed from the data, so that the epochs submitted to further processing would represent the stimuli-related activity of all subjects. Figure 3.12 illustrates the EEG signals afterwards.

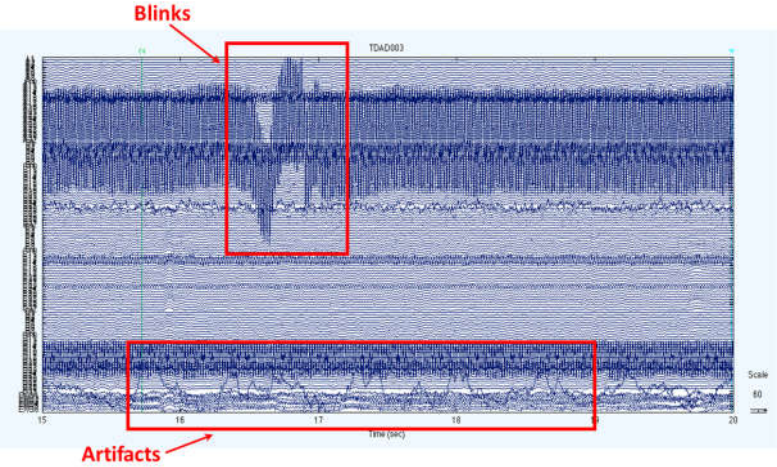


Figure 3.11. Artefacts present in EEG signals. Blinks and muscle contractions.

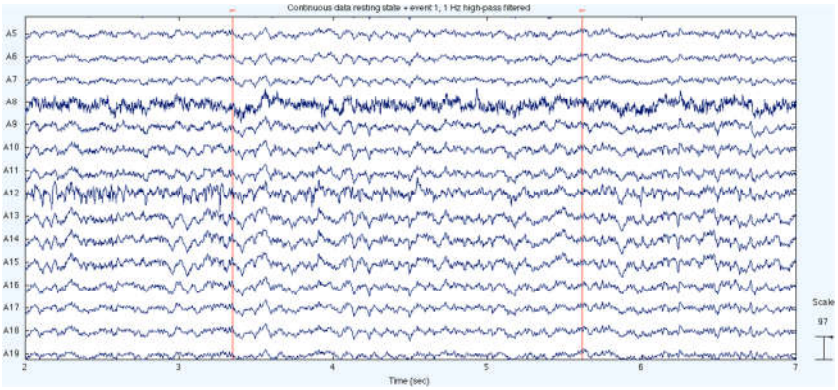


Figure 3.12. EEG signals after removing artefacts by ICA.

Following pre-processing, all channels were transformed into reference-free current source density estimates in order to reduce the effects that volume

conduction might have on connectivity measures (Nunez and Srinivasan, 2006). A spherical spline surface Laplacian procedure, in which the radial current density entering or leaving the scalp beneath electrode sites is estimated by a spline interpolation (Nunez and Srinivasan, 2006), was applied to the brain activity using the Current Source Density Toolbox (CSD Toolbox) (http://psychophysiology.cmpc.columbia.edu). The current source density estimates V at one instant in time is computed from N electrodes using a spherical spline surface Laplacian of the time-series as:

$$V(\boldsymbol{r}) = Q_{m-1}(\boldsymbol{r}) + \sum_{i=1}^{N} P_i K_{m-1}(|\boldsymbol{r} \quad \boldsymbol{r}_i|^2) \qquad (3.1)$$

where $(\boldsymbol{r}_i)$ indicates the Cartesian coordinates of the electrodes sites and $(\boldsymbol{r})$ are the interpolated coordinates. The choice of parameters (head radius=10 cm, m=4, $\lambda = 1.0^{-5}$) was selected according to a high-density Laplacian derivation described in (Tenke et al., 2011; Tenke and Kayser, 2012). The Laplacian reference-free current source density estimates yielded 128 channels ($\mu V/cm^2$ units). SSVEP epochs were averaged across-trials and normalized using a z-score normalization before further analysis.

3.4.2. SSVEP Frequency analysis

Considering the brain as a system where the input is a periodic stimulus, such as SSVEP, we may consider that response also contains periodic elements (Oppenheim et al., 1997). Therefore, transforming the original time-varying EEG signal to the frequency domain, using the Fast Fourier Transform (FFT), may elucidate information about these components. Each raw signal is decomposed into sinusoidal signals of different frequencies using the convolution function illustrated in Figure 3.13, where x(t) is the original signal and f is the frequency of the sinusoidal component to identify. We used the FFT function provided in the Signal processing Matlab's toolbox. This function returns the power spectral density (PSD), which is computed using a multitaper method (Thomson, 1982). This method first divides the signal into overlapping windows, signal segments that are used in the convolution function, and individual FFT responses are computed. Subsequently, the windowed FFTs are averaged to obtain the spectral

estimates. The parameters used were: Hamming window of 2s with 50% overlap and computed for a time window between 1000 to 7500 ms post-stimulus. The frequency components of the signal are 20, 80 and 120 Hz. The FFT illustrates the significant amplitude of the PSD at those frequencies.

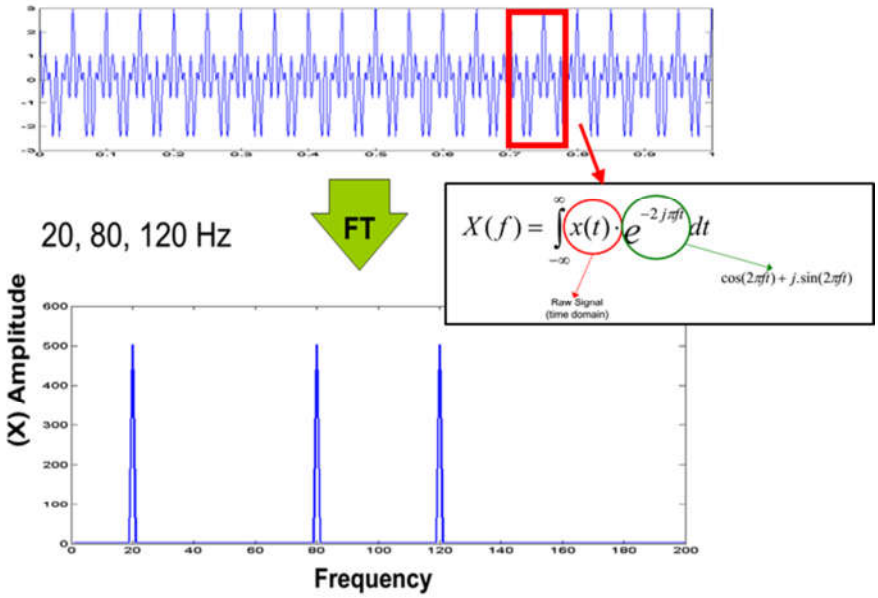


Figure 3.13. The Fast Fourier Transform.

The frequency analysis was applied over all the EEG channels, across all the subjects, in each stimulus. The statistical analysis and results are provided in Chapter 4 and Chapter 5. In this section, we illustrate the effect of the analysis techniques over the EEG signal.

In order to validate the periodic nature of the SSVEP response, the individual FFT of each adult subject is used to compute the grand average FFT, for each stimulus, such it is demonstrated in Figure 3.14. The luminance stimuli present the first high amplitude around 7.5 Hz, which is known as the fundamental frequency or first harmonic. The FFT also presents peaks at the multiples of the fundamental frequency, known as second harmonic (15 Hz), third harmonic (22.5Hz) and so on. From the Figure, we can also observe that SSVEP response is produced over the occipital area. The central region has a predominant

oscillatory response at second harmonic. Meanwhile, the surrounded area has an oscillatory response at the first harmonic.

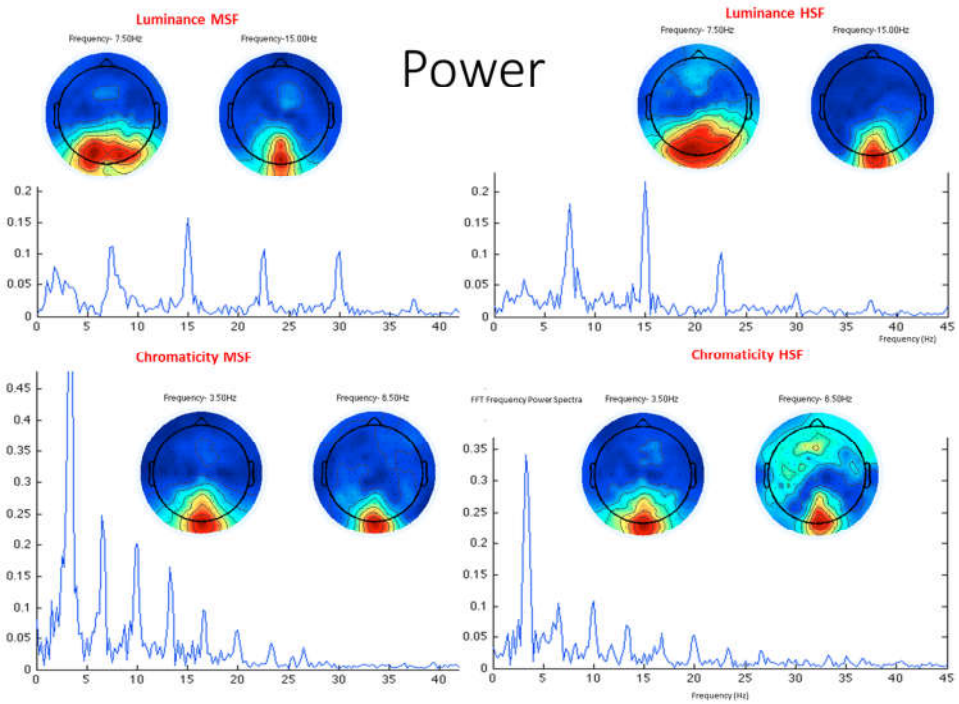


Figure 3.14. Grand average FFT, for each stimulus in adult subjects. The scalp show the areas with response at first and second harmonic

The chromatic stimuli produce SSVEP response around the fundamental frequency of 3.5Hz, and its multiples. The oscillatory responses of the first and second harmonic (7Hz) are generated at the central part of the occipital area. On the contrary to the luminance stimuli, the first harmonic of the SSVEP response in chromatic stimuli is significantly higher than any other harmonic.

Therefore, when the subject is watching flickering stimuli at a temporal frequency 1F, we can detect the harmonic components of the steady-state VEPs in the frequency domain. The steady-state VEPs resulting from repetitive stimulation have the same fundamental frequency as the stimulation but also include higher harmonics (Luck, 2005). The first harmonic (1F) refers to the brain response at the stimulus presentation (fundamental) frequency. The second harmonic (2F) refers to the brain response at twice the input

(fundamental) frequency. The third (3F) and fourth harmonics (4F) indicate the brain response at three and four times the fundamental frequency, respectively, and so on. Neither the neural mechanisms that generate the harmonic responses nor the behavioural aspects that would be associated with each of the harmonics are well understood yet. However, it is suggested that differences in the harmonic responses may be a biomarker for neurodevelopmental and neurocognitive disorders such as autism (Pei et al., 2014) and schizophrenia (Kim et al., 2005).

3.4.3 ERP analysis

Although it was not the main purpose of this study, the quality of the EEG dataset allowed analysing the signals in the time domain. The Figure 3.15 shows the EEG signal of 5 occipital electrodes, in the interval between -150ms to 400ms with respect to the stimulus onset, computed as the average of all the individual signals across the adult subjects. The scalps illustrate as the magnitude at each electrode changes along the time. The luminance stimuli elicited an initial response in the extricate area that it is contracted into the central occipital. Meanwhile the response with chromatic stimuli starts in the central area and expands towards extricate areas.

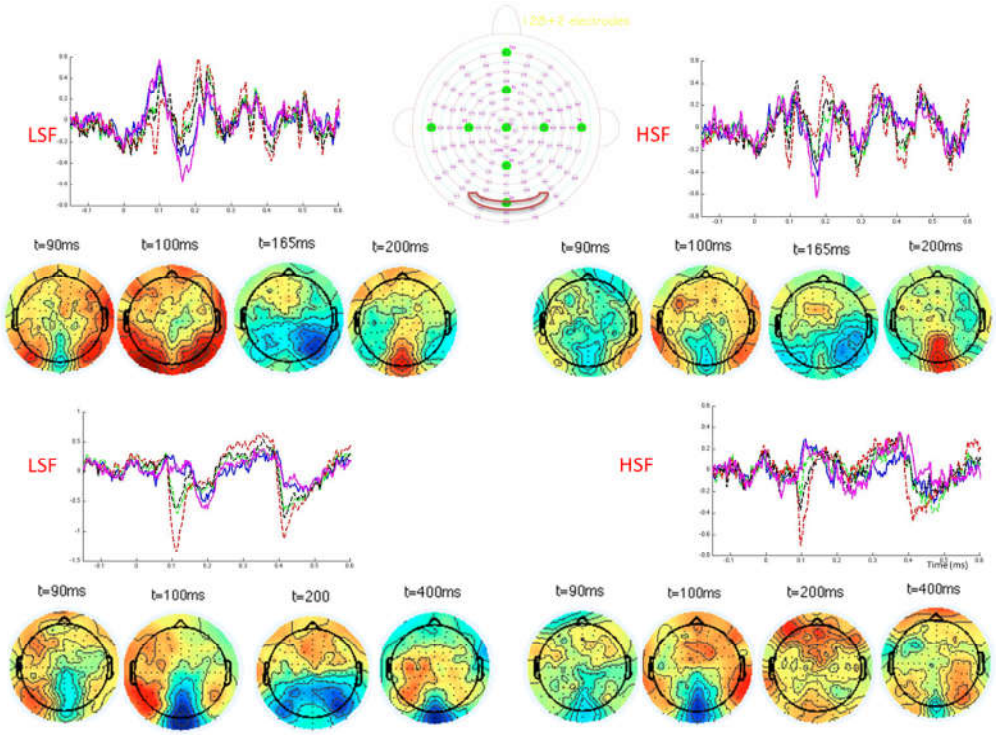


Figure 3.15. Grand average VEP, for each stimulus in adult subjects. The scalp shows the EEG magnitude at specific time points, for each stimulus.

As suggested by Luck (2005), we perform Event-Related Potential (ERP) analysis by focusing on a specific component. We extract information about the peak latency and amplitude analyses of the largest component (C1) of the EEG time series after the stimulus onset, in each epoch. Latency and amplitude of C1 were extracted in the interval 70–120 ms. at Oz channel location for each subject and visual stimulus. It is important to acknowledge that in the current study, due to the nature of the SSVEP paradigm, ERPs can only be calculated from the first part of the stimulus train, and that ERPs were calculated from only small numbers of trials. Statistical results are provided in Chapter 4 and Chapter 5.

3.4.4. Time – Frequency analysis

As it was observed in the previous sections, frequency analysis can provide information about neural networks that maintain an oscillatory response at specific frequency, which are synchronized with respect to the stimulus. However this strategy lacks of information about how this synchronization

evolves with respect to the time. On the other hand, ERP analysis provides information about the behaviour of the EEG signal along the time, but there is no information about the oscillatory nature of the response. Hence, to investigate how the frequency content of brain activity changes over time, the signal was transformed from the time domain into both the time and frequency domains simultaneously by means of wavelet transforms. Whereas Fourier analysis only provides information about frequency content, using wavelet transforms to describe a signal allows to represent not only the temporal changes in the magnitude of the power-spectrum, but also the phase spectrum, which is needed to characterize the neural synchrony between distinct recording sites (Jean-Philippe Lachaux et al., 1999).

The Wavelet Transform of a time series x_n acquired with sampling time steps δ_t and length of N data points (n=0, 1, …, N-1) can be written as:

$$W^x(n,s) = \sqrt{\frac{\delta_t}{s}} \sum_{n'=0}^{N-1} x(n') \psi_0 \left(\frac{(n\prime)\delta_t}{s} \right) \qquad (3.2)$$

where ψ_0 is known as the mother wavelet function and * is the complex conjugated. In the present study, the Morlet wavelet function was used, which is defined as:

$$\psi_0(n) = \pi^{-1/4} e^{i\omega_0 n} e^{-n^2/2} \qquad (3.3)$$

where ω_0 is a dimensionless frequency parameter, also called *scale*.

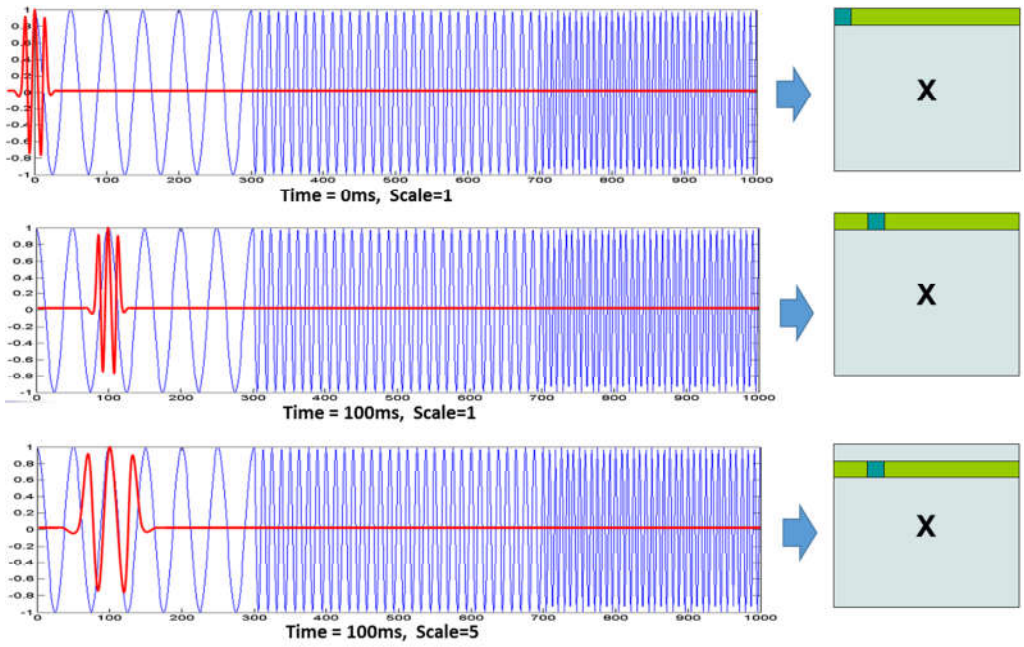


Figure 3.16. Wavelet transform. The time-frequency is performed by computing the convolution of the wavelet mother function and the time series. The mother wavelet is scaled and shifted along the raw signal. The convolution is stored in a matrix.

Figure 3.16 exemplifies how the time-frequency analysis is performed. Wavelet analysis computes the convolution of the EEG signals $x_i(n)$ with scaled and shifted versions of the mother wavelet function. The absolute value of this convolution results in time-frequency (TF) representations of the EEG signals, which is stored in a matrix. The scale is selected based on the frequency of analysis without compromising information relevant to different domains (Torrence and. Compo, 1998).

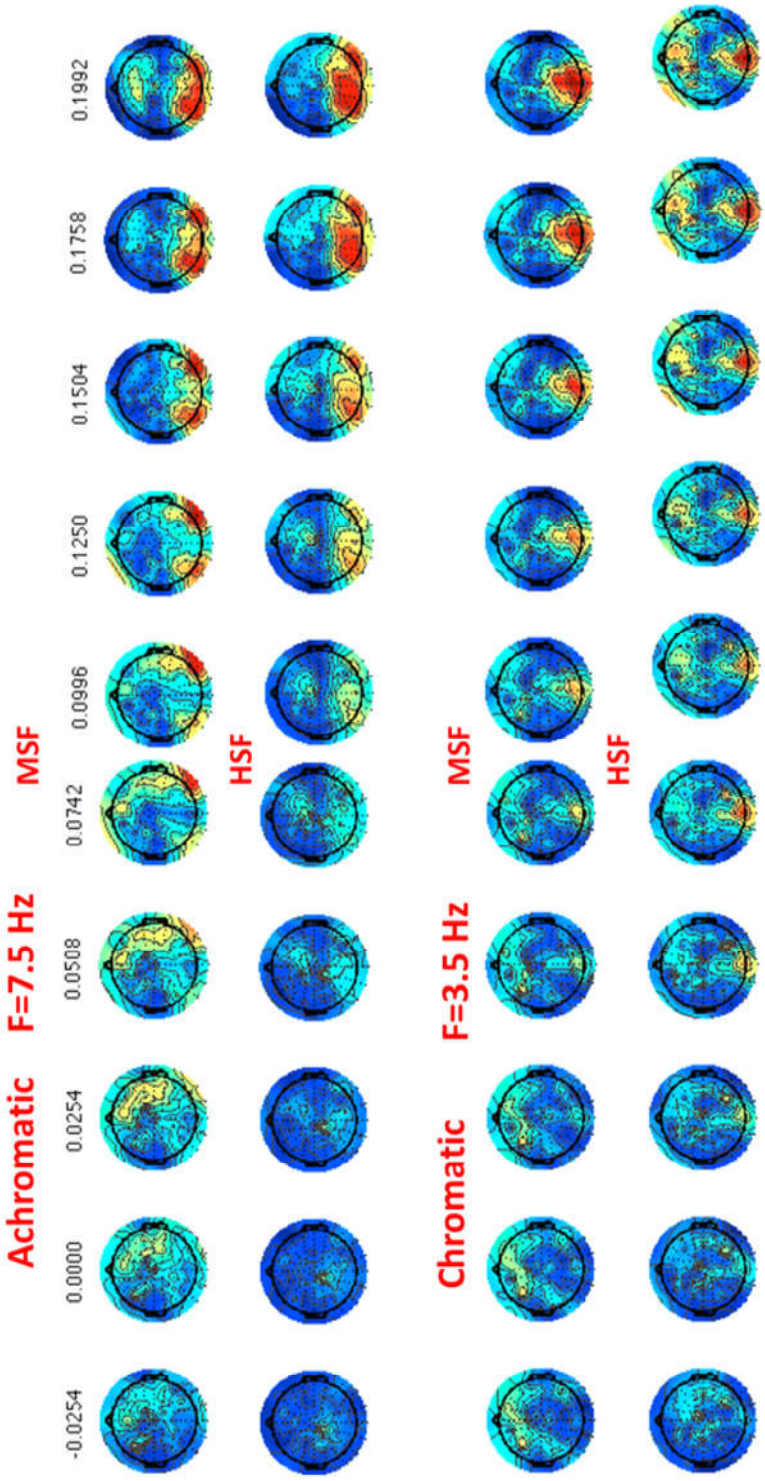


Figure 3.17. Time-frequency analysis performed over the grand average of adult subjects. We obtained the areas syncronized at the fundamental frequencies in each stimulus., at different points of time.

Figure 3.17 illustrates the analysis performed over the grand average of the EEG data in adult subjects. In each stimulus, the wavelet transform is performed over all the channels at the fundamental frequency, and the result is plotted over the scalp figure, at specific time points. We can observe that luminance MSF produces response in the extricate regions at the fundamental frequency, around 75ms. The response with luminance HSF stimulus is elicited around 100ms. The chromatic stimuli produce response at the central part of the occipital area at 3.5Hz.

3.4.5 Time-dependent network analysis

Estimation of the temporal variations in the functional connectivity of neuronal assemblies is a topic of much interest in neuroscience research. The basic idea is that brain dynamics may provide valuable information regarding the frequency-specific synchronization over short periods of time of specific neuronal groups involved in task or stimulus-related processing networks (Lachaux et al., 1999) (Dimitriadis et al., 2012). The application of measures of phase synchrony such as Phase Locking Index (PLI) over short segments of recorded brain activity, has shown to provide important information about time evolution of functional connectivity in a time series at an specific frequency of stimulation (Sazonov et al., 2009) (Dimitriadis et al., 2010).

The PLI method first computes the Wavelet Transform as described in 3.4.4. The instantaneous phase $\varphi^{x_i}(n,f)$ is then calculated as follows:

$$\varphi^{x_i}(n,f) = arctan\frac{imag(W^{x_i}(n,s))}{real(W^{x_i}(n,s))} \qquad (3.4)$$

Finally, the quantification of the phase-locked activity between two brain signals is computed by applying the phase-locking index (PLI), which consists in averaging the instantaneous phase differences as follows (Dimitriadis et al., 2010):

$$PLI\left(x_k(f,n), x_l(f,n)\right) = \left|\frac{1}{N\Delta s}\sum_{n=1}^{N}\sum_{s=s_1}^{s_2} exp\left(i\left(\varphi^{x_k}(f,n,s) \quad \varphi^{x_i}(f,n,s)\right)\right)\right| \qquad (3.5)$$

where N is the number of samples in the time series), ${}^{S_1}/_{S_2}$ indicates the scale limits, Δ represents the range, and f the frequency band under study. PLI function returns a value close to 1 if the phase difference between the two analysed signals has little variation, indicating that they are highly synchronized. If the PLI value is close to zero means that the phase difference varies largely between the two analysed signals, indicating that are not synchronized at all.

This procedure returns the PLI-values organized in a $[NxN]$ matrix in which each node corresponds to the strength of the functional connection between a specific pair of signals. This matrix can be displayed as a structural graph named the functional connectivity graph, where each node represents the recording channel and edges indicate the weighted value between pairs. The application of a networks-based topological metric for weighted connectivity graphs can characterize the functional connectivity graphs. Here, measures of global efficiency as described in (Dimitriadis et al., 2010) were used to characterize functional connections. The global efficiency of the networks is defined as:

$$GE = \frac{1}{N}\sum_{i\epsilon N}\frac{\sum_{j\epsilon N, j\neq i}(d_{ij})^{-1}}{N-1} \qquad (3.6)$$

Where N represents the number of nodes that constitute the network, E represents the number of edges, and w_{ij} indicates the weight between a pair of nodes. The subgraph G_i was defined based on the group of nodes that were direct neighbours of the ith node. Specifically, GE is the inverse of the average of the shortest path length between each pair of electrodes. GE reflects the global efficiency of the parallel transmission of information in the network (Achard and Bullmore, 2007)(Latora V and Marchiori M., 2001) .

Initially, the number of channels available for every recorded EEG signal was 128. However, pruning was necessary in order to reduce computing time, given that connectivity measures are computed between each pair of electrodes. The total number of channels was reduced from 128 to 60. The remained electrodes were relatively evenly spaced and distributed across the regions that were assumed to correspond to the international 10-20 standard recording positions.

The synchronization measure was applied to every possible pair of the remaining electrodes.

Figure 3.18 illustrates the network analysis carried out over the EEG signals of adult subjects, in MSF luminance and chromatic stimuli. Matrices show the connectivity across all the channels and it is observed that response is focused over a specific area, which is central occipital region. Contrary to the luminance response, which magnitude is maintained high, the chromaticity reduces the magnitude, but the connectivity is distributed over other areas in the matrix. From the individual matrices of each subject, the most significant connections were selected by thresholding. The connections that were consistent across subjects are plotted over the scalp figure.

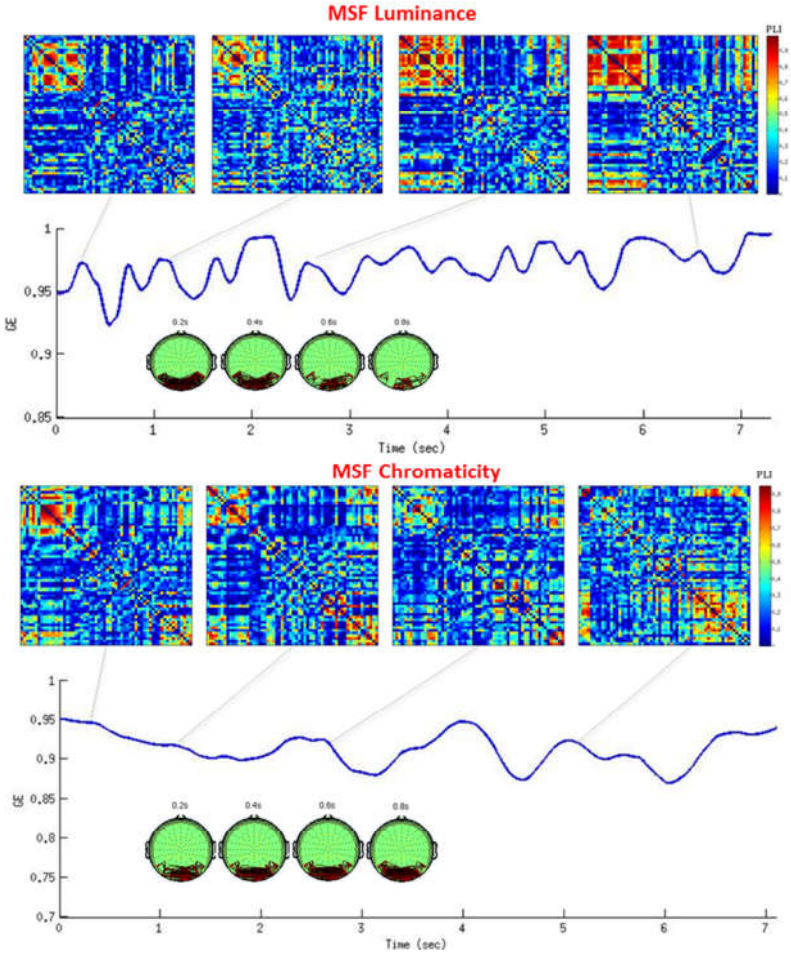


Figure 3.18. Time dependant analysis for MSF stimuli. Matrices show the connectivity degree among all the channels, at specific points of along the time. Scalps show the consistent networks across the adult subjects.

In summary, the overall approach of the thesis is to explore EEG measures associated with the dynamics of functional connectivity (i.e. power of SSVEP responses at the driving frequency and its harmonics, ERP analysis of the first major peak C1, and time evolution of networks co-operative behaviour) that best reflect the functional changes occurring in the brain during early visual perception. More description of the measures and considerations of their application to EEG signals are discussed in more detail within each experimental chapter.

Chapter 4

Investigating age-related changes in networks dynamics during visual perception

4.1 Development of brain function

Typical brain function is the result of a series of dynamic neural processes that occur at different levels of cellular organization during development. These processes begin before birth involving synaptogenesis, myelinogenesis and synaptic pruning that modify the structure of the developing brain to form specialized processing areas and to establish anatomical connections to achieve integrative processing functions (Stiles and Jernigan, 2010; Deoni et al., 2015). Local and long-range interactions will shape the circuitry of the brain, which emerges as a self-organising system that connects cortical and subcortical systems to build the networks required to perform all functions (Cabral et al, 2014). The human brain reaches full maturity when its structural and functional characteristics interact to create an efficient processing system with the synaptic connectivity required for normal brain function (Pereda, 2014; Stiles and Jernigan, 2010). The myelination process that allows neurotransmission by connecting spatially separate neurons is completed in subcortical regions and primary sensory and motor areas of the cortex by the end of the second year (Deoni et al., 2015), myelination of parietal and frontal regions of the cortex continues into early adulthood (Miller et al., 2012). Synaptic overproduction peaks at age 4 and then a slow reduction of synapses takes place until adolescence (Huttenlocher and Dabholkar, 1997). The rate of synaptic pruning differs among brain regions. For example, the visual cortex reaches its maximal

synaptic production at approximately age 2 and then slows to reach the adult level in early adolescence (Huttenlocher and Dabholkar, 1997). Cortical thinning occurs at a constant rate in childhood, particularly between 5 and 11 years of age, across distinct regions of the brain (Spear, 2013). Therefore, it seems that brain regions processing more basic functions such as perception and motion mature first, followed by higher-level areas involved in top-down processing (Connor et al., 2004). This hierarchical development of the brain may provide the starting point to explore how functional connectivity develops.

4.2 Functional connectivity in brain development

Functional assessment of cortical networks in the developing brain can be performed using non-invasive neuroimaging techniques, such as functional MRI, PET, MEG and EEG. Although the brain signals recorded using these techniques are also affected by non-neuronal factors, the application of suitable measures of neuronal synchrony can provide meaningful information on functional connectivity. The fMRI technique is commonly used to identify the structural and functional networks activated during perceptual and cognitive tasks, which include cortical and subcortical regions. Although EEG measures cannot provide direct information on subcortical structures, its high temporal resolution allows studying the dynamics of functional networks at fast time scales in different frequency bands. To assess functional connectivity between recording sites, coherence and correlation have been the most used methods. These methods combine the effects of amplitude and phase in the interactions between two signals, which make them suitable for analysing time-locked events (Lachaux et al., 1999). For phase-locked events, such as the brain responses to rapidly repeated stimuli, EEG phase synchrony methods allow to quantify frequency-specific synchronization between two signals. This measure has been interpreted as a quantification of neural integration in the framework of functional connectivity (Lachaux et al., 1999).

Networks metrics have recently been used to investigate functional connectivity from the systems perspective, where local connections and long-range

connections interact within a functional network (Chavez et al., 2010). To explore how functional connectivity change over time, we used complex network analysis because has proved to reflect the interplay between functional segregation and integration within different functional networks underlying mental processes when applied to EEG time series data (Dimitriadis et al., 2010). This method estimates two measures of connectivity within the network, global and local efficiency, and reveals their interplay within the functional network over time, via time series data. The global efficiency (GE) is a measure of synchronous information exchange in the network. It is estimated by computing the path length, strength and weight of the functional connection between each pair of nodes within the network. Local efficiency (LE) is a measure of the effect of an individual node over its neighbouring nodes. High values of global efficiency indicate more functional integration, while lower values of global efficiency indicate less functional integration. This approach is particularly interesting since the interplay between functional segregation and integration within functional networks has been suggested to provide valuable information about brain function (Tononi et al., 1994; Friston, 2002; Sporns, 2003).

Therefore, investigating the interplay between functional segregation and integration within functional networks over time in children and adults could help to characterize the dynamic organization of coordinated activity as a function of brain development. This would be useful when evaluating the dynamics of brain activity in pathological conditions at early stages of development. Some other methods have recently been applied to EEG signals to evaluate fluctuations in synchronization patterns. Entropy and complexity measures aim to reflect the transient formation and dissolution of local connections within a long-range functional network. They indicate how predictable the synchrony values are in a signal. Steady patterns of synchrony values make a signal very predictable, which is considered to have low complexity. In contrast, spontaneous fluctuations indicate high variability and more complexity in the signal. For our work, we used approximate entropy (ApEn) as a measure of signal complexity (Pincus, 1991) because of its capability

to distinguish changing complexity in EEG data from both adults and children (Lee et al., 2013).

According to previous theoretical studies (Tononi et al., 1994; Friston, 2002; Sporns, 2003), local interactions within segregated brain regions are integrated into long-range interactions to achieve perceptual or cognitive tasks through functional networks, where functional segregation and functional integration co-exist. Information processing carried out by segregated local networks within specialized areas of the brain will be integrated into larger ones across distant brain regions to perform higher level processing functions. The function of local networks might therefore affect the capacity of processing of a long-range functional network. The development of functional connectivity would therefore be quantified by measuring the interplay between segregation and integration within and across brain systems.

Electroencephalographic studies of brain activity in children and adults have investigated this hypothesis by measuring synchronization at the local and long-range scales. For instance, by measuring changes in the electrical activity of a group of 227 children at two different age points (5 and 7 years of age), a research group (Boersma et al., 2011) found reduced brain synchronization when children were older. This was observed during resting state using measures of brain networks, such as clustering, weight and path length. They found higher clustering and path lengths with smaller weight dispersion in the older children, which was interpreted by the authors as reduced functional connectivity within a more efficient network structure across development. Clustering and path length indicated the number of interconnected nodes and the strength of the connection respectively, with the weight dispersion being the range of variability of the synchronization between every node in the network. A previous resting-state functional connectivity MRI (rs-fcMRI) study (Fair et al., 2009) found no differences in clustering and path length across age (7-31 year), but did find local sub-networks within large-scale networks behaving different between children and adults. Synchronization of local connections decreased,

while integration between separated areas increased with age. The author and co-workers suggested a shift from local to distributed organization across development. Similar results, local synchronization in children and global synchronization in adults, have been reported using traditional measures of EEG synchronization (power and coherence) in a group of 20 children (aged 6-11 years) and 23 adults (aged 18-23 years) by Srinivasan (1999). Findings of all the aforementioned authors have been associated with neurobiological processes of brain development, an overproduction of neurons in childhood that form neuronal networks with activity locally synchronized, and to the excess of neurons and connections selectively pruned as the brain matures to achieve integrative function.

Some other EEG studies have investigated brain development by measuring the rhythms of the brain. It has been reported that children show an adult-like alpha frequency (10 Hz) by 10 years of age (Niedermeyer and Lopes da Silva, 1999). Overall, EEG power seems to be higher in children than in adults at all frequency bands, with lower frequencies reaching adult levels in posterior regions earlier than other regions and frequencies (Barriga-Paulino et al., 2011). After evaluating the EEG recordings of five age groups (from 7 to 60 years), another research group (Anokhin et al., 1996) found that synchronization decreased with age at lower frequency bands, while high frequencies increased from adolescence to adulthood. In order to characterize the development of functional networks in a specific temporal frequency in younger children, a recent study (Birca et al., 2010) used Steady-State Visual Evoked Potentials (SSVEPs), which have proved to elicit both local and distributed networks (Srinivasan et al., 2006). Birca and colleagues applied measures of magnitude and phase alignment on the SSVEPs elicited by an intermittent photic stimulation of 5 Hz, in a group of 46 children (aged 3-16 years) and 8 adults. They found reduced SSVEP magnitudes and increased phase alignment with age across multiple brain regions, including occipital, parietal and frontal regions, which was interpreted by the authors as the development of functional integration between separated regions required to process visual stimuli.

Studies on age-related changes in EEG signal variability have reported contrasting findings. For instance, an study applying correlation and power analysis (Anokhin et al., 1996) reported increased neural complexity of brain signals across development (between 7-25 years or age), with higher values in complexity over the frontal associative cortex. By measuring phase synchrony, another research group (Nenadovic et al., 2011) examined the dynamics of coordinated activity in regions of the default network in typically developing children (aged 11 months- 17 years) during resting state. They reported lower variability in the synchronization patterns as age increases.

Development of functional cortical networks has been commonly assessed during the resting state, with the assumption that the study of one global network provides information about the general state of functional integration in the brain. However, in order to study age-related changes in functional connectivity, the lower processing levels of the cortex may provide a more precise trajectory of network development. The organization of the visual cortex undergoes accelerated early development in childhood, which includes the formation of local anatomical networks and long-range connections that develop to a more efficient functional network when children grow older (Spear, 2013). At the local scale, neurons in each visual area share inputs, outputs, and response properties. At each successive level, neuron will respond to more complex stimulus properties. For example, the neurons of striate cortex (V1) show preferential responses for stimulus attributes such as spatial frequency and orientation (Livingstone & Hubel, 1984), while the neurons in extra-striate visual areas mainly respond to colour (V4) (Zeki et al., 1991) and motion (V5) (Watson et al., 1993). Although many more areas have been identified, their functional properties are not completely characterized.

Related to this work, it has been suggested that, at lower levels of processing, luminance and chromatic visual information processing differs between children and adults (Crognale, 2002). The visual evoked potentials (VEP), elicited following the presentation of visual stimuli, have been used to assess the

development and maturation of cortical function. Transient flash (luminance) and pattern stimulation are the most common types of stimuli used to elicit VEPs in clinical applications (Vialatte et al., 2010). The rapid presentation of visual stimuli is thought to elicit frequency-specific synchronization in the brain, which has encouraged its use in the study of functional connectivity. The brain responses to fast stimulation (>3 Hz) are known as steady-state visual evoked potentials (SSVEPs) (Srinivasan, 2006), see Chapter 2 of this thesis for more description on SSVEPs. Although SSVEPs elicited by intermittent photic stimulation has been used to study developmental change of functional brain networks (Birca et al., 2010), it is unclear whether the neuronal activity elicited by SSVEPs using different types of visual stimuli (i.e. gratings, colored checks) may reflect age-related changes in the development of brain function. Thus, this study aimed to investigate age-related changes in the coordinated activity of functional networks involved in the processing of luminance- and chromatic-defined gratings by employing SSVEP paradigms.

Luminance-defined gratings have been used to evoke responses within the magnocellular pathway (M-pathway), where cells respond preferentially to high luminance contrast, low spatial resolution and fast temporal frequency (Tobimatsu and Celesia, 2006). When visual stimuli are presented at low temporal frequency, visual evoked potentials (VEPs) are generated by the brain and are commonly analysed in the time domain. The averaged VEP responses are time-locked to stimulus onset, with three distinguishable components in the waveform: N75, P100 and N145. The latency and amplitude of these components have been studied to evaluate age-related brain function. For instance, the major VEP responses to luminance stimuli at low spatial frequencies have been found in children as young as 3 months old (Crognale et al., 1998). When visual stimuli are presented repetitively at a temporal frequency faster than 3 Hz, a steady-state visual evoked potential (SSVEP) is elicited, and evaluated in the frequency domain by means of the FFT. Fast Fourier Transform (FFT) is a mathematical tool used to analyse the amplitude and phase of the harmonic components of the SSVEP, at the frequency of stimulation (F) and its harmonics (2F, 3F, etc)

(Tobimatsu et al., 1995). SSVEP power at the second harmonic (2F) has shown a band-pass function with a peak at 4 cpd, whereas at the fourth harmonic (4F) SSVEP power showed a high-pass function (a decrease observed only to low spatial frequency), in a study testing responses to a range of spatial frequencies (0.5 to 8.0 cpd) in 13 adults (Arakawa et al., 1999).

Isoluminant chromatic-defined gratings (Red-Green) have shown to preferentially activate the parvocellular pathway (P-pathway), which is highly sensitive to colour, high spatial frequency and low temporal frequency (Tobimatsu et al., 1995). A major VEP component (N1) has been found around 120 ms with peak amplitude at 2 cpd in the occipital region, higher spatial frequencies elicited lower N1 amplitudes (Tobimatsu et al., 1995). The chromatic VEP waveforms are adult-like until the age of 12-14 years (Crognale, 2002). From this age, the latencies of the major component slowly increase, before that, there is a slowly decrease in latency until adult-like waveforms are attained (Crognale, 2002). Arakawa and colleagues (1999) found that SSVEP power at the second harmonic (2F) showed a low-pass function, decreasing at spatial frequencies above 4 cpd. They found no significant difference in SSVEP power at 4F between spatial frequencies.

As described above, SSVEPs are differently influenced by luminance and chromatic gratings as reflected on the harmonic components (2F and 4F) of SSVEP power. ERP amplitudes and latencies are also differently affected by luminance and chromatic gratings, and by age. It appears that luminance and chromatic gratings elicit activity from different neuronal populations in the visual cortex. This has given us the basis for the current study.

In this study we explored age-related changes in functional connectivity. The SSVEP paradigm would allow eliciting frequency-specific synchronization and evaluating the underlying functional networks. Luminance and chromatic gratings were used in order to elicit different functional networks. Luminance gratings would preferentially stimulate the magnocellular pathway and may

allow investigating local connections within primary visual cortex V1. Conversely, the parvocellular pathway would be preferentially stimulated using chromatic gratings, feeding to V4 and may be allowing contribution from V1 to investigate functional integrative processing. It has been reported that the combined use of high-contrast luminance- and isoluminant chromatic- gratings can allow studying the function of both pathways electrophysiologically (Tobimatsu et al., 1995). We used two different spatial frequencies in each case to investigate whether different functional networks are involved in their processing.

Our selection of visual stimuli rests upon a simple and fundamental argument; the onset of even a simple visual stimulus modify the activity of a widespread neural network including striate and extrastriate visual areas (Makeig et al., 2002; Milne et al., 2009). Therefore, evoked responses are well suited to the investigation of neural integration within widespread networks, because can be modulated by the physical properties of visual stimulus, including spatial frequency, luminance and colour (Strasburger et al., 1993; Reagan, 2009; Pei et al., 20014; Tobimatsu and Celesia, 2006; Tobimatsu et al., 1995). Functional mapping of cortical visual pathways suggests that, at the lowest levels of visual processing, there are two anatomically and physiologically independent pathways that develop at different rates and process complementary types of visual stimulus information: the magnocellular and the parvocellular pathways (Dobkins et al., 1999; Hammarrenger et al., 2003; Crognale, 2002). It is thought that the integrity of these pathways may play an important role in normal visual perception, and in neurological and psychiatric disorders including autism and schizophrenia (McCleery et al., 2007; Fujita et al., 2011; Butler and Javitt, 2005; Kim et al., 2005). Both the magnocellular and parvocellular visual pathways begin in the retina and project to the striate cortex, via the lateral geniculate nucleus (LGN) to subsequently interact with other visual processing streams (e.g., the dorsal stream area V5/MT or the ventral-stream area V4), to perform high-level visual processing (Binkofski and Buxbaum, 2013; Denison et al., 2014). Electrophysiological studies to characterizing the functional properties of

these pathways have demonstrated that they consist of cells with distinct spatial, temporal, luminance, and chromatic stimulus preferences (Hubel and Livingstone, 1990;) as well as response dynamics (Lee et al., 1990; Reagan, 2009). Magnocellular cells are preferentially activated by low spatial frequency stimuli, show great sensitivity to luminance contrast, are unresponsive to colour contrast and are characterized by high temporal resolution. In contrast, parvocellular cells are strongly activated by stimulus of high spatial frequency, are sensitive to colour contrast and are characterized by low temporal resolution. Magnocellular cells display transient responses to a luminance change, whereas the responses of parvocellular cells to luminance change are more sustained (Lee et al., 1990; McKeefry et al., 1996). The transient responses of magnocellular cells provide temporal resolution needed for motion detection and global form (Maunsell, et al., 1990). The colour sensitivity and higher spatial resolution of the parvocellular cells are well suited to evaluating detailed form information such as textures and colour, which may be essential to object identification (Vuilleumier et al., 2003).

It is thought that, in the extrastriate visual cortex and highest levels of visual processing, there are segregated contributions of magnocellular and parvocellular pathways, which allows the investigation of neural integration (Cloutman, 2013). It is common to analyse the functioning of the two systems within primary visual cortex (V1) and their integration with higher cortical areas (e.g., V4 or V5) using gratings defined by either luminance or colour contrast at various spatial frequencies (Tobimatsu et al., 1995). The parvocellular pathway projects to area V4 that is particularly concerned with colour and detailed form information, and subsequently connects to the inferior temporal cortex (Tobimatsu and Celesia, 2006). In contrast, the magnocellular pathway projects to area V5/MT that is thought to play an important role in detecting motion and processing of global structure, and terminates in the posterior parietal cortex. Luminance-defined gratings have been used to preferentially activate the magnocellular cells within V1, whereas colour-defined stimuli have been used to preferentially activate the parvocellular system (Tobimatsu et al., 1995,

McCleery et al., 2007; Fujita et al., 2011). Luminance- and colour- defined gratings of different spatial frequencies therefore provide an ideal stimulus to investigate neural integration during visual perception. In this study, the functioning of the magnocellular and parvocellular systems within primary visual area (V1) and their integration to higher visual areas (e.g. V4) were evaluated using luminance (high contrast sinusoidal gratings) and chromatic (equiluminant red–green sinusoidal gratings) stimuli, respectively.

We hypothesized that four different networks will be elicited by luminance and chromatic gratings of two different spatial frequencies. Stimulus-specific patterns of functional connectivity would be reflected as responses to the different stimuli within networks involving either the magnocellular or parvocellular system. Thus, the presence of EEG indexes corresponding to functional networks would indicate that a measure is well suited to the investigation of neural integration because successfully reflects some features of brain organization, and the absence of such indexes may suggest that a measure may not be well-defined. The hypotheses of this study are listed below.

1. Luminance-defined gratings: Due to the preferential response to low spatial frequencies of the magnocellular pathway, and that it has been suggested to mature earlier than other visual areas, and because ERP responses to luminance gratings at low spatial frequencies have been found in young children, then the values of functional connectivity elicited by medium spatial frequency gratings will be similar between children and adults. On the contrary, high spatial frequency will generate between group differences.
2. Chromatic-defined gratings: Due to chromatic gratings processing can be considered an integrative process, and functional integration has been suggested to develop with age, then values of functional integration will be reduced in children, showing an increase in adulthood.

4.3 Methods and materials

Eleven healthy adult volunteers (mean age: 30.08 years, range: 23.7–37.9 years, 7 female) and 30 typically developing children (mean age: 11.09 years, range: 7.3–17.5 years, 7 female) participated in this study. Children and adults were recruited by distributing flyers at local mainstream schools and Sheffield University. All participants reported normal vision or corrected to normal. These subjects did not have any history of epileptic seizures or psychiatric disorders. Adults and children, along with their parents, were informed of methods and purposes of the examination and they provided written consent. The experimental procedure of this study was reviewed and approved by the Ethics Committee of the University of Sheffield. Participants were divided into four age groups as follows: Adults (n=11), 13 to 17 years (n=7), 10 to 12 (n=9), and 7 to 9 years (n=14). The children were divided into these three age ranges (approximately early, middle, and late childhood) so that maturational changes could be investigated.

Subjects description

Age groups	N	Mean age (years)	Min-Max (years)
1	14	8.63	7.30-9.91
2	9	11.65	10.25-12.91
3	7	15.29	13.91-17.50
4	11	30.08	23.70-37.90

Table 4.1. Demographic characteristics of the participants.

As described in the General methods section, the electroencephalogram (EEG) was recorded continuously using the Active––Two Biosemi EEG system (Bio-semi, Amsterdam, Netherlands) with 128 channels covering the entire scalp. Signals were downsampled at 512 Hz in a bandwith filter of 0–40 Hz. Epochs of EEG starting at 500 ms before stimulus onset and ending 8000 ms post-stimulus were extracted. Stimuli consisted of sinusoidal vertical gratings of two different spatial frequencies, such it is described in Chapter 3.

In addition, approximate entropy was calculated to quantify the predictability of the EEG signal in the time domain. The approximate entropy was calculated according to the algorithm introduced by (Pincus, 1991):

$$ApEn(N, m, r) = \Phi^{m}(r) \quad \Phi^{m+1}(r)$$

where $\Phi^{m}(r)$ is defined as

$$\Phi^{m}(r) = \left(N \quad (m \quad 1)\right)^{-1} \sum_{i=1}^{N-(m-1)} lnC_{m,i}(r)$$

The minimum value for ApEn is 0, suggesting a completely predictable sequence. The value of the approximate entropy depends on the following parameters: the length of the epoch (N), the number of previous values used for the prediction of the subsequent value (m), and a filter factor (r). In this study, N=4000, m=2 and r=20% of the standard deviation of the original data series, because theoretical considerations suggested these parameters as a good starting point in EEG signals (Pincus, 1991; Lee et al., 2013).

4.4 Results

4.4.1 SSVEP responses in the time domain: Amplitudes and Latencies

Luminance stimuli

The SSVEPs responses shown in Figure 4.1 consist of averages from 7 to 14 participants per age group (N=41, see Table 1). The morphology of the SSVEPs responses at occipital electrode Oz showed a marked negative deflection (C1) after stimulus onset followed by a steady-state neural response across age groups at both mid and high spatial frequencies.

Repeated-measures ANOVAs with between-subject factor of age group (four levels: 7-9, 10-12, 13-17, adults) and within-subject factor of spatial frequency (two levels: mid, high) were performed separately on the amplitude and latency values of the C1 component.

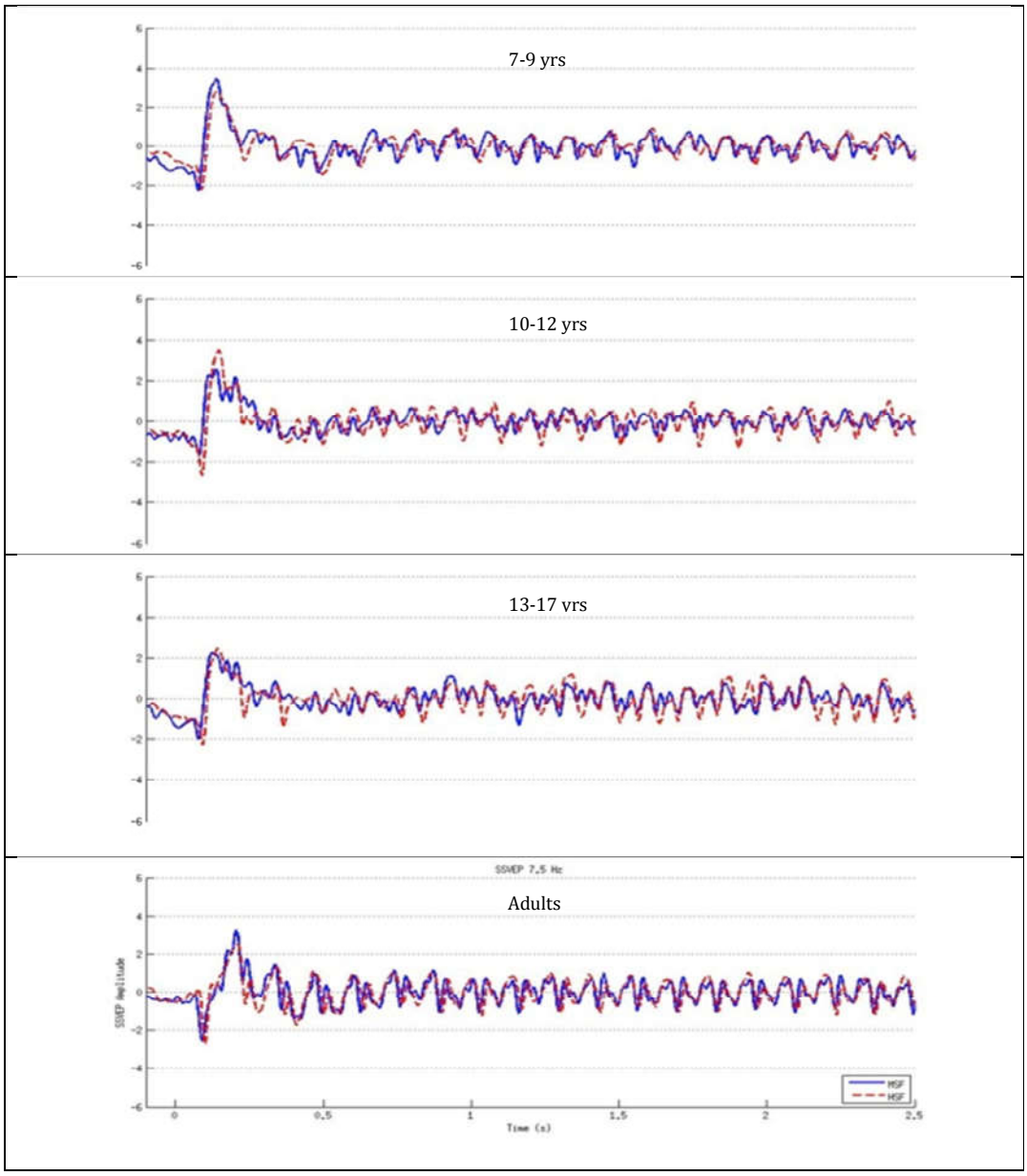


Figure 4.1. Time-domain grand averaged waveforms for luminance gratings. Both spatial frequencies (MSF= 2.8 cpd and HSF=6 cpd) elicited quasi-sinusoidal.

C1 Amplitude

As can be inferred from visual inspection on Figure 4.1, no significant variations in C1 amplitude were found across age groups. Neither spatial frequency nor age produced any significant main or interaction effects, all $F < 2.39$, all $p > 0.13$.

C1 Latency

There was a significant within-subjects effect of spatial frequency ($F(1,37) = 26.494, p < 0.001$) and a significant effect due to age group ($F(3,37) = 7.379, p = 0.001$). There was interaction between spatial frequency and age group ($F(3,37) = 3.399, p = 0.028$). One-way ANOVAs followed by Tukey post-hoc tests were then used to analyze the effect of age group factor on each spatial frequency separately. Paired comparisons indicated that the mid spatial frequency latency values of the adult group were significantly different from the 13-17-years child group ($p = 0.004$), but that the children's groups were not significantly different from each other ($p = 0.28$ to 1). Paired comparisons also revealed that the high spatial frequency latency values of the adult group were significantly different from the 7-9 and 10-12-years child group ($p \leq 0.019$), but that the children's groups were not significantly different from each other ($p = 0.08$ to 0.657). Furthermore, these analyses showed that C1 latency following presentation of mid spatial frequency was earlier than C1 latency of high spatial frequency across age groups, as may be seen in Figure 4.2.

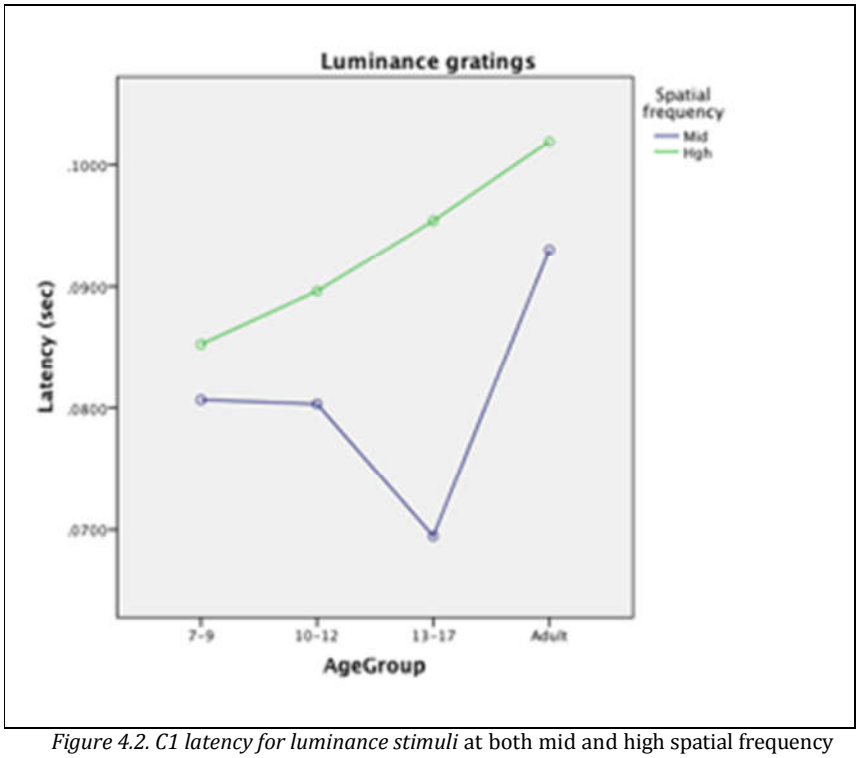


Figure 4.2. C1 latency for luminance stimuli at both mid and high spatial frequency of gratings.

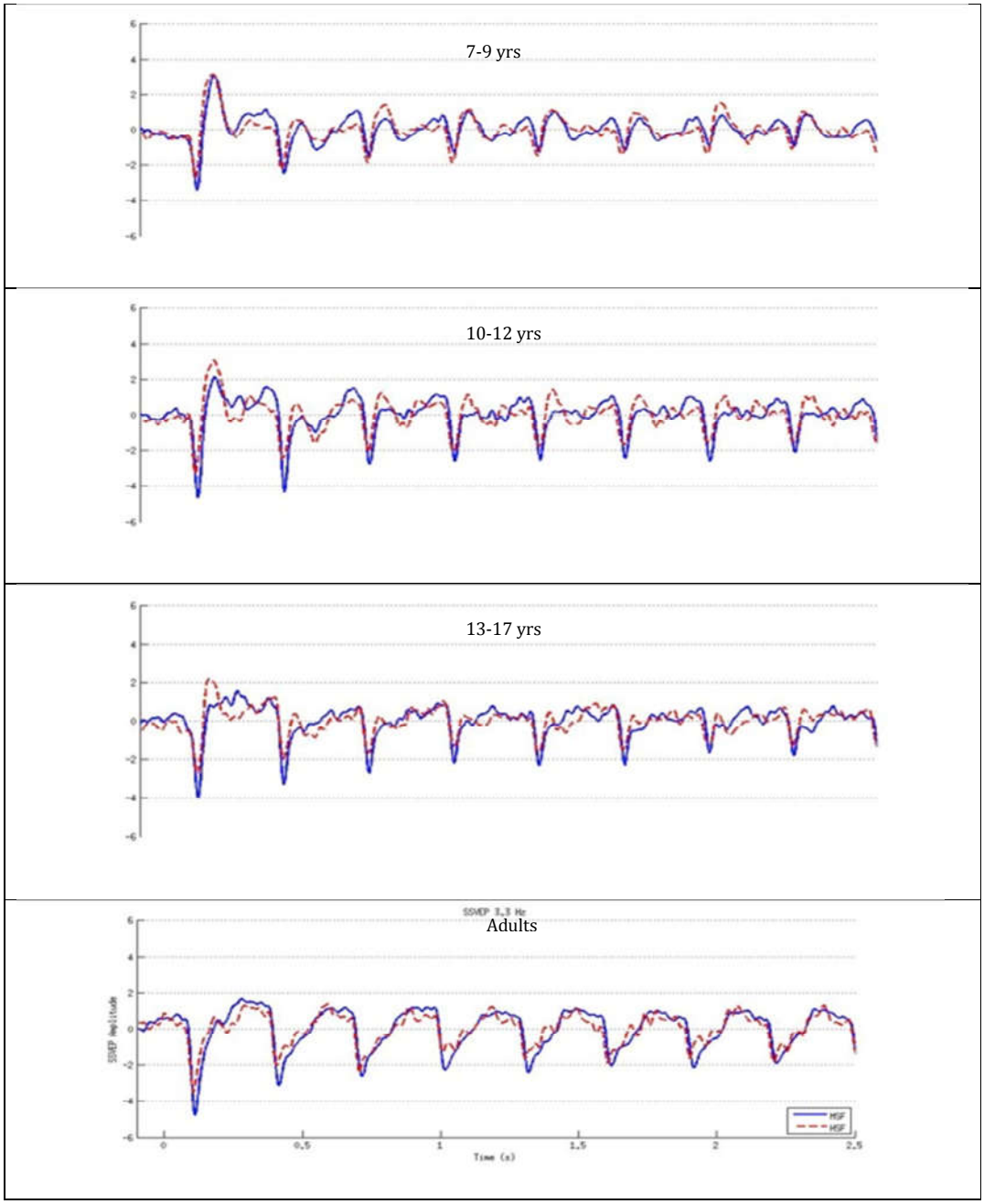


Figure 4.3.. Time-domain grand averaged waveforms for chromatic gratings. Both spatial frequencies (MSF= 2.8 cpd and HSF=6 cpd) elicited a major negative component in response to stimulus onset in all age groups.

Chromatic stimuli

Chromatic stimuli elicited a large major negative wave after stimulus onset across groups (Figure 4.3). It may be seen different morphologies across age groups; while the 7-9 years group showed a more transient-like response, the effect was diminishing across age, so that adults showed no marked change to the offset. Statistical analyses were carried out as described above upon the C1 amplitude and latency values.

C1 Amplitude

There was no significant main effect of age ($F(3,37) = 1.493, p = 0.233$). There was no interaction effect of spatial frequency by age group ($F(3,37) = 1.349, p = 0.273$), but there was a main effect of spatial frequency ($F(1,37) = 28.951, p < 0.001$). These analyses did not find age-related changes on C1 amplitude, but showed larger (more negative) C1 amplitude at MSF than HSF across age, Figure 4.4.

C1 Latency

There was a significant main effect of spatial frequency ($F(1,37) = 15.25, p < 0.001$). There was no interaction effect of spatial frequency by age group ($F(3,37) = 0.582, p = 0.646$). There was no main effect of age ($F(3,37) = 2.517, p = 0.073$). These analyses did not find significant age-related changes on C1 latency, but showed earlier C1 latency at HSF than at MSF across age groups, Figure 4.5.

Validation by regression analyses

Linear regression analyses, as described in (Annaz et al., 2010), were conducted to independently investigate developmental changes on luminance and chromatic stimuli. The independent variable was age and the dependent variables were either the C1 amplitude or latency values.

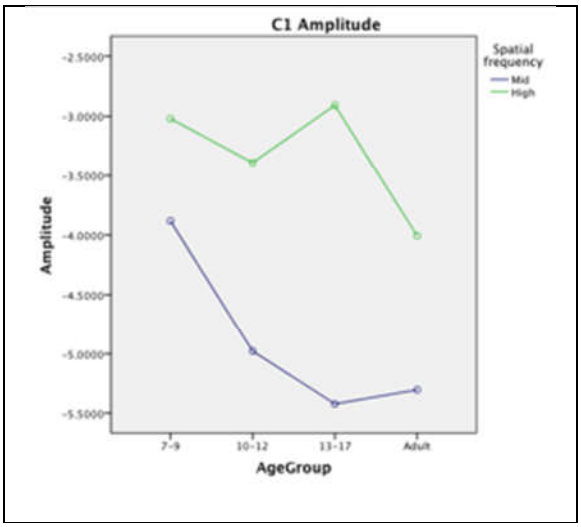


Figure 4.4. C1 Amplitude for chromatic stimuli at both mid and high spatial frequencies of gratings.

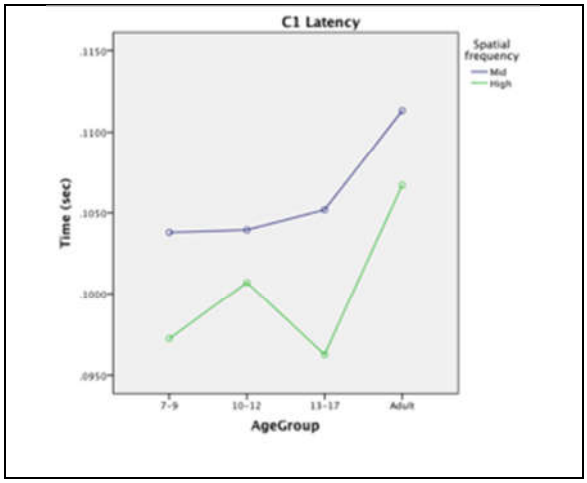


Figure 4.5. C1 latency for chromatic stimuli at both mid and high spatial frequencies of gratings.

C1 Amplitude

Regression analyses showed that age did not predict C1 amplitude in any of the testing conditions (all $R^2 < 0.062$, all $F < 2.531$, all $ps > 0.12$).

C1 Latency

For luminance stimuli, regression analyses showed that C1 latency increased with increasing age at both spatial frequencies (mid: $R^2 = 0.151, F(1,39) = 6.962, p = 0.012$; high: $R^2 = 0.348, F(1,39) = 20.825, p < 0.001$). These analyses showed C1 latency related to age as was also reflected by the analyses of variance ANOVAs. For chromatic stimuli, regression analyses also showed that C1 latency increased with age at both spatial frequencies (mid: $R^2 = 0.152, F(1,39) = 7.014, p = 0.012$; high: $R^2 = 0.117, F(1,39) = 5.174, p = 0.029$). This result is different to that found by the ANOVAs (which was close to significance at p = 0.073). Given that regression analysis is more sensitive (Annaz et al., 2010) to developmental changes, it is safe to conclude that C1 latency does increase with age. Figure 4.6 depicts the developmental trajectories linking latency and chronological age for mid and high spatial frequencies. The regression lines represent a linear model fit of all participants (87 to 454 months).

To summarize, analysis of amplitude and latency values of the first ERP component C1 showed that, for both chromatic and luminance stimuli, C1 latency increased with age at both spatial frequencies of the gratings. They also showed C1 amplitude not related to age.

4.4.2 SSVEP responses in the frequency domain

Luminance stimuli

The power spectrum (μV) estimates, obtained after averaging the trials in the time domain, are showed in Figures 4.7 and 4.8 for MSF and HSF respectively. Topographic maps show the distribution of SSVEP activity at the stimulation frequency. SSVEP harmonics (F and 2F) responses were analyzed using repeated measures analyses of variance with factors as described below.

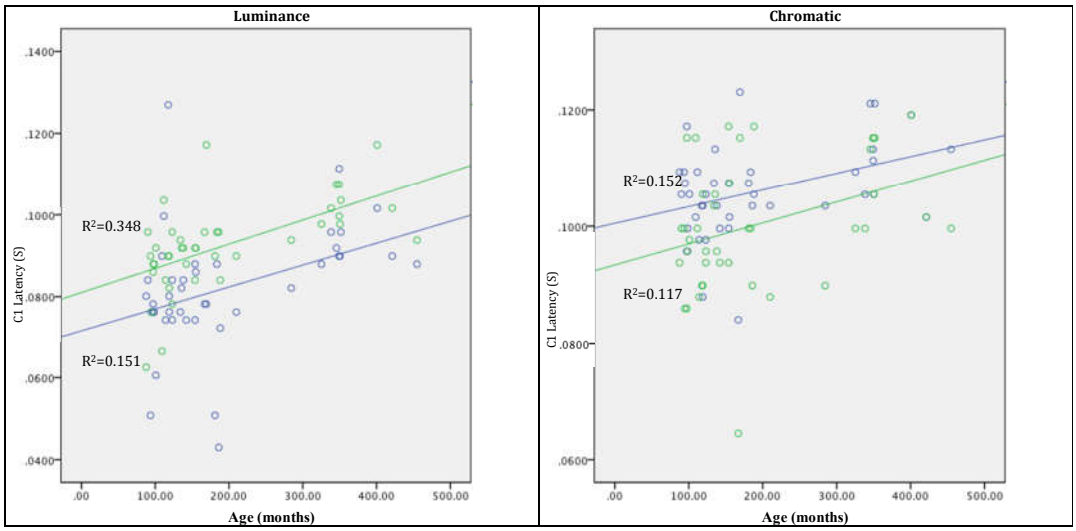


Figure 4.6. Developmental trajectories for C1 latency on luminance and chromatic stimuli showed increased latency with age at both spatial frequencies (MSF, blue; HSF, green). R^2 values indicate the proportion of variance explained by each trajectory.

Neither the main effect of spatial frequency, nor the interaction between spatial frequency and age group reached significance, both $F(1,37) < 1.49$, both $p > 0.23$). The main effect of spatial frequency did not reach significance for the adult sample. There was no interaction effect between harmonic and age group ($F(3,37) = 2.206, p = 0.1$), but there was a significant main effect of harmonic components ($F(1,37) = 4.789, p = 0.035$). There was also a significant effect of age $F(3,37) = 9.89, p < 0.001$.

In order to determine the effects of age on the different components of steady-state VEPs, between-groups analyses of SSVEP responses (peak power) were analyzed using repeated-measures ANOVAs with between-subject factor of group (four levels: 7-9, 10-12, 13-17, adults) and two within-subject variables (Harmonic components: F vs. 2F, Spatial frequency of gratings: mid vs. high).

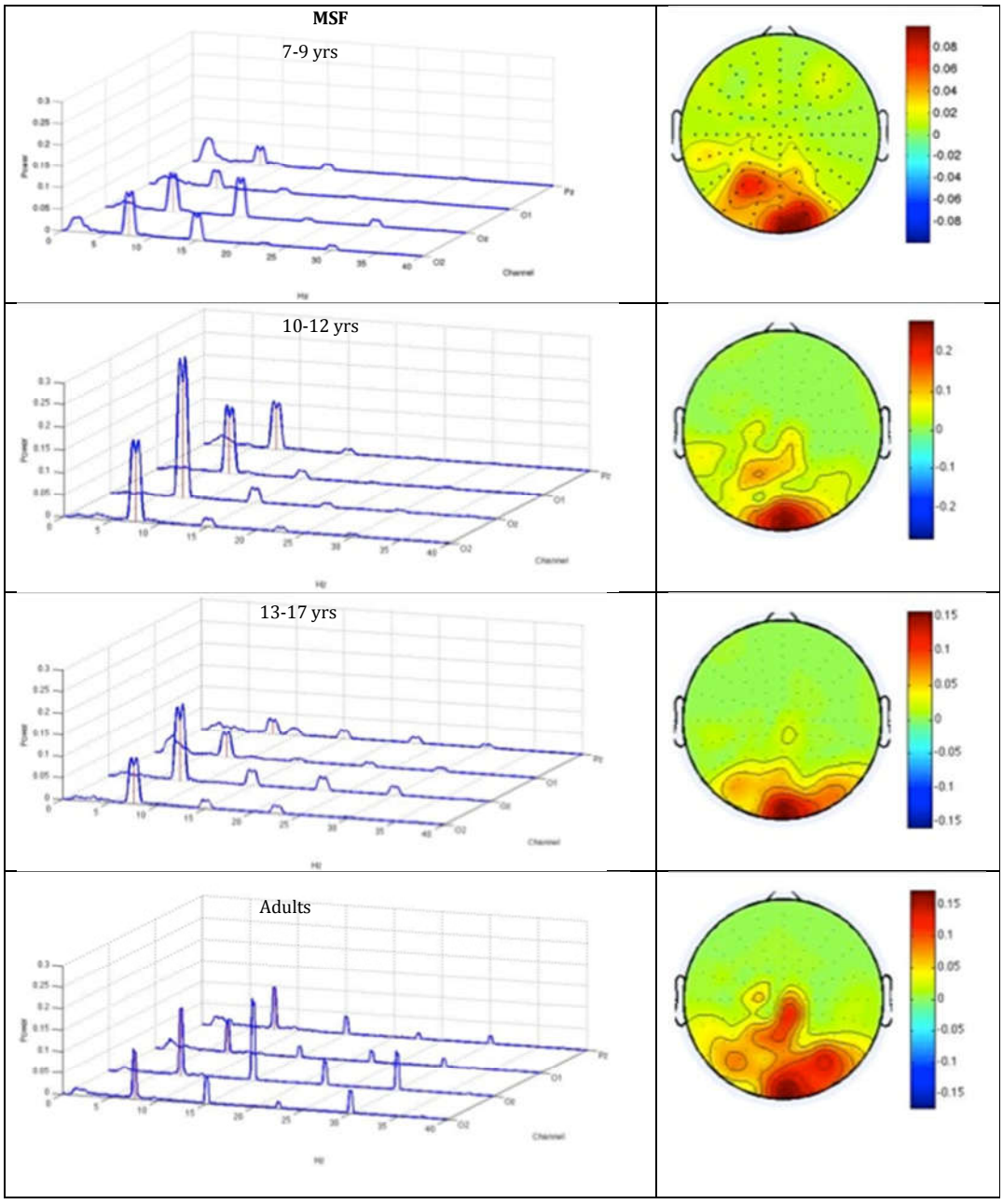


Figure 4.7. Spectrograms of the age groups for luminance MSF=2.8 cpd at four occipital electrodes. The red vertical lines indicate the frequency of stimulation. The topography map shows the power distribution (μV) obtained from all 128 recording sites at the stimulation frequency.

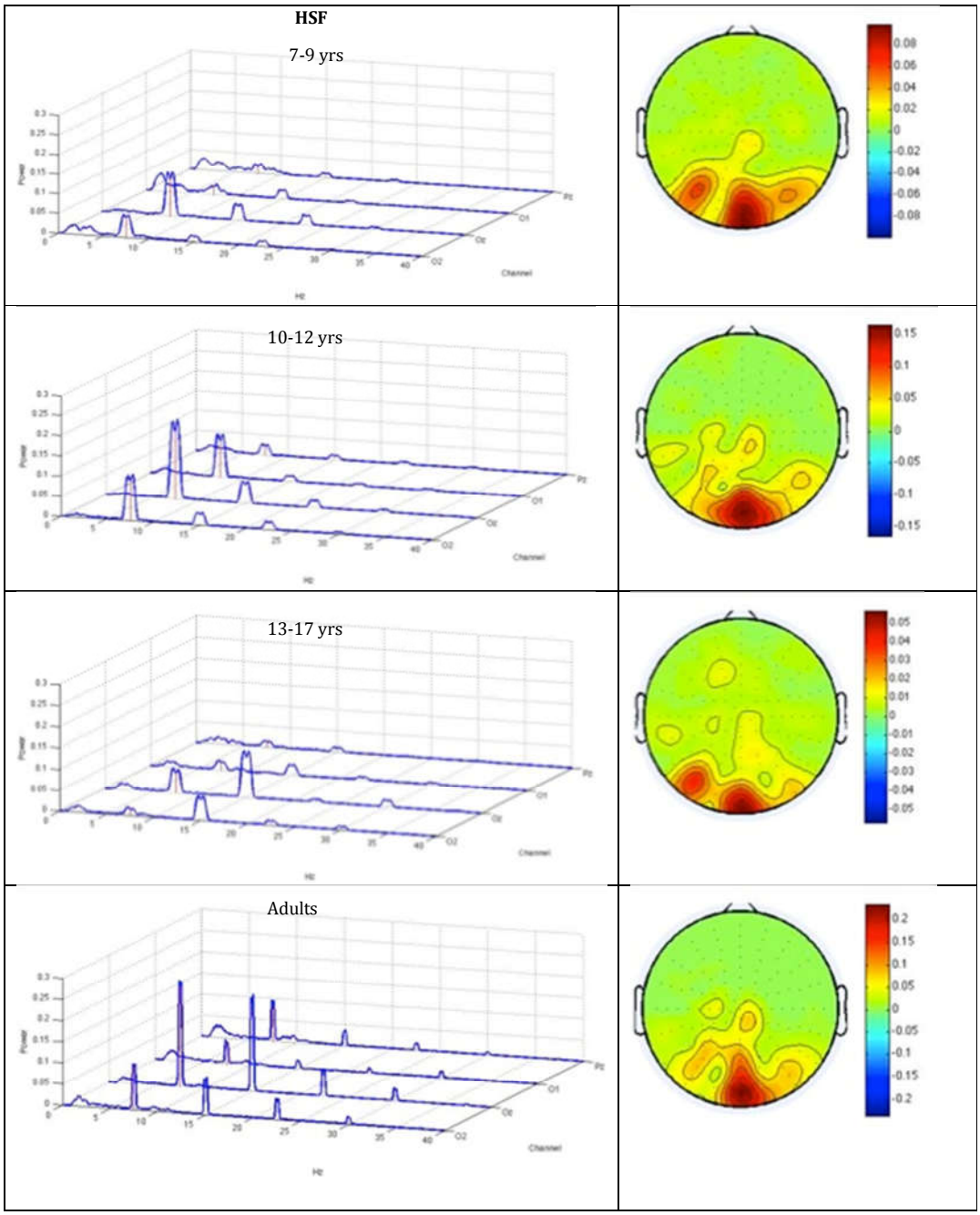


Figure 4.8. Spectrograms of the age groups for luminance HSF=6 cpd at four occipital electrodes. The red vertical lines indicate the frequency of stimulation. The topography map shows the power distribution (μV) obtained from all 128 recording sites at the stimulation frequency.

Table 4.2 summarizes the results of the three-way ANOVA. These analyses showed that power at first harmonic was greater than power at second harmonic. They also showed that, across groups, the mid spatial frequency response was of approximately equal amplitude to the high spatial frequency

response, providing an opportunity for evaluation of harmonic effects free of spatial frequency confound. One-way ANOVA followed by Tukey post-hoc tests were then used to analyze the effect of age group factor on the SSVEP power collapsed over spatial frequency and harmonic. Paired comparisons of the four age groups indicated that the SSVEP power of the adult group were significantly different from each of the child groups ($p \leq 0.017$), but that the children's groups were not significantly different from each other ($p = 0.476$ to 1). These analyses showed that children had smaller power than adults did.

Source of variation	d.f.	Sum of squares	F statistics	P-values
Spatial frequency (Mid vs. High)	1	0.006	0.741	0.395
Harmonic component (F vs. 2F)	1	0.076	4.789	0.035
Age group (7-9, 10-12, 13-17, Adults)	3	0.582	9.890	0.001
Spatial frequency * age group	3	0.037	1.485	0.235
Harmonic component*age group	3	0.105	2.206	0.104
Spatial frequency*Harmonic component	1	0.003	0.252	0.619
Spatial Frequency*Harmonic Component* age group	3	0.022	0.666	0.578
Residual	37	0.726		

Table 4.2. Analysis of variance for the effect of age on the SSVEP power. A*B indicates interaction between the effects of A and B.

Chromatic stimuli

Figures 4.9 and 4.10 shows the neural responses occurred at the fundamental frequency and its harmonics at both spatial frequencies (MSF, HSF) across age groups. A three-way analysis of variance was carried out to examine the effects of repeated measures upon the SSVEPs power. Spatial frequency (mid vs. high) and harmonic components (F vs. 2F) were the within-subjects variables and age group was the between-subjects variable. These analyses revealed that power depended on age, harmonic components and spatial frequency of the gratings, as reflected in significant main effects of spatial frequency ($F(1,37) = 28.1, p < 0.001$), harmonic components ($F(1,37) = 19.522, p < 0.001$), and age group ($F(3,37) = 10.585, p < 0.001$). There was interaction between spatial frequency and age group ($F(3,37) = 12.011, p < 0.001$), and a significant age group x harmonic interaction ($F(3,37) = 10.454, p < 0.001$. There was also a significant harmonic x spatial frequency interaction($F(1,37) = 13.483, p = 0.001$), and

there was interaction between spatial frequency, harmonic and age group ($F(3,37) = 8.593, p < 0.001$).

Two separate two-way ANOVAs were used to follow up the main effect of spatial frequency upon harmonic components separately. For the first harmonic, there was more power following mid spatial frequency than high spatial frequency across age groups ($F(1,37) = 21.654, p < 0.001$). Paired comparisons indicated that power at mid spatial frequency of the adult SSVEPs were significantly different from each of the child groups ($p < 0.001$), but that the children's groups were not significantly different from each other ($p = 0.963$ to 1).

Paired comparison also revealed that high spatial frequency values of the adult's power were significantly different from the 7-9 year child group ($p = 0.036$), but that the children's groups were not significantly different from each other ($p = 0.991$ to 1). For the second harmonic, there was also more power following mid spatial frequency than high spatial frequency across age groups, as reflected by a significant main effect of spatial frequency ($F(1,37) = 4.79, p = 0.035$).

Neither the main effect of age group, nor the interaction between spatial frequency and age group reached significance, both $F < 1.34$, both $p > 0.27$. A follow up analyses of the main effect of harmonic components revealed larger power at the first harmonic for the two spatial frequencies (both $F > 8.87$, both $p \leq 0.005$). At mid spatial frequency, the adult group had significantly larger power than children's groups ($p < 0.001$).

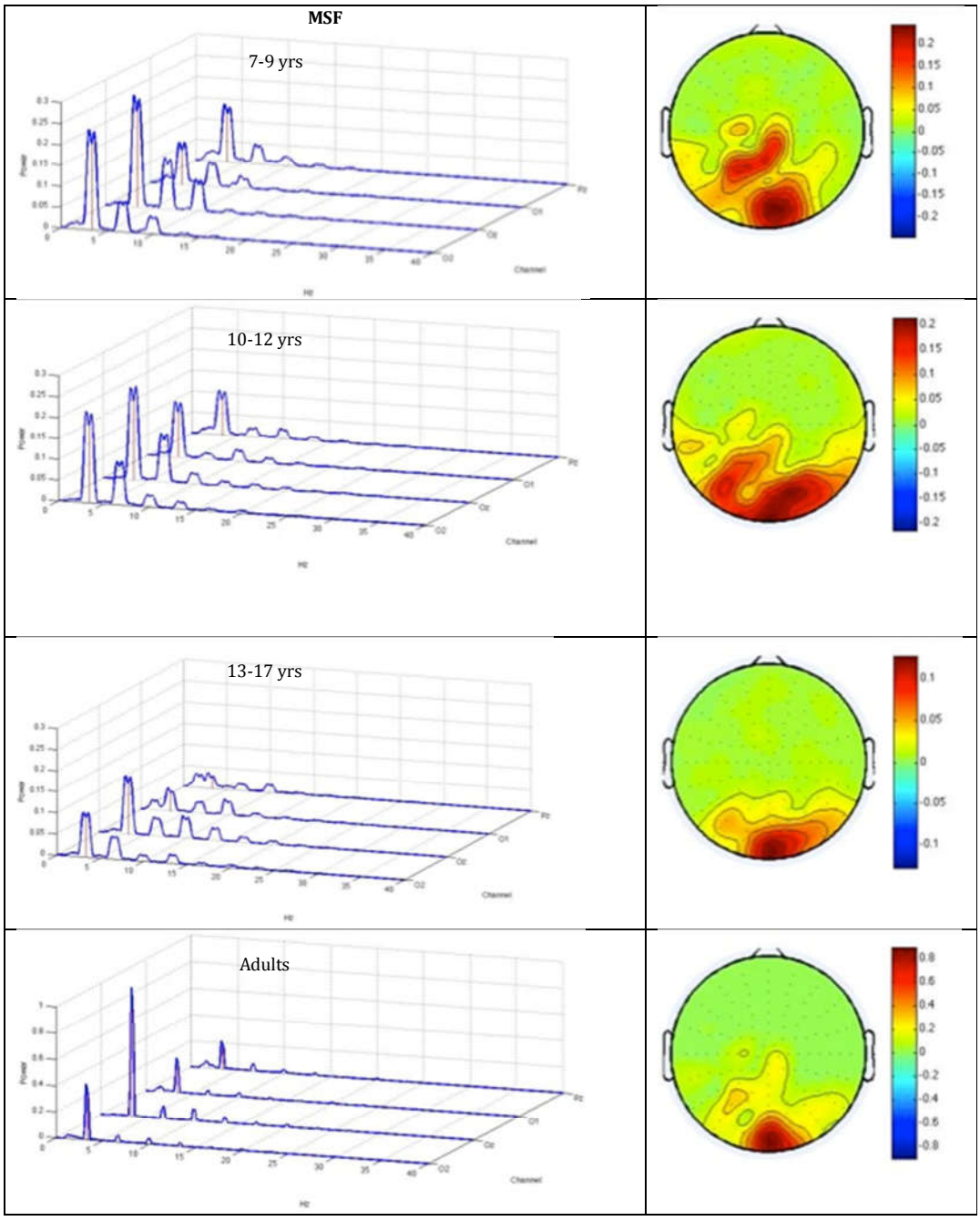


Figure 4.9. Spectrograms of the age groups for chromaicity MSF. Power spectrum of occipital electrodes (O2, Oz, O1 and Pz) averaged across the epoch from all age groups and the topography map of the PSD distribution obtained from all 128 recording sites for MSF=2.8 cpd. The red vertical lines indicate the components of the stimulus frequency.

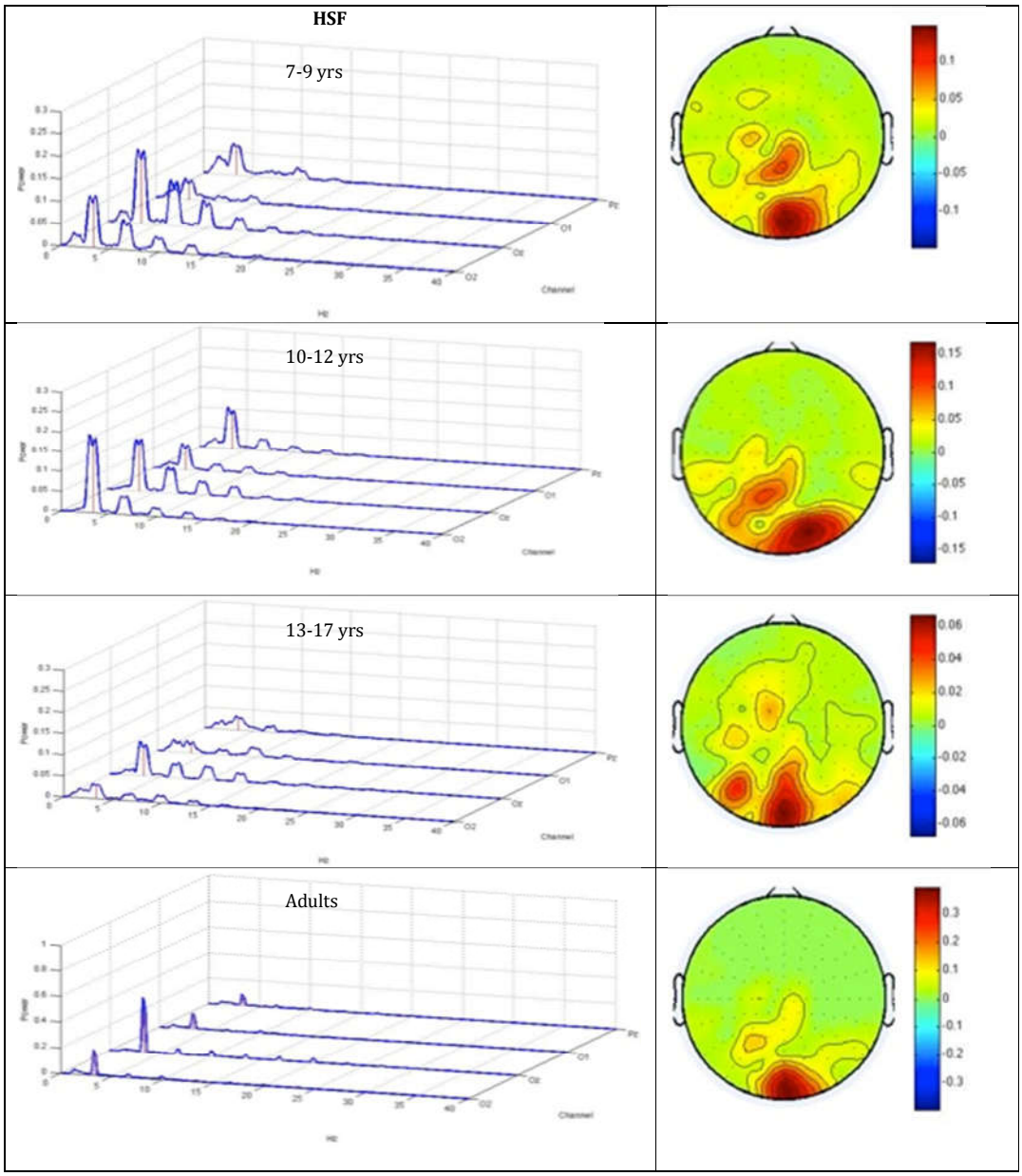


Figure 4.10. Spectrograms of the age groups for chromaicity HSF. Power spectrum of occipital electrodes (O2, Oz, O1 and Pz) averaged across age group and the topography map of the PSD distribution obtained from all 128 recording sites for HSF=6 cpd. The red vertical lines indicate the components of the stimulus frequency.

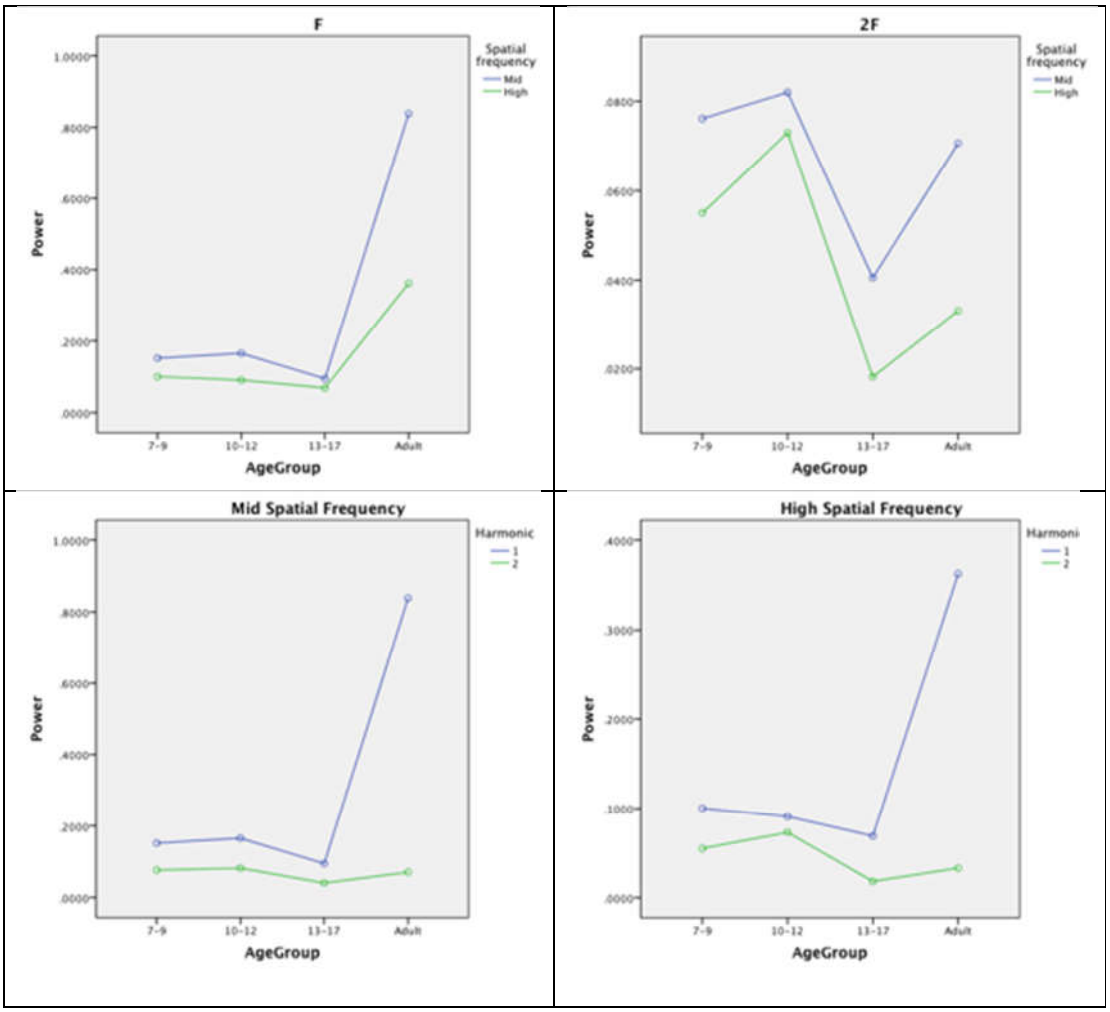


Figure 4.11. Cross-effect of spatial frequency and harmonic components.

To summarize, the effect of harmonic components and spatial frequency of the gratings depended on age group: in children, very small difference in power between the two harmonics and the two spatial frequencies; in adults, however, using MSF led to much higher F power than using HSF. Figure 4.11 shows the effect of age on harmonic components and spatial frequencies. Table 4.3 summarizes the results of the three-way repeated-measures ANOVA.

Source of variation	d.f.	Sum of squares	F statistics	P-values
Spatial frequency (Mid vs. High)	1	0.309	28.1	0.001
Harmonic component (F vs. 2F)	1	1.218	19.522	0.001
Age group (7-9, 10-12, 13-17, Adults)	3	1.860	10.585	0.001
Spatial frequency * age group	3	0.397	12.011	0.001
Harmonic component*age group	3	1.957	10.454	0.001
Spatial frequency*Harmonic component	1	0.174	13.483	0.001
Spatial Frequency*Harmonic Component* age group	3	0.332	8.593	0.000
Residual	37	2.167		

Table 4.3. Analysis of variance for the effect of age on the SSVEP power for chromatic gratings. A*B indicates interaction between the effects of A and B.

Validation by regression analyses

Linear regression analyses, as described above were conducted to independently investigate developmental changes on the luminance and chromatic stimuli. The independent variable was age and the dependent variables were the power values. For luminance stimuli, these analyses showed that SSVEP power at second harmonic increased with age at both spatial frequencies, (MSF: $R^2 = 0.270, F(1,38) = 14.089, p = 0.001$; HSF: $R^2 = 0.290, F(1,38) = 15.509, p < 0.001$). For the first harmonic, SSVEP power increased with increasing age at high spatial frequency ($R^2 = 0.199, F(1,38) = 9.461, p = 0.004$), but not at mid spatial frequency ($R^2 = 0.04, F(1,38) = 1.565, p = 0.219$).

For chromatic stimuli, these analyses showed that SSVEP power at first harmonic increased with increasing age at both spatial frequencies (mid: $R^2 = 0.517, F(1,38) = 40.647, p < 0.001$; high: $R^2 = 0.195, F(1,38) = 9.226, p = 0.004$). By contrast, for the second harmonic, age did not predict increase of power on either spatial frequency (mid:$^2 = 0.003, F(1,38) = 0.118, p = 0.733$; high: $R^2 = 0.052, F(1,38) = 2.098, p = 0.156$). Figure 4.12 shows the developmental trajectories linking SSVEP power and chronological age for mid and high spatial frequencies. The regression lines represent a linear model fit of all participants (87 to 454 months).

In sum, both ANOVA and regression analyses showed age-related changes in SSVEP power for both luminance and chromatic stimuli. As regression proved a reliable developmental analysis (Annaz et al., 2010), it can be concluded that second harmonic increased with age for luminance stimuli at both spatial frequencies of gratings. First harmonic increased with age only at high spatial frequency, as was not related to age at mid spatial frequency. Furthermore, these analyses showed that first harmonic increased with age for chromatic stimuli at both spatial frequencies of gratings. Second harmonic was not related to age.

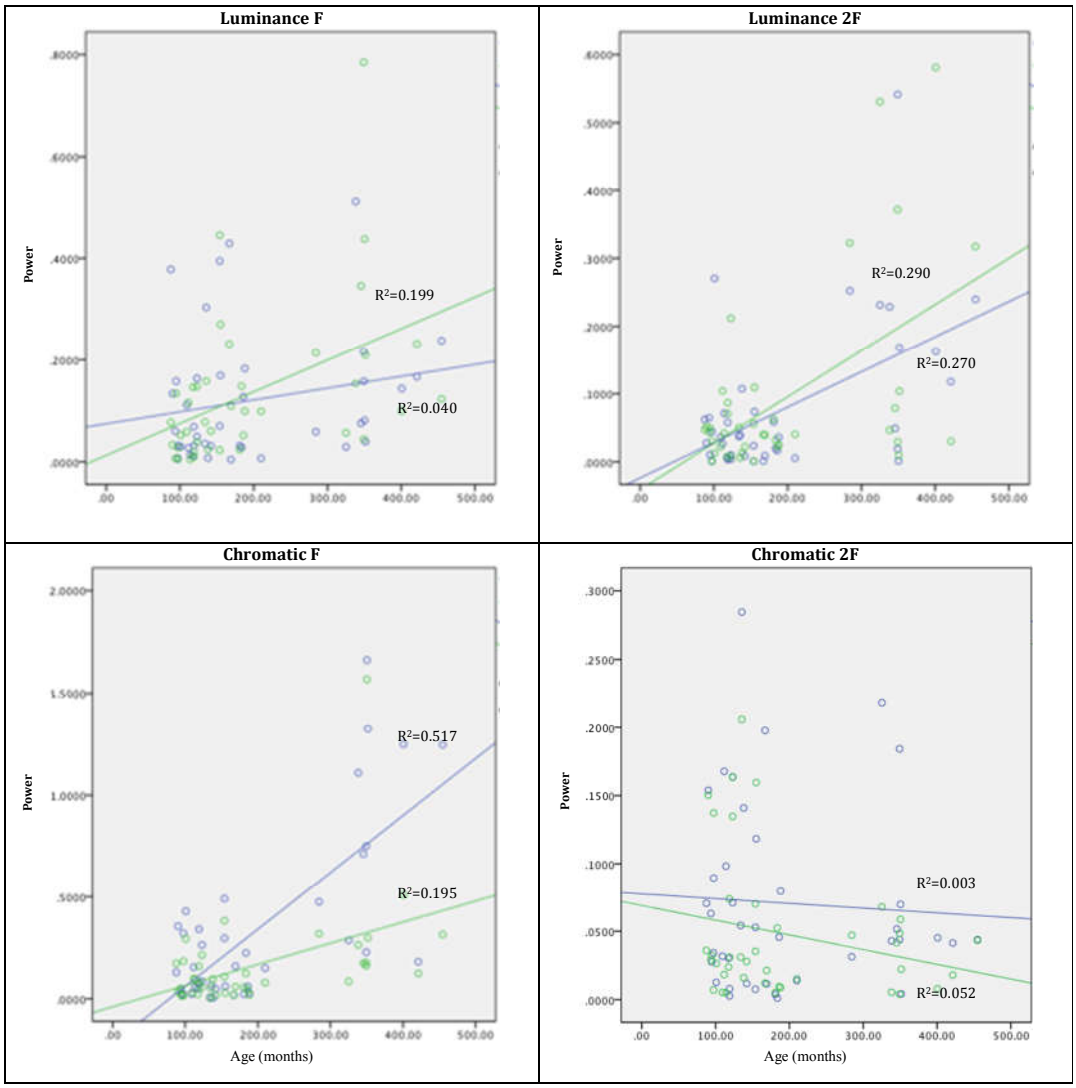


Figure 4.12. Developmental trajectories for SSVEP power on luminance and chromatic stimuli for first (F) and second (2F) harmonics are shown for MSF (blue line) and HSF (green line). R^2 values indicate the proportion of variance in power explained by age.

4.4.3 Time-dependent network analysis

The dynamical changes in spatial frequency processing are provided in this section where grand average network-metric time series at each age group are shown for luminance and chromatic stimuli.

Luminance stimuli

The time-varying structures for mid and high spatial frequency gratings are shown in Figure 4.13 at both the fundamental frequency (F=7.5 Hz) and second harmonic (2F=15 Hz) across groups.

In order to determine the effects of age on the time-varying networks, between-groups analyses of signal variability (ApEn) were analyzed using repeated-measures ANOVA with between-subject factor of group (four levels: 7-9, 10-12, 13-17, adults) and two within-subject variables (Spatial frequency of gratings: mid vs. high, Harmonic components: F vs. 2F). Neither age group nor spatial frequency produced any significant main or interaction effects, all $F < 1.31$, all $p > 0.28$. There was a significant main effect of harmonic ($F(1,36) = 478.269, p < 0.001$). These analyses showed that ApEn was larger at second harmonic than at first harmonic across age groups, Figure 4.14.

Chromatic stimuli

The time-varying structure of spatial frequency processing are shown in Figure 4.15 for mid and high spatial frequency, at both the first harmonic (F=7.5 Hz) and second harmonic (2F=7.5 Hz) across age groups. As above, a three-way repeated measures ANOVA was used to investigate effects of age upon the networks dynamics. Spatial frequency (mid vs. high) and harmonic (F vs. 2F) were the within-subjects variables, and age group was the between-groups variable.

There was a significant main effect of spatial frequency ($F(1,36) = 28.338, p < 0.001$). There was no significant main effect of harmonic ($F(1,36) = 1.992, p = 0.167$), but there was a significant interaction between harmonic and age group ($F(3,36) = 4.175, p = 0.012$). Neither the main effect of age group, nor the interaction between spatial frequency and age group reached significance, both $F < 2.346$, both $p > 0.08$. As may be seen in Figure 4.15, children had larger ApEn in the first harmonic, but adults had larger ApEn in the second harmonic.

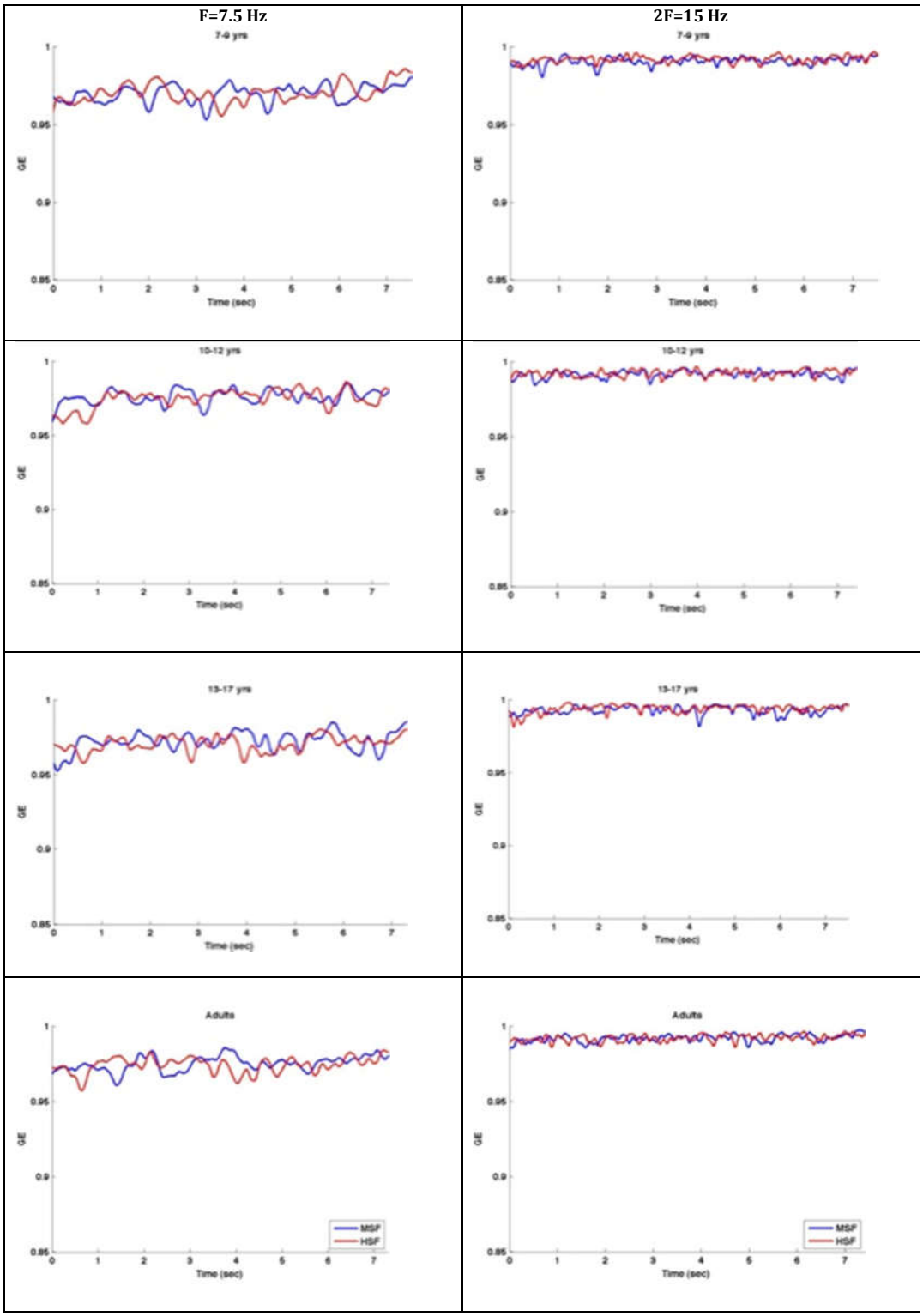


Figure 4.13. Time-evolution of the network metrics with luminance stimuli for all age groups at mid (MSF) and high (HSF) spatial frequency for the fundamental frequency (F=7.5 Hz) and second harmonic (2F=15 HZ).

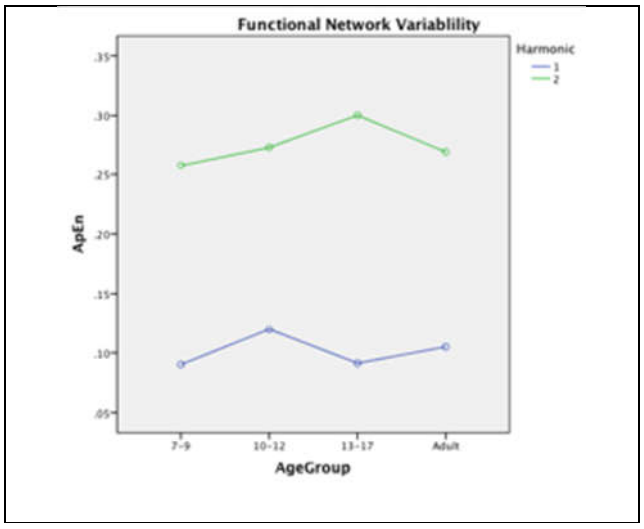


Figure 4.14. The effect of harmonic upon the network variability showed greater variability for the second harmonic (2F).

One-way ANOVAs followed by Tukey post-hoc tests were then used to analyze the effect of age group factor on the ApEn averaged over spatial frequency for each harmonic separately. Paired comparisons of the four age groups indicated that the first harmonic of the adult group was not significantly different from all children groups ($p \geq 0.326$), and that the children's groups were not significantly different from each other ($p = 0.226$ to $p = 0.95$). Paired comparisons also revealed that the second harmonic of the adult group was not significantly different from the children groups ($p \geq 0.069$), and that the children's groups were not significantly different from each other ($p = 0.574$ to $p = 0.913$). Nevertheless, the interaction effect demonstrated a switch in development indicating that ApEn becomes greater for 2F in adulthood.

Validation by regression analyses

Linear regression analyses, as described in (Annaz et al., 2010), were conducted to independently investigate developmental changes on the luminance and chromatic stimuli. The independent variable was age and the dependent variables were the ApEn values. Regression analyses showed a significant age effect (R^2=0.098, F(1,38)=4.15, p=0.049) at the second harmonic of high spatial frequency for chromatic stimuli only. These analyses showed a small effect (only

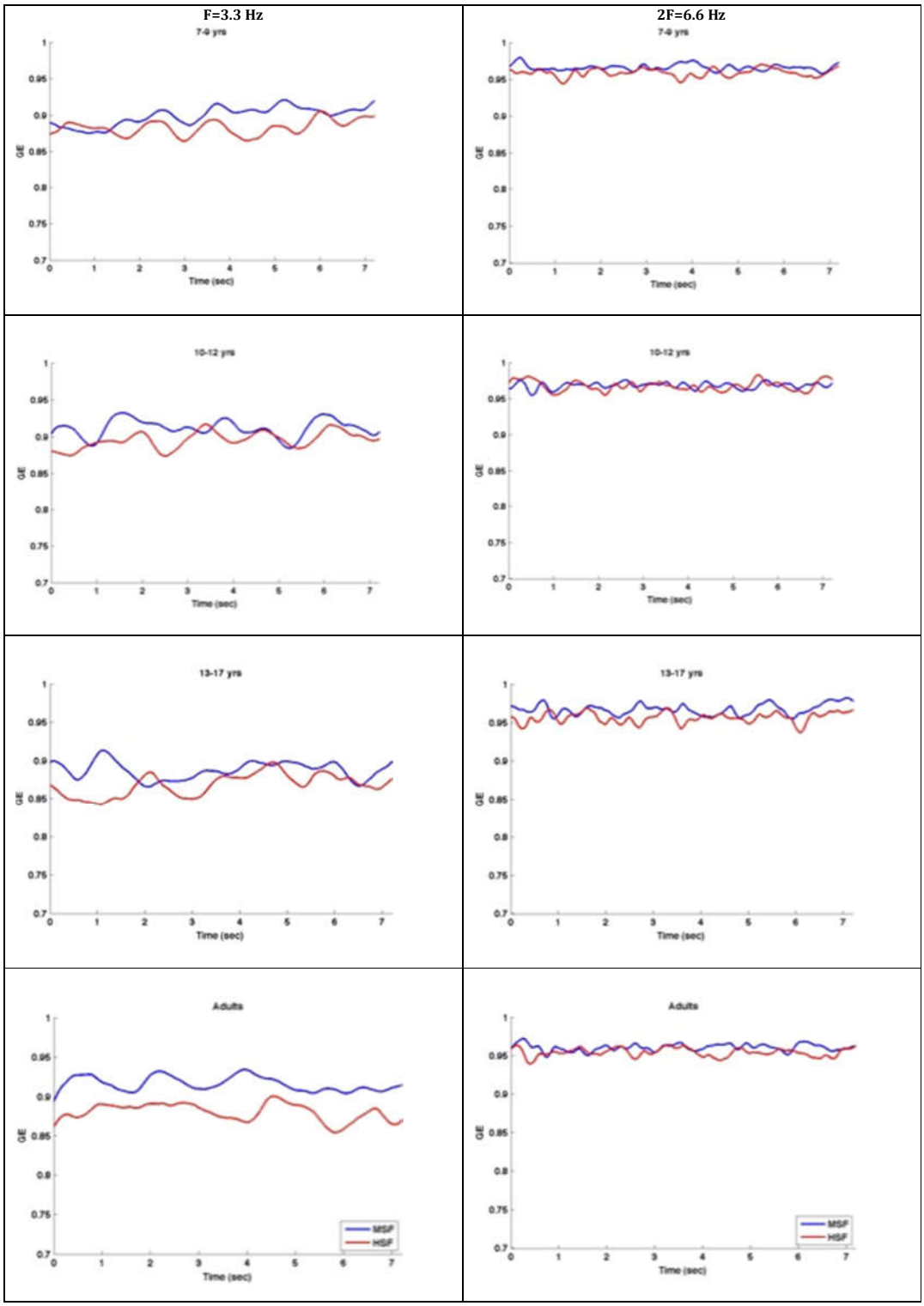


Figure 4.15. Time-evolution of the network metrics with chromatic stimuli for all age groups at mid (MSF) and high (HSF) spatial frequency for the fundamental frequency (F=3.3 Hz) and second harmonic (2F=6.6 HZ) during chromatic gratings.

9.8% of variance explained), therefore may not be reliable, and because the regression analyses were not corrected for multiple comparisons, this result should be treated with caution, Figure 4.16. Figure 4.17 depicts the developmental trajectories linking approximate entropy and chronological age for high spatial frequency. The regression line represent a linear model fit of all participants (87 to 454 months). As can be seen in Table 4.4, no other relationships reached significance.

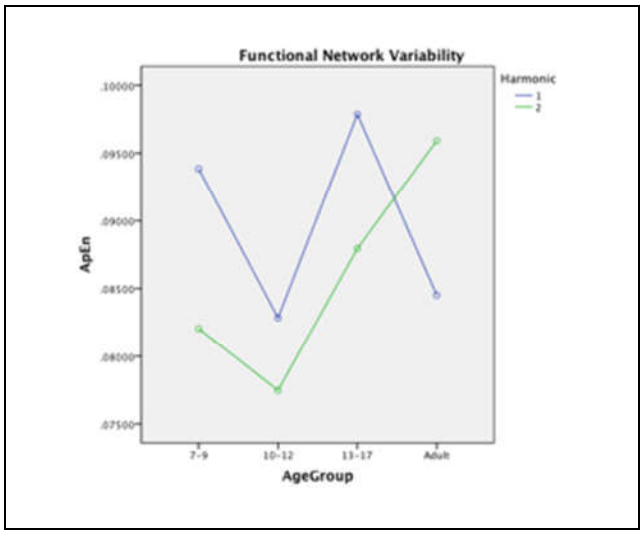


Figure 4.16. The effect of harmonic on the network variability with chromaticity.

Source of variation	R^2	d.f.	Sum of squares	F statistics	P-values
H1_Luminance_Mid	0.055	1	0.006	2.218	0.145
H1_Luminance_High	0.022	1	0.001	0.868	0.357
H2_Luminance_Mid	0.006	1	0.001	0.217	0.644
H2_Luminance_High	0.001	1	0.000	0.038	0.846
H1_Chromatic_Mid	0.080	1	0.001	3.325	0.076
H1_Chromatic_High	0.000	1	0.000	0.003	0.960
H2_Chromatic_Mid	0.081	1	0.001	3.343	0.075
H2_Chromatic_High	0.098	1	0.002	4.150	0.049 *
Residual		38	0.017		

Table 4.4. The relationship between chronological age and ApEn reached significance only for chromatic gratings at high spatial frequency.

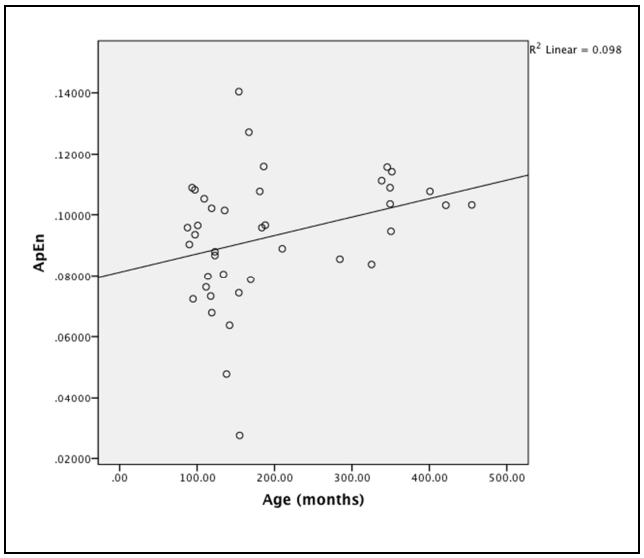


Figure 4.17. Relationship between chronological age and ApEn on chromatic gratings at high spatial frequency. R^2 value indicate the proportion of variance in ApEn explained by age.

GE changes with age

A comparison of the GE values across conditions (MSF/HSF) was carried out using the T-tests on the variables of first harmonic (F) and second harmonic (2F). Results are presented as probability levels across time as shown in Figure 4.18. For the first harmonic, only the adult group showed a consistently significant difference between conditions across time under chromatic stimulation ($p<0.005$). These analyses showed that for the adult group, GE proved a reliable index of spatial frequency processing.

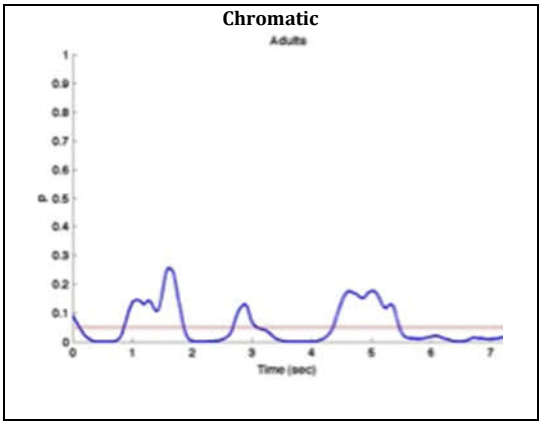


Figure 4.18. The probability values of the time-varying metric GE for chromatic stimuli were significant different ($p<0.05$) across conditions (MSF/HSF) only in the adult group at the fundamental frequency (F= 3.3 Hz) of stimulation. The red line shows the p value p=0.05.

In summary, these analyses showed that C1 latency increased with age for both luminance and chromatic stimuli, whereas C1 amplitude was not related to age. SSVEP power increased with age at high spatial frequency gratings, whereas no age-related differences were found for mid-spatial frequency gratings in the luminance stimuli. For chromatic stimuli however, SSVEP power increased with age at both mid- and high- spatial frequency gratings. The network metrics showed higher levels of global efficiency at mid- spatial frequency than those for high- spatial frequency in chromatic stimuli, this reached significance only in adulthood. Children showed higher values of approximate entropy than adults only in the chromatic condition.

4.5 Discussion

This study explored age-related changes in functional connectivity, aiming to characterize and quantify the development of brain function. Using a SSVEP paradigm, sinusoidal gratings defined by either luminance or color contrast were presented in a flashing manner in order to elicit frequency-specific synchronization of neuronal activity. Two spatial frequencies were used in each case to compare the coordinated activity generated by them in the brain of children and adults. The SSVEP paradigm generates brain responses at the temporal frequency of stimulation (fundamental frequency) and its multiples (harmonics). In this study, SSVEP responses were analyzed at the fundamental frequency (F) and second harmonic (2F) in order to explore their effects in brain activity across age. Thirty participants were divided in four age groups attempting to analyze critical developmental stages: early childhood (7-9 years), middle childhood (10-12 years), adolescence (13-17 years), and adulthood. Statistical analyses were applied between groups (ANOVA) and across age (Linear Regression).

The first main hypothesis in this study that children will have similar functional connectivity than adults when processing medium spatial frequency of

luminance gratings was proven. SSVEP power analysis was performed over the steady-state response of the recorded signals. For the temporal frequency of stimulation (F), reduced power was found in children when compared to adults at high spatial frequency. For the second harmonic (2F), increased power was observed with age at both spatial frequencies. Given the lack of relationship between SSVEP power and age in the mid spatial frequency luminance condition, it appears as though networks underpinning mid- spatial frequency luminance change may have reached adult levels by the childhood ages measured here. Our results are in agreement with previous ERP and behavioural findings of an earlier maturation of the mechanisms specialized for processing low frequency input in the visual system, while sensitivity to high spatial frequencies continues increasing across development (Crognale et al., 1998; Howard and Reggia, 2007). Low spatial frequency processing is thought to be an important aspect of global processing, such as face recognition (Goffaux & Rossio, 2006). It has been reported that children process faces holistically, just like adults, by age 6 or perhaps younger (Pellicano & Rhodes, 2003). Low spatial frequencies are believed to provide information about the general characteristics of objects, such as shape, size, and large contours (Vlamings et al., 2010). Thus, the integrity of low spatial frequencies processing mechanisms is crucial for healthy development of brain function. As our results suggest that children may have reached adult levels in the processing of medium spatial frequency, it might be an indicator of the integrity of functional connectivity associated to early visual channels in neurodevelopment, which would be an important consideration for future studies, especially when pathologies are being evaluated.

The second main hypothesis in this study that children will have reduced functional integration than adults when processing chromatic gratings was proven. SSVEP power was found to increase across age at both spatial frequencies for the temporal frequency of stimulation (F). Lower variability of the global efficiency (GE) time series associated to chromatic processing at both spatial frequencies was observed in adults compared to those of children. Our results support recent findings on functional organization changes between

children and adults (Nenadovic et al., 2011; Fair et al., 2009; Boersma et al., 2010). Nenadovic et al., (2011) found more transient changes in functional connections in children than in adults within the default network. Synchronization within brain areas was reduced, while coordinated activity between separated brain areas was increased in studies reported by the other research groups (Fair et al., 2009; Boersma et al., 2010). Thus, our findings provide evidence for more organized activity in adults than in children within functional networks underpinning chromatic processing, which might indicate that "functional brain networks develop from local to distributed organization" (Fair et al., 2009).

Analyses carried out upon C1 amplitude and latency of the ERP signal showed no age-related changes in the amplitude of C1. By contrast, latency of C1 was longer across age. Recent evidence has suggested that age-related changes in networks dynamics as measured by ERP components latencies, may be an indicator of effective perception of information (Pinal et al., 2015) in integrative functions. The authors argue that longer latencies suggest slower processing speed, which would improve the processing of detailed information contained in a complex stimulus or task. In an adult sample, they reported longer ERP latencies with increasing age during working memory tasks. This result seems to be in line with the statement of the neural efficiency hypothesis, which proposes that less efficient information-processing systems requires the activation of more neurons or brain cortical circuits to process data, some of which are redundant to processing performance. Traditionally, earlier latencies have been associated to faster information processing carried out by cortical circuits. However, a different line of work suggests that the neuronal activity that generates ERPs is modulated by long-lasting postsynaptic potentials of subcortical transmitter systems reaching the cortical neurons. In our work, we discuss that the longer C1 latency in adults than in children during visual perception may support the information-processing efficiency theory. The earlier latencies in children might reflect a better developed sensory network, as children have an overabundance of neurons and synapses along the visual pathways before the pruning of

redundant synaptic connections and cortical thinning, but delayed development of integrative processing (Swick et al., 1994; Zanto et al., 2010). Although longer latencies with age have also been reported for luminance- (Celesia et al., 1987; Tobimatsu et al., 1993) and chromatic gratings (Crognale, 2002), we must be cautious when interpreting our findings because of the small number of ERPs analysed in this study.

Our results showed that the harmonic 2F was differently affected by luminance and chromatic stimulation. SSVEP power at 2F increased with age for luminance gratings at both spatial frequencies, while no effect was found for chromatic stimuli at neither of the spatial frequencies. These results are compatible with those reported by Arakawa and colleagues (1999), who reported that the fourth harmonic was less affected by chromatic stimuli than for luminance gratings. Analysis of approximate entropy (AP) showed increased variability at high spatial frequency for chromatic gratings. Although this could indicate more complexity in functional connectivity, it might be due to an increase in the temporal frequency, rather than to a developmental change. The 2F component is important, as it is though affected in neurological conditions such as Alzheimer's disease (Celesia et al., 1993) and autism (Pei et al., 2014).

Therefore, developmental change within the network dynamics of visual perception was apparent from childhood to adulthood. Networks processing luminance change and chromatic change showed development over the time-course studied here. This was evidenced by the increase in C1 latency across the developmental period and also by the increase in SSVEP power across the developmental period. However, given the lack of relationship between SSVEP power and age in the mid spatial frequency luminance condition, it appears as though networks underpinning mid- spatial frequency luminance change may have reached adult levels by the childhood ages measured here. This suggests that although there may be functional reorganization across development, medium spatial processing would be a useful biomarker of typical brain function.

Chapter 5

Exploring the networks dynamics in autism spectrum disorder

On the issue of early identification of biomarkers for autism, it has been noted that, "... a single measure at a single time point can be very misleading..." (Gordon, 2011) as it may not reflect the functional mechanisms of the condition over the course of development. In this context, this experiment seeks to identify potential differences in networks dynamics between children with and without autism across childhood.

5.1 Autism: Is it in the brain?

Autism is the most severe form of a spectrum of pervasive neurodevelopmental disorders known as autism spectrum disorder (ASD). It is a complex condition characterized by alterations in social interaction, impaired communication abilities, and stereotyped patterns of behaviour (DSM V, American Psychiatric Association, 2013). Symptoms can manifest in distinct levels of severity, which result in a substantial heterogeneity in the diagnosis (Geschwind and Levitt, 2007). Autism affects 1 in every 100 children and adolescents, with males being affected three times more often than females (Baird et al., 2006). Evidence for atypical development in the autistic brain includes structural and functional abnormalities in cortical and subcortical systems, which disrupt the dynamics of brain development, such is described in the following section.

5.1.1 Evidence of the autistic brain

One of the first structural evidences of atypical brain in autism was provided by Kanner (1943), who reported enlarged heads in some of the affected children. The advancement of imaging technology, such as computed tomography (CT), Positron Emission Tomography (PET) and Magnetic Resonance Imaging (MRI), provided a window into the structure and function of the brain. CT studies revealed an enlargement of the lateral ASD brain ventricles (Balottin et. al., 1989). Some MRI studies have suggested that in children with ASD (ages 18 months to 4 years), the brain is bigger by about 5% to 10% (Hazlett et al., 2005; Sparks et al., 2002). By the same age, white matter would have increased disproportionately compared to grey matter enlargement (Hazlett et al., 2005; Courchesne et al., 2001). It is not clear if the growth of white matter persist into adulthood, but grey matter has been reported to increase by about 6%–12% in adults (Courchesne et al., 2001). The parietal, temporal and frontal regions seem to be the more enlarged regions, with cortical thinning reported in adulthood (Hadjikhani et al., 2005; Piven et. al., 1996; Raine 2006). Children (age 8-12 years), on the other hand, seem to have increased cortical thickness all over the cortex, particularly in the parietal and temporal cortices (Hardan et al., 2006).

Postmortem studies have related cortical thickness to high neuronal density, whereas enlargement of white matter has been related to an excessive number of neurons (Bailey et al., 1998). Casanova et al. (2002) suggested an abnormal development of the neocortex at the level of minicolumns. Within the first two years of age, in normal development, neurons will organize in minicolumns, which will be separated enough to allow dendrites from different cells bundled together and axonal fascicles extend throughout several layers of the cortex (Amaral et al., 2008). In the autistic brain, however, it has been observed an atypical number and width of minicolumns, with reduced space between them (Casanova et al., 2006). These structural alterations in early brain development would alter normal function of brain systems at different scales, including lower processing levels of visual perception (McKavanagh et al., 2015).

5.1.2 Non-invasive techniques to detect Autism

Autism is not usually detected before the first two years of age (Lord et al., 2006). Parents, however, may be noticed some signs of developmental disruptions (e.g. passivity, irregular sleeping and eating behaviours) at a younger age, in children that later are diagnosed with autism (Landa, 2008). Efforts for an earlier diagnosis include the identification of a biological marker that may serve as the perfect complement to traditional behavioural and developmental screening. Currently, there is no blood test or non-invasive biological test that can be used at earlier age. A promising biological test would be the electrical activity of the brain, as measured by EEG, which can be noninvasively recorded from the scalp of very young children. Evaluating EEG brain activity in children with or without autism would help to characterize and quantify levels of atypical development in the autistic brain.

In spite of the fact that EEG does not provide direct information on subcortical structures (Wood & Allison, 1981), artificially activating the visual cortex with a periodic stimulus (i.e. to generate a SSVEP) will elicit functional networks that could provide indirect information on subcortical structures if the stimulation frequency is low, or on extracortical structures if the frequency of stimulation is higher than 3 Hz (Srinivasan et al., 2007). Functional networks are generated within brain systems at different scales as it decodes stimulus-produced activations of neurons. Following the hierarchical organization of the brain (Van Essen et al., 1992), neurons at lower processing levels of the cortex will fire according to the stimulus properties to which they are sensitive, and their responses will be send to higher levels of processing (Schechter et al., 2003). The magnitude and frequency of neuronal firing will shape the electrical activity recorded from the scalp by the EEG, then the EEG activity becomes, basically, an indirect measure of information processing carried out by the stimulated structure. Steady-state visual evoked potentials (SSVEPs) have been widely used to entrain neuronal assemblies eliciting functional cortical networks. The frequency of stimulation (Srinivasan et al., 2006) and complexity of the visual task (Silberstein et al., 1990) generate effects on the size, distribution, and

dyamics of the functional networks, which would allow to evaluate local functional segregation and distributed functional integration within brain networks. SSVEPs are traditionally analysed in the frequency domain over a small number of long trials (~10 12-sec trials) (Pei et al., 2014), and are less affected by blinks, and muscle and eye movements than other EEG techniques. The SSVEP technique is therefore a potentially useful technique to identify differences in the brains of people with and without autism.

5.2 Visual Perception in Autism

Although the specificity, severity, and consistency of visual abnormalities in ASD still are debated, there is indicative research where individuals with autism show over-functioning of local networks underpinning low-level perceptual processing (Mottron et al., 2006), and deficits in the integrative mechanisms of functional cortical connectivity (Just et al., 2004; Happe & Frith, 2006). Belmonte et al. (2004) suggested that superior or weaker processing abilities in autism depend on the complexity of the functional network required to process a visual task. Taken together, these observations lead to a disrupted functional connectivity theory (Kana et al., 2011).

Studies on altered connectivity of functional networks in ASD has provided no conclusive evidence on the development of brain dysfunction, there is a lack of consensus in terms of the scale of affected networks and their underlying neural mechanisms (Koh et al.,2010; Vlamings et al., 2010, Rajendran et al., 2007). One reason for this is because the development of integrative processing, where lower processing levels of the cortex feed higher levels of information processing to achieve more complex tasks, has not been systematically explored. The purpose of the present study is therefore to explore the networks dynamics of low-level functional connectivity and its effects in higher functional networks, according to the hierarchical organization of the brain, in order to identify early differences in the development of brain function in ASD.

5.2.1 Research on altered visual local connectivity

In this work, local connectivity is defined as communication within adjacent segregated populations of neurons (Fries, 2005), such as those networks underpinning early spatial information processing within the primary visual cortex (Tononi et al., 1994). Altered local connectivity has been reported in ASD, in visual system's spatial frequency-based processes, the processes by which neural functional groups encode detailed information or whole representations of an image (Deruelle et al., 2004). A recent electrophysiological study on contrast sensitivity in ASD revealed no difference in the processing of low spatial information (0.8 cpd), but a trend towards differences between individuals with and without ASD in high spatial processing, as showed by similar ERPs to medium (2.8 cpd) - and high (8 cpd) -spatial frequencies when presented luminance-defined gratings at high contrast (90%) (Jemel et al., 2010). The research group interpreted these findings as individuals with ASD employing the same neural mechanisms to process mid- and high- spatial frequency information, which would influence their attention towards detailed aspects of a visual image.

Another study on SSVEP responses to low- contrast (16.6%) luminance-defined gratings of low spatial frequency (1 cpd) reported that latency and amplitude of the first major ERP component, the P1 (the major positive component at around 120 ms) did not differ between adults with and without ASD (Fujita et al., 2011). In addition, analysis of power spectral, with Fourier analysis techniques, over the steady-state responses showed no differences between groups. This group interpreted this finding, as the magnocellular pathway would have been no altered in ASD. A recent SSVEP study (Pei et al., 2014) on spatial frequency processing in children with ASD (aged 5 to 17 years) evaluated function of early visual channels at high contrast (80%) at the frequency of the second- (15 Hz) and fourth- harmonics (30 Hz) in a range of spatial frequencies (2-30 cpd). They reported no differences at low spatial frequency (2 cpd), but abnormal processing at higher frequency information (8.2 – 14.4 cpd) at the second harmonic. Finally, no differences were found at the fourth harmonic. The authors

interpreted these results as evidence for separate early visual channels, with normal response at low spatial frequency (2cpd) and atypical high spatial processing mechanisms in ASD. These findings provided evidence of segregated functional networks elicited by SSVEPs and their suitability to study lower processing levels of the cortex.

A recent assessment of the brains' response to different spatial frequency in a group of 21 adolescents and adults with and without ASD (Keita et al., 2014), used vertically-oriented gratings defined by both luminance (with and without noise) and texture contrast across a range of spatial frequencies (0.5, 1, 2 4 and 8 cpd). An increased sensitivity to high spatial frequencies (>4 cpd) in the luminance gratings was observed in the ASD group, which was interpreted as evidence for detail-oriented perception in ASD. Given the methodological differences to assess low-level processing, it is not clear whether the function of early filtering mechanisms in visual perception is altered in ASD.

5.2.2 Research on visual long-range connectivity

Long-range connectivity is defined, for our purposes, as interactions between not adjacent brain areas (Vissers et al., 2011) with segregated functions that share information with higher areas of the system organization. This large network receives information from lower levels to create higher order processing modules with more complex function (Braddick & Atkinson, 2011). Examples of large networks will be those processing texture, motion and colour (Zeki et al., 1991), which integrate outputs from V1, which encode local properties (i.e. orientation and spatial frequency), and extra-striate cortical visual areas. This consideration will help to explore the development of integrative processing, which is considered deficient in individuals with ASD.

An ERP study carried out by Fujita et al. (2011) evaluated brain responses to luminance- and chromatic-defined vertical sinusoidal gratings at low spatial frequencies (luminance: 1 cpd, chromatic: 2 cpd). They reported no between group differences in the ERP responses to luminance gratings. For the chromatic

gratings, however, there was a longer latency of the C1 component in the ASD group, no difference was found in the amplitude of C1. This result was interpreted as parvocellular pathway impairment in ASD. McCleery et al. (2009) evaluated visual perception in a group of infants at high-risk for autism. They presented luminance- and chromatic-defined gratings to a group of 13 infants (aged 6 months), at low spatial frequency (.27 cpd) and temporal frequency of 4.2 Hz (SSVEP paradigm). For chromatic gratings, no between group difference was reported. However, there was greater sensitivity to luminance gratings in the group of children at high-risk of ASD. These results were interpreted as evidence for an altered processing of low spatial frequency in luminance-defined gratings and were related an abnormal magnocellular pathway.

5.3 Aim of this study

While these studies are in agreement with the notion that visual perception in ASD is altered, it is not clear the specific disturbance in information processing. Is altered local connection of functional networks at earlier stages of processing, or large networks with integrative deficits which are altered in autism? Is the complexity of the stimulus that the autistic brain has to processes which makes its performance superior or weaker? These studies provided no firm conclusions to answer these questions yet. However, they showed that a systematic exploration of the dynamics of the functional networks involved in the processing of distinct properties of visual stimuli might provide a tool towards this endeavour.

Specifically, a systematic electrophysiological assessment of lower processing levels of the cortex would include SSVEP paradigms to help identifying potential changes in the functional networks underpinning visual perception by estimating the ERP and SSVEP responses elicited by this paradigm (Pei et al., 2014; Fujita et al., 2011) and visual stimuli of different complexity (Keita et al., 2014). Therefore, in this study the dynamics of functional connectivity during visual perception were assessed in children with and without autism using a

SSVEP paradigm. Luminance- and chromatic-defined gratings at two spatial frequencies (2.8 and 6 cpd) were used to evaluate functional segregation and integration. Two main hypotheses were generated with respect to networks dynamics changes between groups.

1. Because low and high spatial frequencies are thought to be processed by distinct functional visual channels, will elicit different networks, with higher values at high spatial frequency in ASD participants compared to the typical group if there is a detail-oriented bias in ASD.
2. Because chromatic-defined gratings are considered complex visual stimuli, the levels of connectivity will show reduced values in the autistic group if the mechanisms of neural integration are disturbed in ASD. Reduced functional connectivity will be observed at medium spatial frequency, with no between group difference at high spatial frequency in ASD compared to controls.

5.4 Methods and materials

Data from ten children diagnosed with ASD (mean age: 12.1 years, std: 3.66, range 8.2–17.8 years) were used in this study. All ASD participants were clinically diagnosed with an autism spectrum disorder according to DSM-IV criteria (American Psychiatric Association, 2000). Medical and Developmental history was obtained by the questionnaires described in Chapter 3. The clinical diagnosis was corroborated using the Autism Diagnostic Observation Schedule (Lord et al., 2000) by trained researchers of the Sheffield Autism Research Lab (ShARL, http://autismresearchlab.group.shef.ac.uk). The ASD participants were recruited from autism-specialist schools and NHS-units supporting patients on the autistic spectrum. All participants were administered the General Conceptual Ability score (GCA): ASD (M=99.62, SD=20.02), controls (M=114.80, SD=17.18).

Ten healthy children (mean age: 11.2 years, std: 3.22, range 7.8–17.5 years) were selected from a pool of 30 typically developed children recruited from local mainstream schools and screened for neuropathology with a brief medical

history questionnaire, such it is described in Chapter 3. As similar studies (Pei et al., 2014), children for the typically developed (TD) control group were selected by age-matching, where their inclusion was based on being a suitable match to one of the participants with ASD in terms of chronological age. Participants were not matched on cognitive level because SSVEP testing does not involve instructions or complex tasks. Apparatus, stimuli and analysis techniques used in this study are described in Chapter 3.

5.5 Results

5.5.1 SSVEP responses in the time domain

Luminance stimuli

The time-averaged grand waveforms elicited by mid- and high- spatial frequencies, at posterior electrode site Oz, may be seen in Figure 5.1. The EEG traces showed neuronal activity corresponding to the temporal frequency of stimulation (F=7.5 HZ) for both control and ASD groups. However, as can be seen in this figure, the C1 component of the recorded SSVEP was visibly more pronounced for the control group than for the ASD group at both spatial frequencies. Therefore, amplitude and latency values of C1 were analysed separately and submitted to statistical analyses as described below.

Repeated-measures ANOVAs with between-subject factor of group (two levels: TD, ASD) and within-subject factor of spatial frequency (two levels: mid, high) showed that there was a significant main effect of group ($F(1,18) = 13.38, p = 0.002$). Neither the main effect of spatial frequency, nor the interaction between spatial frequency and group reached significance, both $F < 0.775$, both $p > 0.39$. These analyses showed that C1 amplitude was smaller in the participants with ASD than in the TD group, see Figure 5.2.

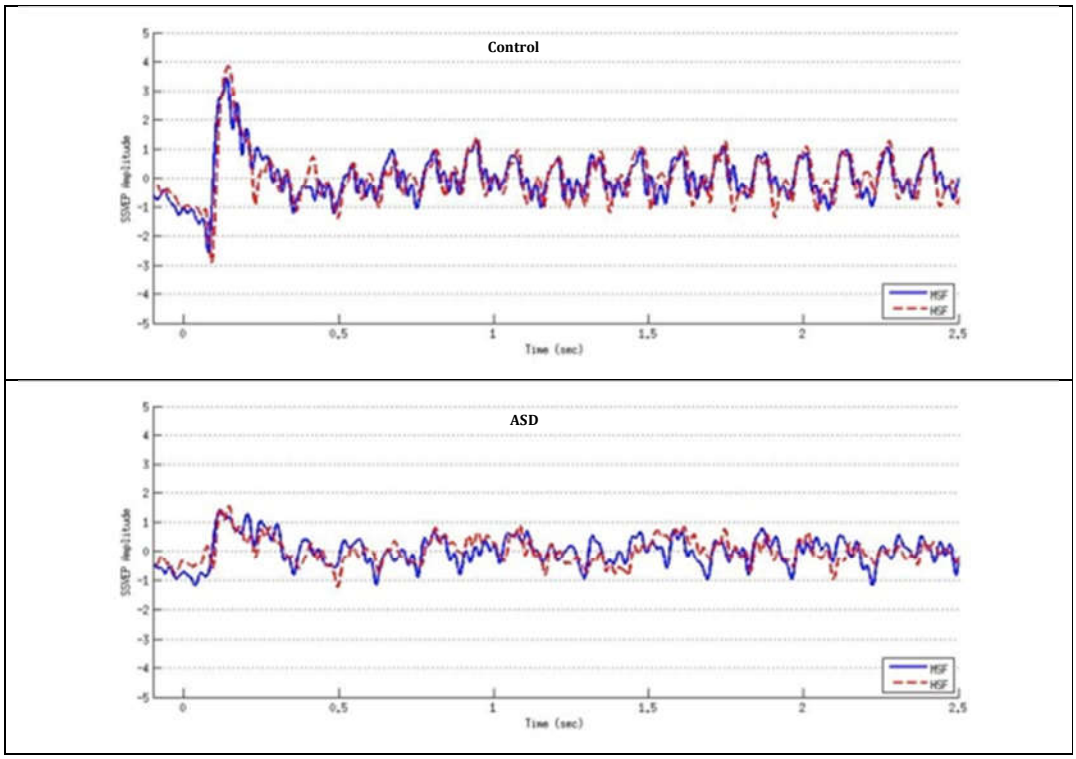


Figure 5.1. Time-domain grand averaged waveforms with luminance of normalized potentials (SSVEPs) for luminance gratings for control and ASD groups.

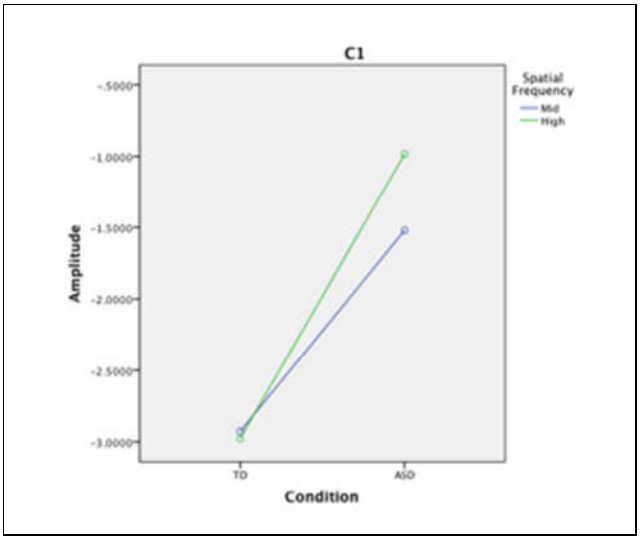


Figure 5.2. C1 amplitude in ASD subjects. C1 amplitude is smaller in the ASD group for luminance stimuli at both mid and high spatial frequency of gratings.

A 2x2 repeated measures ANOVA as described above showed no significant variations in C1 latency across groups. Neither spatial frequency nor group produced any significant main or interaction effects, all $F < 4.328$, all $p > 0.05$.

Chromatic stimuli

Chromatic stimuli elicited well-defined responses to stimulus onset across groups as reflected in the time-averaged grand waveforms shown in Figure 5.3. A prominent first component C1 was observed in both groups. Therefore, statistical analyses were carried out as described above upon the C1 amplitude and latency values.

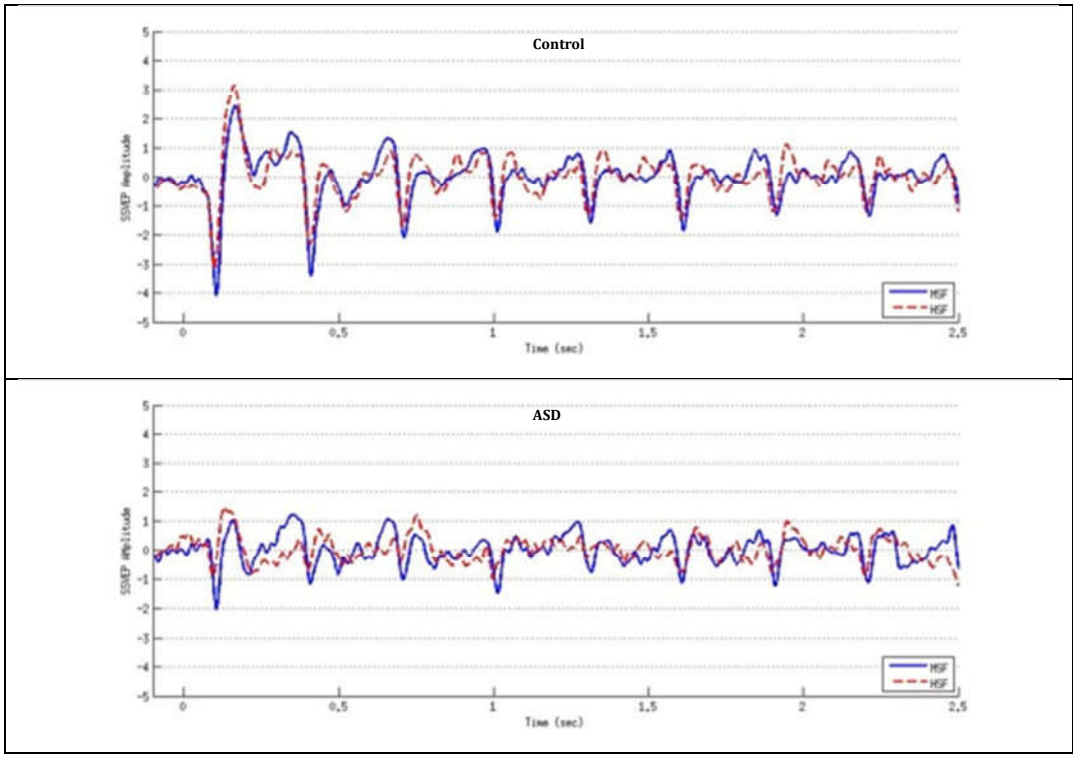


Figure 5.3. Time-domain grand averaged waveforms with chromaticity. Normalized potentials (SSVEPs) for chromatic gratings for control and ASD groups.

There was no significant main effect of group ($F(1,18) = 3.358, p = 0.083$). There was no interaction effect of spatial frequency by group ($F(1,18) = 0.349, p = 0.562$), but there was a main effect of spatial frequency ($F(1,18) =$

17.642, $p = 0.001$). These analyses did not find group-related changes on C1 amplitude, but showed larger (more negative) C1 amplitude at mid spatial frequency than high spatial frequency across groups, Figure 5.4.

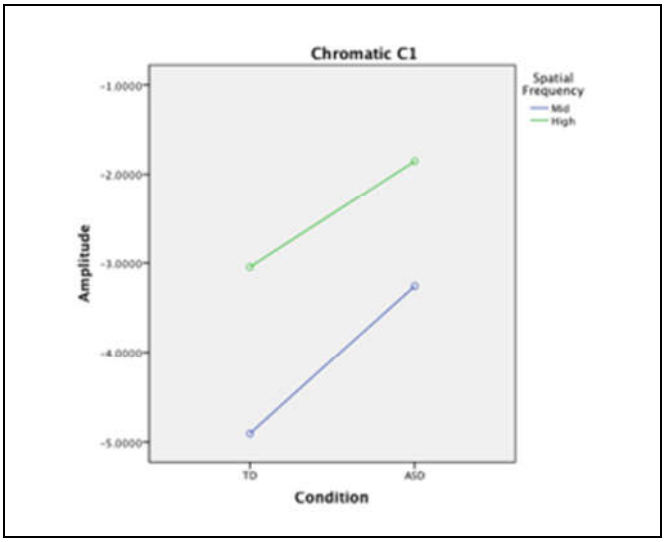


Figure 5.4. C1 Amplitude for chromatic stimuli at both mid and high spatial frequencies of gratings.

No important variations in C1 latency were found across groups. Neither spatial frequency nor condition produced any significant main or interaction effects, all $F < 3.83$, all $p > 0.066$. These analyses showed that C1 amplitude is larger in the TD group than ASD group. This effect reaches statistical significance only in the luminance condition.

5.5.2 SSVEP responses in the frequency domain

Luminance stimuli

As is characteristic of steady-state evoked response (Regan, 1989) the recorded SSVEPs were dominated by periodic components at the frequency of stimulation and its harmonics. Here, the primary neural response occurred at the first harmonic (frequency of stimulation) at both mid-spatial frequency (Figure 5.5), and high- spatial frequency (Figure 5.6) in both TD and ASD groups. As may be

seen, SSVEP amplitudes were maximal at the Oz electrode in both TD and ASD groups.

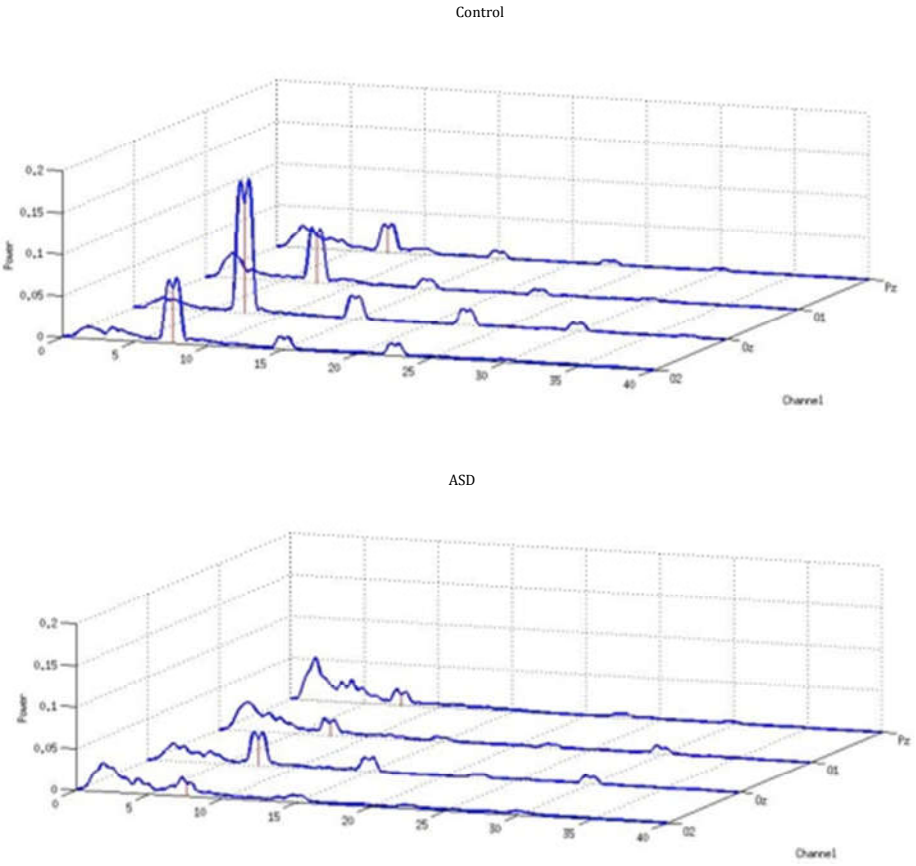


Figure 5.5. Spectrograms of SSVEP responses with MSF luminance at four occipital electrodes for mid spatial frequency are shown for both control and ASD groups. The red lines indicate the frequency of stimulation (F=7.5 Hz).

Based on the focus of activity in both groups at Oz, differences between groups as a function of spatial frequency and response harmonic were quantified at this electrode were the maximum activity was found in the group averages. SSVEP harmonic (F and 2F) power responses were analysed using repeated-measures ANOVAs with between-subject factor of group (two levels: TD, ASD) and two within-subject variables (Harmonic components: F vs. 2F; Spatial frequency of gratings: mid vs. high).

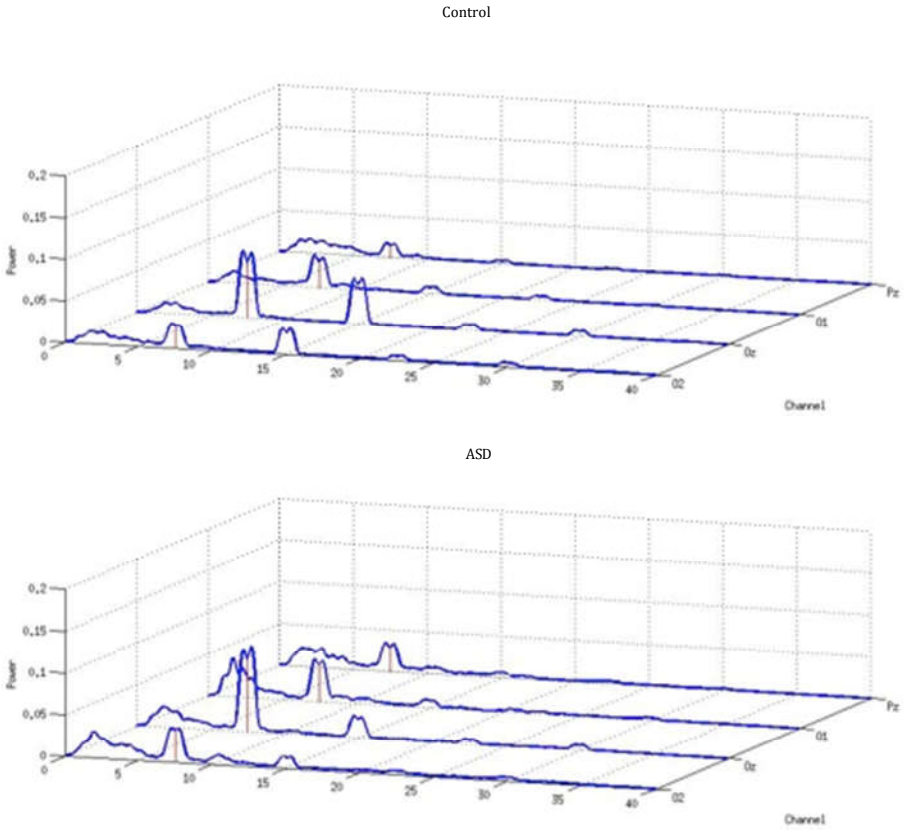


Figure 5.6. Spectrograms of SSVEP responses with HSF luminance at four occipital electrodes for high spatial frequencies are shown for both the control and ASD groups. The red lines indicate the frequency of stimulation (F=7.5 Hz).

There was no significant main effect of group ($F(1,18) = 2.084, p = 0.166$). Neither the main effect of spatial frequency, nor the interaction between spatial frequency and harmonic components reached significance, both $F(1,37) < 0.663$, both $p > 0.712$. There was no interaction effect between harmonic and group ($F(1,18) = 0.390, p = 0.54$), but there was a significant main effect of harmonic components ($F(1,18) = 8.269, p = 0.010$). There was also a significant interaction between spatial frequency and group ($F(1,18) = 4.805, p = 0.042$). There was an interaction harmonic component spatial frequency group effect ($F(1,18) = 6.501, p = 0.02$). Two separate two-way ANOVAs were carried out to examine the interaction between group, harmonic component and spatial frequency. A repeated-measures factor spatial frequency and a between-subjects factor group were applied upon the first and second harmonic components

separately. These analyses showed that, for the first harmonic, there was an interaction between group and spatial frequency ($F(1,18) = 7.016, p = 0.016$). Two separate independent-samples t-tests were then used to compare ASD group with TD group at each spatial frequency. These analyses showed that, at mid spatial frequency, there was a marginal significance in SSVEP power between groups, ($t(18) = 2.083, p = 0.052$). Power was larger in the TD group than ASD group, Figure 5.7. At high spatial frequency, however, no significant difference on SSVEP power was found between groups, $t(18) = 0.520, p = 0.61$. For the second harmonic, there was no interaction between group and spatial frequency, ($F(1,18) = 0.447, p = 0.512$).

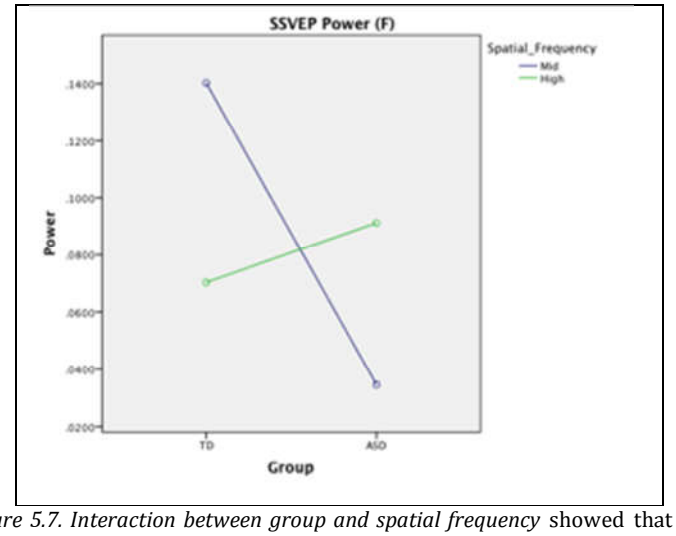


Figure 5.7. Interaction between group and spatial frequency showed that SSVEP power in the TD group was larger than ASD group for the first harmonic, but similar for the second harmonic.

Chromatic stimuli

The group averages in Figures 5.8 and 5.9 show that SSVEP amplitudes were maximal at the Oz electrode in both control and ASD groups for mid- and high spatial frequency. As described above, a three-way analysis of variance was carried out to examine the effects of repeated measures upon the SSVEP power. Spatial frequency (mid vs. high) and harmonic components (F vs. 2F) were the within-subjects variables and group was the between-subjects variable.

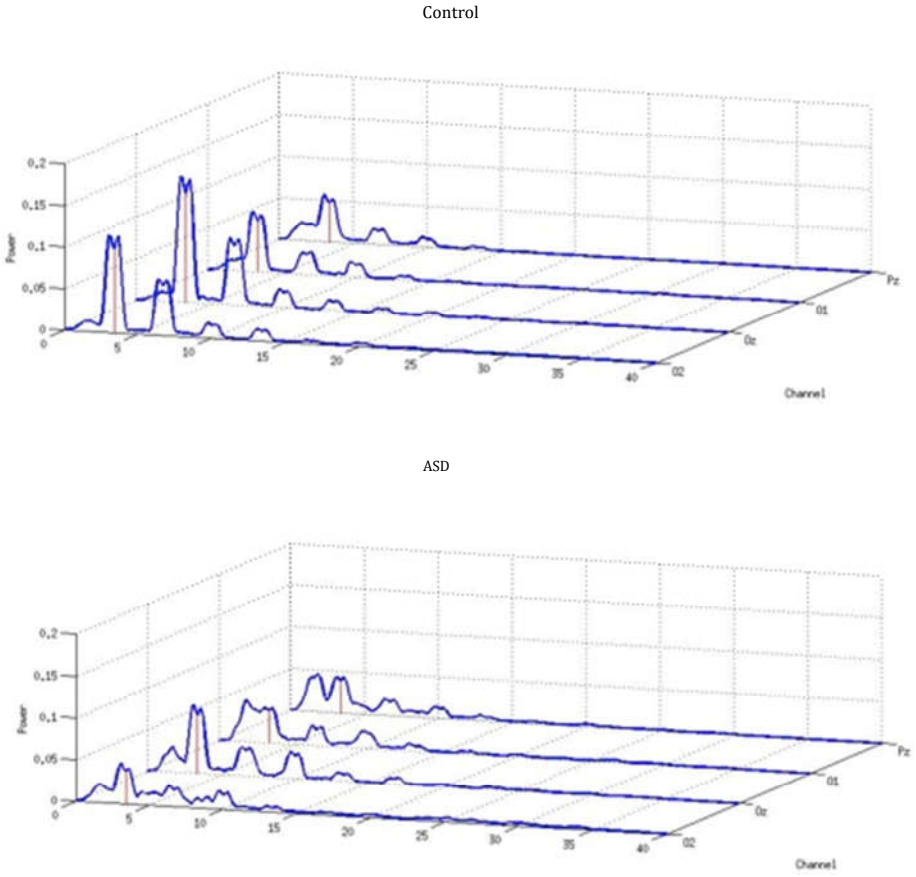


Figure 5.8. Spectrograms of SSVEP responses with MSF chromaticity at four occipital electrodes for TD and SD children.

There were significant main effects of spatial frequency ($F(1,18) = 12.085, p = 0.003$), and harmonic components ($F(1,18) = 6.718, p = 0.018$). There was no significant effect of group ($F(1,18) = 3.350, p < 0.084$). There was a marginal interaction between spatial frequency and group ($F(1,18) = 4.403, p = 0.05$). There was no significant harmonic x group interaction ($F(1,18) = 0.066, p = 0.8$). There was no significant harmonic x spatial frequency interaction ($F(1,18) = 0.165, p = 0.69$). There was no interaction between spatial frequency, harmonic and group ($F(1,18) = 0.184, p = 0.67$).

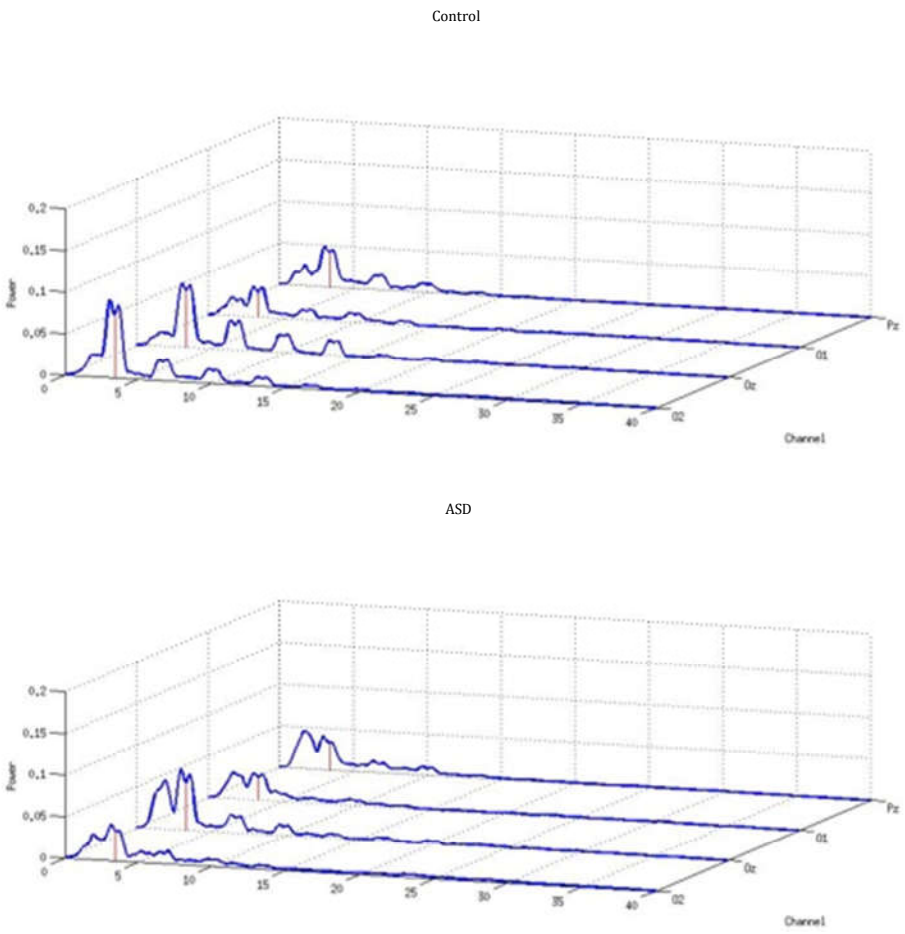


Figure 5.9. Spectrograms of SSVEP responses with HSF chromaticity at four occipital for the control and ASD group.

Two separate independent samples t-tests were used to follow up the interaction effect between spatial frequency and group on the SSVEP power averaged over F and 2F. These analyses showed that SSVEP power at mid spatial frequency was significantly larger in the TD group than ASD group, $(t(18) = 2.232, p = 0.039)$. For high spatial frequency, however, there was no significant difference on SSVEP power between groups, $(t(18) = 0.745, p = 0.529)$.

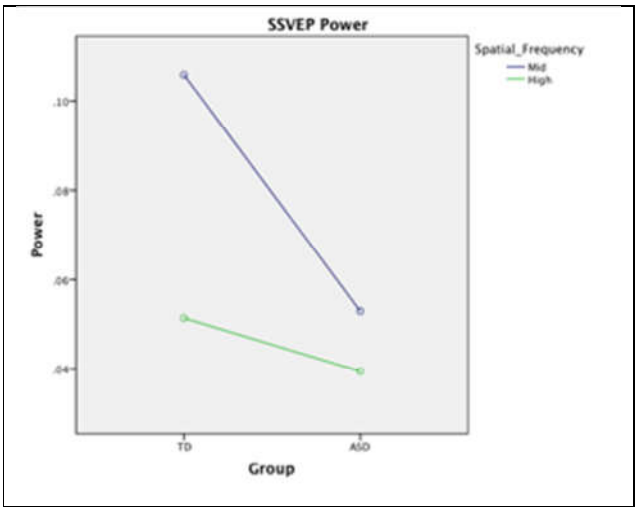


Figure 5.10. Interaction effect between spatial frequency and group showed that, at mid spatial frequency, SSVEP power was significant larger in the TD group than ASD group.

These analyses showed that SSVEP power was larger in the TD group than ASD group. This effect reached statistical significance only in the mid spatial frequency.

5.5.3 Time-dependent network analysis

Luminance stimuli

The dynamical changes in mid and high spatial frequency processing are shown in Figure 5.11 where grand average network-metric time series are shown for both TD and ASD groups at first (F=7.5 Hz) and second harmonic (2F=7.5 Hz).

In order to determine the effects of group on the time-varying networks, between-groups analyses of signal variability were analysed using a repeated-measures ANOVA with between-subject factor of group (two levels: TD, ASD) and two within-subject variables (Spatial frequency of gratings: mid vs. high, Harmonic components: F vs. 2F). There was no significant main or interaction effects of group, all $F < 3.031$, all $p > 0.099$. There were main effects of spatial frequency ($F(1,18) = 5.329, p = 0.033$), and harmonic ($F(1,18) = 100.084, p < 0.001$).

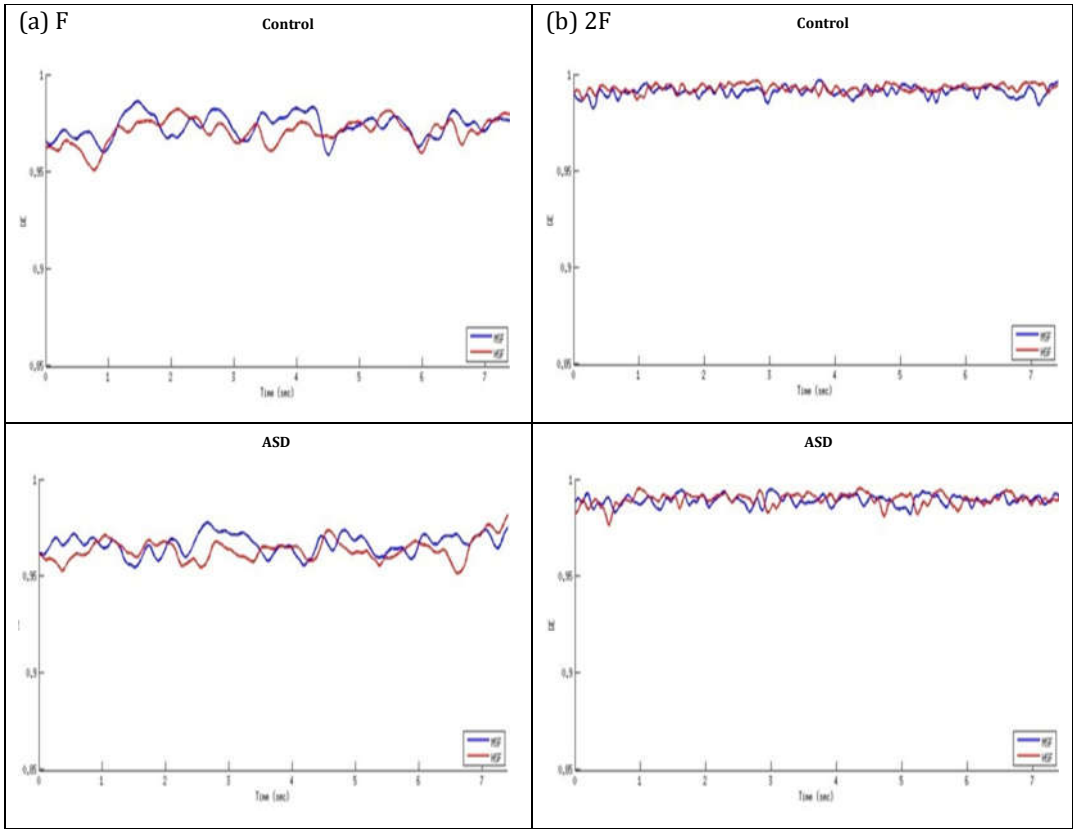


Figure 5.11. Time evolution of global efficiency with luminance for mid (MSF=2.8 cpd) and high spatial frequencies (HSF=6 cpd) for the first harmonic (F=7.5 Hz) and second harmonic (2F=15 Hz) in both control and ASD groups.

There was also a significant interaction between spatial frequency and harmonic, ($F(1,18) = 7.349, p = 0.014$). These analyses showed that approximate entropy was larger at first harmonic than second harmonic across groups. Two separate two-way ANOVAs were used to follow up the main effect of spatial frequency upon approximate entropy on harmonics separately. For the first harmonic, there was larger entropy following mid spatial frequency than high spatial frequency across groups ($F(1,18) = 9.526, p = 0.006$), Figure 5.12. There was no significant interaction between spatial frequency and group, ($F(1,18) = 3.241, p = 0.089$). For the second harmonic, neither the main effect of spatial frequency, nor the interaction between spatial frequency and group reached significance, both $F < 7.96$, both $p > 0.384$.

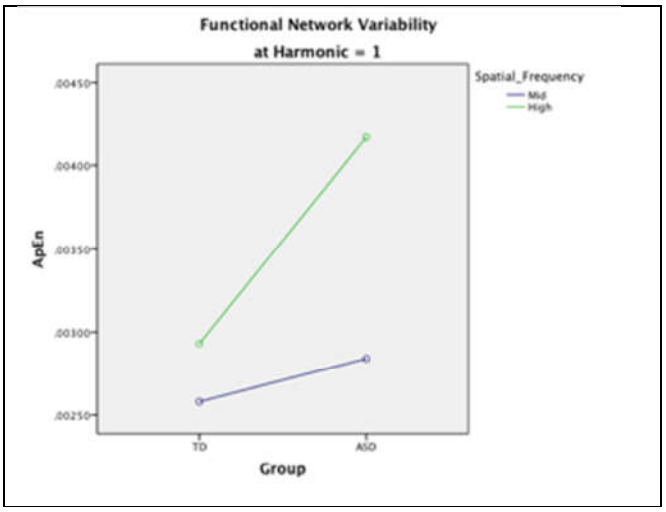


Figure 5.12. Interaction effect of spatial frequency and harmonic on the network variability.

Chromatic stimuli

The time varying structures for mid and high spatial frequency gratings are shown at both the first harmonic (F=7.5 Hz) and second harmonic (2F=7.5 Hz) across groups, Figure 5.13.

GE changes

A comparison of the GE values across groups (TD/ASD) was carried out using the T-tests on the variables of first harmonic (F) and second harmonic (2F) for each condition separately. Results are presented as probability levels across time as shown in Figure 5.14. For the first harmonic, there was no consistent significant difference between groups across time under any condition. For the second harmonic, however, comparison between groups showed a consistent significant difference across time under chromatic stimulation at mid spatial frequency ($p<0.005$).

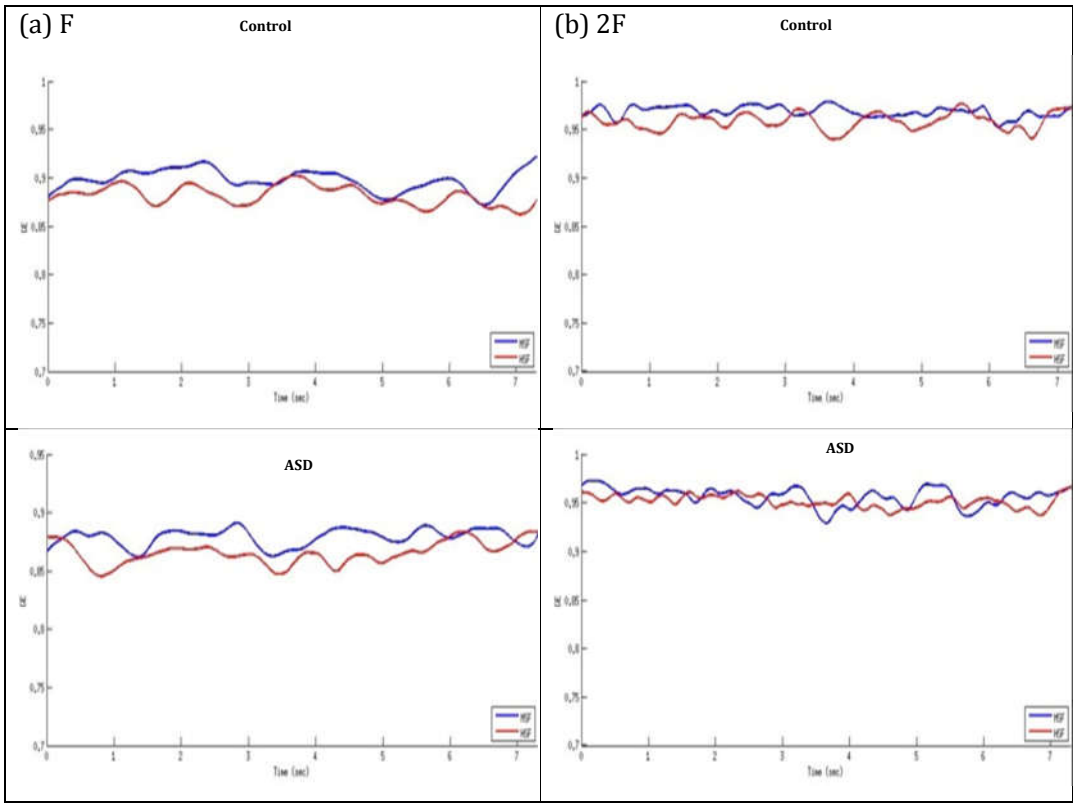


Figure 5.13. Time evolution of global efficiency with chromaticity for mid and high spatial frequencies for the first harmonic (a) and second harmonic (b) in the control and ASD groups.

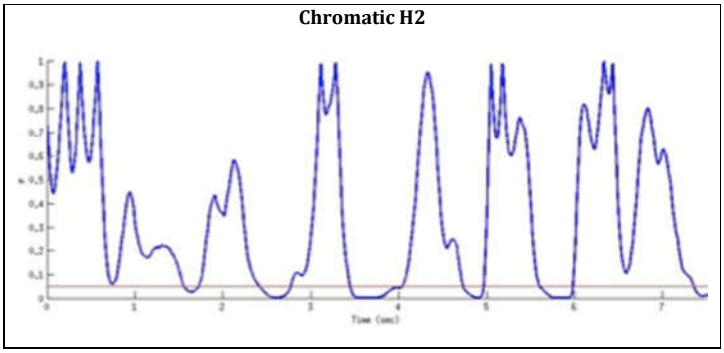


Figure 5.14. The probability values of the time-varying GE for chromatic stimuli were significant different ($p<0.05$) across conditions (TD/ASD) only in the mid spatial frequency at the second harmonic. The red line shows the p value $p=0.05$.

Even though the probability values of the time-varying metric GE did not show consistent significant difference across time at any other condition, there were sporadic time segments where significance was apparent. Therefore, given the

aim of this study is to identify potential differences in brain dynamics between children with and without autism, this intermittent significant difference was investigated. For each participant and condition, the mean value of the time-varying metric GE was calculated and submitted to statistical analyses.

Repeated-measures ANOVAs with between-subject factor of group (two levels: TD, ASD) and within-subject factor of spatial frequency (two levels: mid, high) showed that, there was a significant main effect of group ($F(1,18) = 6.907, p = 0.017$). There was a main effect of spatial frequency ($F(1,18) = 6.472, p = 0.02$), but the interaction between spatial frequency and group did not reach significance, ($F(1,18) = 0.181, p > 0.6$). These analyses showed that global efficiency (GE) was smaller in the participants with ASD than in the TD group. These analyses also showed larger GE at mid spatial frequency than high spatial frequency across both groups (see Figure 5.15).

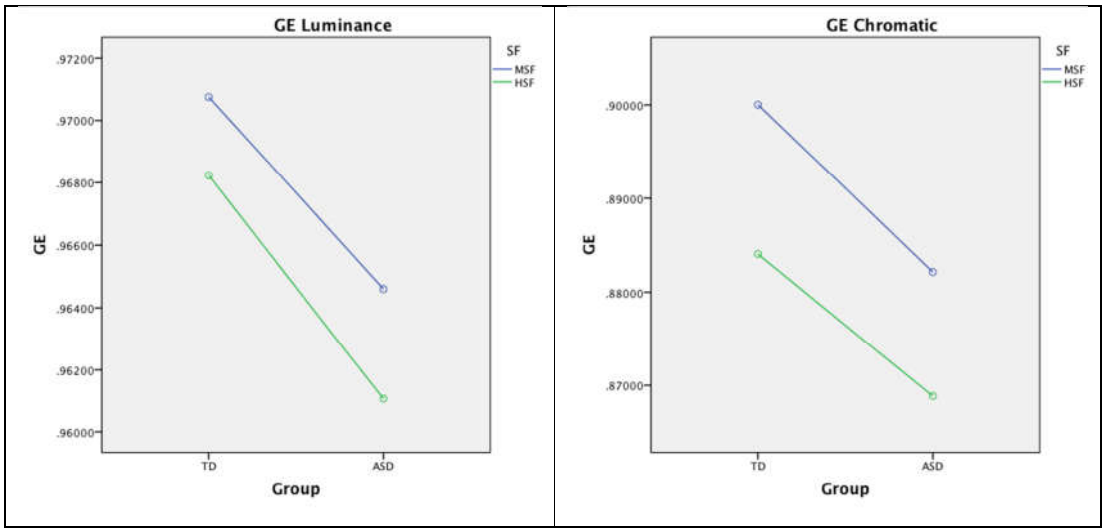


Figure 5.15. The mean values of the time-varying GE for luminance and chromatic stimuli were significant smaller in the ASD compared to controls. Both groups showed a decrease of GE when increasing the spatial frequency of gratings.

There was a significant main effect of group ($F(1,18) = 4.689, p = 0.044$). There was a main effect of spatial frequency ($F(1,18) = 21.21, p > 0.01$), but the interaction between spatial frequency and group did not reach significance, ($F(1,18) = 0.177, p > 0.6$). These analyses showed that global efficiency (GE) was smaller in the participants with ASD than in the TD group. These also showed larger GE at mid spatial frequency than high spatial frequency across both groups.

To summarize, these analyses showed that amplitude of C1 was reduced in the ASD group in comparison to control group. This effect reached statistical significance only in the luminance stimuli. These analyses also showed that latency of C1 did not change across groups and conditions. Analyses in the frequency domain showed reduced SSVEP power in the ASD group compared to the control group. This effect reached statistical significance at low spatial frequency in the luminance condition for the first harmonic. For chromatic stimuli, this effect reached statistical significance at low spatial frequency regardless of frequency of harmonic. Analyses showed more approximate entropy (ApEn) at high spatial frequency across goups. Analyses of global efficiency showed decreased GE in the ASD group compared to TD group across conditions.

5.6 Discussion

The aim of this experiment was to identify changes in the networks dynamics of children with and without autism. Luminance- and chromatic-defined gratings were used to evaluate local and integrative neural mechanisms. Two different spatial frequencies: low (2.8 cpd) and high (6 cpd), were presented in order to evaluate information processing in early visual channels in V1, and their effects in higher visual areas to achieve integrative processing. Stimuli were presented in a SSVEP paradigm, which has been used to assess functional brain networks in a number of perceptual and cognitive tasks, with the advantage of allowing us to evaluate the latency and amplitude of the first major component (C1) and the

power spectral of the steady-state response (Fujita et al., 2011; Pei et al., 2014). Measures of global efficiency and complexity of the SSVEP were also evaluated in order to complete a set of measures of networks dynamics that might provide complementary information regarding potential changes in brain activity between children with and without autism.

The main hypothesis in this study that participants with autism will have higher values of connectivity at high spatial frequency compared to control group was not proved. We found, contrary to suggestion in the literature (Belmonte et al., 2004; Mottron et al., 2006), no evidence for an over-connected network underpinning high spatial frequency processing in individuals with ASD. However, we found evidence for reduced functional segregation of medium spatial filtering mechanisms in ASD. Between-group differences in either ERP or SSVEP responses were not found for high spatial frequency, whether defined by either luminance or colour, this could suggest that networks underpinning high spatial frequency have attained typical function in the autistic group studied here. This result is consistent with previous studies on contrast sensitivity in autism (Kéita et al., 2014, de Jonge et al., 2007), where equal or better performance on high spatial frequency processing was observed in the autistic individuals in simple (luminance-defined gratings) and complex stimuli (texture-defined gratings, motion and form). Although the authors interpreted this finding as a detail-oriented bias in autism (Kéita et al., 2014) and preserved functioning of the magnocellular-dorsal and parvocellular-ventral pathways (de Jonge et al., 2007), it could also reflect, a more general disturbance of the integrative mechanism in ASD because the response at high spatial frequency is typical, but the response at medium spatial frequency is not.

The second main hypothesis in this study that participants with autism will have reduced connectivity during medium spatial processing of complex stimuli (chromatic-defined gratings) compared to control group was proven. A between group difference was found for luminance-defined gratings, with the autistic group demonstrating reduced C1 amplitude, suggesting a functional abnormality

in networks underpinning luminance change. Moreover, there were group differences for the response to spatial frequencies, with the autistic group showing a decrease in SSVEP power to low spatial frequency gratings, whether defined by either luminance or colour, suggesting that individuals with autism may have a limited capacity to process low spatial frequency rather than "reduced efficiency of neuro-integrative mechanisms operating at a perceptual level in autism" (Bertone et al., 2005).

Bertone and colleagues proposed the "complexity hypothesis", which suggests deficient processing of complex but not simple stimuli in ASD, based on psychophysical data demonstrating an increased sensitivity to simple stimuli (luminance-defined gratings) and lower sensitivity to complex stimuli (texture-defined gratings) in individuals with autism. The results of the current study show no evidence of deficits in neural integration after presentation of chromatic-defined gratings, stimuli that would be classified as "complex" by the complexity hypothesis because are processed in multiple brain areas, beyond primary visual area V1 (Zeki et al., 1991). However, our results are consistent with the results of McCleery at al., (2007) who found no difference in sensitivities to chromatic-defined gratings, and abnormal sensitivities to luminance-defined gratings during visual perception in a group of infants with high-risk of autism.

More recently, an electrophysiological study (Jemel et al., 2010) investigated the contrast sensitivity response properties of the ERPs associated with luminance-defined gratings of low- (0.8 cpd), medium- (2.8 cpd) and high- (8 cpd) spatial frequencies in autistic adults. The control group demonstrated distinct segregated networks for mid- and high-frequency gratings, whereas individuals with autism showed similar responses to mid- and high-frequency processing. The authors concluded that this finding provide evidence for altered functional segregation of early visual channels, with a bias towards detail-oriented visual processing. We found, contrary to suggestion in the previous study (Jemel et al., 2010), no evidence for identical sensitivity to mid- and high-frequency

processing in individuals with ASD. However, we do find evidence for reduced functional segregation of low spatial filtering mechanisms in ASD. Furthermore, our data revealed that along with the reduced functional segregation at low spatial information in the luminance gratings, there was a reduced functional integration of chromatic information at low spatial frequency. This could suggest that there is a direct relationship between the degree of long-range functional connectivity and the degree of local functional connectivity, as recently reported by Khan et al. (2013). Khan and colleagues used magneto-encephalography to measure task-related functional connectivity in ASD, and found that "local and long-range functional connectivity is reduced in concert in autism spectrum disorders". However, although we do find that individuals with autism exhibited reduced functional segregation of mid- spatial filtering mechanisms and reduced functional integration between areas involved in mid- spatial frequency chromatic change, which might suggest interplay between these two properties of functional brain organization, further investigation of this topic is needed in order to better understand the deficits we observed here, especially considering the small sample tested in this study.

Therefore, changes within the network dynamics of visual perception were apparent between participants with and without autism. Networks processing luminance change and chromatic change showed atypical development in the autistic participants. We found evidence for altered local functional connectivity within neural assemblies mediating lower processing levels of the cortex, and altered long-range functional connectivity between brain areas beyond the primary visual cortex. Specifically, the reduced perception to low frequency information in ASD can be interpreted as reflecting atypical local connectivity affecting the tuning and response properties or functional segregation of spatial filters mechanisms (Bertone et al., 2005, Tononi et al., 1995). Furthermore, the degree of long-range functional connectivity showed a direct relationship with the degree of local functional connectivity, thus, long-range and local connectivity were reduced in the ASD group.

Different theories at the neurological level have been proposed to explain alterations in functional segregation and integration in ASD (Belmonte et al., 2004; Muller et al., 2008). At a cellular level, the hypothesis of disturbed GABA in the development and refinement of local neuronal circuits attempts to account for the alterations in brain connectivity (Pizzarelli & Cherubini 2011). In particular, alterations in local networks underpinning orientation and spatial frequency perception have been associated with an imbalance in excitatory and inhibitory processes in the brain (Edden et al., 2009). More recently, an SSVEP study on the neuronal responses to texture-defined stimuli reported decreased synchronization of brain activity, which was related to an imbalance in GABAergic mechanisms (Snijders et al., 2013). An alteration on the neuronal processes regulating the excitatory-inhibitory balance may be responsible for reduced functional segregation of low spatial frequency information in ASD (Bertone et al., 2010), and possibly, may be involved in the differential characteristics of autistic performance on higher processing levels, where GABAergic transmission contribute to maintain the functional integrity of the neuronal circuits.

In conclusion, the results of the present study provide electrophysiological evidence for the interplay between functional segregation and functional integration in the autistic brain. Specifically, our results add to the few other functional connectivity studies suggesting that local and long-range functional connectivity is reduced in concert in ASD. Although further studies will be needed to determine whether similar alterations are present in higher cortical areas during visual perception of more complex stimuli as well, our results suggest that alterations in higher-level functions may be, at least in part, due to downstream effects of alterations occurring at earlier stages. These results are consistent with a biological model of inhibitory alteration early in development that might impair local circuits, with a collateral consequence of disturbed synchronization within large neuronal networks in ASD.

Chapter 6

Conclusions

This thesis presents a network dynamics approach to evaluate brain function in typical development and in ASD. The measures applied to identify and characterize the dynamics of functional networks provided information about the functional segregation and functional integration in the brain. Evaluating brain function in children requires techniques that would reflect the dynamical changes in brain activity related to development. The brain function of typically developing children and adults was evaluated to estimate age-related changes in functional connectivity and characterize normal development. This characterization was needed to evaluate atypical development in ASD.

Our literature review showed that brain function would be investigated by assessing functional segregation within local networks and its functional integration into long-range networks to achieve perception and other mental states. The hierarchical organization of the human brain provides the theoretical framework to study the development of brain function. The subcortical structures of the visual cortex, namely magno- and parvocellular- pathways are suggested to develop from childhood to adolescence and have extensively been studied in typical and atypical development. At the lower processing levels of the cortex, the integrity of early visual channels has been a focus of research in atypical development. It has been suggested that alterations in the early mechanisms of spatial information processing may account for the behavioural and cognitive deficits in ASD. Specifically, alterations in low spatial frequency processing would alter the perception of global information in a visual image, whereas alterations in the mechanisms associated to high spatial information

would bias visual perception towards detail-oriented processing. Higher processing levels of the visual system such as colour and motion have been studied to evaluate parvocellular (P) and magnocellular (M) pathways respectively. These subcortical pathways feed higher visual streams called the ventral "what" and dorsal "where" streams. The formation of functionally segregated modules and connections in the brain are associated to neurodevelopmental processes that change over the course of development. Therefore, the visual system can provide the structural modules to study the development of early brain function from a network approach.

Traditionally, vision has been assessed from two contrasting points of view, local and holistic. Local view has focused on investigating activation of specific visual areas (functional segregation) such as P and M pathways, visual channels, colour and motion perception. Holistic view has mainly evaluated synchronization (functional integration) between visual areas involved in visual tasks, such as face recognition and object discrimination. However, the results from these approaches are mixed, it is still unclear whether lower processing levels are affected in ASD or it is the higher integrative mechanisms that are affected in this condition. Reasons for this divergence may be methodological differences in the evaluation, which include the participants' age, the stimuli used and the analysis techniques. One more reason is the hypothesis that functional segregation and integration coexist in the human brain, and therefore, they would be studied in concert.

In this work, we adopted this hypothesis to evaluate changes in networks dynamics across development and in ASD. Functional segregation and integration can be evaluated in concert by applying complex networks measures. Information on local activation and integration can be obtained by measuring the strength and distance between pair of nodes within the networks. The nodes would be the recording sites of the electroencephalogram (EEG) signal. This approach has recently been applied to EEG signals from children and adults, but mainly during resting state to study the default network or global brain function.

Findings from these studies are interesting because has been interpreted as reorganization in brain structure and function from childhood to adulthood. However, this approach has no been used to study lower processing levels and the development of integrative processing in visual system using EEG. We are interested on estimating the relationship between functional segregation and integration in typical development to characterize functional networks that later could be used to evaluate ASD. Recently, an fMRI study suggested that the interplay between functional segregation and functional integration may be abnormal in ASD (Khan et al., 2013). Therefore, this was our main interest in this work.

Steady-State Visual Evoked Potentials (SSVEPs) have been widely used to entrain neuronal assemblies eliciting cortical functional networks. The size and distribution of the functional network is related to the frequency of stimulation (Srinivasan et al., 2006). Stronger SSVEP power has been observed at 3Hz, 7-8Hz, and 12-13Hz, with a distribution over occipital, parietal and temporal lobes. Higher frequencies generate responses over frontal lobes as well. The complexity of the visual stimulus and the cognitive task also has an effect on the size, distribution and the dynamics of the functional networks. Although both structured (e.g. gratings) and non-structured (full-field luminance flicker) can elicit widespread synchronous responses, gratings can be used to evaluate local functional networks. SSVEP luminance-defined gratings across a range of spatial frequencies elicit segregated functional responses of spatial filtering mechanisms in primary visual cortex. Functional segregation of spatial information may be altered in ASD, with an over-functioning of high spatial frequencies (>6 cpd), and similar responses to medium (2.8 cpd) and high spatial information (8 cpd). An increase in the complexity of the visual stimuli may influence the sensitivity to spatial information in ASD, diminishing the functional integration of large-range networks. Texture- and chromatic-defined SSVEP gratings have been used to assess functional integration beyond primary visual cortex. SSVEPs are suitable for children participants because are less affected by blinks, and muscle and eye movements. SSVEPs are traditionally analysed in the

frequency domain over a small number of long trials (~10 12-sec trials). Therefore, SSVEP gratings would allows studying the dynamics of functional networks mediating normal and atypical brain development. To achieve this, SSVEP power, Global Efficiency (GE) and Approximate Entropy (APEn) were evaluated in participants EEG. In addition, given the good quality of the recorded data, the major component (C1) of the ERP response (~100 ms) was also evaluated in latency and amplitude.

Two studies presented in Chapters 4 and 5 explored the networks dynamics of functional networks mediating typical brain development and brain function in ASD. Luminance-defined vertical gratings with spatial frequencies of 2.8 and 6 cpd were used to evaluate local functional segregation of early spatial visual channels within the primary visual cortex (V1). The frequency of presentation was 7.5 Hz. Chromatic-defined vertical gratings at the same spatial frequencies (2.8 and 6 cpd) were used to evaluate long-range functional integration beyond V1. The frequency of presentation was 3.5 Hz. These specific spatial and temporal frequencies were chosen because they have proven to elicit strong brain responses and stimulate magno- and parvocellular pathways, also because it appears to influence the atypical responses in ASD. The main findings of these studies are discussed in this chapter within the framework of the functional segregation and integration approach to emphasize common underlying mechanisms.

The main research aim of the first study, reported in Chapter 4, was to identify age-related changes in networks dynamics underpinning luminance and chromatic gratings. Participants were 30 children and 10 adults of the typically developing population. SSVEPs were recorded from the scalp during visual perception and analysed the C1 amplitude and latency, SSVEP power, global efficiency (GE) and approximate entropy (ApEn). Analyses revealed that developmental change within the network dynamics of visual perception was apparent from childhood to adulthood. Networks processing luminance change and chromatic change showed development over the time-course studied here.

Interestingly, functional networks underpinning luminance and chromatic grating were differently affected by development. Functional networks underpinning chromatic gratings showed major changes through development. The global efficiency (GE) time series showed interplay between functional segregation and functional integration within functional networks. Functional segregation within close areas decreased, whereas functional integration between distant areas increased across development. This was evidenced by the fewer and smooth fluctuations in the GE time series of adults, which also showed two distinct functional networks underpinning medium and high spatial frequency without overlapping GE values. In contrast, transient and higher fluctuations were observed in the GE time series of children, where GE values of the two functional networks overlapped due to higher functional segregation of neighbouring nodes and smaller functional integration between distant nodes. Our findings provided evidence for reorganization from local to distributed networks in typical development. Increased functional integration across development was also evidenced by SSVEP power measures. Given that increases in spectral power elicited by visual stimulus are associated with increases in the synchrony and size of the neural networks recruited during visual perception, reduced modulation of these networks might suggest immature mechanisms underlying neural integration in childhood. In contrast, our results showed that networks underpinning mid- spatial frequency luminance change might have reached adult levels by the childhood ages measured here.

The research aim of the second study, described in Chapter 5, was to identify potential changes in networks dynamics between children with and without ASD. Participants were 10 typically developing children and 10 children diagnosed with ASD age matched. Interestingly, our results showed atypical processing of medium spatial frequency in the autistic individuals compared to children without ASD. Reduced SSVEP power suggests decreased synchrony and size of the neural networks recruited during medium spatial processing. This may also be seen in the global efficiency (GE) time series were GE values are

lower in the ASD group. If we consider that the typically developing children in this study were also analysed in the study described in chapter 4, then they would have reached adult levels and perhaps full maturity of the mechanisms of lower processing levels underlying typical brain function. Thus, the atypical processing of medium spatial frequency in the autistic individuals might be related to atypical maturation of the mechanisms underlying lower processing level of the cortex, perhaps involving the magnocellular (M) pathway and early visual channels. Furthermore, atypical processing of medium spatial frequency in the autistic individuals was also observed during chromatic condition. Given the lack of relationship between SSVEP power and groups in the high spatial frequency at either chromatic and luminance conditions, it appears as though networks underpinning high spatial frequency processing may have typical development by the time measure here. Our results showed a direct relationship between functional segregation and integration in the networks dynamics. Atypical functional integration may be due, at least in part, to atypical functional segregation at lower processing levels in visual channels. Therefore, we provide electrophysiological (EEG) evidence for local and long-range functional connectivity reduced in concert in ASD.

6.1 Findings with regard to the research questions

Research question 1

What EEG indices can reflect age-related changes in the network dynamics during visual perception? (Chapter 4)

The result of this study is a potential biomarker of typical brain function in neurodevelopment. Our results suggest that although there may be functional reorganization from local to distributed networks across development, networks underpinning medium spatial frequency change would be a useful biomarker of typical brain function.

Research question 2

Can EEG measures of networks dynamics reflect potential changes in brain function during visual perception in ASD? (Chapter 5)

The result of this study is a potential EEG biomarker to characterize atypical brain function in autism. Our results suggest a direct relationship between functional segregation and functional integration during visual perception; atypical functional connectivity in local lower processing mechanisms might contribute to the disruption in long-range functional integration reported in ASD, because both abnormalities occur in concert in the autistic brain.

6.2 General discussion

The combination of brain dynamics measures and steady-state visual evoked potentials (SSVEPs) allowed the examination of the dynamic interplay between functional segregation and integration in brain networks, over development and in autism spectrum conditions. In the current study, we examined networks underpinning luminance and chromatic change at both mid- and high-spatial frequency. Analyses of SSVEP power, amplitude and latency of the first ERP component (C1) and functional connectivity networks revealed age-related changes in functional networks during visual perception and suggested altered networks dynamics in autism spectrum conditions. Interestingly, these changes were stimulus- and frequency-specific. In the following section, our results are discussed considering these two aspects.

SSVEP analyses may reflect age-related changes of functional networks underpinning luminance and chromatic processing

We investigated developmental changes in steady-state visual evoked potentials (SSVEPs) elicited by chromatic and luminance– defined stimuli at mid- and high-spatial frequency in 30 children (7 to 17 years) and 11 adults, as a function of age. We found that, for luminance stimuli, a lack of relationship between SSVEP power and age in the mid- spatial frequency stimulus suggests that networks underpinning mid- spatial frequency luminance change may have reached adult

levels by the childhood ages measured here. In contrast, SSVEP power elicited by luminance gratings of high spatial frequency increased with age, the highest values were observed mostly by adulthood. These results are in agreement with previous ERP and behavioural findings of an earlier maturation of the mechanisms specialized for processing low frequency input in the visual system, while sensitivity to high spatial frequency continues increasing with age (Crognale et al., 1998; Howard and Reggia, 2007). Therefore, reduced capacity in children to synchronize activity elicited by luminance gratings with high spatial frequency may reflect a late structural and functional maturation of the involved brain regions (Birca et al., 2010).

We also found that the latency of C1 increased with age. We discuss that earlier latencies in children tested here might reflect an overdeveloped sensory network, as children have an overabundance of synapses along the visual pathways before the pruning of redundant synaptic connections and cortical thinning, but delayed development of integrative processing (Swick et al., 1994; Zanto et al., 2010). However, although longer latencies with age have also been reported elsewhere (Celesia et al., 1987; Tobimatsu et al., 1993; Crognale, 2002), we must be cautious when interpreting our findings because of the small number of ERPs analyzed in this study.

Regarding chromatic stimuli, we found that SSVEP power increased with age at both mid- and high-spatial frequency. Increases in SSVEP power elicited by visual stimulus have been related to increases in synchrony and size of the neural networks recruited during information processing (Srinivasan et al., 2006; Herrmann, 2001). Given the reduced modulation of the networks underpinning chromatic change, our results might suggest reduced efficiency of the mechanisms of neural integration operating at a perceptual level (Bertone et al., 2005) in childhood.

We also found lower variability of fluctuations in brain coordinated activity, revealed by entropy measures derived from the phase synchronization displayed in the network-metrics time series, in the adult group as compared to the children group. It is known that fewer fluctuations in synchronization patterns

may reflect increased functional coordination (Nenadovic et al., 2011; Lachaux et al., 1999; Anokhin et al., 1996). Therefore, our results might suggest that functional integration among regions involved in chromatic processing increases as children maturate. This is consistent with previous ERP studies showing that colour vision system matures over development, reaching a peak until late adolescence (Boon et al., 2007; Crognale, 2002).

SSVEP analyses may reveal reduced functional segregation and integration in individuals with autism spectrum conditions

Analyses of SSVEPs elicited by chromatic and luminance stimuli, at mid- and high- spatial frequency, explored the networks dynamics of individuals with and without autism. Measures of SSVEP power, amplitude and latency of the first ERP component (C1) and functional connectivity networks were compared between two groups of participants (8.2–17.8 years), one with autism spectrum conditions and the other with typical development. We found, contrary as suggested by the literature (Vlamings et al., 2010, Belmonte et al., 2004; Mottron et al., 2006), no evidence for enhanced visual processing of high spatial frequency in individuals with ASD. Surprisingly, we found reduced brain activity in response to mid- spatial frequency in autistic participants, compared to the typical control group, during visual processing of both luminance and chromatic stimuli. Although this could be interpreted as a specific abnormality in networks that are tuned to mid- spatial frequencies, it could also reflect a more general disturbance of neural synchrony that is evident only when compared against the effect induced in chromatic processing. Furthermore, although it is commonly assumed that abnormalities in either the magnocellular (McCleery et al., 2007) or parvocellular (Fujita et al., 2011) visual streams are likely to be responsible for abnormal low spatial frequency processing and abnormal chromatic processing, respectively, this only holds within primary visual cortex (V1). Given that these stimuli were presented to activate low- and high- stages of visual processing (e.g. the ventral-stream area V4), the stimuli presented here are well suited to the investigation of neural integration, at the local and long distance

levels. We discuss that our findings provide evidence for reduced functional segregation of visual channels responsible for processing mid- spatial frequency in autism (Jemel et al., 2010), and for reduced functional integration between brain areas involved in the processing of mid-spatial frequency chromatic change in autism. Furthermore, our findings provide electrophysiological evidence for the interplay between functional segregation and integration in the organization of the autistic brain, as has been suggested by other theoretical and MEG studies (Belmonte et al., 2004; Just et al., 2007; Khan et al., 2013). Accordingly, reduced capacity for functional integration between widespread cortical regions may be due, at least in part, to reduced functional segregation of locally specialized cortical regions. Therefore, our results suggest that individuals with autism may have limited capacity to process mid- spatial frequency, and that this early abnormality holds the potential to create a cascade of abnormalities in later processing stages, including (but perhaps not limited to) the mechanisms of neural integration operating at a perceptual level in autism.

In summary, this study adds to the accumulating body of evidence suggesting developmental change within the network dynamics of visual perception apparent from childhood to adulthood. Networks underpinning mid- spatial frequency luminance change may have reached adult levels by the childhood ages measured here, whereas a not yet fully developed mechanism specialized for processing high spatial frequency and chromatic change was also apparent in this group. In contrast, individuals with autism showed intact high spatial frequency processing but abnormal mid- spatial frequency responses to both luminance and chromatic stimuli. Therefore, this study also supports prior reports of reduced functional segregation and integration in autism using simple and complex stimuli. Individuals with autism showed reduced functional segregation of mid- spatial filtering mechanisms and reduced functional integration between areas involved in mid- spatial frequency chromatic change, which might suggest interplay between these two properties of functional brain organization. We suggest that these stimuli therefore may be useful in

characterizing visual processing in typical development and autism, and that a systems-level approach—whereby the functional segregation and integration of widespread brain networks in autism is examined in relation to typical development—may provide a more detailed characterization of autism at the neurological level.

6.3 General conclusions

Our rationale for this study was that different visual stimuli (Luminance- and chromatic- defined gratings) would produce different functional networks within and across the different functional modules of the visual system. It was anticipated that high-contrast vertical gratings would preferentially stimulate the magnocellular (M) pathway and early visual channels, while isoluminant chromatic gratings would stimulate distant cooperative subsystems. Our results showed that SSVEPs measures behaved differently in response to chromatic and achromatic stimulation. Furthermore, spatial frequencies generated different responses in the SSVEPs. This was observed across typical development and in ASD. This might be interpreted as different networks underpinning luminance change and chromatic change. Therefore, SSVEPs have proved to elicit different functional networks involving local and distributed brain systems, which has made possible to study functional segregation and integration across development and in ASD.

Several measures were evaluated to identify networks dynamics underlying typical and atypical development. SSVEP power and global efficiency (GE) provided the most valuable information. Although we also estimated ERP measures, we were cautious when interpreting our results because of the small number of trials, even though they might be in accordance with previous published work. Approximate entropy (ApEn) was also evaluated but did not provide supplementary information to that provided by the global efficiency time series. However, when taken together, all these measures provided support

to each other. Therefore, our results might indeed reflect brain networks dynamics rather than analytical bias.

6.4 Future work

The ultimate goal for ASD researchers is to detect the condition at early stages of life in order to start opportune strategies to contribute to a better outcome for the child. As suggested by the present thesis, the starting point could be to identify EEG patterns of information processing related to typical brain function in the developing brain, to further identify what has gone wrong in atypical brain development. This thesis was designed with this objective in mind. Our results demonstrated that EEG measures could reliably provide information regarding functional connectivity and its dynamics, and how brain dynamics is modulated by stimulus specificity and brain maturation at early stages of visual perception in typical and atypical neurodevelopment.

Although special care was taken when creating the appropriate visual stimuli for eliciting different neural networks, our sample size was relatively small. It is important to highlight that this was an exploratory study; therefore more detailed analyses with a larger sample size would be necessary in order to determine the true potential of the EEG indices presented here. This study confirms that EEG biological markers for ASD are an interesting area for future research. It reinforces the importance of planning and performing systematic studies and also suggests increasing the range of spatial frequencies and the complexity of the visual stimuli used in the current study in order to identify heterogeneity in the autism spectrum.

Although we did not match the participants on cognitive level because SSVEP testing involves no tasks, is relatively unaffected by attention, and at the perceptual level is not affected by IQ (Pei et al., 2014), which makes it well suited to the study of functional connectivity during visual perception in children and individuals with autism and other neurological conditions, this work may be

extended to include thorough control selections in the future. If the sample is to be treated as healthy, a range of demographic and psychometric tests should be applied to a larger sample, in order to identify well-defined matching groups based on quantitative measures of levels of brain maturation, rather than chronological age alone as performed in this study. The same protocol should be followed with the ASD sample in order to identify heterogeneity in the spectrum (Lenroot and Yeung, 2013) that might bias the result.

A challenge for future research is relating the reduced functional segregation of mid- spatial filtering mechanisms we have observed at a perceptual level in ASD to their integration with higher-level functions such as cognition and behaviour. As it is shown in our data and has also been reported elsewhere (Khan et al., 2013; Belmonte et al., 2004; Just et al., 2007), reduced short-range connectivity with concurrent reduced long-range connectivity may be a possible neurological substrate for the cognitive and behavioural deficits in ASD. However, further investigation of this topic is needed in order to better understand at which point of the mechanisms of neural integration the deficits we observe are generated, especially given the small sample tested in this study. The use of more complex stimuli will be necessary to detect cognitive and task-related deficits in autism (Vlamings et al., 2010; Catarino et al., 2013). The method used here to identify atypical functional properties of spatial processing may be useful in determining whether certain spatial frequencies contribute to abnormalities on other tasks, such as ones involving local and global scales. Lower spatial frequencies are thought to be an important aspect of global processing, such as face recognition (Goffaux and Rossio, 2006), identification of general shape, size, and contours of objects (Vlamings et al., 2010), whereas higher spatial frequencies are important for detailed perception. Although we do find abnormal mid- spatial frequency processing and intact mechanisms for high spatial frequency processing, the examination of a larger range of spatial frequencies is needed to determine whether individuals with autism exhibit enhanced high frequency processing (Keita et al., 2014; Vlamings et al., 2010), which might contribute to the detail-

oriented bias in this group (Dakin and Frith, 2005; Happé and Frith, 2006; Mottron et al., 2006; Behrmann et al., 2006).

Finally, we suggest that the type of measures and stimuli used in this study may be applied to earlier periods in development to obtain a more detailed characterization of typical brain function that could be used to identify the risk of autism in younger populations (e.g. infant and toddler), severity of brain dysfunction and for evaluating treatment efficacy.

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Appendix A

Parent Information Sheet

The University Of Sheffield.

Department Of Psychology.

Investigating Subtypes in Autistic Spectrum Disorder

You and your child are being invited to take part in a research project. This project is funded by the Clinical Psychology Unit at the University of Sheffield in collaboration with the Sheffield Autism Research Lab (ShARL). Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. If there is anything that is not clear or if you would like more information, please feel free to contact us in whatever way is most convenient for you. Our contact details are at the end of the information sheet, and include a postal address, email addresses and a telephone number. We will respond to your query as soon as possible. Take time to decide whether or not you wish to take part. Thank you for reading this.

What is the purpose of the study?

We are investigating the brain activity, behaviour and cognition of people with Autistic Spectrum Disorders (ASD) to explore whether there are different subtypes within the spectrum. It is hoped that this will help to gain a better understanding of the disorder. By working with people with and without ASD our researchers can develop better insight into the strengths and impairments associated with ASD.

Why have I been chosen?

If you have received a letter from school, this is because your child's school have given their permission for us to contact you. We are working with a number of schools in the area to recruit children ***with and without*** an Autistic Spectrum Disorder.

Alternatively, we may have got your details from the Child and Adolescent Mental Health Service (CAMHS) that works with your child. This will be because your child has a diagnosed Autistic Spectrum Disorder.

Your cover letter explains where we got your details from. If you feel unsure, please feel free to contact us. We are working with a number of NHS services, schools and non-statutory organisations to contact people in the area.

Who can take part?

We are recruiting children that ***do not*** have an Autistic Spectrum Disorder as well as those who do. Therefore, if you do not feel that your child has an Autistic Spectrum Disorder they can still take part.

If your child has an Autistic Spectrum Disorder then they must have a diagnosis of autism, Asperger's syndrome, atypical autism or pervasive developmental disorder not otherwise specified (PDD-NOS).

Children must be at least five years old to take part. In order to be able to engage with some of the activities, it is important that your child has some expressive language.

Is there any reason why my child should not take part?

Due to the use of flickering stimuli in the electroencephalogram (EEG), people with epilepsy should not take part in this study.

The EEG component of this study requires your child to wear a cap on their head. If you think you're your child would be distressed by wearing this, or by the researchers touching their head and face to set up the equipment, then you may not wish to take part in the study. If you do decide to take part and your child appears uncomfortable on the day, we will discontinue.

You may also wish to consider the length of the study. We expect that most children will need to take breaks during testing. However, if you feel that your child would be very restless or would be distressed by the length of this study then you may not wish to take part.

What will happen if my child does take part?

If you decide that you would like your child to participate then we will ask you to bring your child to the Psychology Department at the University of Sheffield. On the day, we will make sure that your child is happy to participate.

The study is made up of three parts:

1. We will carry out a structured age-appropriate play session. During this time an experimenter will engage your child in conversation and a number of short tasks (e.g. looking at a picture book, describing a picture). This part of the testing will be video recorded.
2. We will ask your child to complete some general ability tests. This consists of solving a number of verbal and non-verbal puzzles with the experimenter. This will take up to 30 minutes.
3. The third part of the study will involve measuring your child's brain waves while they look at patterns that appear and disappear on a computer screen. This will be measured using electroencephalogram (EEG). Here is a picture of what the equipment looks like:

An EEG is used to give a functional display of how brain cells are working. It helps us to understand how the brain works in different conditions. If you would like more information about EEG, please contact us. EEG is harmless and painless procedure which involves placing a flexible head cap onto the head. A small amount of gel is used so your child's hair may get wet in places. Once the cap and sensors have been placed onto the head we will ask your child to sit still on a comfy chair and watch the computer screen. This part will take about 40 minutes: 20-30 minutes to put the cap on, then 10 minutes watching the screen. It will help us if your child does not wear any products in their hair on the day of the testing, and if they can arrive with dry hair.

If they are not comfortable, then they can withdraw at any time. If you or the researchers feel that your child is distressed then the testing can be discontinued.

How long will it take?

The study will take about two hours in total. An appointment will be made at a time that is convenient for you. It is possible to book two one-hour sessions if you feel that this would be more manageable for your child. This may be two sessions spread out across one day with a break in the middle, or it may be two sessions on different days.

As a parent what will I have to do?

As your child is under 18, you will need to give written consent for them to take part. If you are coming to the University, then this will involve signing a consent form on the day. If your child is going to participate at school we will need to have received your signed consent form before testing.

We will also ask you to complete some questionnaires about your child's developmental history and behaviour. If you are not going to be present when your child takes part then we will send these to you and arrange for you to return them to us.

Do I have to take part?

It is up to you and your child to decide whether or not to take part. If you choose not to take part this will not have any penalty or negative effects for you or your child. If you do decide to take part

you will be asked to sign a consent form. You and your child are free to withdraw at any time without giving a reason. This will not have any negative effects for either of you.

Where will the research take place?

In some cases we may test in your child's school if there are facilities available and a number of children taking part. You will be informed if this is the case nearer the time.

If we are not visiting your child's school, or if you would prefer for your child to participate outside of school time, then we will make an appointment for you to visit us at the Department of Psychology. This is part of the University of Sheffield located on Western Bank.

Will my costs be covered?

Some participants will be required to travel to the university or to a NHS centre in order to be tested. If this is the case and you are concerned about the cost of this, please get in touch. We have a very small budget available to help with travel costs, and may be able to make a contribution to help.

What are the possible disadvantages and risks?

There are no known risks to your child for any of the procedures involved. Disadvantages to you are the amount of time that you would be required to spend at the Psychology Department and the fact that your child's hair may become messy with gel deposits. This gel will wash out easily with shampoo.

What are the possible benefits?

There are no direct benefits of taking part for you or your child. However you may find it interesting to learn how researchers use non-invasive techniques to study brain activity. Most participants find this fun or interesting. You will also leave with the knowledge that the experience has contributed to furthering the process of autism research.

Will you be able to diagnose my child with ASD?

Tests will be administered for research purposes only. We are not able to provide your child with a diagnosis of autism. If you are concerned about your child's development, we recommend that you consult your General Practitioner.

What will happen to my information?

All information that is collected about you and your child during the study will be kept strictly confidential. Your data will only be used within the Sheffield Autism Research Lab and will be kept confidential within this research team. You will not be identified in any reports or publications that emerge from this research.

Paper records and video recordings will be stored securely with the Department of Psychology. These will be destroyed once they are no longer being used by ShARL for the research that you have consented to.

What happens if the research study stops earlier than expected?

In the unlikely event that the research ends prematurely, your data will still contribute to the larger study taking part in ShARL. We will contact you should this occur.

What if something goes wrong?

If you are unhappy about the treatment that you receive from the researchers, you can contact the project supervisor Dr Elizabeth Milne on (0114) 222 6658. If you feel that your complaint has not been handled to your satisfaction, you can contact the University's Registrar and Secretary. In the unlikely event of an adverse incident during your child's visit, we will report this according to University guidance.

If you have been recruited through an NHS service, you can contact the team in your NHS trust that manages complaints.

DETAILS WILL BE ENTERED HERE FOR THE RELEVANT TRUSTS

What will happen to the results of the research project?

The results of the study will be analysed by Holly Norbron, Jen Gallagher, Dr Elizabeth Milne and other members of ShARL, and will be prepared for publication in relevant scientific journals and written up as two doctoral-level theses contributing to a professional qualification. No information about any individuals will be available from this report. The results are likely to be published up to two years after the study takes place. We will not be providing individual feedback on your child, however you will be able to obtain a summary of the findings of the project from any of the researchers involved if you so wish.

Your data will also contribute towards a larger project taking place within ShARL. These results will also be submitted for publication in scientific journals.

Who has ethically reviewed the project?

The project has been reviewed by the Department of Psychology ethics subcommittee at the University of Sheffield. It has also been reviewed by relevant NHS ethics committees and the National Autistic Society.

Contact information

Researchers: **Holly Norbron** and **Jen Gallagher**,
Trainee Clinical Psychologists at the University or Sheffield.
Clinical Psychology Unit
University of Sheffield
Western Bank
Sheffield
S10 2TN

Appendix B

Letter to parents

The University Of Sheffield.

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f
sychology.

Dear Parent,

We are writing to you because your child's school have given their consent for us to contact you regarding participation in an ongoing research programme. This research is being conducted as part of a Doctoral Thesis in Clinical Psychology at the University of Sheffield in collaboration with the Sheffield Autism Research Lab (ShARL).

We are interested in recruiting participants both with and without a diagnosis of autism. Autism affects many people in the UK, roughly 1 in every 100 people have the disorder. While there is no known cure for autism, early diagnosis, improved understanding and treatment can make a big difference in how people with autism and their families cope.

We have enclosed an information sheet giving details of an ongoing research study in the department. If you would like your child to take part, please complete the form below and return it to us in the pre paid envelope. The research will take place at your child's school and an appointment will be made for them to attend a session during school time. After reading the information enclosed, if you would like your child to participate, please complete the consent form and return to us in the envelope provided. Your child will not be able to take part without your prior consent. It is not necessary for you to be present at the session; however, if you would like to attend, please contact us and we will try to accommodate this.

On returning the form below, your details will also be added to a database within the autism lab and you may be invited to take part in future research projects within the department. Your data will be kept strictly confidential and you can ask to have your name removed from the database at any time, to do so please contact Elizabeth Milne (see contact details below).

Children who take part in our studies usually find them interesting and enjoyable and will be contributing to something that is very important. Our research would not be possible without the support of parents. We hope you consider assisting us in gaining a better understanding of autism.

Thank you for your interest.

Holly Norbron and Jen Gallagher
(Trainee Clinical Psychologists)
HNorbron1@Sheffield.ac.uk
JGallagher2@sheffield.ac.uk

Sheffield Autism Research Lab
Director - Dr Elizabeth Milne
E.Milne@sheffield.ac.uk
(0114) 2226558
Department of Psychology
University of Sheffield
S10 2TN
www.autismresearchlab.group.shef.ac.uk

1. Child's Name: _____

2. Child's gender: _____

3. Child's Date of Birth: _____

4. Does your child attend school? YES NO (go to Q5)
4a. If yes, which school? _____
4b. Is this a mainstream school, or a special school? _____

5. Does your child require one-to-one support in school? YES NO

6. Does your child have a developmental disorder? YES NO (go to Q7)
6a. If so, what is the diagnosis? Please give as much detail as possible, i.e. the exact diagnosis, any co-morbid diagnoses (i.e. more than one type of diagnosis).
_____
_____
_____

6b. Who made the diagnosis? _____
6c. When was the diagnosis made? _____

7. Does your child have any other medical diagnosis? YES NO (go to Q8)
7a. If yes, please list here.
_____
_____

8. Does your child take regular medication? YES NO
8a. If yes, please list the medication here
_____
_____
Please provide the contact details via which you would like us to contact you regarding this and future research

Parent / caregiver name: _____

Address:_____

Tel:_____

Email:_____

Appendix C

Letter to parents from CAMHS

The University Of Sheffield.

Department Of Psychology.

Dear Parent,

We are contacting you because you have a child with an autistic spectrum disorder and the CAMHS team you are involved with have given their consent for us to contact you regarding participation in an ongoing research programme. This research is being conducted as part of a Doctoral Thesis in Clinical Psychology at the University of Sheffield in collaboration with the Sheffield Autism Research Lab (ShARL).

We are interested in recruiting participants both with and without a diagnosis of autism. Autism affects many people in the UK, roughly 1 in every 100 people have the disorder. While there is no known cure for autism, early diagnosis, improved understanding and treatment can make a big difference in how people with autism and their families cope.

We have enclosed an information sheet giving details of an ongoing research study in the department. If you would like your child to take part, please complete the form below and return it to us in the pre paid envelope. We will then contact you to arrange an appointment for you and your child to attend. At this appointment, you will be asked to sign a consent form before your child can participate in the study.

On returning the form below, your details will also be added to a database within the autism lab and you may be invited to take part in future research projects within the department. Your data will be kept strictly confidential and you can ask to have your name removed from the database at any time, to do so please contact Elizabeth Milne (see contact details below).

Children who take part in our studies usually find them interesting and enjoyable and will be contributing to something that is very important. Our research would not be possible without the support of parents. We hope you consider assisting us in gaining a better understanding of autism.

Thank you for your interest.

Holly Norbron and Jen Gallagher
(Trainee Clinical Psychologists)
HNorbron1@Sheffield.ac.uk
JGallagher2@sheffield.ac.uk

Sheffield Autism Research Lab
Director - Dr Elizabeth Milne
E.Milne@sheffield.ac.uk
(0114) 2226558
Department of Psychology
University of Sheffield
S10 2TN
www.autismresearchlab.group.shef.ac.uk

1. Child's Name: _____

2. Child's gender: _____

3. Child's Date of Birth: _____

4. Does your child attend school? YES NO (go to Q5)
4a. If yes, which school? _____
4b. Is this a mainstream school, or a special school? _____

5. Does your child require one-to-one support in school? YES NO

6. Does your child have a developmental disorder? YES NO (go to Q7)
6a. If so, what is the diagnosis? Please give as much detail as possible, i.e. the exact diagnosis, any co-morbid diagnoses (i.e. more than one type of diagnosis).
_____
_____
_____

6b. Who made the diagnosis? _____
6c. When was the diagnosis made? _____

7. Does your child have any other medical diagnosis? YES NO (go to Q8)
7a. If yes, please list here.
_____
_____

8. Does your child take regular medication? YES NO
8a. If yes, please list the medication here
_____
_____

Please provide the contact details via which you would like us to contact you regarding this and future research

Parent / caregiver name: _____

Address:_____

Tel:_____

Email:_____

Appendix D

Consent Form

Participant Consent Form (Parent version)

Title of Research Project: Investigating Subtypes in Autism Spectrum Disorder

Name of Researcher: Holly Norbron and Jen Gallagher (Trainee Clinical Psychologists)

Participant Identification Number for this project: **Please initial box**

1. I confirm that I have read and understand the information sheet dated 20th January 2012 explaining the above research project and I have had the opportunity to ask questions about the project. ☐

2. I understand that mine and my child's participation is voluntary and that we are free to withdraw at any time without giving any reason and without there being any negative consequences. In addition, should me or my child not wish to participate on the day or decide not to answer any particular question or questions, we are free to decline. ☐

3. I understand that mine and my child's responses will be kept strictlyconfidential within the autism lab. I give permission for members of the research team to have access to mine and my child's anonymised responses. I understand that neither my name nor my child's will be linked with the research materials, and we will not be identified or identifiable in the report or reports that result from the research. ☐

4. I agree for my child to be video recorded for the purpose of accurate assessment. ☐

4. I agree for the data collected from me to be used in future research being conducted in the autism lab. ☐

5. I agree for my child to take part in the above research project. ☐

_____ _____ _____

Name of Participant Date Signature
(*or legal representative, i.e., parent*)

_____ _____

_____ Lead Researcher	Date	Signature

Please contact us if you have any further questions:

Holly Norbron (Trainee Clinical Psychologist)
HNorbron1@Sheffield.ac.uk

Jen Gallagher (Trainee Clinical Psychologist)
JGallagher2@Sheffield.ac.uk

Clinical Psychology Unit
The University of Sheffield
Western Bank
Sheffield
S10 2TN
Tel: 0114 2226558

Appendix E

Developmental History Questionnaire

Participant Code: _____
Participant Date of Birth: _____
Today's Data: _____

Please answer this series of questions about your child's developmental history. If you would rather not answer a particular question, feel free to miss it out. The first six questions ask about developmental history, i.e. when particular milestones were achieved. Please provide as much detail as possible. If you can't remember the time-course of these events, then just state this. The last two questions ask about current language abilities. Please provide examples if possible. If you are not sure how to interpret any of the questions, feel free to ask the experimenter.

History

1) How old was your child when they first started to communicate using single words? If you can't remember exactly, please simply indicate whether you feel that this was or was not at an appropriate age compared with other children.

_____

_____

2) How old was your child when they first started to communicate using short phrases? If you can't remember exactly, please simply indicate whether you feel that this was or was not at an appropriate age compared with other children.

_____

_____

3) Did you ever notice that your child regressed in their use of language, i.e. 'lost' certain words from their vocabulary, or stopped using language that had previously been used? _____

If YES, at what age did this occur? _____

Please elaborate if possible

_____

_____

_____

Have you felt that your child regressed in other areas of their ability? E.g. making eye contact, showing you how they feel (facial expressions), using gestures (e.g. pointing), showing you their toys etc. By regression, we mean that your child seemed to

develop skills in a normal way, but later lost these skills. For example, a child who gestured a lot when they were 2 years old, but who stopped using gestures when they were 3 years old. Note that this is different from a child who never learned to make eye contact.

If YES, at what age did this occur? _____

Please elaborate if possible

_____

_____

_____

4) How old was your child when you first became concerned about their development?

_____

_____

5) How old was your child when they were given a diagnosis of ASD?

_____

_____

Current

1) Does your child currently show difficulties, compared to other children of the same age, in **understanding** spoken language? If so, please provide a description and example if possible.

_____

_____

_____

2) Does your child currently show difficulties, compared to other children of the same age, in **producing** spoken language (talking)? If so, please provide a description and example if possible.

_____

_____

_____

Appendix F

Brief Medical History Questionnaire

BRIEF MEDICAL HISTORY
QUESTIONNAIRE

CONFIDENTIALITY – The medical questionnaire and the information contained within will be treated as a confidential document.

Participant code:	Today's date:
Date of Birth:	Male/female (delete as appropriate):
Was your child born prematurely? Yes/No (delete as appropriate): If yes, at how many weeks?	How much did your child weigh at birth?
Completed by Parent/caregiver (delete as appropriate):	

If ASD diagnosed only	
Given clinical diagnosis:	Co-morbid diagnoses:

Please answer ALL of the following questions:

Medical Conditions					
You are asked to indicate whether your child currently has or has ever had any of the following medical conditions:					
1	Epilepsy; fits, blackouts, fainting turns or unexplained loss of consciousness	**Yes**	☐	**No**	☐
2	Head injuries leading to loss of consciousness requiring hospital admission	**Yes**	☐	**No**	☐
3	Recurrent headache or migraine	**Yes**	☐	**No**	☐
4	Anxiety/depression, phobias, mental breakdown or stress related problems	**Yes**	☐	**No**	☐
5	Visual impairment needing glasses/contact lenses	**Yes**	☐	**No**	☐
6	Injury or surgery to eye(s) including laser eye surgery or any other type of refractive surgery	**Yes**	☐	**No**	☐
7	Any visual defect e.g. scotoma, blindness in one eye, night blindness, colour blindness, reduced visual field, blurred vision or detached retina	**Yes**	☐	**No**	☐

Family History
Does anyone of your child's close relatives have a mental health condition (e.g. schizophrenia, autism spectrum disorder or any other)? If YES please give details and relationship.