Neural correlates of developmental dyslexia

A multimodal study of typical and atypical reading

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Developmental dyslexia is the most common neurobehavioural disorder that can affect up to 17% of school-aged children. It is characterised by literacy difficulties in accurate and fluent word recognition. Previous research has made significant progress into studying the behavioural, cognitive and neurobiological causes of the reading disability. More recently, important advances into understanding the neural circuits and the aetiology of dyslexia has come from, respectively, brain imaging and genetic findings. Although neuroimaging studies have been able to identify the cortical areas involved in the reading deficit, the high heterogeneity in dyslexic traits still remains poorly investigated. The aim of this research was to expand on the previous findings by offering a thorough investigation of brain networks underlying complex symptoms of dyslexia. For this reason, a multimodal cognitive, genetic, structural and functional neuroimaging approach was employed. First, different dyslexic functional networks were identified by studying MEG resting state functional connectivity and network topology. In particular, an un-biased graph theory measure, the minimum spanning tree, was used to portray frequency specific connectomes in relation to fluency and genetic components. Second, a novel MEG paradigm was designed to delineate time-specific neural correlates and dynamic oscillatory activity in the first stages of reading processing, the prelexical orthographic visual word recognition, in typical and dyslexic readers. Third, structural connectivity, by means of diffusion tensor imaging (DTI), was investigated in typical and atypical readers. The outcome of each of these studies demonstrates how a correlational cognitive-genetic-neuroimaging method is fundamental to be able to distinguish neural mechanisms and brain networks underpinning different dyslexic phenotypes. Overall, the results of this work provide a strong case for the design and the application of a multimodal (cognitive, genetic, structural and functional neuroimaging) approach to examine neurobiological correlates of typical and atypical reading development, and better define the dyslexic continuum.

Keywords: developmental dyslexia, magnetoencephalography (MEG), connectivity, brain networks, diffusion tensor imaging (DTI).

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Table of contents

Nomenclature

Acronyms / Abbreviations

ABC	Aston Brain Centre
AF	Arcuate Fasciculus
ASEBA	Achenbach System of Empirically Based Assessment
BC	Betweenness Centrality
BOLD	Blood Oxygen Level Dependent
BPM	Brief Parental Monitoring
CR	Corona Radiata
DDAU	Dyslexia Development Assessment Unit
DLD	Developmental Language Disorder
DMN	Default Mode Network
DSM	Diagnostic and Statistical Manual of Mental Disorders
DTI	Diffusion Tensor Imaging
ERD	Event Related Desynchronisation
ERF	Event Related Field
ERP	Event Related Potential
ERS	Event Related Synchronisation
FA	Fractional Anisotropy
FC	Functional Connectivity
FG	Fusiform Gyrus

fMRI	functional Magnetic Resonance Imaging
GWAS	Genome Wide Association Study
HPI	Head Position Indicator
HS	Hemispheric Specialisation
ICA	Independent Component Analysis
IFG	Inferior Frontal Gyrus
IFOF	Inferior Frontal Occipital Fasciculus
ILF	Inferior Longitudinal Fasciculus
IQ	Intelligence Quotient
ITN	International Training Network
LCM	Lexical Categorisation Model
LCMV	Linearly Constrained Minimum Variance
MCP	Multiple Comparison Problem
MEG	Magnetoencephalography
MMN	Mismatch Negativity
MOG	Middle Occipital Gyrus
MRI	Magnetic Resonance Imaging
MST	Minimum Spanning Tree
MTG	Middle Temporal Gyrus
PDE	Phonemic Decoding Efficiency
PET	Positron Emission Tomography
PLI	Phase Lag Index
REC	Research Ethics Committee
RSFC	Resting State Functional Connectivity
RSN	Resting State Networks

RT	Reaction Time
SAM	Synthetic Aperture Magnetometry
SLI	Specific Language Impairment
SQUID	Superconducting Quantum Interference Device
SS	Standard Score
SSS	Signal Source Separation
STG	Superior Temporal Gyrus
STS	Superior Temporal Sulcus
SWN	Small World Network
Th	Tree Hierarchy
TOWRE	Test of Word Reading Efficiency
tSSS	temporal extension of the Signal Source Separation
VE	Virtual Electrodes
VWFA	Visual Word Form Area
VWFS	Visual Word Fusiform System
WM	White Matter

List of figures

List of tables

Chapter 1

Introduction

Chapter summary

The present chapter introduces the neurosciences of reading and provides a theoretical background for the hypotheses developed in the experimental chapters (Chapters 3, 4, 5, 6 and 7) of this thesis. It starts with the description of the neural mechanisms involved in reading and reading development. Following this, it illustrates the aetiology of developmental dyslexia and the various theories advanced to explain the reading disorder. To conclude, it introduces the rationale behind this work and lists the overall aims and objectives of this thesis.

1.1 Reading in the brain

Reading and writing are relatively recent cultural conventions that have developed around 5000 years ago (Dehaene, 2009). According to Dehaene (2009) the fact that the *Homo sapiens* was not evolutionarily and genetically predisposed to read defines a 'reading paradox'. From this perspective, it is clear that in developing the new cognitive skill of reading, the brain had to rely on pre-existing cortical circuits such as those specialised for object recognition. This theory has been defined as the 'recycling hypothesis' in the studies of evolution of reading (Dehaene, 2009; McCandliss et al., 2003). In other words, the human brain is unique in its ability to adapt new functions through learning (Dehaene et al., 2004).

For a long time the main question posed by scientists investigating visual word recognition has been how can the human brain understand individual words' meaning from around 50,000 possibilities in less than half a second (Grainger and Holcomb, 2009). Cognitive neurosciences have shown how the reading system can be sub-divided into four components responsible for pre-lexical, orthographic, phonological and lexico-semantic processes, that work in concert to analyse efficiently the printed linguistic information.

Furthermore, brain studies have successfully identified the key cortical regions for fluent reading. Almost two decades ago, the first reading neuroimaging structural, using functional magnetic resonance imaging (fMRI) and positron emission tomography (PET), and neurophysiological, using electroencaphalography (EEG) and magnetoencephalography (MEG), studies proposed that printed word and word-like (pseudowords) stimuli elicited activation in the left lateralised system, composed by a ventral (extrastriate areas and inferior occipitotemporal sites) and a dorsal stream (temporo-parietal circuit composed by angular supramarginal gyri and the inferior parietal lobule), (Fiez and Petersen, 1998; Nobre et al., 1994; Price et al., 1996; Puce et al., 1996; Pugh et al., 2000; Salmelin et al., 1996; Schlaggar and McCandliss, 2007; Shaywitz et al., 1998; Tarkiainen et al., 1999).

It has been repeatedly confirmed by now, that an efficient neural reading network is composed by three major cortical hubs or circuits in the left hemisphere (Finn et al., 2014; Martin et al., 2015), see Figure 1.1.

1. Left temporo-parietal (TP) network – involved in phonology processes.
2. Left ventral occipito-temporal (VoT) cortex, which includes lateral extrastriate, inferior fusiform and inferior temporal regions, including the Visual Word Form Area (VWFA) – responsible for visual orthographic word recognition.
3. Left inferior frontal gyrus (IFG) and precentral gyri – associated with speech production.

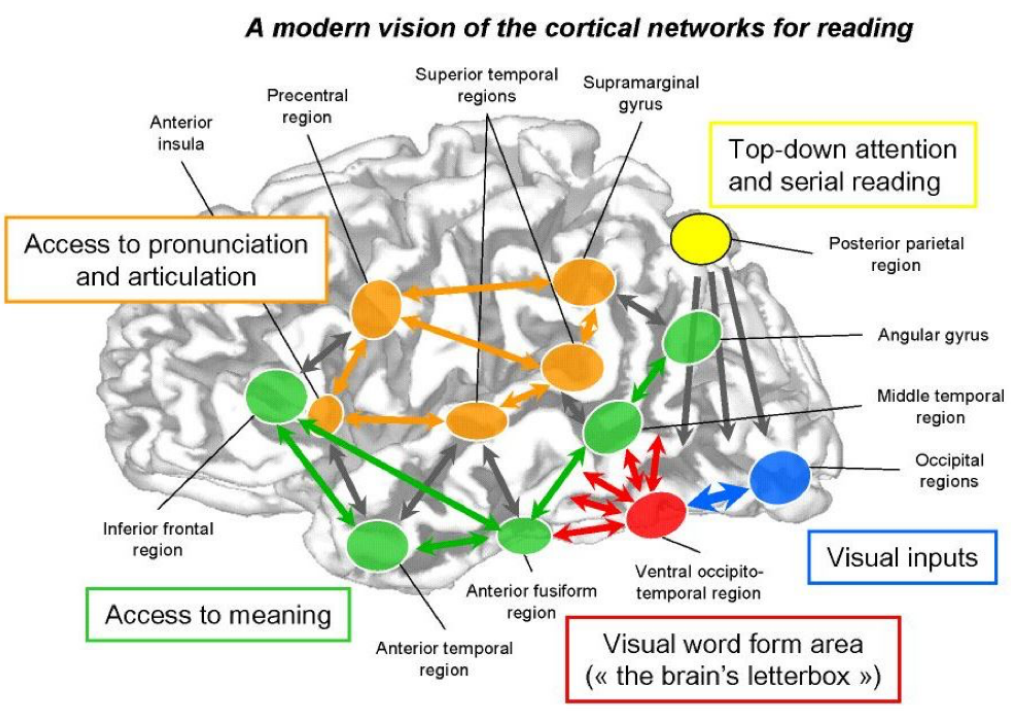


Fig. 1.1 The reading functional network. This is a schematic representation of the cortical regions that support the route from the visual word input till the comprehension. According to this neural model, the visual word form identification (or the visual form of letter strings), is carried out by the "brain's letterbox", or Visual Word From Area (VWFA), in the ventro occipito-temporal cortex. The VWFA is 'functionally specialised' for extracting abstract and invariant representation of letter strings, and it is only activated for visual and not spoken words (*unimodal activation*). Similarly, the superior temporal gyrus is only activated for spoken words. This modality (visual form) specific information can then access *heteromodal* components of the language network in the left hemisphere, such as the inferior frontal gyrus (Broca's area) and the temporo-parietal region (Wernicke's area), activating distributed associations that lead to the meaning encoding. In this image these regions are colour coded, based on their encoding function, such as word meaning, phonology and articulation. Efficient reading development involves efficient bi-directional communication between these areas. *Adapted from Booth et al. (2003); Dehaene (2009); Dehaene et al. (2004).*

Functional imaging studies have been able to pinpoint how silent reading mainly activates posterior parts of the left hemisphere (left angular and supramarginal gyri), whereas reading out loud engages more frontal areas (speech production or Broca's), activating thus the whole temporo-frontal network (orange network in Figure 1.1). According to Booth et al. (2003) neural processing routes that lead to the understanding of meaning of the word, contain both *unimodal* or functionally specialised regions of interest and *heteromodal* components as well. For instance, functional neuroimaging has shown how phonological representation of spoken words activates unimodal auditory areas (superior temporal gyrus), the orthographic representation of written words activates unimodal visual areas of the fusiform gyrus (VWFA), and how cross-modal paradigms measuring phonological/orthographic representations activate heteromodal regions in the angular and supramarginal gyri (Booth et al. (2003) and Figure 1.1). Important understanding of association between letters and speech sound, as the basis of reading, came from fMRI studies. Van Atteveldt et al. (2004) found that unimodal (auditory and visual) presentation of letters activated lateral and inferior occipito-temporal cortex, while the posterior superior temporal sulcus (STS) and gyrus (STG) who had a heteromodal response. The authors suggested the STS and STG as candidate regions for multisensory convergence (Van Atteveldt et al., 2004).

The increased interest and number of brain studies, mainly in the fMRI literature, investigating the emergence of reading as a cognitive skill, have offered the opportunity to perform meta-analyses and outline commonalities across neuroimaging findings. For instance, Martin et al. (2015) reviewed and summarised findings of forty fMRI studies comparing children and adult's neural activation during reading. The authors showed how distinct patterns of reading-related brain activation characterise child and adult readers. While in children there is evidence of high convergence in the left superior-temporal and bilateral regions, in adults higher activation was detected in bilateral posterior occipito-temporal, cerebellar and left dorsal precentral regions. When overlaid statistical maps of reading related areas from the two groups (adults and children), commonalities were found in the occipito-temporal, inferior frontal and posterior parietal regions, shown in Figure 1.2 (Martin et al., 2015). In a recent study, (Liu et al., 2018) used large-scale functional brain network and graph theory analysis to study change in configuration in young adult and child reading networks (i.e. rhyming and meaning judgement tasks). The authors found that while adults had higher inter-regional connectivity and nodal degree (local synchronisation) in occipital regions, children exhibited stronger inter-regional connectivity in temporal regions, and only adult network configuration showed different activation patterns between tasks Liu et al. (2018). These results suggested that reading development (from child to adult fluency) is represented by increased reliance on regions of visual orthographic processing and decreased reliance on auditory phonological processing (Liu et al., 2018). In other words, the reading functional brain networks become more specialises over development, by shifting from subcortical-cortical and intra-subcortical connectivity to intra-cortical connectivity (Liu et al., 2018). Using a similar approach, Smith et al. (2018) used a longitudinal study of young readers (8-14 years) to show how changes in functional segregation, or changes in regional specialisation according

to graph theory, predict changes in the pseudoword decoding as a measure of fluent reading. These findings all indicate that brain connectivity changes dynamically in relation to increased reading skills.

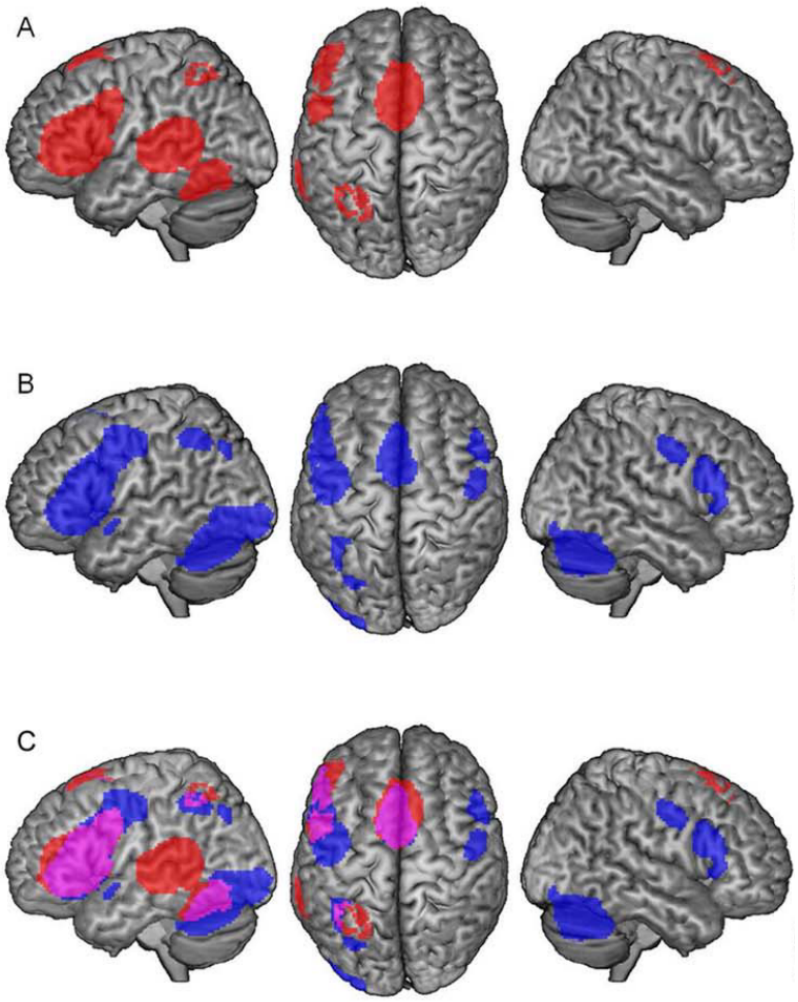


Fig. 1.2 Results of the meta-analysis of 40 fMRI studies investigating brain activation during reading in child and adult readers. A) Cortical regions activated in children during reading (in red). B) Brain areas activated during reading in adults (in blue). C) Cortical maps of overlapping child-adult regions are marked in pink/purple. It has been suggested in other functional connectivity studies (Liu et al., 2018) that the adult reading network relies more on visuo-orthographic and visual spatial attention regions (occipito-temporal areas), which indicates that the adult reading is more automated in nature. *Adapted from Martin et al. (2015).*

While structural MRI studies have helped localise the specific cortical regions involved in the reading processing (for instance Houdé et al. (2010)), M/EEG studies have helped to understand, with millisecond precision, the temporal dynamics of the information processing in the visual word recognition. The M/EEG reading literature has shown how time specific neural signatures or components called event related potentials (ERPs), or in case of MEG, event related fields (ERFs) are able to provide insightful information about the time-course of the neural activity in the reading architecture (Dien, 2009). In the cascade of events, by the first 100 ms of reading comprehension the extrastriate occipital cortex is activated with the P100 component. Following this, around $\sim$150 ms the inferior occipital cortex is responding selectively to the word shape like stimuli with a positively oriented component (P150); the visual word form area (VWFA) situated in the in the left ventro-occipito-temporal (vOT) cortex, mediates the pre-lexical orthographic analysis (Dehaene et al., 2005) and within this region a negative N170 component peaks at around 150-180 ms from stimulus onset (Dien, 2009). Later evoked activity, peaking from 200 ms onward (P200, N400) has been associated to more anterior language areas (left supramarginal gyrus and the anterior part of the fusiform gyrus) and thus, related to phonological and semantic processing leading to comprehension (Dien, 2009; Grainger and Holcomb, 2009). These studies show how the analysis of reading event-related components (ERPs and ERFs) provides a comprehensive time-lined neurocognitive model of the visual word recognition. In the cascade of events leading to fluent reading, the ventral occipito-temporal cortex (vOT) has a pivotal role in visual and orthographic word processing (Cohen et al., 2003). This ensemble of areas has been named the visual word form system (VWFS), (Vinckier et al., 2007). This region of interest has been of particular importance in neuroimaging studies, that tried to define better its word specificity and role in the reading acquisition (Brem et al., 2006).

To explore further the role of the ventral stream combined fMRI and ERP studies have investigated VWFS in relation to changes in development (Brem et al., 2009) and in the passage from adolescent to the adult reading brain (Brem et al., 2006). Children, adolescents and adults were tested with the same implicit visual word processing paradigm with both modalities (fMRI and ERP). The ERP analysis showed the specificity of N1 or N170 for words, localised in source space over the left occipito-temporal cortex. Furthermore, the time scale and the amplitude of the N1 peak activation differed between children (197-257 ms), adolescents (150-210 ms) and adults (146-206 ms), (Brem et al., 2009). Other studies have linked changes in the ERP signatures, precisely, decrease in amplitude with age, to an increased functional efficiency (Parviainen, 2006). On the other hand, Brem et al. (2009) found a dissociation in the fMRI modality, where the whole brain analysis did not detect any VWFS specificity. Instead, the cortical pattern of activation was posterior and bilaterally distributed. The authors concluded how this inter-modal (fMRI vs ERP) dissociation is due to different methodological sensitivity. While the ERP analysis, or neurophysiology in general (M/EEG) is the appropriate technique to measure the event-related transient activity (in this case N1), fMRI response was permeated with sustained activity and top-down processes providing images of more distributed sources (Brem et al., 2009).

These studies (Brem et al., 2006, 2009; Parviainen, 2006) have demonstrated that the specialised tuning of low level processing is better measured with time sensitive techniques (M/EEG) and how important it is to try to integrate a multimodal approach, to investigate maturational changes in the reading network. Given that MEG is well-suited measure of transient activity in the developing reading brain (Eulitz et al., 2000; Pulvermüller et al., 1997) an important research question would be to define neurophysiological correlates of impaired visual word recognition, or developmental dyslexia, which will be discussed below.

1.2 Developmental dyslexia

Developmental dyslexia is defined as a "specific learning disability of neurobiological origin characterised by difficulties in accurate and/or fluent word recognition and poor spelling and decoding abilities" (Lyon et al., 2003). The British Dyslexia Association (BDA) has adopted Rose (2009)'s definition of developmental dyslexia as a learning difficulty that affects skills of accurate and fluent word reading and spelling; is characterised by difficulties in phonological awareness, verbal memory and verbal processing speed; and it is not specific to lower intellectual abilities. As a disorder, the reading disability, is best thought of as a continuum, with no clear cut off points and it can present itself with a range of comorbidities or co-occurring developmental difficulties (aspects of language, motor co-ordination, mental calculation, concentration and personal organisation), (Rose, 2009). It has been shown how dyslexia is potentially the most common neurobehavioural disorder affecting children with prevalence ranging from 5 to 17% (Shaywitz et al., 2008; Shaywitz and Shaywitz, 2005). Moreover, often dyslexia (50% of the cases) also meets some of the diagnostic criteria of specific language impairment (SLI), attention-deficit hyperactivity disorder (ADHD) and dyspraxia (Habib, 2000; Paracchini et al., 2016).

1.2.1 Neural systems of dyslexia

Here, a historical journey into the neuroimaging of dyslexia is outlined. There is a century old evidence of first studies about the neurological origin of the 'visual word blindness', when Morgan (1896) and Hinshelwood (1917) observed similar symptoms in adolescents and dyslexic children (review by Habib (2000)). Similarly, in lesion studies Déjerine (1895) was proposing that the left angluar gyrus might play a crucial role in the 'visual word blindness'. Following this, post-mortem pathological studies showed brain abnormalities in the temporo-parietal regions in dyslexic readers (Habib, 2000; Lyon et al., 2003). Subsequently, the spotlight moved onto studying language lateralisation and brain asymmetries (Galaburda et al., 1987, 1978; Geshwind, 1968). For instance in one of the first neuroanatomical studies, Galaburda et al. (1985) have observed how dyslexic brains showed neuronal ectopias [1] and architectonic dysplasias in perisylvian regions of the left hemisphere. This finding was

[1] An ectopia is a displacement or malposition of an organ or other body part, which is then referred to as ectopic. Most ectopias are congenital, but some may happen later in life. source: *Wikipedia*

further confirmed in independent animal studies, showing that fine similar cortical ectopias in in-utero silenced DYX1C1 (one of the most studies gene candidate for dyslexia) adult rat brains (Kere, 2014; Threlkeld et al., 2007).

The early neuroimaging studies investigating abnormalities in dyslexic brains, explained the impaired sensory processing (auditory or visual processing) linked to dyslexia, as the result of reduced cortico-cortical connectivity. In one of the fist PET studies, Paulesu et al. (1996) showed reduced activation of the left insula, as a bridge between anterior and posterior language/phonological areas in dyslexics. The authors proposed to define dyslexia as a 'disconnection syndrome' (Paulesu et al., 1996). In a diffusion tensor imaging (DTI) study Klingberg et al. (2000) found bilateral temporo-parietal microstructure anomalies in poor compared to good readers. To study how dyslexia changes across orthography transparency (deep or shallow) and across languages, Paulesu et al. (2001) used a PET study in English, Italian and French dyslexic participants. Although Italian participants performed better at the reading cognitive measures, relying on a 'facilitated' or transparent orthography, all the participants exhibited reduced activation in the left hemisphere, with a peak in the middle, inferior and superior temporal gyri. This study corroborated the 'biological unity' of developmental dyslexia across languages and highlighted the importance of neuroimaging into studying the neurobiology of dyslexia (Paulesu et al., 2001; Schlaggar and McCandliss, 2007).

Following this, the increased number of available neuroimaging techniques (PET, DTI, MRI, M/EEG) and, therefore, studies investigating brain activation in dyslexia provided converging evidence of a left hemisphere posterior dysfunction in both dorsal and ventral sites across several reading tasks, (for complete reviews on the subject see Habib (2000); Paulesu et al. (1996); Pugh et al. (2000, 2001)). According to the literature, the ventral circuit composed by lateral extrastriate areas and left occipito-temporal regions that usually are activated during word and pseudoword reading (Fiez and Petersen, 1998; Puce et al., 1996), has been proposed to be dysfunctional in the readings disability (Pugh et al., 2000; Salmelin et al., 1996; Shaywitz et al., 1998). Additionally, abnormal activation in the dorsal stream or temporo-parietal circuit has been linked to severe reading deficit, during decoding and analysis in the language processing (Pugh et al., 2000). Moreover, the anterior circuit localised around Broca's area in the inferior frontal cortex, associated with sequencing in speech and articulation but also silent reading and naming, has been linked to impaired reading too (Salmelin et al., 1996; Shaywitz et al., 1998).

While the structural imaging (PET and f/MRI) has been very accurate into identifying spatially the reading network, the dynamic time-course of the neural processing of the reading disability has been better captured by M/EEG studies. Therefore, ERP studies have been an useful method to measure the neural timing of auditory, visual and sensory cognition in the posterior circuits linked to developmental dyslexia (Taylor and Keenan, 1990). For example, in a review Leppänen and Lyytinen (1997) have summarised the validity of measuring steady state responses, hemispheric differences in auditory ERPs (P1-N1-P2 and P3 components), as well as the application of ERPs in the prediction of developmental language and dyslexia disorders risk factors and the use of mismatch negativity (MMN)

to probe differences in neural basis of the deficit. The authors concluded that differences between typical and language-disordered readers in each of the observed phenomena was related to variations in some aspects of cognitive processing. For instance, ERP latency differences were linked to timing deficit or slow temporal processing in dyslexics compared to typical readers; N1 amplitude differences were related to arousal/attentional factors and to the tuning of the auditory system; and differences in MMN indicated different processing in sensory memory functioning (Leppänen and Lyytinen, 1997). In addition, it has been demonstrated how measuring ERPs in newborn babies, is a valid tool to identify differences in cortical responses in infants with and without risk for dyslexia (Leppänen et al., 1999). This makes ERP studies a very powerful tool for detection of early biomarkers or predictors of the disorder.

Further evidence of differences in the time course of cortical activation in dyslexics and typical readers came from MEG studies. Using a passive reading paradigm, Salmelin et al. (1996) demonstrated how dyslexic readers failed to show or had a delayed word-specific visual response (here defined M1 and later named the left N170 by Bentin et al. (1999)) in the left inferior temporo-occipital cortex, and in the left temporal lobe for later evoked activity (200-400 ms). Following this, in a MEG study Helenius et al. (1999) explored the dissociation between local feature and pre-lexical word analysis in dyslexic and typical readers. The authors used Gaussian noise patches to manipulate the difficulty of visual word recognition (feature analysis vs letter-string processing) and observed how the time course of neural sources of activation varied between dyslexic and typical readers. The results indicated that although the early visual responses to stimuli (0-100 ms) were temporally and spatially localised in the postero-medial occipital areas in both groups, letter-specific activation ($\sim$ 150 ms) in the left inferior occipito-temporal area was undetectable in most dyslexic readers (Helenius et al., 1999).

Following these pioneer M/EEG studies investigating differences in the fast visual specialisation in typical and atypical readers, more neurophysiological studies focussed their interest on developmental changes linked to the first stages of reading. Specifically, the pre-lexical orthographic processing phase has been largely investigated as the modulation of the N170 cortical response, as an index of word reading automaticity (Maurer et al., 2005a; Sánchez-Vincitore et al., 2017). This left lateralised VWFA's brain signature has also been largely applied to study different trajectories of typical and atypical (dyslexic) reading acquisition (Maurer et al., 2005b, 2011) and the effects of fluency training in children with dyslexia (Fraga González et al., 2016b).

Much progress has been made since the definition of the 'congenital word blindness'. An even longer path has to be walked to be able to fully describe the specific contribution of underlying causes of developmental dyslexia. For instance, it still has to be fully ascertained which brain features predispose the reading delay and which are the result of long-term unsuccessful reading, or how to measure the pathway genes-brain-reading disability, and how to classify better the high heterogeneity within the dyslexic spectrum. The overreaching goal of this PhD thesis is to introduce a comprehensive multimodal approach into investigating neural correlates of different pathways in (a)typical reading.

1.2.2 Functional and structural networks in dyslexia

The main challenge in the last two decades of neuroimaging in dyslexia has been to describe better the 'left posterior hemisphere underactivation' (Pugh et al., 2000; Richlan et al., 2009; Shaywitz and Shaywitz, 2005; Temple, 2002). It has been accepted by now, that the dorsal (temporoparietal) system is responsible of mapping letters (grapheme) or 'visual forms' onto their phonology in an attentionally controlled manner, and how this function is fundamental in the early phases of the reading acquisition (Richlan et al., 2009; Sandak et al., 2004). This system includes the angular gyrus, the supramarginal gyrus and the posterior part of the superior temporal gyrus (Wernicke's area) and it is highly activated when decoding pseudowords (Landi et al., 2013). The ventral stream is composed by the left inferior occipitotemporal/fusiform (vOT) regions, also known as the visual word fusiform system (VWFS) or visual word form area (VWFA), and by more anterior regions in the middle and inferior temporal gyri (Landi et al., 2013). This stream's main function is fast automatic processing of familiar visual words and frequent letter strings (Richlan et al., 2009).
It has been suggested how the primary dysfunction of the dorsal system in the grapheme to phoneme translation, is the primary deficit in developmental dyslexia (McCandliss et al., 2003; Pugh et al., 2000), followed by the left ventral underactivation (Richlan et al., 2009). In addition, other studies have shown overactivation in frontal and right hemisphere regions in dyslexic readers, as compensatory mechanisms for the left posterior dysfunction (Richlan et al., 2009). In particular, overactivation in the left inferior frontal regions has been linked to articulary word guessing due to the increased effort in word recognition, and the right temporoparietal engagement to mechanisms of pure compensation (Pugh et al., 2000; Sandak et al., 2004; Shaywitz and Shaywitz, 2005).
In a quantitative meta-analysis of fMRI studies investigating functional connectivity in typical and atypical readers, Richlan et al. (2009) showed how peaks of underactivation were found in left inferior parietal, superior temporal, middle and inferior temporal, and fusiform regions. Additionally, the authors observed how underactivation in the inferior frontal gyrus was associated to overactivation in the primary motor cortex and the anterior insula (Richlan et al., 2009). In a subsequent analysis, the same research group followed up on reviewing the developmental model of dyslexia. The results were of opposite nature, pointing towards an early engagement of the left occipito-temporal cortex in the reading acquisition and failure of such an activation in dyslexia. The authors concluded that in younger dyslexic readers the main deficit would be in the temporo-parietal phonological dysfunction, whereas in the older/adult dyslexics would be of a viso-orthographic or left occipitotemporal dysfunction (Richlan et al., 2011).
In sum, fMRI studies in dyslexic readers demonstrate *hypoactivation* in the left hemispheric temporo-parietal, occipito-temporal and inferior frontal networks and *hyperactivation* in corresponding righ-hemispehric regions (Ozernov-Palchik and Gaab, 2016; Richlan et al., 2011).

Although fMRI connectivity studies are a powerful method for describing brain networks and their hypo/hyper functioning, the vast majority of connectivity studies in dyslexia has mainly focused only on regions part of the left-hemisphere functional reading network or on reading task-related

activations (Finn et al., 2014). Some evidence about anatomical proprieties came from studies investigating structural connectivity with diffusion imaging. Diffusion Tensor Imaging (DTI) studies have shown how the sub-cortical white matter architecture differs between dyslexic and typical young readers (Vandermosten et al., 2012a). By investigating the diffusion white matter proprieties, DTI studies have consistently reported lower fractional anisotropy (FA) values of diffusivity in the left temporoparietal and frontal areas, corroborating the hypothesis of these areas being 'disconnected' in dyslexia (Vandermosten et al., 2012b). In the white matter structural network, these connecting fibres are the left arcuate fasciculus and corona radiata (dorsal pathway) and the inferior longitudinal and fronto-occipital fasciculus (ventral pathway). Changes in structural connectivity within the structural network have been linked to improvement in the reading skills, as well as considered to be valid predictors of poor or good reading skills, when measured in pre-readers (Vanderauwera et al., 2017; Wang et al., 2017). When looking at the white matter structural networks, Lou et al. (2019) looked at the correlation between topological proprieties and the severity of dyslexia. By applying a network-based analysis the authors found that network topological parameters, such as clustering coefficient, local efficiency, transitivity, and global efficiency positively correlated with literacy skills in dyslexic children, and explained variance in literacy skills (literacy ability, phonological ability, and rapid automatic naming) beyond white matter connectivity measures (Lou et al., 2019). This study highlighted the importance of studying whole brain network topology and how topological proprieties could represent potential correlates of differences in reading ability.

Similarly, studying resting state functional connectivity (RSFC) can help to better understand the causative or consequential aspects of reading disability, by exploring the whole brain integration and synchronisation (Rubinov and Sporns, 2010). In addition, task-based studies carry the intrinsic challenge of creating age and reading level appropriate paradigms (Koyama et al., 2011). Generally speaking, this spontaneous or 'intrinsic activity' (He et al., 2008) plays an important functional role by providing scaffolding for long range neural communication and top-down constraints to sensory, cognitive or motor engaging activity (Mantini et al., 2007). Several studies have proven the correlation between low frequency fluctuations in resting fMRI (De Luca et al., 2006) and correspondent temporal dynamics of neurophysiological (M/EEG) activity (de Pasquale et al., 2010; Scholvinck et al., 2010). These spontaneous fluctuations are organised in specific functional anatomical networks, named Resting State Networks (RSNs), (De Luca et al., 2006; Mantini et al., 2007). While it has been extensively shown that different cortical networks are activated at rest (RSNs) or in task-free condition, and during cognitively engaging activities, it still needs to be understood how reading related regions interact with the rest of the brain network. In terms of brain network analyses (i.e. connectomics) the RSNs are densely intra-connected modules with a core group of hub areas that convey information between RSNs (Bailey et al., 2018; van den Heuvel and Sporns, 2011). It follows that functionally related brain regions might correlate in their spontaneous activity, measured by resting state functional connectivity (Vogel et al., 2013). For instance, with a network analysis approach Vogel et al. (2013) showed how regions preferential with reading are also activated in a number of different tasks and how

this configuration changes over development. This study showed how the functional organisation of regions involved in single word reading, are not specific but useful to reading processing. In particular by using graph theory community description and modularity optimisation, no specific community structure was identified but it rather mapped onto different other systems (fronto-parietal, cingulo-opercular, and default mode network), (Vogel et al., 2013). Using a similar approach Bailey et al. (2018) studies how brain's network architecture related to reading in children aged 8-11. By applying a connectomics approach, this study showed how the reading network falls in domain-general RSNs such as attention and executive functions: the dorsal attention network, for instance, encompasses the visual word form area (VWFA) which is very important to reading but seems not to be specific to, (Bailey et al., 2018; Vogel et al., 2014). In addition Bailey et al. (2018) showed how nodes or areas that are key in dyslexia had increased hubness or participation in the network configuration. These studies seem to support the hypothesis that connectivity within RSNs correlates with reading skill and that abnormalities in dyslexia can be described by increased hubness or traffic load onto the the core areas that connect RSNs, (Bailey et al., 2018; Vogel et al., 2013).

Furthermore, in the neurophysiology framework, it has been demonstrated how investigating the task-free oscillatory networks (such as MEG RSFC), allows to map frequency-specific age related changes (Khan et al., 2018). This makes the MEG an ideal tool for defining dynamic proprieties of functional networks in developmental studies.

Overall, there is a a paucity of studies exploring both fMRI and M/EEG whole brain task-free proprieties in developmental dyslexia. So far, resting state network studies have shown how children with dyslexia exhibit increased connectivity from the reading network to the limbic system and DMN (Finn et al., 2014), how the RSFC profiles correlate with reading skills in children (Koyama et al., 2010), as well as a bilateral overactivation in frontal areas (Dimitriadis et al., 2013; Pagnotta et al., 2015), and increased local processing at the cost of less integrated network organisation (Fraga González et al., 2014; Liu et al., 2015). Undoubtedly, the potential of the RSFC network analysis in developmental dyslexia has yet to be fully exploited.

Lastly, to be able to consistently explain the variable functionality of different patterns in connectivity (correlational in fMRI and effective in M/EEG), it is not enough to constrain it to a fixed structural architecture (being the DTI structural connectivity undirected), (Park and Friston, 2013). To gap the bridge between 'structural' and 'functional' connectivity, the application of network theory seems to offer the perfect platform to integrate this dyadic information. Graph theory application or analysis of network topology in the brain allows to define organisational proprieties of the networks of interest, with the ultimate goal of integrating and summarising functional and structural qualities (Bullmore and Sporns, 2009; Park and Friston, 2013).

1.2.3 Cognitive mechanisms of dyslexia

Preeminent and relevant to the neuroimaging studies mentioned above, theories of dyslexia are described below. It is important to highlight that none of these models can individually explain

the phenotypic variability in the dyslexic continuum and that the proposals here illustrated are not mutually exclusive. Before describing major theories proposed to explain neural mechanisms of dyslexia, it is important to clarify the relationship between IQ and dyslexia. According to the official definition of the disorder, dyslexia is defined as a reading difficulty irrespective to the IQ. Nevertheless the role of IQ in the diagnostic process of dyslexia is still source of debate. Interesting insights came from fMRI studies exploring differences in brain activation in dyslexics with high and low IQ. Tanaka et al. (2011) carried our a fMRI study comparing phonological processing in two independent but matched groups of dyslexic children: *discrepant* (poor reading and normal IQ) and *non-discrepant* (poor reading and and low IQ). Behaviourally the discrepant and non-discrepant dyslexic groups did not differ in phonological discrimination, nor in the atypical brain functioning (Tanaka et al., 2011). This study supports the idea that the relationship between IQ and the phonological awareness deficit, typically linked to dyslexia is quite weak (Tanaka et al., 2011), confirming that deficits in dyslexia cannot be explained by low or high IQ.

Phonological Theory

Etymologically, phonology derives from the combination of Greek words *phono-* meaning "voice, sound" and *-logia* which means "a speaking, discourse, treatise, doctrine, theory, science". The predominant cognitive model in dyslexia advanced the hypothesis that developmental dyslexia is due to a specific impairment in the representation, storage and retrieval of speech (Ramus et al., 2003; Snowling, 1995). It has been consistently reported that dyslexic readers have difficulties with phonological awareness and limitations of verbal short term memory (Snowling, 1998, 1995). The phonological theory of dyslexia postulates that, in order to fluently read out lout, spoken words can be disentangled in elemental sub-components of speech or 'phonemes' and, in this manner, the letters (or 'graphemes') represent the sounds to be sounded out (Lyon et al., 2003; Shaywitz et al., 2008; Snowling, 1998). If the sounds are not efficiently represented in the grapheme-to-phoneme translation, reading skills fail to develop properly (Ramus et al., 2003). Shaywitz and Shaywitz (2005) described how large-population studies in children and adolescent (a)typical readers point towards the phonological as a primary deficit in dyslexia. In brief, this model proposes that since reading is a product of decoding and comprehension (Gough and Tunmer, 1986), a lower linguistic level (phonology) decoding deficit would prevent, higher order processes such as comprehension (Shaywitz and Shaywitz, 2005). Behavioural evidence for the phonological theory came from experimental studies measuring both phonological awareness, non-word reading tasks but also by showing the effects of remediation of phonological skills on the improvement of the reading ones (Shaywitz et al., 2008; van Ijzendoorn and Bus, 1994). Furthermore, it has been suggested that the phonological deficit has its neural origin in the congenital dysfunction in the left hemisphere perisylvian areas that underlie phonological representations and connect orthographic representations (Ramus et al., 2003).

While the correlation between phonological awareness, as 'the active manipulation of sounds', and the reading ability has been repeatedly shown, its direct or proximal cause of poor reading has

been less clear (Castles and Coltheart, 2004). It has been proposed that the acquisition of reading skills does not affect the level of phonological awareness but it affects the way in which children preform phonological awareness tasks (Castles and Coltheart, 2004). This argument has opened the debate about the *casual* role of phonological deficit in dyslexia and new venues into studying causes of dyslexia (Ramus and Szenkovits, 2008; Vidyasagar and Pammer, 2010). For instance Vidyasagar and Pammer (2010), have shown how visuo-spatial attention is fundamental for reading. According to the recycling hypothesis, learning how to read has taken advantage of pre-existing neural mechanisms, that have adapted for a different cognitive function (Dehaene et al., 2004; Vidyasagar and Pammer, 2010). It is thus possible that in reading the same top-down mechanisms, responsible for visual search in object recognition, are activated during letter recognition (Vidyasagar and Pammer, 2010). This perspective does not exclude the phonological deficit hypothesis but increases the level of complexity when studying the neural causes of developmental dyslexia. An interesting perspective into defining the relationship between speech processing and phonological deficits came from Ramus et al. (2013). This study looked at different models to define the relationship between phonological deficits, specific language impairment (SLI), and dyslexia: the 'severity model' where the same speech processing and phonological deficits cause SLI also cause reading deficits in dyslexia; the 'additional deficit model' similar to the severity model, which hypothesises that there is a shared underlying phonological deficit in dyslexia and SLI, where SLI is just a severe version of it; and the 'component model' in which dyslexia and SLI are considered to be entirely distinct disorders (Ramus et al., 2013). By testing a big sample of children with SLI and dyslexia, the authors performed factor analysis to compare individual performance at a wide range of tests with predictions of each model. The results showed a strong overlap between SLI and dyslexia, as two distinct disorders that are frequently comorbid, showing that the multiple-component model best explains the relationships between different dimensions, and SLI and dyslexia (Ramus et al., 2013).

Sensory processing

According to the rapid temporal processing hypothesis, first advanced by Tallal (1980), there is a high correlation between the ability of dyslexic readers to correctly process phonemes, and the performance during the discrimination and temporal order perception in auditory processing. According to this theory, dyslexic readers will struggle in processing auditory stimuli that have short duration and occur in rapid succession (Casini et al., 2018; Tallal, 1980). The slow temporal processing would, in turn, be the underlying or basic deficit at the auditory level that triggers poor phonological development and performance (Tallal et al., 1993). Following this proposition, several studies have shown how the temporal dimension of the stimuli is such only when they are considered in the dynamic dimension or changing in time (Talcott et al., 2002, 2000; Witton et al., 2002, 1998).
Talcott and Witton (2002) proposed a 'sensory-linguistic' approach to study the sensory mechanisms involved in the reading development. Focusing only on single-word reading, can provide more information on subsequent processes, but most of all, the evidence of the potential sensory impairments

is at that basic word-recognition level (Talcott and Witton, 2002). Within this framework a range of different experimental studies (acoustic and visual frequency stimuli modulation, psychophysical coherent and incoherent motion) have demonstrated how sensory processing does have a role in the development of literacy skills and it is impaired in dyslexic readers (Talcott et al., 2002, 2000; Witton et al., 2002, 1998).
Moreover in vision the correlation between coherent motion and orthographic skills implies a role of dynamic visual processing in the reading ability (Stein, 2000; Talcott and Witton, 2002). The neurobiological basis of this phenomenon are explained by the *magnocellular theory*. The visual magnocellular system is responsible for timing visual events (Stein, 2001) and is required to acquire good orthographic skills (Stein, 2001). Briefly, *parvo* and *magno* cells carry visual signals from the retina to the brain. Where the majority of these ganglion cells is parvo (in latin means small) and responsible for coding colour and fine details, the magno (big) cells are larger and more myelinated, and relevant for timing events in visual word processing (Stein, 2000). This theory hypothesises that the magnocellular pathway is disrupted in dyslexic readers, leading to a cascade of events that starts with deficiencies in visual processing, and going through the posterior parietal cortex leads to abnormal binocular control and visuospatial attention (Ramus et al., 2003; Stein, 2001; Talcott et al., 2000). Supporting evidence of the magnocellular deficit came from anatomical studies (Livingstone et al., 1991), psychophisical studies (Talcott and Witton, 2002) and brain imaging (Eden et al., 1996).

Attention

The deficits in sensory sequence processing and the M-cells (magnocellular) theory led Hari and Renvall (2001) to propose that dyslexics might suffer from a prolonged 'attentional dwell' time in the rapid stimulus sequence, in both visual and auditory tasks. By measuring an attentional blink in dyslexic and typical readers the authors showed how the attentional allocation is prolonged by $\sim$30% in dyslexics (Hari et al., 1999). Henceforward it has been proposed that dyslexic readers have a 'sluggish attentional shifting' (SAS), (Hari and Renvall, 2001). Other studies have demonstrated how the attentional blink, as the inability to identify and decode the second of two rapidly sequences of targets, is impaired in dyslexic children, measured during an object recognition task (Visser et al., 2004). In comparing different aspects of attention in typical and atypical young readers Skarżyński (2014) described how dyslexic children showed deficits in alertness, shift of attention, divided attention, flexibility and visual search. Additionally, the critical role of attention in reading is also underlined by the high rates of co-occurrence or comorbidity between dyslexia and attention deficit/hyper activity disorder (ADHD) (Shaywitz and Shaywitz, 2008).
Furthermore, ERP studies have shown how the attention modulates the initial stages of the word processing (Nobre et al., 1994; Ruz and Nobre, 2008). Overall, these studies suggest that attention does play a key role in developmental dyslexia.

As mentioned above, theories here exposed are not an exhaustive list of the cognitive and theoretical propositions explaining reading disability in the literature. This is beyond the purposes of

this thesis, since the focus will be on investigating the neural correlates of developmental dyslexia. Nevertheless, from the perspective of a multimodal approach into studying developmental disorders, such as dyslexia, it is crucial to describe mechanisms of co-occurrence or comorbidity between dyslexia and associated disorders. For instance, it is well known by now that dyslexia and Attention Deficit Hyperactivity Disorder (ADHD) co-occur more frequently (25%-40%) than expected by chance (Boada et al., 2012), as well as SLI (Ramus et al., 2013). Studying comorbidity, especially in development, is very important to be able to identify and understand the etiological and pathogenic mechanisms that underlie sets of symptoms (Boada et al., 2012). To be able to determine factors that can explain complex disorders, a multiple deficit model is required, that in turns allows to identify cognitive risk factors for each disorder (Boada et al., 2012). Similarly, as mentioned previously, it has been discussed for a long time how to classify the continuum between SLI and dyslexia (Bishop and Snowling, 2004). Today the debate is not about diagnostic criteria or definitions for specific learning disorders anymore, but on how to use this shared etiology to better identify risk factors or predictors of learning disorders. To be able to define *trajectories* of typical and atypical reading development, several studies have focused on longitudinal investigation of children with family risk of dyslexia. Snowling and Melby-Lervåg (2016) reviewed 95 publications on oral language deficits in familial dyslexia and observed how children at family risk of dyslexia do experience delayed language as infants and toddlers. Further, longitudinal studies showed how family risk of dyslexia was predictive of significantly poorer phonological awareness and literacy skills in at school age (Snowling and Melby-Lervåg, 2016). The authors suggested that phonological processing deficit could be conceptualised as an endophenotype of dyslexia that increases the continuous risk of reading difficulties (Snowling and Melby-Lervåg, 2016). More recently, Snowling et al. (2019) did a retrospective longitudinal study, by comparing children diagnosed at age 8, either for dyslexia or Developmental Language Disorder (DLD) and compared with their cognitive skills at age three. This study found how children with a poor language outcome (DLD) showed a wider range of impairments in the preschool period (poor pre-reading skills, executive and motor problems; while children with the dyslexic outcome had more specific deficits (Snowling et al., 2019). This study elegantly showed how phonological deficits are shared risk factors for dyslexia and DLD, and how important it is to look at shared etiological pathways when studying learning difficulties (Snowling et al., 2019). Finally, comorbidity could be potentially explained by shared genetic variance, as explained in the next paragraph.
The objective of this section was to introduce the overarching theoretical framework for the present interest of this thesis into investigating some aspects of reading, such as the the prelexical orthographic stage of the visual word recognition. The next section will feature the neurobiology of dyslexia, exploring the genetic influence in the aetiology of the reading disability.

1.2.4 The genetics of dyslexia

Studying human genetics is a powerful tool to analyse brain development, since the advances in the field have shown how human population shows a mutation in every gene, providing thus the oppor-

tunity to identify disease-causing genes essential for development (Hu et al., 2014). Modern DNA sequencing has allowed computationally based gene identification to rapidly identify small subset of candidate disease genes (Hu et al., 2014; Ng et al., 2010). Thus, genetic advances have helped to better study genetic influences on brain development. For instance, the understanding of neural migration from the genetic point of view, has helped to better define causes of brain malformations, such as lissencephaly (loss in gyral patterning with thickening of the cortex), (Hu et al., 2014). It has also been shown how mutations in many different genes often cause similar phenotypes. This would suggest an overlap in cellular mechanisms and pathways, (Hu et al., 2014). Moreover, the absence of structural malformations in most of the developmental disorders (epilepsy, autism, ADHD, and intellectual disability) it seems that brain connectivity would be the most susceptible aspect of neurodevelopment (Hu et al., 2014).

Notwithstanding the large amount of theories proposed to explain the causes of the reading disability, the etiology of dyslexia still remains unknown (Habib, 2000; Paracchini et al., 2007). The many theories advanced are not exclusively able to explain the variability of dyslexic phenotypes and it has been recognised that neurological basis of dyslexia has a strong genetic contribution. One of the main purposes of performing genetic studies is to pinpoint the biological basis of the neural deficits (Paracchini et al., 2016). Despite the fact that is has been acknowledged, by now, that dyslexia is caused by genetic factors, (Paracchini et al., 2007), confirmed by twin studies (Gayán and Olson, 2001; Harlaar et al., 2005), the specific measurable genetic influences on the underlying neural mechanisms still remain relatively unexplored (Paracchini et al., 2016).

One of the first evidences of dyslexia or 'congenital word-blindness', as a heritable disorder goes back to Hinshelwood (1917). Following this, genetic contribution to dyslexic aetiology was described early on by Hallgren (1950). Since then multiple loci for dyslexic susceptibility have been identified, confirming that heritability in dyslexia ranges from $\sim$40% - 70% (Schlaggar and McCandliss, 2007; Schumacher et al., 2007). Given the high heterogeneity of the disorder, it is quite challenging to define specific reading or cognitive phenotypes to characterise the optimal sample of investigation (Paracchini et al., 2007). It has been proposed that the most sensible approach to study the neurobiological basis of dyslexia is to investigate the association between normative reading behaviours (phonological, orthographic, phonemic awareness and word recognition skills), and genetic expression within families (Castles et al., 2006; Fisher and DeFries, 2002; Schlaggar and McCandliss, 2007). However, this type of longitudinal research is often very challenging due to the interference of the environmental constraints. For this reason, genetic dyslexia-susceptible studies have become quite common.

In the past decade, the advances in technology have allowed both linkage and association genetic studies, using both quantitative and qualitative approaches, to pinpoint the chromosomal regions (X, 1, 2, 3, 6, 15) that contain dyslexia susceptibility genes (Wysocka et al., 2010). The most relevant are four candidate dyslexia susceptibility genes: DYX1C1, KIAA0319, DCDC2 and ROBO1, (Galaburda

et al., 2006; Schumacher et al., 2007). Proteins encoded by these genes have been directly or indirectly linked in pathways of cortical neuronal migration and other neurodevelopmental processes, such as axon growth (Galaburda et al., 2006). Specifically, ROBO1 has a role in neural migration and axon growth, DCDC2 in the neural migration of the neocortex and development of the corpus callosum, KIAA0319 is indirectly linked to pathways involved in cell adhesion (Galaburda et al., 2006). Similarly, common genetic variants associated with reading and language disorders can be used to explain shared risk factors or comorbidity between, for instance dyslexia and SLI (Paracchini, 2011). Newbury et al. (2011) tested for the same association in SNPs in two cohorts, SLI and dyslexia. KIAA0319 was the only gene that showed association with reading measures in both dyslexia and SLI (Newbury et al., 2011).

The proposed model of 'dyslexia susceptibility' offers a theoretical platform to integrate genetic, neurodevelomental changes and the observed perceptual/cognitive deficits in dyslexia, as confirmed by animal and human studies, (Ozernov-Palchik and Gaab, 2016). Albeit the improvement in understanding the molecular mechanisms linked to the reading impairment, establishing functional candidate genes to specific neural dyslexic patterns, and therefore identifying specific at-risk predictors before the start of formal education, still represents a challenge in the current literature (Ozernov-Palchik and Gaab, 2016; Schlaggar and McCandliss, 2007). One way to approach this issue would be to carry out longitudinal neuroimaging developmental studies of individuals at risk, such as Guttorm et al. (2003); Lohvansuu et al. (2018). Or to study common variants in large cohorts, across languages and nations, to be able to identity common variants linked to the reading disability, such as the two most important GWAS in the literature Becker et al. (2014); Gialluisi et al. (2018). Both of these modalities of investigations are quite challenging due to time consuming and long term researchers and participants commitment.

Dyslexia is part of language-related disorders that are complex and result from the interplay of multiple factors that are both of genetic and environmental origin (Paracchini, 2011). The identified genes, mentioned earlier, represent only a very small component of causative elements. It follows, that a multimodal approach is essential. Thus, an alternative would be to focus more on underlying neurobiological mechanisms specific to candidate genes that have a distinct phenotype, significantly correlated to dyslexia, paired with neuroimaging. For instance, functional neuroimaging studies of autism have started to factor in genetic variants to evaluate their impact on cognition (Paracchini, 2011). An example of neuroimaging genetics study is described in Chapter 6 of this thesis.

1.3 Overall aims and objectives of this thesis

This thesis has an overall objective to conduct a multimodal investigation of neural correlates in typical and atypical young readers. Given the high heterogeneity within the dyslexic continuum, more parsimonious approaches are needed to define different neural patterns of activation underlying these reading phenotypes.

In particular, it intends to tackle different aspects of structural and functional cortical underpinnings in relation to reading skills and genetic contributors. Further, given the lack of MEG RSFC studies investigating dynamic proprieties of network changes in developmental dyslexia, it aspires to expand upon current neuroimaging investigations by proposing a fine-grained method to distinguish specific sub-types in the definition of the disorder spectrum.
Specifically, it focuses on variability of (dys)fluency traits, considered one the most persistent symptoms of the reading disability (Ferrer et al., 2015; Fraga González et al., 2018a; Shaywitz and Shaywitz, 2008).

Thus, the specific aims are:

1. To define the high variability *within* the dyslexic spectrum, by exploring the MEG RSFC and network topology in relation to specific dyslexic sub-types, defined by cognitive and genetic components.In this case, the cognitive component is the level of fluency in single word-reading (above and below threshold) and the genetic one, is carriers and non-carriers of a dyslexia-related genotype, PCSK6 (described in detail in Chapter 6).

2. To examine the neural specialisations of previously proposed brain signatures linked to the first stages of visual word recognition,such as the N170 in the left visual word fusiform system, by employing a newly developed MEG paradigm to probe, both sensor and source level, differences in visuo-orthographic processing in skilled and impaired young readers.

3. To investigate differences in the structural dorsal connectivity in dyslexic and typical readers and to explore interactions between reading skills and measures of diffusivity in impaired readers.

In sum, the work described in this thesis is a multi-level corollary of studies investigating neurphysiological (MEG), structural (DTI), genetic, cognitive and behavioural correlates of developmental dyslexia in children aged 10-12 years old. It additionally offers a primary example of network analysis application to better describe proprieties of functional networks in dyslexia, based on reading skills and genetic influences.

Chapter 2

General Methods

Chapter summary

The following chapter will describe the methods applied in the experimental Chapters 3, 4, 5, 6 and 7. It includes description of recruitment procedures of both adult and child participants, cognitive and behavioural measures utilised in the assessment of developmental dyslexia, and the neuroimaging (MEG and MRI) techniques used throughout this thesis. Following this, magnetoencephalography (MEG) system, preprocessing pipeline, source reconstruction methods and the connectivity estimates will be detailed. Then, a brief introduction to network analysis and graph theory will be illustrated. To conclude, the diffusion tensor imaging (DTI) will be introduced.

2.1 Introduction

The following chapter aims to introduce the materials and methods exploited throughout the work described in this PhD thesis. It starts with the description of the developmental population of interest, hence the participants' recruitment. Following this, cognitive and behavioural measures utilised for the group of children with dyslexia are described. Then, neuroimaging techniques, data acquisition and data analyses are outlined. Lastly, general overview of connectivity and network analyses are described.

2.2 Participant recruitment

Generally, volunteers that took part to any of the experimental studies described in this thesis have been screened for eligibility to participate to a neuroimaging study. Following Aston Brain Centre's guidelines, ethically approved questionnaires that interrogated medical history of previous major or recent surgery interventions, potential pregnancies, psychiatric disorders, metal fragments or implants in the body, first generation dental work, permanent make-up or tattoos, were administered to all the participants before scanning. These were the general exclusion criteria for participating to all the neuroimaging studies described in this thesis. Note that some of these features would not prevent volunteers to take part to the MEG part, as in case of tattoos for instance. Nevertheless, the choice was to extend the criteria to all the neuroimaging modalities employed in this work (MEG, MRI, and DTI) to prevent incomplete data sets. A summary table of participants and methods for each of the experimental chapters (Chapter 3, 4, 5, 6, and 7), is illustrated in Table 2.1.
Each of the neuroimaging protocols carried out in this thesis, has been reviewed and approved by the Aston University Research Ethics Committee (REC), with ethics approval numbers 408, 1220. Prior to scanning, written consent was obtained from all the participants. For those below the age of 17, both parental and child written consent and assent forms were completed. Additionally, participants and their caregivers were informed about the Aston Brain Centre data treatment policy for research purposes only.
Furthermore, before starting the MEG or MRI procedures, every participant was informed of their rights and advised that they could interrupt the study at any point, if feeling any distress or discomfort. Additionally, all the child and adult volunteers were repeatedly, in written and verbal form, assured that participation to the study was completely voluntary.

Study/Chapter	Participants		Methods	
	Typical/Fluent	Atypical/Dysfluent	Cognitive	Neuroimaging
RSFC and network topology in developmental dyslexia (Chapter 3)	N=17	N=14	TOWRE	MEG - PLI and MST MRI - T1
"Super Mario": a novel MEG paradigm to investigate visuo-orthographic processing in reading (Chapter 4)	N=10		Pilot study	MEG-LCMV and ERP MRI- T1
"Super Mario" in typical and atypical young readers (Chapter 5)	N=15	N=8	Dyslexic group: TOWRE BAS Reading and Spelling	MEG-LCMV and ERP MRI-T1
Resting state functional connectivity and network topology in dyslexia related genotype: a MEG case-study (Chapter 6)	N=7	N=7	Dyslexic group: Groups defined by genotype PCSK6^{-} PCSK6^{+}	MEG-PLI and MST MRI-T1
Diffusion Tensor Imaging (DTI) study in developmental dyslexia - the left arcuate fasciculus (Chapter 7)	N=16	N=12	Dyslexic group: TOWRE BAS Reading and Spelling	DTI-fractional anisotropy (whole brain and AF)

Table 2.1 Summary table of studies and participants in this thesis. This table illustrates the number of participants included in each study, the cognitive batteries used, and the neuroimaging methods applied. In the first MEG study (Chapter 3) investigating resting state functional connectivity (RSFC) in children with dyslexia, the performance at the Test of Wording Reading Efficiency (TOWRE) was used to divide the participants in dysfluent and fluent dyslexic readers. Functional connectivity was measured using the Phase Lag Index (PLI), and network topology was described using the Minimum Spanning Tree (MST). The mean age for the both dysfluent and fluent groups was 13 years old. The second MEG study is described in Chapter 4, and it is the "Super Mario" pilot study involving 10 typical readers (mean age= 24 years old). This study was carried out to pilot the paradigm, and to test the MEG analysis (described in Chapter 4). For this reason, there were no 'atypical' group and cognitive measures to be included. In this case sensor level analysis was based on time-locked evoked potentials (ERP), and source-level was performed using the Linear Constrained Minimum Variance (LCMV) beamformer. The third MEG study is described in Chapter 5, and it investigates the early visual processing in typical and dyslexic readers. For the dyslexic group correlation analysis was carried out with measures of fluency (TOWRE), reading, spelling, and the performance (accuracy) at the "Super Mario" paradigm. The typical and dyslexic group were matched by mean age (11 years old). Same as in Chapter 4, sensor level analysis was based on time-locked evoked potentials (ERP), and source-level was performed using the Linear Constrained Minimum Variance (LCMV) beamformer. The fourth MEG study, investigates differences in resting state functional connectivity (RSFC) and network topology, in a group of dyslexic children, stratified by the genetic component (PCSK6, described in Chapter 6). In this case, the groups were matched by age (12 years old), TOWRE, reading, and spelling scores. The analysis applied was the same as Chapter 3, PLI and MST. The last neuroimaging study (Chapter 7) is a diffusion tensor imaging (DTI) study, investigating differences in white matter architecture, by measuring fractional anisotropy, in the whole brain and in the left arcuate fasciculus (AF), in typical and atypical readers. Typical readers' mean age was11 years old, and dyslexic readers' mean age was 10 years old. Additionally, for the dyslexic group measures of fluency (TOWRE), reading, and spelling were correlated with DTI connectivity indices.

2.2.1 Pilot study participants

For the MEG pilot study described in Chapter 4, healthy adult volunteers were recruited among Aston Brain Centre staff and Aston University postgraduate students. The inclusion criteria were no history of psychiatric illness or neural disorders such as epilepsy, correct or correct-to-normal vision, no history of developmental disorders (such as attention deficit hyperactivity disorder (ADHD), specific language impairment (SLI), dyslexia, autism spectrum disorders (ASD)). The exclusion criteria were the same as listed in the previous section.

2.2.2 Typically developing children

For the control group young volunteers in school age (6 to 17 years old) with no history of educational assessment, developmental, psychiatric or neural disorders, correct or correct-to-normal vision, were recruited to take part to a brain study, involving MEG, MRI and DTI imaging. The recruitment was carried out via the University newsletter and the Aston Brain Centre's Facebook and Twitter profiles. All the children who participated in any of the neuroimaging studies were compensated with two Amazon vouchers (£20). For this study, the MEG protocol was approved by the Aston University Ethics Committee (ethics number 1220). Same as before, the exclusion criteria applied were general contraindications for neuroimaging procedures such as pregnancy, history of epilepsy, claustrophobia, metal and contraceptive implants in the body, current medication for psychiatric disorders or neural conditions (e.g. epilepsy).

2.2.3 Children with diagnosis of dyslexia

The Aston Brain Centre (ABC), along with being a research facility, hosts the Dyslexia and Development Unit (DDAU) which provides a service for assessing specific or general learning difficulties. Families who were addressed to the ABC for educational assessment purposes were approached by one of the trained researchers and invited to take part to an ongoing study investigating genetic influence in people at risk for dyslexia. Prior to this the families were contacted, either via phone or email, by the ABC admin staff to survey whether they preferred not to be approached by the research staff to be potentially recruited to take part to the ABC research.
If they agreed, after obtaining written consent from both parents and young participants, DNA (saliva sample), cognitive and behavioural measures, were collected. At this stage, none of the researchers were aware of the assessment outcome and families were assured that the participation to all the studies was completely voluntary and for research purposes only. At this stage, the children who took part to the first study were rewarded with a £10 Amazon voucher.

Subsequently, the participants and their families interested into participating further in the brain research were invited in the following three to six weeks, to come back to the ABC to take part to the neuroimaging study. For taking part to the neuroimaging study, all participants were compensated with an Amazon voucher (£20) and were offered a picture of their brain. To take part children

had to be between 6 and 17 years old and have an official diagnosis of dyslexia. The diagnosis of dyslexia was based on the Neuropsychologists' report, based on the standardised measures of British Assessment subscales of reading and spelling. Generally, if participant's standardised performance was two standard deviations under the population mean (age corrected), and co-occurrent and co-morbid deficits (ADHD, autism, or specific language impairment) were excluded, the diagnosis of dyslexia was reported. The general exclusion criteria were the same as for the control group, such as pregnancy, history of epilepsy, claustrophobia, metal and contraceptive implants in the body, and other developmental or psychiatric disorders. For the studies involving only dyslexic participants, described in Chapter 3, 6 and 7, the MEG and DTI protocols, as well as additional cognitive and behavioural measures were approved by the Aston University Research Ethics Committee, under the ethics number 488.

2.3 Phenotypic measures

The following section will describe the phenotypic measures used during the educational assessment by the neuropsychologist to ascertain children's cognitive and educational attainment and additional cognitive and behavioural measures collected by the researchers. A schematic summary of the phenotypic measures used throughout this thesis is shown in Table 2.2.

2.3.1 Cognitive and reading skills

The use of cognitive and behavioural measures is a well established and standardised method to understand and define phenotypic differences between typical and a-typical development. For chapters 3, 5, 6 and 7 some of the psychometric measures applied by the neuropsychologist to determine developmental dyslexia or specific learning impairment and to estimate the educational attainment were utilised in this thesis. For instance, measures of reading and spelling were employed to explore additional reading skills within a dyslexic sample stratified by fluency measures in fluent and dysfluent participants (Chapter 3), or to extrapolate the 'reading level' of the dyslexic group, as a whole (Chapter 5 and 7). Within the context of the educational assessment, different age-appropriate cognitive measures of verbal and non verbal skills were employed by the educational psychologist to assess if the child referred had specific or more generalised learning difficulties (Table 2.2). These included measures of:

1. verbal and non-verbal IQ (similarities,vocabulary, matrix reasoning and block design)
2. short and long term memory (immediate and delayed recall of words and digits)
3. language skills (alliteration, rhyme, reading, spelling and writing).

Phenotypic measures	Cognitive/Behavioural domain	Psychometric Batteries
IQ	similarities vocabulary matrix reasoning block design	WISC or WASI
Memory	immediate recall (words/digits) delayed recall (words/digits)	BAS or WIAT
Language skills - Phonology	allitteration rhyme	BAS or WIAT
Reading skills	spelling reading	BAS or WIAT
Fluency	speeded word decoding non-word decoding	Sight Word Efficiency (SWE) Phonemic Decoding Efficiency (PDE)
Handedness	hand preferenece questionnaire measure of relative handedness L/R ratio	Annett Handedness Questionnaire Peg-Q pegboard
Behaviour	personality traits ADHD symptoms	Brief Parental Monitoring Barkley Parents Questionnaire
Family History	Prevalence of dyslexia in the close and wider family	Parents family history questionnaire

Table 2.2 This table lists the phenotypic measures employed by the educational psychologist during the educational assessment (IQ, memory, language and reading skills) with the respective sub-tests used. The last column refers to the psychometric batteries employed. Additional measures collected by the research team are listed: reading single-word fluency measures (TOWRE battery is composed by the sub-tests SWE and PDE), handedness, behaviour and family history. Detailed description of each of these measures follows below.

The standardised psychometric batteries used for the cognitive testing were the British Ability Scales (BAS-II; Elliott (1996)), the Wechsler Intelligence for Children (WISC-IV; Wechsler (2003)), the Wechsler Abbreviated Scale of Intelligence for older children (WASI-IV; Wechsler (1999)) and the Wechsler Individual Achievement Test Second Edition (WIAT-II; Wechsler (2005)).
According to client's age and specific reasons of referral (suspicion of language impairment, dyscalculia, working memory or ADHD problem), the psychometric batteries used by the neuropsychologist to assess these problems oftentimes differed from case to case. Therefore, standardised age-corrected scores were transformed into z-scores, to allow comparisons between different sub-scales, having in that way the same mean and standard deviation (sd), (i.e. WASI and BAS reading and spelling sub-tests).
For the purposes of this thesis non-verbal IQ, total scores of reading and spelling measures were employed to describe the dyslexic group in Chapter 3, 5, 6 and 7. In addition, the researchers administrated the Test of Word Reading Efficiency (TOWRE), that focuses on the word-level reading ability for sight word reading and phonemic decoding. TOWRE is a standardised measure of fluency and accuracy in single-word reading, it is composed of 2 sub-tests, 'Sight Word Efficiency' (SWE) or the ability to read aloud single words and 'Phonemic Decoding Efficiency' (PDE), the ability to read pronounceable non-words. Each subtest (SWE or PDE) consists of a list of words that gradually increases in number of letters and difficulty. Participants were asked to read aloud as many words as they could in 45 seconds time (Torgeson et al., 1999). The total number of words in each subtest provides a measure of accuracy and fluency. The raw scores of the PDE and SDE are also highly correlated and their sum provides a composite total standard score (PDE+SDE), corrected for age. Therefore, the TOWRE offers an efficient and quick way to measure reading fluency.

2.3.2 Behavioural measures

It has been mentioned in the previous chapter that developmental dyslexia often comes with high rates of comorbid or co-occurrent behavioural problems.
For the study of genetic influences in children at risk for dyslexia two behavioural parental questionnaires were utilised. The Achenbach System of Empirically Based Assessment (ASEBA) is a assembly of different instruments for assessing problems, competencies and adaptive functioning. The different batteries were created as a sensible tool to identify syndromes' co-occurring problems, when in 1952 the Diagnostic and Statistical Manual for Mental disorders (DSM-I) had only two diagnostic criteria to describe child psychopathology (Achenbach et al., 2004). The ASEBA sub-test employed was the Brief Parental Monitoring (BPM) for ages 6 to 18 questionnaire which investigates externalizing, internalizing and attention problems. Additionally to check whether there were co-occurent ADHD symptoms in the dyslexic group, the Barkley Parent questionnaire for hyperactivity, inattention and opposite defiant disorder was administered (Barkley and Murphy, 1998). For the purposes of this thesis, these behavioural measures were not included in the analysis, since the small sample size

(N=14). Nevertheless, it was relevant to collect this information in sight of a larger sample analysis and potential co-occurring deficits in the dyslexic spectrum of interest.

DNA collection and analysis

As mentioned above, families who visited the ABC for educational assessment were also invited to take part to a larger cross-sectional study, that investigates the relationship between specific genes (DCDC2 and PCSK6) and dyslexia. After obtaining written consent to take part to the study a saliva sample, using Genotek kit was collected from each participant, see Figure 2.1. The saliva sample was then labelled, sealed and sent to collaborators (Dr Silvia Paracchini) at the University of St Andrews in Glasgow (UK) who proceeded to carry out the genotyping and DNA sequencing. For the DNA analysis, the group led by Dr Silvia Paracchini performs TaqMan technology with an off the shelf assay using a ViiA7 qPCR instrument (LifeTechnologies, Paisley, UK). The genetic information from a sub sample of the children who participated in this study, was part of the MEG-genetic study, described in Chapter 6.

Handedness

Since the beginning of studies in developmental dyslexia and the hypothesis of an abnormal hemisphere lateralisation in dyslexics, cognitive neuroscientists speculated that among dyslexic readers left handedness would be more frequent than expected (Paracchini et al., 2016). Since then the neurobiology has tried to explain the relationships between genetic influences, brain asymmetries and handedness in dyslexic readers. To estimate measures of relative handedness, the PegQ peg-board was utilised, as shown in Figure 2.2. Participants were asked to move the wooden pegs from one row to the other, the test was repeated three times for each hand. PegQ measures the time taken for the participant to move the 10 pegs from one row to the other, with the left and right hand measured separately (Annett, 1985). Additionally, Annette handedness preference questionnaire was administered to ascertain the hand preference from each subject, (Annett and Kilshaw, 1983).

Fig. 2.1 Genotec saliva collection kit

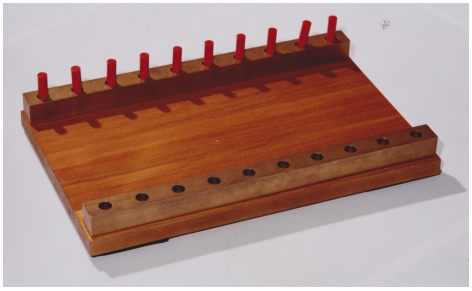


Fig. 2.2 PegQ board used to measure relative handedness.

2.4 Magnetoenecephalography

In 1968 David Cohen published a paper, demonstrating how it was possible to measure alpha activity from the scalp using a device described as 'a magnetic detector in a multilayer shielded chamber' (page 784 in David (1966)). Two years later, Zimmerman et al. (1970) introduced the Superconductive Quantum Interference Device (SQUID), a much more accurate and sensitive magnetic detector, that could measure tiny magnetic fields, much less than one-billionth the strength of the Earth's magnetic field. The demonstration of these experiments led to the use of biomagnetism to measure magnetic fields that are generated by electric currents in living tissues or bioelectrical sources and to the development of the MEG research field (Sternickel and Braginski, 2006).
The first MEG device designed started with 5-7 channel in the mid-1980s, 30-40 sensors in the 1990s, to finally introduce the first MEG helmet in 1992 (Vrba and Robinson, 2001). Modern MEG systems use SQUIDs to detect magnetic fields generated by the electric current in the brain and convert those into 'recordable' electric voltages (Sternickel and Braginski, 2006). SQUIDs are usually used in combination with superconducting pickup coils, acting like antennae. These sensors are mounted in the MEG helmet called dewar, which is cooled by liquid helium and keeps the detectors in super conducting mode. Throughout the years of application and extended use, biomagnetism has proven to be an efficient tool in brain research for the non-invasive estimation of oscillatory neural activity with high temporal resolution. In clinical research and use, it has been shown to be a powerful method to estimate neural dynamics in a range of neural and psychiatric disorders such as epilepsy, brain tumours, dementia, schizophrenia, autism spectrum disorder (ASD), pain research and many others (Agirre-Arrizubieta et al., 2014; Körber et al., 2016; Uhlhaas et al., 2017). For a recent review on the MEG clinical relevance and practise refer to Hillebrand et al. (2018).
Compared to EEG, the advantages of using MEG to measure brain activity are the more accurate source localisation, since the MEG does not take into consideration anatomical layers (skull) in between the sub/cortical origin of the signal and the sensors (Vorwerk et al., 2014); an improved signal to noise (SNR) ratio into capturing activity at higher frequencies (gamma-band) (Lopes da Silva, 2013); the lower contamination of muscular artefacts since the the separation between cortical and muscular and/or cardiac activations is facilitated in MEG acquisition because it does not need a reference as in EEG (Uhlhaas et al., 2017). In addition, it has been shown how MEG can detect deep and gyral sources and how the sensitivity to pick deeper sources depends on the source depth and not on orientation (Hillebrand and Barnes, 2002). Moreover, the preparation prior to the recording session is much shorter and less tedious when compared to the EEG procedures. On the other hand, the MEG is also very sensitive to head movement, which requires subjects to sit very still for long periods of time, during the recordings. This might be challenging sometimes, especially when carrying out developmental studies, hence the recordings might be affected by movement artefacts. Nevertheless, modern MEG system, as the one used in this thesis, have developed efficient movement compensation methods, discussed further in this chapter.

2.4.1 Elekta Neuromag Triux System

All the MEG data in this thesis were acquired with a whole-head 306-channel neuromagnetometer (Elekta-Neuromag, Finland). The system provides a high spatial coverage, given by the coil combination of 204 planar gradiometers and 102 magnetometers. The gradiometers are highly sensitive to focal activity, whereas the magnetometers are more sensitive to deeper or widespread sources. To avoid external noise, the MEG device is situated in a magnetically shielded room (MSR), manufactured by Vacuum Schmelze GmbH (Hanua, Germany), in the Aston Brain Centre, Aston University in Birmingham (UK) Figure 2.3.

Fig. 2.3 Elekta Triux MEG machine in the magnetic shielded room (MSR) in the Aston Brain Centre, Aston University, Birmingham (UK).

2.4.2 Preprocessing

The preprocessing pipeline described below has been employed in chapters 3, 4, 5 and 6.

MaxFilter and head movement compensation

Elekta Neuromag system is provided with a specialised software, MaxFilter, that filters out internal and external interference, reduces measurement artifacts, transforms the data between different head positions and compensates perturbations due to magnetized material on the head and head movements. MaxFilter operates based on the signal source separation (SSS) method, that separates the signal coming from the sensor array (gradiometeres and magnetometers) from the sphere described by the shape of the helmet (Taulu and Simola, 2006; Taulu et al., 2005).

In brief, SSS uses fundamental properties of electromagnetic fields and harmonic function expansions to separate measured MEG data into three components:

- signal originating inside the sensor array,
- signal originating outside the sensor array and,
- noise and artefacts generated by the sensors or sources of interference located very close to them.

The way the SSS denoising works is by discarding the harmonic function components of the signal originating outside the sensors (high spatial frequencies) and by reducing the noise originating within the sensors (Taulu and Simola, 2006) and Figure 2.4. Particularly to be able to identify and suppress interference arising from the sensor array or very close to it, MaxFilter applies the temporal extension of the SSS method (tSSS). tSSS is able to denoise signal coming from magnetized pieces in proximity or on the subjects head such as dental work or braces or by pacemakers or stimulators attached to the subject (Taulu and Simola, 2006).

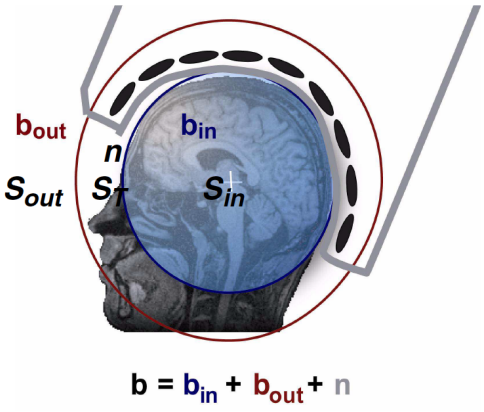


Fig. 2.4 The geometry of Signal Space Separation (SSS). This image shows the components that the signal source separation (sss) method used to measure brain signal. The geometry of this type of Maxwell filtering divides the 'space' into: one hypothetical spherical shell inside of the sensor array (S_{in}) that encloses the brain, and an additional one enclosing all MEG sensors (S_{out}). The radii of the shells are calculated, as the smallest and largest distances from the origin to the sensors location. b_{in} are the brain signals originating inside of the sensor arrays (S_{in}), b_{out} are the external disturbances arising outside of the sensor array (space S_{out}) and n are noise and artefacts generated by the sensors and sources of interference located very close to the sensors (space S_T). The interference is suppressed by omitting the harmonic function components corresponding to highly spatial frequencies, discarding the S_{out} component, and by reducing the S_T-space component n. This method is based on Maxwell's equations, the operation is Maxwell filtering, hence the name MaxFilter. *Adopted from Elekta (2010).*

All the MEG data acquired in this thesis were sampled at 1 kHz and were subsequently pre-processed with MaxFilter software (Elekta Neuromag Oy, version 2.2.10). To ensure the removal of artifacts/noise originating outside and inside/close to the MEG sphere, tSSS was applied with a sliding window of 30 seconds and a sub space correlation limit of 0.9 (Taulu and Simola, 2006).

MaxFilter also provides online head movement tracking function. In addition, the software includes movement compensation method (MaxMove) which relies on the acquisition of the head position coils (HPI), that monitor distance of head movement within the sensors. Based on continuous monitoring HPI coils, the software measures the head position of the subject within the helmet. This information can be subsequently used to compensate for movement or apply 'movement correction' (MC) in the preprocessing phase of the data. When MC is active MaxFilter, first performs SSS or tSSS and thereafter transforms the data, based on HPI continuous positions, to a static reference position (Elekta, 2010). The combination of tSSS and MC has been proven to be very useful in clinical measurements or developmental studies, where head movement is quite common (Nenonen et al., 2012). In participants whose head movement exceeded 5/6 mm and was below 8 mm, tSSS and MC were applied.

2.4.3 MEG and MRI co-registration

In order to perform an accurate source reconstruction of the MEG data, T1 structural MRI images were acquired for each subject. In addition prior to every MEG recording session, locations of five head position (HPI) coils were attached to the participants scalp (on the forehead and behind the ears) and recorded with respect to three anatomical landmark points (nasion and two periauricular points) using a 3-D head digitizer (Isotrak, Polhemus, Colchester, VT, USA). Additional scalp surface points (~450) were digitized to create a tridimensional headshape subsequently used for the co-registration. The way co-registration between MEG 3D headshape and the weighted T1 MRI image works is by applying multiple iterations of a surface matching algorithm with the calculation of the least error, described in (Adjamiana et al., 2004). For this process to work efficiently it is fundamental that fiducial points (nasion and two peri auriculars) and additional landmarks, such as eyebrows, cheeks and the nose, are clearly marked during MEG head digitisation step.

2.4.4 MEG Source reconstruction

The high temporal resolution of the MEG allows to detect, within a millisecond time scale precision temporal dynamic brain activity involved in sensory, motor and cognitive processes. The best way to summarise this rich information coming from a wide array of sensors or detectors, is to reconstruct the generators or sources of these signals (Uhlhaas et al., 2017).
While measuring brain activity on a sensor level helps to understand real time task-specific changes in different brain regions, determining functional 'integration' or connectivity is to measure how

different brain regions communicate or interact between each other (Schoffelen and Gross, 2009). Predicting functional connectivity from sensor level recordings is very challenging due to field spread contamination (a single source affecting multiple sensors). Contrarily, source projection offers a better window to measure oscillatory activity, reducing the effect of field spread, enhancing the spatial localisation and making the results easier to interpret and to compare to fMRI studies, for example Brookes et al. (2011); de Pasquale et al. (2010); Schoffelen and Gross (2009). Generally, the objective of source level connectivity analysis is to measure the information flow and interaction between different brain areas (Schoffelen and Gross, 2009).
To estimate neural sources of activity, or simply, to reconstruct 3D images of the brain activation from a two dimensional MEG measurement represents an inverse problem (Lima et al., 2006). The inverse problem is theoretically insolvable or mathematically 'ill-posed', since it has no unique solution (Hincapié et al., 2017; Larson et al., 2014).
Nevertheless, it is possible to make certain assumptions that can model a plausible forward solution. Providing a forward solution means to calculate magnetic fields, constrained on the assumption of 3D current distributions (an unique problem), and of matching iteratively these to measurements until an acceptable match/solution is achieved. Forward solutions are performed by localisation algorithms that act like spatial filters or beamformers (Sternickel and Braginski, 2006). Generally, beamformer solutions rely on the the premise that different cortical areas cannot be perfectly and linearly correlated in their activation, for some examples see Gross et al. (2001); Hillebrand and Barnes (2005); Hillebrand et al. (2005); Singh et al. (2002); Vrba and Robinson (2001).

Following this, several source reconstruction algorithms have been developed in the past couple of decades trying to provide better solutions for the inverse problem (Brookes et al., 2008; Hillebrand et al., 2005; Sekihara et al., 2002). These can be summed up in three main groups of application: the equivalent dipole approach, the linear distributed sources approach and the spatial filtering approach. The illustration of the first two methods is beyond the purpose of this thesis, for extensive review see Fukushima et al. (2015). In the spatial filtering approach, which is the method used in this thesis (chapters 3, 4, 5 and 6), an optimal spatial filter maps sensor-level measurement to the current source amplitude, using each single point of a spatial grid or atlas (Fukushima et al., 2015; Hillebrand et al., 2012).

Atlas (AAL) based beamformer was applied to estimate resting state functional connectivity in chapters 3 and 6. Detailed description can be found below and in Hillebrand et al. (2012). Another well-established method of spatial filtering is the linear constrained minimum variance (LCMV) beamformer (Van Veen et al., 1997). Spatial filter derives from the weighted sum of of the data recorded at different sites, or sensors. The weights minimise the power output by the linear constraint, which imposes the beamformer (or spatial filter) to pass brain signal from a specified location while ignoring activity from different locations (Van Veen et al., 1997). The resulting power map is then normalised by the noise power, as a function of location, and it results in a neural activity index map. The inverse operator, thus, minimises the variance at the filter output subject to a linear constraint

(Hincapié et al., 2017). This also assures that the stopband response is small in locations different to the one being filtered (Hincapié et al., 2017). LCMV does not require any a priori assumptions about the number of active sources, since it is based on the spatial covariance of the electrical activity. LCMV was applied for the source reconstruction in Chapters 4 and 5.

There are numerous ways of measuring functional connectivity and the MEG field is far from reaching consensus on best or most recommended methods (Gross et al., 2013). Nevertheless, the literature seems to agree that there is no best, or most reliable, method to estimate functional connectivity. The choices should, instead, be dictated by the most suitable analysis framework to the nature of the data acquired. Thorough reviews about limits and pitfalls of different source reconstruction methods, and best practise in the MEG connectivity field are provided by Bastos and Schoffelen (2016); Colclough et al. (2016); Hincapié et al. (2017); Palva et al. (2018). In the case of MEG source analyses applied in this thesis, for Chapter 3 studying RSFC, Phase Lag Index (PLI) has been chosen as the preferred method to measure functional connectivity. For Chapters 4 and 5, the LCMV beamformer was applied to source localise the activity during "Super Mario" task. In the first instance, the choice of the PLI was driven by the hypothesis of reproducing the analysis pipeline described by Hillebrand et al. (2012), with child data. Secondly, the choice of the LCMV source localisation was dictated by previous and similar studies of evoked/induced activity, not having a specific frequency or ROIs based hypothesis (Hincapié et al., 2017).

2.4.5 Resting State Functional Connectivity- RSFC

A wide number of neuroimaging studies has shown how the brain is one complex system composed by large-scale distributed networks whose intrinsic organisation and functional integration is at the basis of behaviour and cognition (de Pasquale et al., 2010; Fox et al., 2005; Mantini et al., 2007; Raichle et al., 2001). These networks are highly activated in task-free conditions and have been defined resting state networks (RSN), for more details refer to Fox and Raichle (2007); Fox et al. (2005); Mantini et al. (2007). Given its high temporal resolution MEG-source analysis provides the perfect tool for measuring resting state functional connectivity (RSFC) and to understand intrinsic large-scale oscillatory activity with real-time precision (Baker et al., 2014; Brookes et al., 2011; Colclough et al., 2016). For instance, studying MEG RSFC has been proven to be able to define neurophysiological differences between healthy and clinical populations (Stam et al., 2014; Uhlhaas et al., 2017; Uhlhaas and Singer, 2006); or how spontaneous oscillatory activity exhibits frequency-specific spatial patterns of activity (Hipp et al., 2012). As described in the next paragraph, the topology of the connectivity in the brain is distributed according to small world networks' proprieties (SWN, e.g. Bassett and Bullmore (2006)), which makes some connections (hubs) more important than others (Uhlhaas et al., 2009). Following this theory, to be able to process information both architectures of connections (structural connectivity), that coordinate and bind functions through convergence/divergence, and dynamics allowing self-organisation of spatio-temporal activity, need to interact proficiently (Uhlhaas et al., 2009). High temporal resolution techniques such as EEG and MEG are very useful into

measuring neural activity with specific temporal patterning, as network oscillations, synchronisation, and phase locking (Uhlhaas et al., 2009). There have been identified specific cortical oscillations associated with cognitive behaviours: slow oscillations (theta rhythm, 4-8 Hz) have been associated with frontal midline and subregions of the prefrontal cortices implicated in behavioural monitoring and valuation of responses outcomes, and top-down processing; reduced alpha (8-13 Hz) is associated with attention; while beta band (15-30 Hz) is generally thought of as the preparation and inhibitory control of the motor system, sensory-motor integration, and top-down signalling; highest frequency oscillations (e.g gamma band, 40-80 Hz) have been identified as an 'attentional finger print' too (Wang, 2010). These are just examples of neural frequency-related brain dynamics, during cognitive tasks. Nevertheless, brain's neurophysiology is way more complicated than that. For example circuit synchronisation, depends on synaptic inhibition, hence the constant interaction between excitatory and inhibitory neural populations, that is based on cross-frequency coupling or interactions (Popov et al., 2018b; Wang, 2010). For these reasons, it is important to be able to study the resting state functional connectivity (RSFC) or task-free conditions, to be able to better understand what might drive or underlie cognitive functioning.

For the analysis of resting state functional connectivity (RSFC) in Chapters 3 and 6, an atlas-based beamformer solution was applied to measure functional connectivity using phase lag index (PLI). To define a set of atlas based Regions of Interest (ROI), helps to better interpret the oscillatory dynamics in anatomical space, provides a robust way to perform statistical analyses, and a common platform to compare results obtained with other modalities, such as MEG task-positive or DTI data (Hillebrand et al., 2012). Moreover, using Phase Lag Index (PLI) as a frequency specific estimator of functional connectivity prevents volume conduction potential biases. This method has been proven to be able to efficiently measure frequency-band dependent patterns of eyes-closed resting state functional networks, and to be more robust compared to other connectivity measures, such as coherence (Hillebrand et al., 2012). By using different M/EEG data sets, (64 coupled oscillators, EEG with absence seizure, data from Alzheimer patients and controls, and two MEG data sets) Stam et al. (2007) have described how compared to imaginary coherency and phase coherence, the PLI as a measure of asymmetry of the distribution of phase difference, is a better estimate of true synchronisation, and less sensitive to common sources or volume conduction problems. Details of the analysis pipeline and Phase Lag Index (PLI) computation can be found in Chapter 3. In brief, the beamforming technique is used to estimate time-series for a set of atlas ROIs and, following, mean PLI or 'weighted degree' is calculated for the estimation of functional connectivity (FC) between these regions, Figure 2.5 illustrates the Hillebrand et al. (2012) analysis procedure.

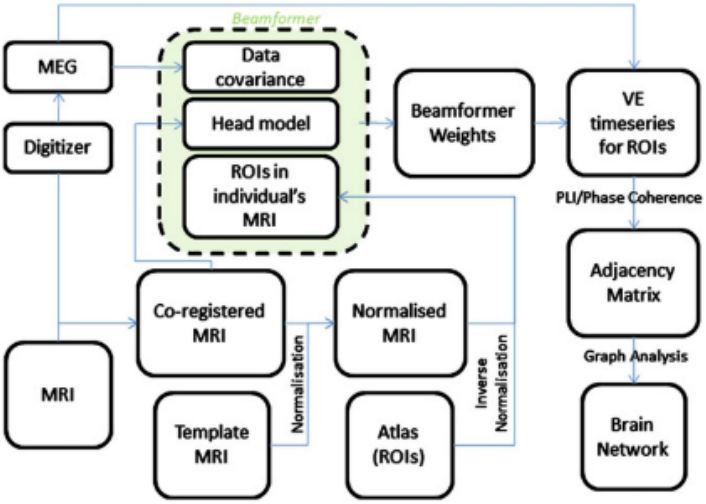


Fig. 2.5 This figure shows the MEG RSFC analysis steps: in the preprocessing phase, individual MRIs are MEG co-registered, and further used to define the atlas ROIs using a Talairach system (or atlas by Gong et al. (2009). The volume conduction model is based on the co-registered MRI and the frequency specific data covariance matrix is then used to compute the beamformer weights in order to create virtual electrode (VE) time series for the ROI voxels. For each frequency band of interest, PLI between different ROIs is estimated and the resulting adjacency matrix can be further utilised for network analysis, see next paragraph. *Adapted from Hillebrand et al. (2012)*

2.5 Network analysis

The increased number of whole-brain connectivity studies investigating functional associations and structural proprieties (anatomical connectivity) has opened the way to the application of network theory in the study of normal, impaired and developing brain function (Mišić and Sporns, 2016; Rubinov and Sporns, 2010; Sporns et al., 2005; Stam and van Straaten, 2012). Network brain analysis arises from the necessity to adopt a simple and 'unbiased' method able to describe the main features of different cortical processes, that can integrate information across different neuroimaging modalities (functional and structural MRI, EEG, MEG etc.) conditions, behavioural states and neurological/psychiatric diseases (Bullmore and Sporns, 2009; Newman, 2010; Stam and van Straaten, 2012). Mathematically, complex systems in nature can be represented by a collection of nodes (vertices) and links (edges) between pairs of nodes (Rubinov and Sporns, 2010). The exact 'mathematical formalism' or theoretical framework that the modern network theory builds upon, is graph theory (Stam and van Straaten, 2012).

2.5.1 Graph Theory

Graph theory originated from Euler's work, in particular the solution to the 'seven bridges Köninsberg problem', (Euler, 1736). Early applications of graph theory in neuroscience used the small world network (SWN) and the scale-free concepts to describe structural and functional networks (Bullmore and Sporns, 2009; Sporns and Zwi, 2004). Small world networks (SWN) efficiently combine local specialisation and global integration by following a power law degree (nodal) distribution (Barabasi Albert-Laszlo et al., 1999; Stam, 2014; Strogatz, 2001). According to Barabasi Albert-Laszlo et al. (1999) nodes or vertices connectivity in large networks follows a scale-free power law distribution where, in the network expansion, the addition of new vertices is preferentially 'aggregating' or connecting to nodes already well connected, (for a complete overview of network science refer to Park and Friston (2013); Stam (2014); Stam and van Straaten (2012). Small world and scale free characteristics have been associated to optimal cognitive functioning. A deviation from this 'optimal' SWN and scale-free topology has been linked to impaired cognitive functioning and neuropsychiatric disorders (Alexander-Bloch et al., 2010; Stam, 2014). In graph theory, small networks are represented ad sets of network nodes or vertices and their network connections (edges), as illustrated in Figure 2.6 (Stam and van Straaten, 2012).

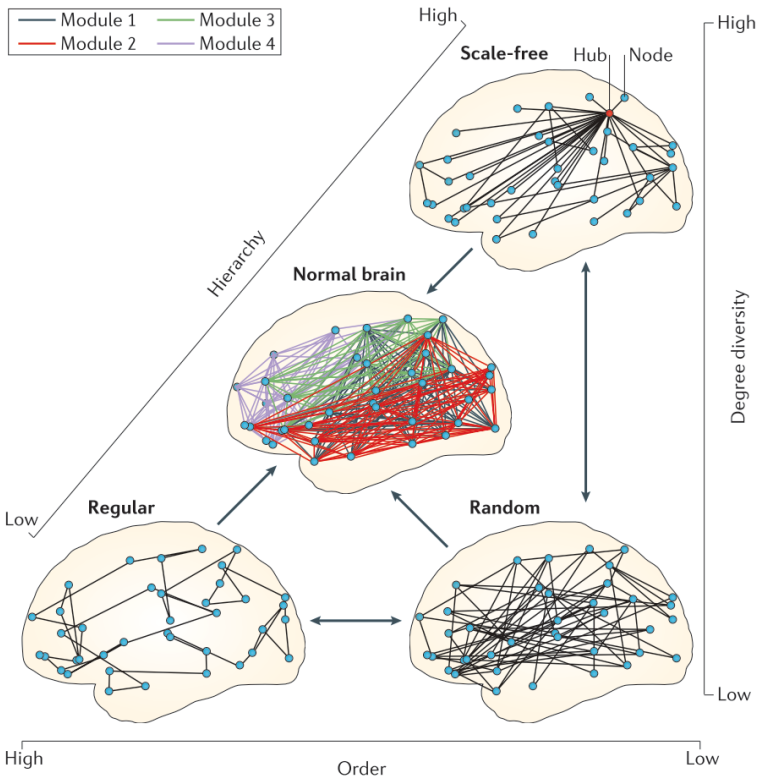
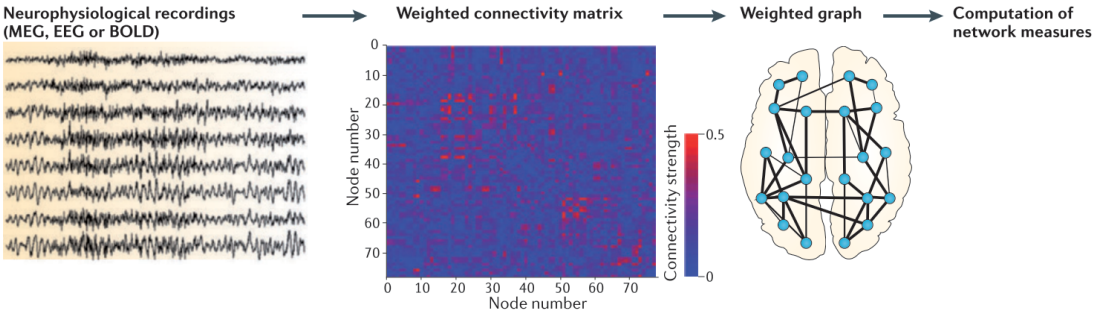


Fig. 2.6 Schematic representation of the Small World Network (SWN) proprieties and scale-free distribution in the study of brain networks. The organisation of normal brain networks. Normal brain's network configuration has an intermediate morphology between regular (highly ordered), random (disorganised) and scale-free (rich nodal configuration or 'hubness' and high hierarchical distribution) networks. According to the scale-free SWN-ness the result of the optimal normal brain functioning is the combination of these three elements, which result in a heriarchical modular network. *Adapted from Stam (2014).*

Following this, it is possible to illustrate mathematically the network or graph proprieties of functional connectivity matrices, Figure 2.7. In this thesis, the large-scale network dynamics connectivity estimates of ROIs, as PLI in this case, can be conceptualised as values (nodes) reflecting the importance of that region in the functional network, see Hillebrand et al. (2012); Stam and van Straaten (2012) for an example .



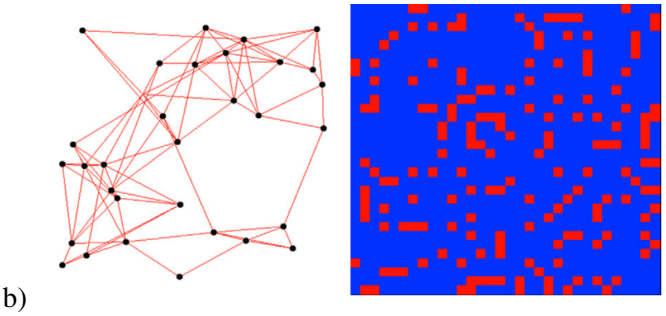


Fig. 2.7 From empirical data to network analysis. a) In principle to be able to apply network analysis to the empirical data it is important to define nodes and edges. In case of M/EEG a node can be a sensor or a source. Links are defined by the measures of synchronisation between two nodes (MEG sources). Therefore, the empirical data can be represented by an NxN connectivity matrix (Figure 2.7 a) and each matrix cell representing the presence or not of strength of connection or relation among nodes (connectivity). This matrix can then be analysed as a weighted graph. b) Mathematical representation of the PLI adjacency matrix on the right and the derived undirected and unweighted graph, on the left. This graph has the same number of nodes as the same number of rows and columns in the matrix *Adapted from Stam (2014); Stam and van Straaten (2012).*

Graph metrics, or topology indices such as clustering coefficient, path length and efficiency measures are used to characterise small-world proprieties of the network (Bassett and Bullmore, 2006), whereas metrics such as degree, betweenness, closeness and eigenvector centrality are used to identify the crucial areas within the network (De Vico Fallani et al., 2014). To be able to reconstruct the network based on pairwise estimates of functional connectivity, and extract relevant metrics that describe topological properties of the network, a lot of arbitrary decisions have to be made. For

example, the application of appropriate statistical procedures and thresholding of the graph metrics (graph size or number of nodes), may give rise to false positive results or make different size networks not comparable, for an extensive review refer to De Vico Fallani et al. (2014). One way to address this issue is to implement graph theory measures that do not imply any thresholding, such as Minimum Spanning Tree (MST), which is the network analysis method applied in this thesis, in chapters 3 and 6.

2.5.2 Minimum Spanning Tree

To describe topological distributions of the resting state functional connectivity (RSFC) examined in this thesis (chapters 3 and 6), Minimum Spanning Tree (MST) was implemented. MST is defined as an a-cyclic subgraph of the original network, that offers a very elegant solution to traditional network approaches methodological limitations, such as comparability between different network sizes or different number of nodes, for an extensive review refer to Tewarie et al. (2014b, 2015). In brief, MST is mathematically defined as the sub-network that connects all the nodes of the tree while minimising the link weights and without forming loops (Tewarie et al., 2015). The assumptions underlying the MST application are that all the nodes of the tree are connected and that their edges or links are unique. This means that the MST analysis is insensitive to traditional 'scaling' or thresholding required in other graph theories applications, since it is a hierarchical and ordered reconstruction of the strongest connections in the tree of interest (Tewarie et al., 2015).
MST has been shown to be a reliable graph theory method to explore life-span and developmental changes in functional network topology in young children as well as in developmental dyslexia, and in neuropsychiatric disorders (Boersma et al., 2013; Demuru et al., 2013; Fraga González et al., 2018b; Smit et al., 2016; Tewarie et al., 2014a). More details about the MST algorithm, different MST metrics and MST literature can be found in the methods section of Chapter 3.

2.6 Diffusion Tensor Imaging - DTI

While functional connectivity studies have defined links between brain activation and cognitive functions, structural connectivity investigations have explained the underlying anatomical architecture of these functional networks (Mišić and Sporns, 2016; Park and Friston, 2013; Sporns et al., 2005). In vivo imaging, the neuroimaging technique used to describe large-scale structural neural proprieties is called Diffusion Tensor Imaging.

Diffusion Tensor Imaging, hereafter DTI, is an MRI derived method that provides important information about the microstructural and physiological proprieties of the tissues of interest (Basser and Pierpaoli, 1996). The effective diffusion tensor (D) derived for a voxel, provides information about features of anisotropic and isotropic proprieties of the fiber-tract organisation (Basser et al., 1994; Basser and Pierpaoli, 1996). In neuroimaging DTI is used for white matter (WM) tractography. In principle, since water 'diffusion' or movement has been found to be much faster along the direction of the white matter fibres than in the perpendicular one (Basser and Pierpaoli, 1996), the difference between perpendicular and parallel to the fibres motion is at the basis of diffusion imaging (Assaf and Pasternak, 2008). Therefore, DTI is a technique for quantification of white matter proprieties (Basser and Pierpaoli, 1996) and can be used to compare the integrity between WM tracts across subjects. This type of tractography technique allows to compare regions with highly aligned and hence myelinated (more integrated) axons, with regions with less myelination, and thus less integrated or coherent, organisation of axons (Dougherty et al., 2005).
The tensor decomposition extracts the 'diffusivities' of the fibre, that are used to calculate summation indices, such as fractional anisotropy (FA), fibre direction and coherence index (Assaf and Pasternak, 2008; Basser et al., 1994; Basser and Pierpaoli, 1996; Dougherty et al., 2005). The specific physical properties of the calculation of the gradients are beyond the scope of this thesis, for a technical review refer to Beaulieu (2002). Generally, FA has so far been one of the most applied DTI measures when it comes to brain tractography (Assaf and Pasternak, 2008). FA is a measure of microstructural features of a voxel and it represents the orientation of of diffusion (iso/aniso - tropic). Chiefly, it is assumed that high FA values within a voxel mean high 'diffusionality' or direction diffusion, as the ones observed in white matter fibres (Dougherty et al., 2005).

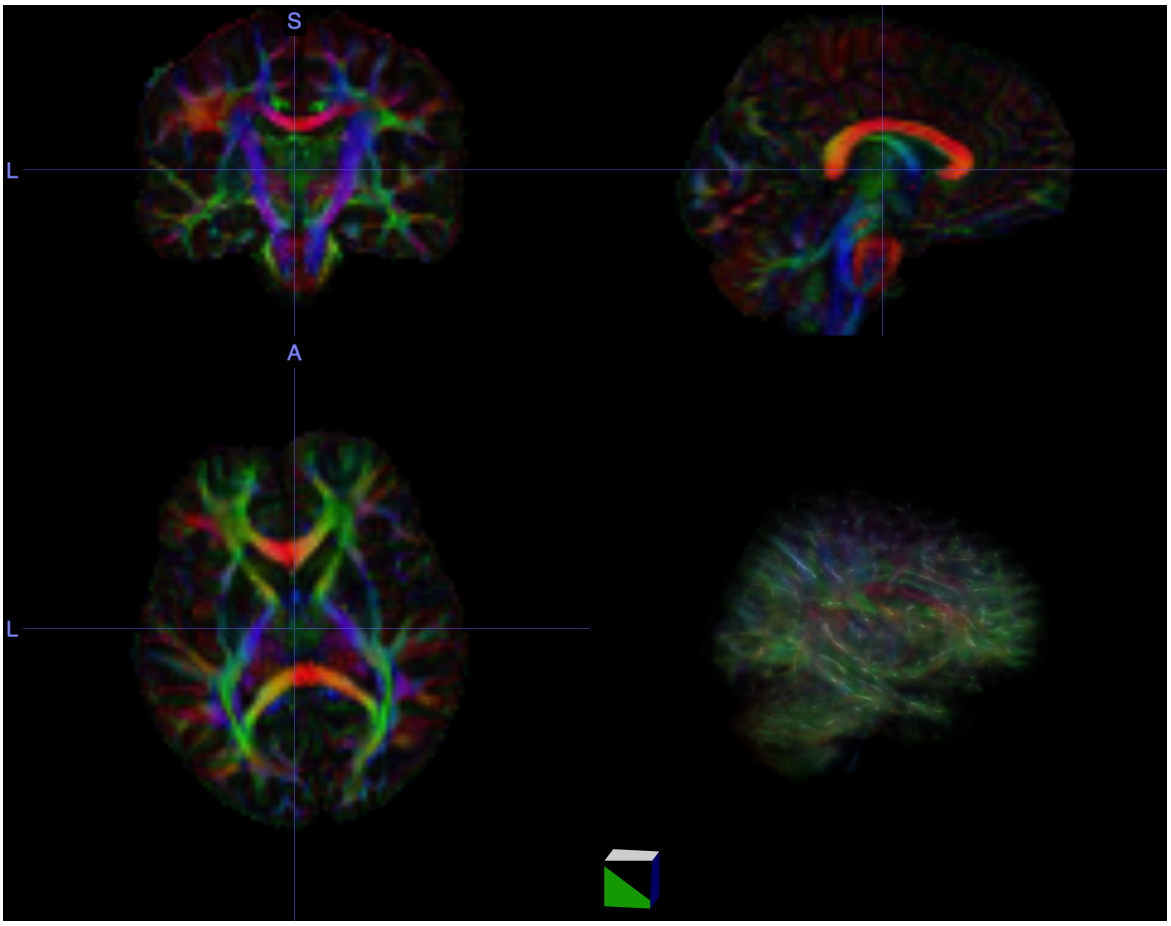


Fig. 2.8 This picture shows how DTI raw data looks like after acquisition. This DTI sequence was acquired on a 14 years old boy.

DTI has the big advantage over other MRI techniques, such as fMRI, because it can discriminate between the tissue structure of the white matter, that means that different patterns of water diffusion (*anisotropy*) will indicate differences in the fibre proprieties, where more myelination, higher FA values means more integration, and less myelination, lower FA means less coherence in the axon organisation (Dougherty et al., 2005).
Although a relatively young neuroimaging technique, DTI has been widely applied in brain research to test effects of disease or condition on specific structural ROIs (Deshpande et al., 2015). This methodology has allowed to study WM architecture and integrity of normal and pathological (multiple sclerosis, stroke, ageing, dementia, schizophrenia, etc.) conditions , (Assaf and Pasternak, 2008). Moreover, it has been proven to be a sensitive measure of life-span structural changes, in terms of axon packing and myelination, especially in studies looking at developmental changes in brain maturation (Asato et al., 2010; Lebel and Beaulieu, 2011). In this thesis, DTI has been applied in chapter 7 to study the diffusion proprieties in one of the core structures of the reading network, the left arcuate fasciculus (FA). In particular, FA values in AF have been compared between typical and atypical young readers. The details of the DTI data acquisition and analysis can be found in chapter 7.

2.7 Conclusion

This chapter aimed to provide a general introduction to the methods employed in this thesis. It described participants, phenotypic measures, different neuroimaging modalities utilised, followed by data acquisition and general analysis procedures. It serves as a general guide of methodology used in each of the experimental chapters.

Chapter 3

Resting state functional connectivity and network topology in developmental dyslexia

Chapter summary

This chapter will describe a study investigating MEG resting state functional connectivity (RSFC) of different dyslexic reading phenotypes. Based on cognitive measures of fluency (TOWRE) a group of dyslexic young readers was divided in fluent and dysfluent sub-groups. By applying an atlas-based (AAL) MEG beamformer approach, a detailed anatomical mapping of neurophysiological patterns for different cortical (alpha, beta, theta and delta) rhythms was obtained in source space. Phase Lag Index (PLI) was used to estimate functional connectivity. Subsequently, to describe the network topology the Minimum Spanning Tree (MST), an a-cyclic subgraph that connects all nodes of the tree in a unique way and forms the backbone of the functional network, was reconstructed. The topology of the MST was characterised using different global (diameter, leaf and tree hierarchy) and local measures (eccentricity, degree and betweenness centrality). Overall, this study shows how fluency can be used as a neural classifier of RSFC and network topology in different sub-types of developmental dyslexia. This is a first example of a source space MEG study investigating intra-dyslexia variability based on single word reading profile.

3.1 Introduction

Reading is a complex process which requires distant areas to work efficiently in synchrony. It has been widely accepted, by now, that developmental dyslexia is linked to reduced neural synchronisation in regions of the left hemisphere reading network (for detailed metanalyses see Martin et al. (2016); Richlan (2012); Richlan et al. (2009)). In particular, activation in left posterior temporal, inferior parietal lobule and left frontal gyrus seems to be reduced in impaired readers (Richlan, 2012).
Recent advances in neuroimaging methods focusing on functional connectivity, showed how dyslexia is not merely represented by 'underactivation' of the left reading network but it is also characterised by reduced connectivity between these regions (Koyama et al., 2013; Quaglino et al., 2008; Wise Younger et al., 2017). Furthermore, DTI studies showed how fibre tracts connecting some of these core regions have reduced structural connectivity in dyslexics compared to their peers (for more details about DTI studies in dyslexia see Chapter 7 and review by Vandermosten et al. (2012a)).
Nevertheless focusing the connectivity analyses only on task based or specific regions of interest comes with the risk of neglecting important information about whole brain network integration and synchronisation (Rubinov and Sporns, 2010). Within the brain networks framework, Stam et al. (2016) used a dynamical model to understand how aspects of structure-function relations are detected by different connectivity measures (functional and effective connectivity), and if the structural degree of brain areas might influence the strength of functional interactions in absence of direct links. The authors looked at similarities between modularity for structural network, functional, and effective connectivity by using a simple model derived from infection (susceptible–infected–susceptible) dynamics (Stam et al., 2016). This study is relevant to this chapter, since the ROIs extracted used the same AAL, described below (Gong et al., 2009). The results showed how functional connectivity measure, based upon the correlation coefficient of integrated node activation time series, was very similar to the ones obtained with BOLD time series (Stam et al., 2016). In addition, the effective connectivity network was shaped by strong connections between high degree hubs, with a similar pattern to MEG PLI networks observed previously (Stam et al., 2016; Tewarie et al., 2014a). These results suggested that the structural degree product of two areas might be strongly predictive of the intensity of the traffic between them (Stam et al., 2016).
To better understand the relationship between task-free (RSFC) and brain networks active during task performance, Di et al. (2013) studied meta-analytic coactivation task-based patterns, and compared them to the RSNs. The authors showed how if two regions had higher correlation in the resting-state network, they also had higher coactivation strength in the task conditions Di et al. (2013). However, the network configuration analysis revealed different configuration in task-based coactivation vs task-free, where the coactivation network had higher global efficiency, lower mean clustering coefficient, and lower modularity, when compared to the RSNs (Di et al., 2013). There was also a low correlation of node degrees between the two networks; this has been explained as a hub 'shift' between task performance and resting state (Di et al., 2013). In sum, task-free network exhibited greater small-worldness, facilitating global information transmission, while the task-free one had higher modularity

(the extend the brain network can be divided into sub-communities), (Di et al., 2013).
Furthermore, task-based connectivity studies have several methodological challenges, especially when applied to developmental populations where it is difficult to constrain task specific activations from underlying developmental and cognitive mechanisms (Church et al., 2010). Therefore the challenge for task-based neuroimaging studies focusing on reading is to design an 'ideal task' that would be able to probe the reading network, taking into account age and educational level dependencies (Koyama et al., 2010). Investigating resting state functional connectivity (RSFC) or task independent activity might offer a solution to these problems, since it helps to understand how these areas communicate between each other and their underlying cortical architecture (van den Heuvel and Hulshoff Pol, 2010).
Resting state functional connectivity (RSFC) fMRI studies of reading have shown a relationship between resting state networks (RSNs) and reading ability, where reading putative areas were positively correlated between each other at rest (Koyama et al., 2011; Schurz et al., 2015; Zhao et al., 2011). For instance Koyama et al. (2011) demonstrated how RSFC-behavioural measures positive correlation in children was based on increased connectivity between inferior parietal cortex (reading ROI) and subcortical attention regions (thalamus), this association was specific for children and was absent in adult skilled readers.
Differently, the same type of study has shown contrasting patterns of RSFC in developmental dyslexia. In a task-independent fMRI study Koyama et al. (2013) showed how reduced intrinsic functional connectivity in the dorsal attentional network (left inferior parietal sulcus and middle frontal gyrus), was associated with dyslexia. Furthermore, in a fMRI task-positive and task-independent study in typical and impaired readers, Schurz et al. (2015) found how dyslexics exhibited reduced functional connectivity in left posterior temporal areas and the left inferior frontal (IFG) gyrus, suggesting a permanent disruption between frontal and temporal areas both during task demands and at rest. The opposite pattern of increased functional connectivity, was found between reading-related areas and posterior/midline cortical areas (precuneus/cuneus) of the default mode network, (Schurz et al., 2015). Additionally, Vogel et al. (2012) found an association between reading abilities and the abnormal resting state functional connectivity between visual form areas and the dorsal attentional network, being more related to spatial and feature attention processes. In a whole-brain fMRI connectivity study of younger and older typical and dyslexic readers, Finn et al. (2014) showed how deficits in connectivity related to dyslexia are not constrained to reading areas only, and involve a wider network of regions: such as decreased connectivity in the visual pathways, increased connectivity to limbic regions and the DMN, reduced and altered connectivity to the VWFA and, persistent connectivity to a left-hemisphere anterior language region.
Overall, these studies suggest that RSFC in dyslexia presents a complex mosaic of areas activated, partially overlapping but not limited to those typically associated with reading (Fraga González et al., 2018b; Richlan et al., 2011).

3.1.1 Current study

While the MRI (fMRI and DTI) literature has widely explored task-free functional connectivity in developmental dyslexia (for extensive reviews refer to Richlan (2012); Richlan et al. (2009)), there is still paucity of M/EEG studies looking in that direction.

Notably, some EEG studies have found differences in long-range connectivity patterns suggesting reduced global efficiency in dyslexics compared to non impaired readers (Dimitriadis et al., 2013; Fraga González et al., 2016a, 2018b; Pagnotta et al., 2015; Vourkas et al., 2011). For instance, in a MEG study Dimitriadis et al. (2013) showed how children with reading difficulties display significantly reduced global efficiency and reduced beta band local efficiency in the left temporoparietal regions at sensor-level. Global efficiency was estimated using a distance matrix, produced from mapping functional connectivity weights to pairwise distances, and it corresponds to the inverse of the harmonic mean of the shortest path length between each pair of nodes. Or simply, global efficiency is the total efficiency of parallel transformation transfer (Dimitriadis et al., 2013). Local efficiency, was calculated as a measure of fault tolerance of the network, or how well each subgraph exchanges information when the indexed node is eliminated, (Dimitriadis et al., 2013). This study applied the weighted phase lag index (wPLI) as measure of functional interdependencies between MEG sensors, in a sliding window fashion (Dimitriadis et al., 2013).

Particularly relevant for the current chapter is the study by Fraga González et al. (2016a) who compared EEG resting state connectivity between a group of dyslexic and typical young readers (3rd grade), using Phase Lag Index (PLI) to estimate functional connectivity, and Minimum Spanning Tree (MST) to describe network topology. The authors found that, although there was no difference in PLI connectivity measures, the MST had a peculiar profile in impaired readers. The analysis showed how the dyslexic group had a less integrated network, based on the lower leaf fraction and higher MST diameter (Fraga González et al., 2016a). In a recent investigation, the same pipeline was applied to study EEG RSFC in adulthood. In the comparison of PLI and MST between dyslexic and adult readers Fraga González et al. (2018b) found significant results in alpha band (8-13 Hz). Specifically, MST analysis revealed higher global values (kappa, mean, degree) in dyslexic participants, which suggested a more integrated global network at the disadvantage of specialised sub-networks (Fraga González et al., 2018b).

These studies represent a valid case to support the application of network analysis into investigating the widespread organisation of functional connectivity in developmental dyslexia. Within this framework, MST analysis offers a robust tool to measure potential differences in configuration of reading functional networks, when the connectivity strength stays preserved (Fraga González et al., 2018b; Stam et al., 2014). Furthermore, evidence from task-dependent studies shows how functional connectivity estimates correlate with levels of reading ability (Milne et al., 2003; Vourkas et al., 2011). For example, in a task-dependent EEG connectivity study Žarić et al. (2017) showed how different levels of (dys)fluency among a group of 9-year old dyslexic readers exhibited different patterns of functional connectivity.

What still remains unexplored is the possibility to study different patterns of RSFC and network topology *within* the dyslexic spectrum, in MEG source space. The aim of the current chapter is, therefore, to describe dyslexia's intrinsic heterogeneity by examining resting state functional connectivity (RSFC) and network topology (MST) in dyslexic young readers with different reading profiles. Specifically, the main goal of this study is to examine whether different levels of fluency (single-word and non word reading), could be used as classifiers of MEG RSFC and minimum spanning trees (MST) phenotypes in a group of children with a recent diagnosis of developmental dyslexia.

3.2 Method

3.2.1 Participants

As described in chapter 2, the recruitment of dyslexic participants was carried out by approaching families referred to the DDAU on the day of their visit to the ABC for educational assessment purposes. Here, participants were invited to, first of all, take part to the behavioural-genetics longitudinal study investigating associations between dyslexia-related genotypes (DCDC2 and PCSK6), reading scores and behavioural measures, for an example see Scerri et al. (2011, 2017). If participants consented to be recruited in this first part, a saliva sample, measures of hand preference and fluency testing (word and non-word reading TOWRE test by Torgeson et al. (1999)) were collected. Following this, children were invited to return to the ABC, for a second visit, to take part to the neuroimaging study. Throughout three years of recruitment (October 2015 - October 2018), approximately 35% of families approached agreed to take part in the neuroimaging study. To take part to the study, the inclusion criteria were normal, correct-to-normal vision, no history of epilepsy, psychiatric disorders, age between 6 – 17 years old. Overall, thirty-seven (n=37) participants (mean age 12 years old) took part in the current study. Among these, thirty-one (n=31) were included in the current analysis: 3 participants were excluded because did not have diagnosis of dyslexia and 3 for excessive head movement during the MEG recordings, for descriptive measures see Table 3.1. One quarter of the participants (n=8) also took part to an additional MEG study, described in chapter 5, either prior or after in a pseudo-randomised fashion, the resting state paradigm.

Following this, the whole group (n=31) was divided in two sub-groups, fluent (n=17) and dysfluent (n=14) dyslexic readers, based on their performance at the Test Of Word Reading Efficiency (TOWRE) by Torgeson et al. (1999). TOWRE focuses on the word-level reading ability, for sight word reading and phonemic decoding. More details about the administration of this test can be found in the Phenotypic measures section in the methods chapter (chapter 2).

In sum, the whole dyslexic sample was stratified based on the total standard score at TOWRE (TOWRE ss). Total Word Reading Efficiency is given by the sum of standard scores at the sub-tests of Phoneme Decoding Efficiency (PDE) and Sight Word Efficiency (SWE). PDE measures the ability to decode non-words or how efficiently one can read unfamiliar words, whereas the SWE is a test that measures single word reading as a whole units (Tarar et al., 2015). The dysfluent group comprised all the

participants whose TOWRE ss < 89, considered below average; and the fluent group included all the participants whose TOWRE ss > 89, as an above-average performance.

3.2.2 MEG data acquisition and preprocessing

The detailed procedure of MEG data acquisition and initial preprocessing procedures are fully described in chapter 2, including the pre-MEG head digitisation and HPI coil registration.
For this particular task, to make sure the instructions were understood properly, prior to the MEG recording, all children had a 'mock' run through the experiment outside the MSR. Six minutes of alternating (1,5 min each) eyes-open (EO), eyes-closed (EC) resting state paradigm were recorded. Subjects were asked to sit comfortably in a semi-reclined chair and stay as still as possible (avoiding head movements, excessive blinking, swallowing or teeth clenching). During the eyes-closed condition they were instructed to relax and think about anything in particular and let their mind wander spontaneously. During the eyes-open condition they were asked to look at the fixation cross projected centrally on a grey screen. The task was structured as follows: 90 seconds eyes-closed, 90 seconds eyes-open, 90 seconds eyes-closed, 90 seconds eyes-open. Short periods of eyes closed/open were alternated because it has been noticed that children cannot sit still for long periods without moving too much and/or falling asleep.
All the data was preprocessed using the MaxFilter software (Elekta Neuromag Oy, version 2.2.10) with the tsss (temporal extension of signal source separation) method and for participants whose head movement exceeded 6 mm, movement correction was additionally applied. Subsequently, MEG individual T1 MRI images coregistration was carried out, using an in-house Elekta pipeline, described in the methods section (chapter 2).

3.2.3 MEG source analysis

The analysis pipeline is illustrated in Figure 3.1. The first step was to project the sensor level MEG signal in a set of source time series, using beamformer weights. For each subject, a set of 116 regions of interest (ROIs) were defined using Gong et al. (2009) atlas. Then, the projection of cortical and sub-cortical atlas ROIs onto the individual T1s was visually inspected to make sure that the spatial normalisation between the AAL voxels and individual MRI images was accurate. For further analysis, subcortical ROIs of the AAL were discarded, resulting thus in a set of 78 virtual electrodes (VE) or ROIs in the template space. Following this, beamformer weights were applied to extract each VE time series in the 0.5-48 Hz frequency band, same as in (Ponsen et al., 2013). Following this, for each subject 5 artefact-free epochs of 16.384 samples ($\sim$16 sec) of data were selected using Elekta Graph interface and converted to ASCII files in order to be analysed further with the Brainwave (Version: 0.9.152.12.26) freely available software [1].

[1] BrainWave: http://home.kpn.nl/stam7883/brainwave.html

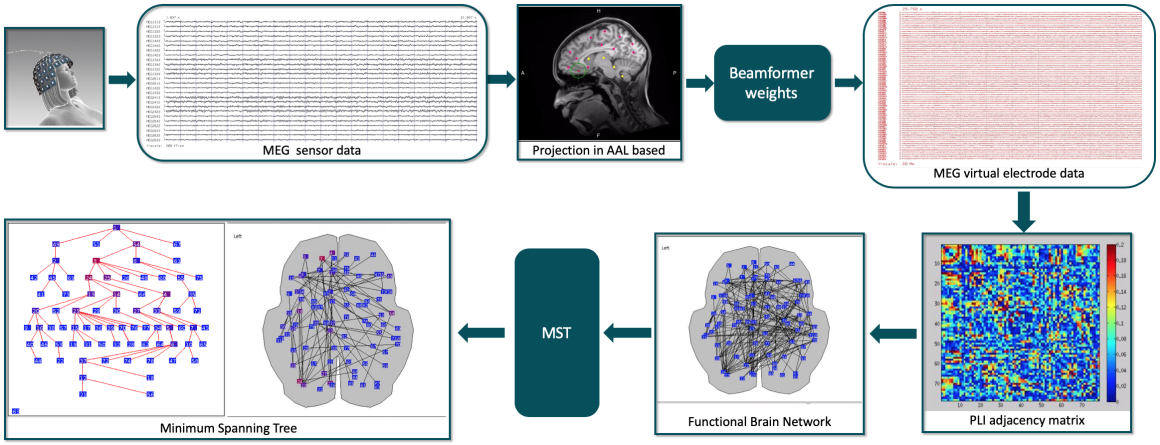


Fig. 3.1 MEG Resting State Functional Connectivity (RSFC) analysis pipeline. MEG sensor level data was projected into individual source space, using an atlas based beamformer of 78 regions of interest (ROIs) or virtual electrodes (VE). The 78 ROIs were based on predefined adult atlas by Gong et al. (2009) and used as a spatial filter. The MEG sensor-level data was projected onto individual, subject-specific, T1 images by using the 78 ROIs AAL. Then, beamformer time series were computed for each VE of the AAL for 4 frequency bands: delta (1-4 Hz), theta (4-8 Hz), alpha (8-13 Hz) and beta (13-30 Hz). Following this, functional connectivity between ROIs was estimated using the Phase Lag Index (PLI), for each frequency band separately as in Hillebrand et al. (2012). PLI is a measure of phase synchronisation and it measures the asymmetry of the distribution of phase difference between two signals (Stam et al., 2007). The PLI adjacency matrix was then used to reconstruct the minimum spanning tree (MST) or the backbone of the functional network.

3.2.4 Spectral power and Phase Lag Index (PLI) analysis

Within BrainWave, epochs were converted into the frequency domain using the Fast Fourier Transform (FFT) implemented in the software (resolution ¼=0.25 Hz). Following this, for each participant and across the whole spectrum (0.5-48 Hz) mean, over epochs and subjects, power was computed for each ROI, as described in Ponsen et al. (2013). Then, for four frequency bands of interest: delta (1-4 Hz), theta (4-8 Hz), alpha (8-13 Hz) and beta (13-30 Hz), Phase Lag Index (PLI) was applied to estimate functional connectivity between the 78 regions of the atlas (Gong et al., 2009). Phase Lag Index (PLI) is a measure of the asymmetry of the distribution of phase difference between two signals (Hillebrand et al., 2016; Stam et al., 2007). PLI has been proven to be more robust to spurious connections compared to other connectivity measures such as coherence or imaginary coherence (Stam et al., 2007). The PLI is non-zero when there is an asymmetry in the distribution of the instantaneous phase difference and, therefore, it only quantifies non-trivial connections, the equation is described in 3.1. As well described in Stam et al. (2007) and Ponsen et al. (2013), PLI reduces the effects of volume conduction by ignoring zero and π phase differences between pairs of signals.

$$PLI = |< sign\left[(sin\left(\Delta\Phi\left(t_k\right)\right))\right] >| \tag{3.1}$$

Where the phase difference is defined in the interval $[-\pi, \pi]$ and $<>$ is the mean value (Ponsen et al., 2013). The PLI ranges between 0 and 1 $[0 \leq PLI \leq 1]$. When the PLI is non-zero, it means either no coupling or coupling with a phase difference centred around 0 mod π. PLI strength or size is directly proportional to the increase of this number, for more details about the computation of the PLI see Stam et al. (2007).

The mean PLI was computed for all possible pairs of ROIs, resulting in a 78 x 78 adjacency matrix. Next, for each ROI, the mean PLI between that ROI and all the other ROIs was computed, as a measure of overall connectivity of a region or, according to Rubinov and Sporns (2010), node strength in terms of graph theory. Then, details of connectivity patterns between regions were examined (Ponsen et al., 2013). The mean PLI values for each ROI were compared between the fluent and dysfluent group by means of permutation analysis, decribed by Nichols and Holmes (2001).

3.2.5 Minimum Spanning Tree-MST

The minimum spanning tree (MST) is an a-cyclic sub-network of the original 'weighted' network that connects all the nodes of the tree (network) without forming loops and has the minimum total weight of all possible spanning trees (Tewarie et al., 2015). The MST is constructed using the Kruskal algorithm (Kruskal, 1956). The algorithm orders the weights of all links in descending order and starts the construction of the tree with the largest link weight and adds the following, in descending order, until all the nodes N are connected in an acyclic sub-network that consists of M=N-1 links, that means it has a N-1 fixed density. During the process of adding new nodes, if the addition of a new node forms a loop, the link is discarded, see Figure 3.2 for more detail. In the application here, the algorithm orders the distance of all links in a descending order, starting from the 'largest link weight' (Tewarie et al., 2015). The link or edge weight is defined as the inverse of the connectivity estimate (1/PLI), as described in Tewarie et al. (2014a).

The MST reconstructed here represents the sub-network with the strongest or maximum connectivity indices. After the construction of the tree, all the edges are assigned value of 1.

There are numerous MST metrics that can be used, both at the global and local level, to describe the network topology (Tewarie et al., 2015). In this study the following global MST descriptives were used: Leaf number (L), diameter (d), tree hierarchy (Th), degree (k), eccentricity and betweenness centrality (BC). Summary of the measures can be found in Table 3.1.

MST parameter	Symbol	Description
Leaf number	L	Number of end nodes (i.e.nodes with degree k=1) represents the dimension from linear to star graph
Diameter	d	Longest shortest path d of an MST. The value of the d decreases when the L increases.
Tree Hierarchy	Th	Quantifies the trade-off between large scale integration in the MST and the overload of central node
Degree	k	Number of links for a given node.
Eccentricity	Ecc	Longest shortest path from a reference node to any other node in the MST. The eccentricity of the whole MST is the difference between the eccentricity values of the nodes with the largest and smallest eccentricity in the tree.
Betweenness Centrality	BC	Fraction of all shortest paths that pass through a particular node.

Table 3.1 Summary of the MST global and local descriptives used in this study

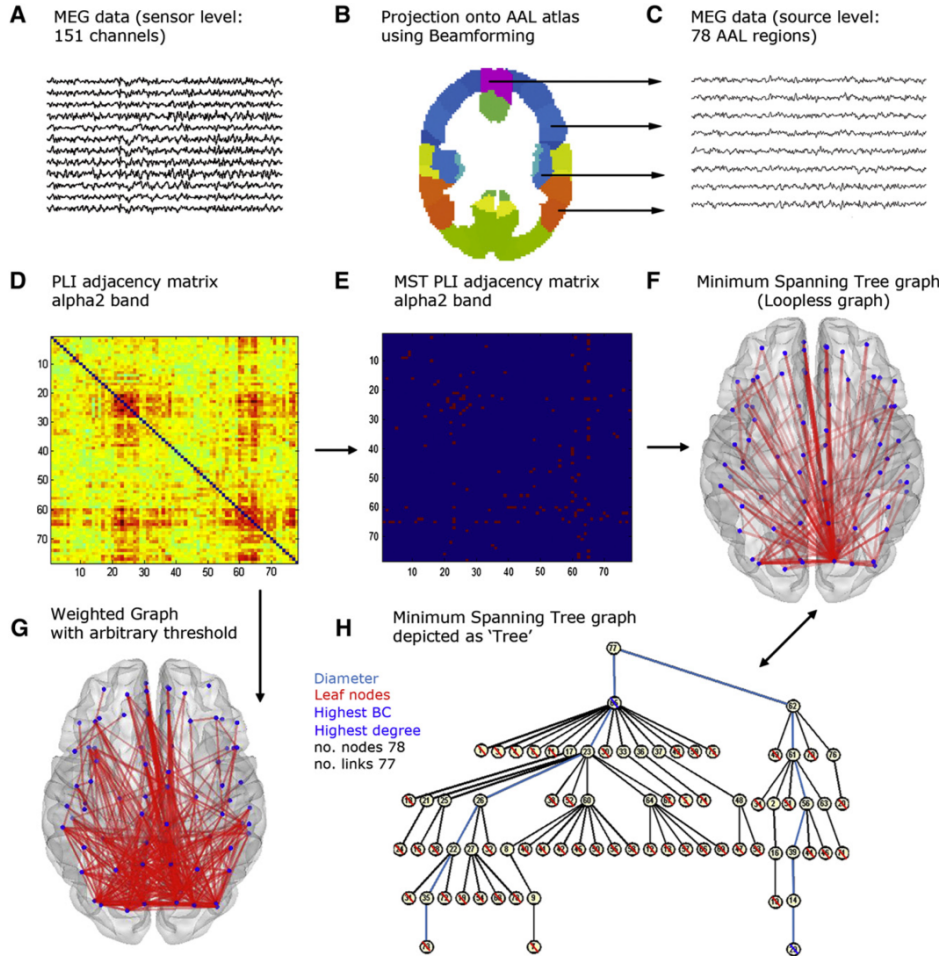


Fig. 3.2 Minimum Spanning Tree (MST) reconstruction pipeline. Analysis pipeline of the AAL beamformer and the construction of the MST. From MEG sensor level data (A), projected into the source space via an atlas based beamformer to construct 78 VE or ROIs (B and C), (D) PLI 78x78 reconstructed adjacency matrix, used as a connectivity metric and the construction of the MST by using the application of the Kruskal algorithm (F and H). *Adapted from Tewarie et al. (2014a).*

3.3 Results

For the comparison between behavioural measures of standardised scores of IQ, reading, spelling, mean power and mean PLI in alpha, beta, theta and delta frequency bands four one-way ANOVAs were carried out in SPSS (BM Corp. Released 2017. IBM SPSS Statistics for Mac, Version 24.0. Armonk, NY: IBM Corp), with significance threshold set at p-value < .05. Following these comparisons, regional measures of mean power, PLI and MST local estimates of degree, betweenness centrality and eccentricity were performed via non-parametric permutation t-testing (number of permutations = 5000), described in Nichols and Holmes (2001).

3.3.1 Behavioural measures

Mean age, non-verbal IQ and standard scores of reading and spelling testing batteries were compared between the fluent (N=17) and dysfluent (n=14) groups. In this case, comparing subscales of the TOWRE sub-tests was not applicable (n.a.) since the stratifying of the two sub-groups was done accordingly. The mean and the standard deviations values are illustrated in Table 3.2. Significant differences were detected in age, with the fluent group being marginally younger (p-value = .018) and in the verbal IQ, where the dysfluent group exhibited significantly lower scores (p-value= .026). The two groups did not differ in the reading and spelling normative scores, suggesting that fluency can be a suitable classifier of dyslexic sub-samples.

	Fluent Mean (SD)	Dysfluent Mean (SD)	p-value
N	17	14	
Sex ratio (m:f)	(12:5)	(10:4)	
Age	10.58 (2.19)	13.06 (3.4)	**.018**
handedness (L:R:A)	0:13:4	9:1:4	
IQ (non-verbal)	109 (13)	93 (16)	**.026**
PDE	98 (10)	80 (6)	n.a.
SWE	105.5 (11)	73 (7)	n.a.
Reading (BAS or WIAT)	98 (12.86)	81.5 (15.82)	.088
Spelling (BAS or WIAT)	88.55 (10.24)	78.63 (14.25)	.099

Table 3.2 Demographic and behavioural measures of the fluent and dysfluent dyslexic readers. The two groups differed significantly in age and IQ. PDE and SWE are the fluency (TOWRE) sub-tests which measure respectively, single word and non-word reading speed and accuracy. The norms based on Torgeson et al. (1999)'s manual indicate that an overall total scaled score < 70 is considered very poor, 70-79 poor, 80-89 below average, 90-110 average, 111-120 superior, >130 very superior. For the spelling and reading batteries, standard (z-transformed) scores of either BIAT or WIAT are reported, with the mean=100 and sd=15.

3.3.2 Spectral power and connectivity

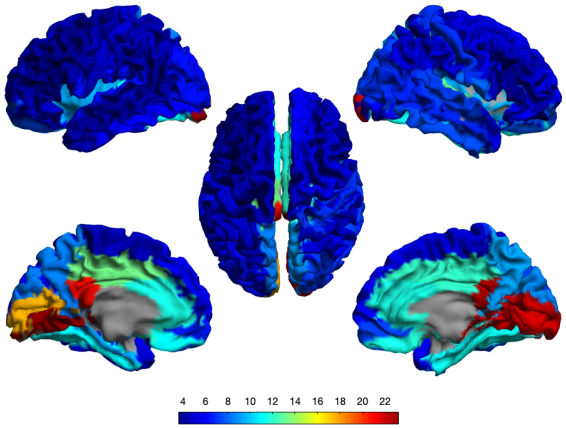
Mean power of the whole spectrum of interest (0.5-48 Hz) and mean PLI for the alpha (8-13 Hz), beta (13-30 Hz), theta (4-8 Hz) and delta (1-4 Hz) were compared with four one-way ANOVAs. The results are shown in Table 3.3. No significant differences were detected in this case (p-value >.05).

		Fluent (N=17)		Dysfluent (N=14)		
		Mean	(SD)	Mean	(SD)	p-value
Power		10.33	(11.76)	6.35	(2.345)	.228
Delta	PLI	.1100	(.0142)	.1128	(.012)	.570
Theta	PLI	.0896	(.0117)	.0877	(.008)	.612
Alpha	PLI	.0865	(.0170)	.0872	(.008)	.898
Beta	PLI	.0491	(.0057)	.0497	(.004)	.766

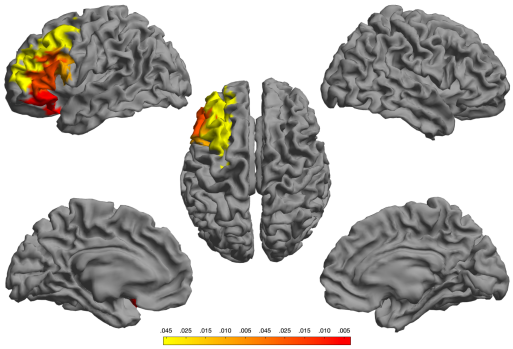
Table 3.3 Overall power mean and global frequency specific (delta, theta, alpha, and beta) PLI values for fluent and dysfluent dyslexic groups. Mean and standard deviation (SD) values of power and PLI values for the frequency bands of interest (1-4 Hz, 4-8 Hz, 8-13 Hz and 13-30 Hz) in fluent and dysfluent dyslexic readers. There were no significant differences detected in the comparisons between the two groups (p-value >.05)

Local power estimates

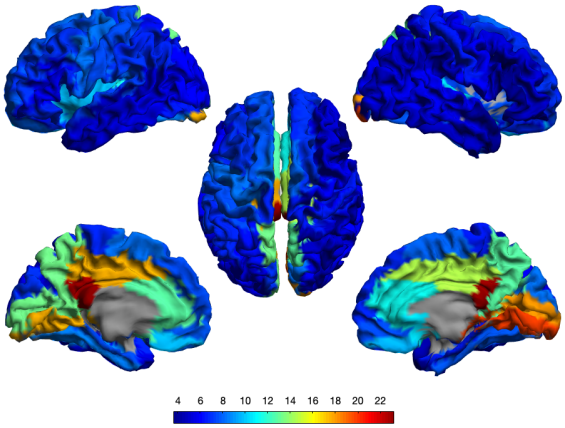
Permutation t-testing (number of permutations = 5000) showed significant differences in power distribution in the left frontal regions. Specifically, an increase in power was found in the left inferior orbital (p-value<.05, t-value=2.6), mid left frontal (p-value<.05, t-value=3.6), left inferior fronto-opericular (p-value<.05, t-value=3.3) and left triangular frontal cortex (p-value<.05, t-value=2.9), Figure 3.3.



a) Dysfluent



b) difference



c) Fluent

Fig. 3.3 Mean power spectrum (4-22 Hz) in dysfluent and fluent dyslexic groups. From the top a) mean power distribution of the dysfluent dyslexic group; b) Significant (p-value < .05) increase in power was detected in the following ROIs of the atlas: Left inferior orbital, mid left frontal and left inferior fronto-opericular the colour scheme is based on t-values in significant regions (more red is increased absolute t-values); c) mean power distribution for the fluent dyslexic group.

3.3.3 MST

Global measures

Four one-way ANOVAs were used to compare means of MST leaf number, diameter, tree hierarchy, mean degree, betweenness centrality and eccentricity for each of the frequency bands (delta, theta, alpha and beta). For the alpha frequency band, significant differences were found in diameter (p-value = .038) and degree estimates (p-value =.036). In beta frequency band mean Tree hierarchy (Th) was significantly higher (p-value = .043) in the fluent group compared to the dysfluent one. The results of the four one-way ANOVAs are shown in Table 3.4. Furthermore, functional connectivity estimates based on PLI did not show any significant effect on both global (mean PLI) and regional permutation analysis. The significant differences detected at the global level in alpha and beta frequency band were followed up by local or regional MST analysis.

		Fluent (N=17)		Dysfluent (N=14)		
		Mean	(SD)	Mean	(SD)	p-value
Delta	Diameter	.2186	(.0227)	.2149	(.0195)	.636
	Leaf Number	.5258	(.0248)	.5278	(.0261)	.834
	Tree Hierarchy	.3875	(.0173)	.3785	(.0161)	.147
	Degree	.1075	(.0144)	.1088	(.0129)	.793
	Eccentricity	.1674	(.0176)	.1635	(.0156)	.528
	Betweenness Centrality	.6844	(.0324)	.7008	(.0273)	.143
Theta	Diameter	.2104	(.0296)	.2193	(.0245)	.377
	Leaf Number	.5247	(.0226)	.5173	(.0319)	.453
	Tree Hierarchy	.3771	(.0242)	.3702	(.0215)	.419
	Degree	.1094	(.0139)	.1099	(.0197)	.923
	Eccentricity	.1644	(.0134)	.1666	(.0198)	.712
	Betweenness Centrality	.6809	(.0911)	.7025	(.0398)	.417
Alpha	Diameter	.2108	(.0171)	.2054	(.0161)	**.038**
	Leaf Number	.5243	(.0162)	.5265	(.0265)	.774
	Tree Hierarchy	.3782	(.0199)	.3709	(.0292)	.412
	Degree	.1077	(.0094)	.1194	(.0195)	**.036**
	Eccentricity	.1602	(.0146)	.1552	(.0152)	.361
	Betweenness Centrality	.6987	(.0334)	.7178	(.0445)	.182
Beta	Diameter	.214	(.0142)	.2106	(.0212)	.602
	Leaf Number	.5187	(.022)	.5105	(.0325)	.411
	Tree Hierarchy	.3834	(.0234)	.3652	(.0246)	**.043**
	Degree	.1107	(.0146)	.1105	(.0215)	.972
	Eccentricity	.1634	(.0123)	.1586	(.0209)	.438
	Betweenness Centrality	.6806	(.0399)	.7053	(.0519)	.146

Table 3.4 Frequency (delta, theta, alpha and beta) specific MST global metrics values (mean and sd) and differences between the fluent and dysfluent dyslexic groups. Significant differences (p-value = .038) between the two groups were detected in alpha frequency band (8-13 Hz) where the fluent dyslexic group exhibited longer diameter. In the MST analysis the diameter is the longest shortest path of a network. In addition, dysfluent dyslexic readers had a significantly higher (p-value = .036) degree values. Global degree is calculated on the values of the maximum degree values and represents the global estimate of number of neighbours. In the beta (13-30 Hz) frequency band the fluent dyslexic group had significantly higher values (p-value =.043) in tree hierarchy (Th). Th is a measure of balance between diameter reduction (from line-like tree to more star-like tree) and the overload prevention (Boersma et al., 2013).

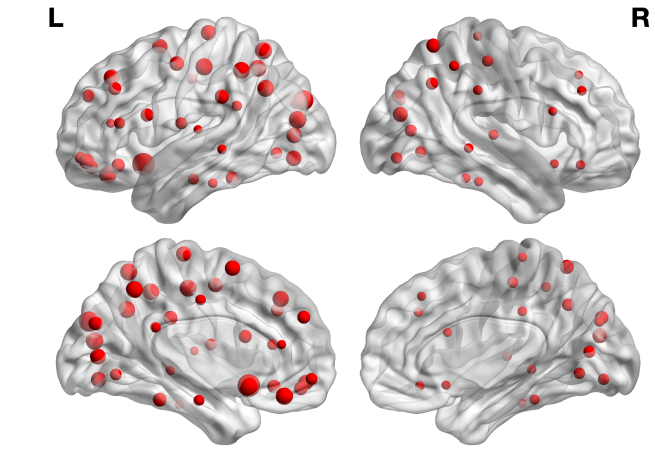
MST regional metrics

As mentioned above, to further explore the global differences in the MST global outcome of diameter, degree and tree hierarchy in alpha and beta frequencies, regional MST analysis was applied for local measures of MST degree, MST betweenness centrality (BC) and eccentricity, using permutation (number of permutations=5000) t-testing to compare dysfluent and fluent dyslexic readers.

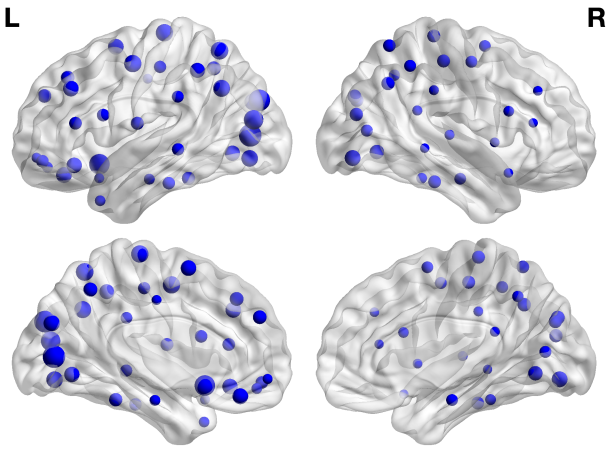
Degree

In the alpha frequency (8-13 Hz) dysfluent dyslexic readers showed significantly lower values of degree in right (supplementary) motor area (p-value <.025, t-value=-2.8). Close to significance higher values of degree were observed in the left postcentral cortex (p-value <.05, t-value= 1.8), and in the right frontal triangular cortex (p-value<.05, t-value=-1.6). Figure 3.4 depicts the nodes with the highest (above - average, mean >50%) values of degree of the dysfluent dyslexic group (red nodes), fluent one (blue nodes), and below (in green) the nodes where significant differences were detected.

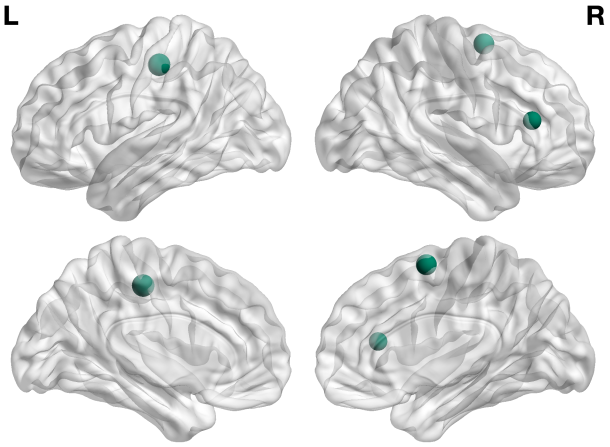
In the beta frequency band (13-30 Hz) the dysfluent group showed significantly lower values of degree in the left lingual, right inferior parietal and supra-marginal right cortex (p-value<.025, t-values=-1.9;-2.5;-2.6). In addition, close to significance higher values of degree were detected in left rectus and orbital medial frontal cortex (p-value<0.5, t-values=1.7;1.6). Lower degree values were also detected in left precentral, left mid occipital and right frontal superior (p-value <.05, t-values=-1.7;-1.6;-1.6) of the atlas, Figure 3.5.



a)



b)



c)

Fig. 3.4 Alpha (8-13 Hz) local MST degree results. a) Distribution of the strongest (above-average) degree values in the dysfluent readers network (red nodes). b) Distribution of the above-average degree values in the fluent dyslexic readers (blue nodes). c) ROIs were significant difference between dysfluent and fluent dyslexic readers was observed (green nodes). Here, while close to significant higher values of degree were detected in the left postcentral cortices (t-value= 1.8) and lower in the right frontal triangular cortex (t-value=-1.6). Significantly lower (p-value<.025) values of degree were detected in the right motor cortex t-value=-2.8). The size of the nodes corresponds to the degree value (blue, red) and t-values (green).

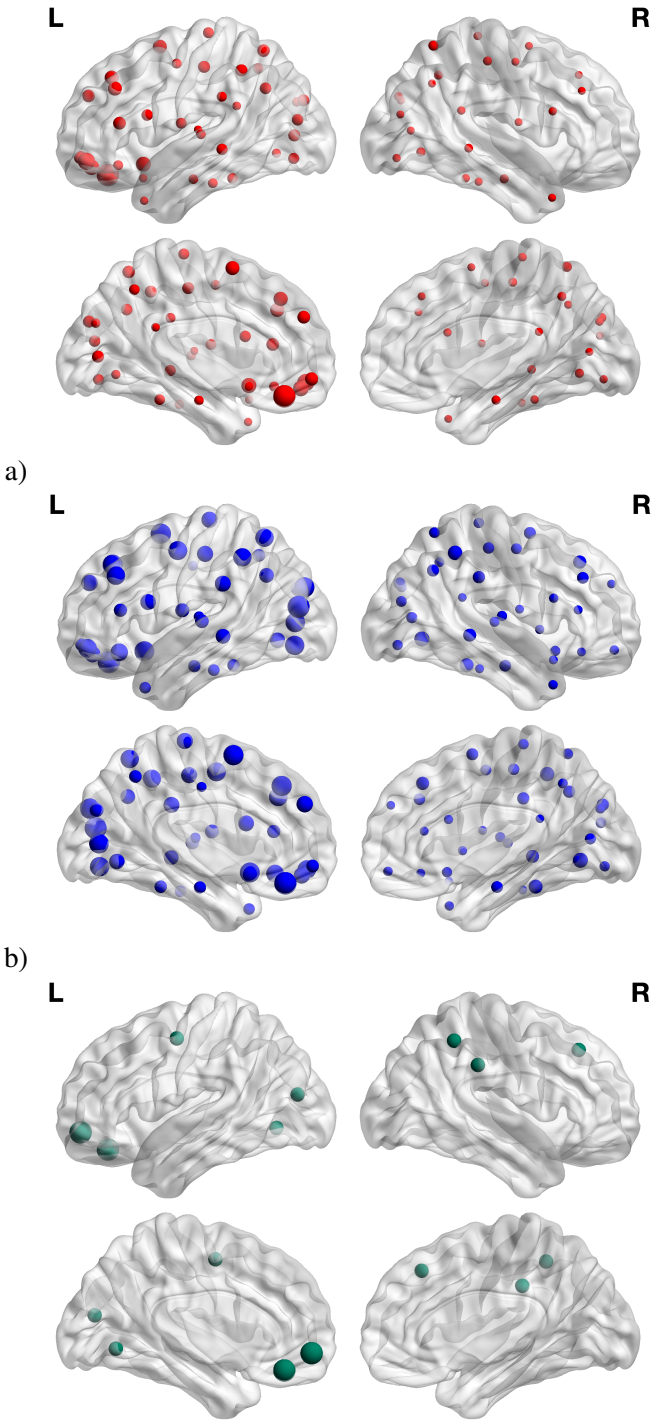


Fig. 3.5 Beta (13-30Hz) MST local degree results. a) Top dysfluent dyslexic readers above-average nodal distribution. b) Fluent dyslexic group (blue) above-average degree distribution. The size of nodes (blue and red) reflects the degree value. c) Significant ROI (green). The dysfluent group showed significantly lower values of degree in the left lingual, right inferior parietal and supra-marginal right cortex (p-value<.025, t-values=-1.9,-2.5,-2.6). In addition plotted here, other regions that were close-to-significance threshold, such as left rectus, orbital medial frontal, left precentral, left mid occipital and right frontal superior (p-value<.05, t-values=1.7,1.6,-1.7,-1.6,-1.6). The size of the nodes reflects t-values.

Betweenness Centrality

As before, local betweenness centrality (BC) measures between the groups was measured via means of permutation t-testing (number of permutations = 5000) for alpha (10-13 Hz) and beta (13-30 Hz) frequency bands separately.

In the alpha frequency band (8-13 Hz), the dysfluent group had significantly higher BC values in the left inferior parietal and posterior cingulum (p-value <.025, t-values=1.8,3). Furthermore, close to significance values were detected in the left insula and right superior temporal regions (p-value<.05, t-values=1.6,1.6). The results are illustrated in Figure 3.6 (the edges in the figure are derived from the reconstructed BC matrix).

In the beta frequency band (13-30 Hz), the results showed that the dysfluent group had significantly higher values in the left rectus, left fusiform and right superior parietal (p-value<.025, t-values=2.2,2,2.8). In addition, higher BC values were detected in left orbital medial, middle, and inferior frontal (triangularis) cortex, right superior temporal pole and precuneus regions (p-value<.05, t-values=1.8,1.8,1.9,1.6,1.8). Same as above, the results are illustrated in Figure 3.7, where in both groups the distribution of the above average BC values is pictured as nodes and the edges (links) are weighted according to the BC connectivity matrix. In the lower plot, based on t-values. The edges in the difference plot were derived from the difference BC matrix.

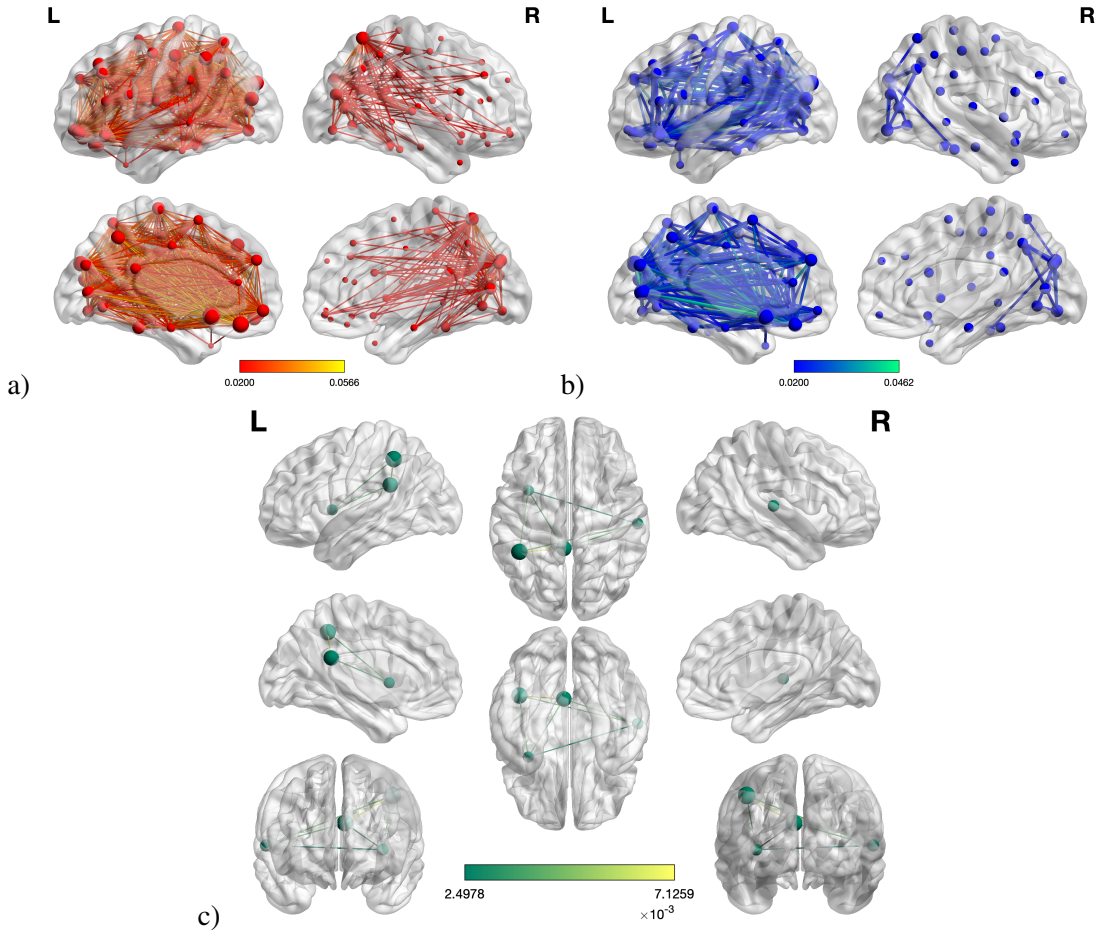


Fig. 3.6 Alpha (8-13Hz) band betwenness centrality (BC) regional results. a) Above-average BC values (hubness) in the dyslexic dysfluent group (red-orange) b) Above-average BC distribution of values in the fluent dyslexic group (blue-green) In both groups a) and b) the distribution of the above average BC values is >50% mean values and is pictured as nodes. The edges (links) are weighted according to the BC connectivity matrix. c) Significant ROIs (green). Higher BC values were detected in the left inferior parietal, posterior cingulum (p-value<.025, t-values=1.8,3) and in the left insula cortex (p-value <.05, t-value=1.6) and in the right superior temporal cortex (p-value<.05, t-value=1.6). Here, the size of the BC nodes represents the size effect, bigger the nodes larger the difference between the groups was based on t-values.

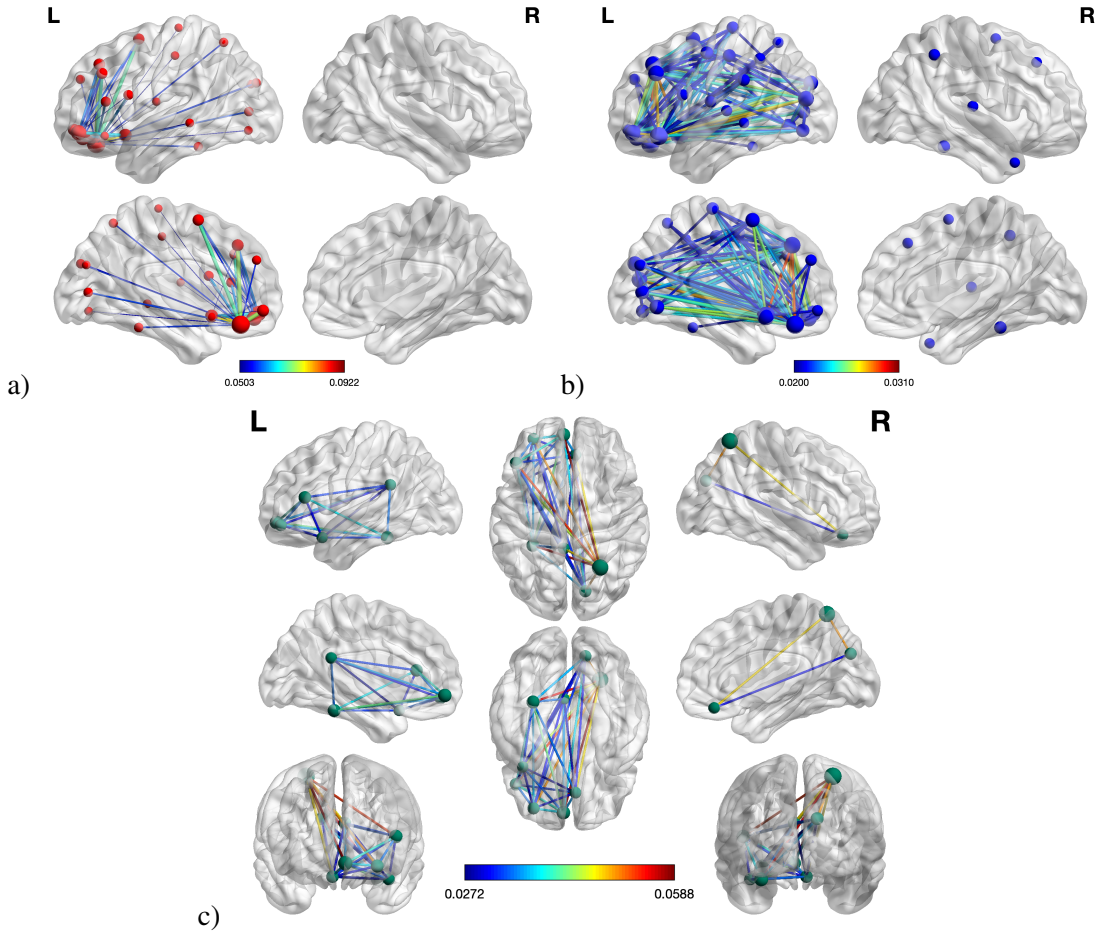
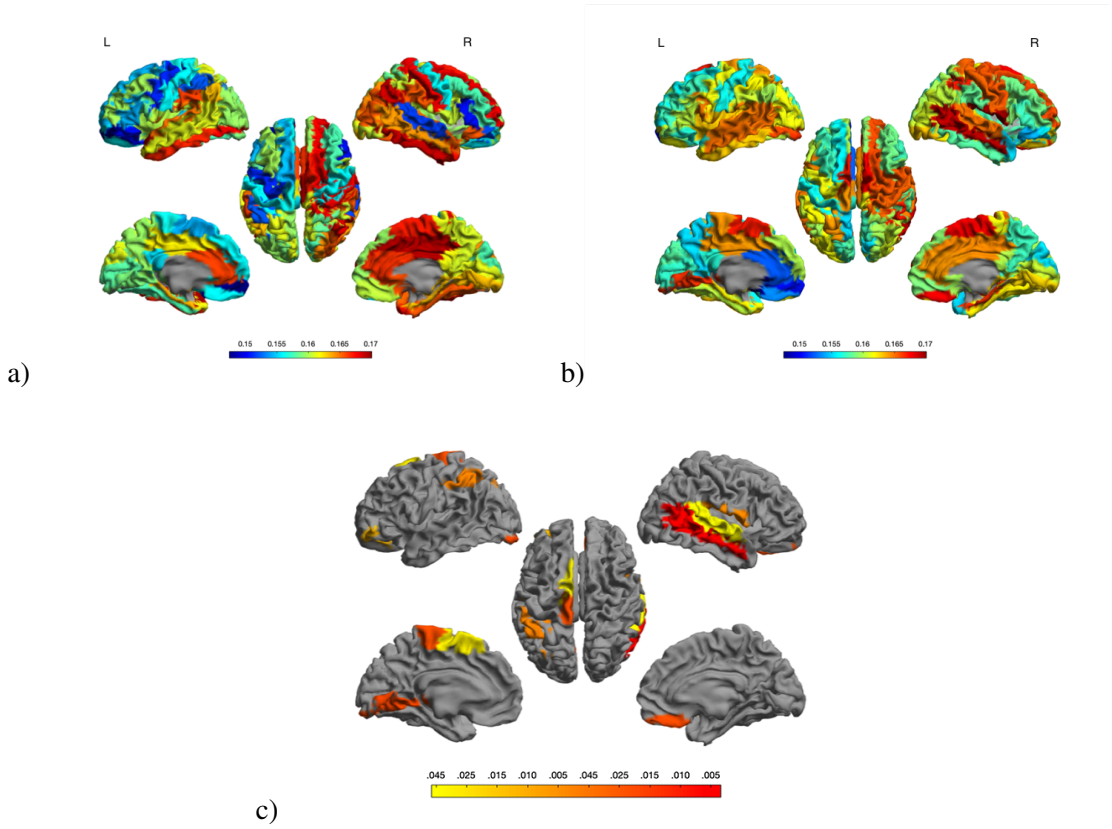


Fig. 3.7 Beta (13-30 Hz) band MST regional values in betwenness centrality (BC) in dysfluent and fluent dyslexic readers, and significant ROI in beta (13-30 Hz) frequency band. a) Local beta band estimates of BC in dysfluent dyslexic readers b) Fluent dyslexic readers local BC values (blue nodes). As above, the BC nodes pictured here represent the above-average (>50% mean) values in each network. c) significant increase of BC in dysfluent dyslexics was found in the left rectus, left fusiform and right superior parietal (p-value<.025, t-values=2.2,2,2.8). In addition close-to-significance higher values of BC in dysfluent readers were detected in the left orbital medial, middle, and inferior frontal (triangularis) cortex, left superior temporal pole and precuneus regions (p-value<.05, t-values=1.8,1.8,1.9,1.6,1.8). The size of the green nodes is based on t-values.

Eccentricity

Differences in MST eccentricity between-groups were measured with permutation t-testing (number of permutations = 5000). In the alpha frequency band (8-13 Hz), the dysfluent dyslexic readers displayed significantly lower eccentricity values in left middle-orbital frontal, superior motor, precentral, inferior parietal and lingual regions (p-value<.05, t-values=-1.8,-2.7,-1.6,-1.6,-2.2). in the right hemisphere, significantly lower values of eccentricity were found in the rectus, opercular rolandic and mid and superior temporal regions (p-value<.05, t-values=-2,-2.2,-2.9,-2.4), Figure 3.8.



a) b)

c)

Fig. 3.8 Alpha band (8-13 Hz) regional distributions of eccentricity in dysfluent, and fluent dyslexic readers and ROIs where significant differences were detected. a) Mean eccentricity values of ROIs in the dysfluent dyslexic network (top left) and b) Mean eccentricity values of ROIs in the fluent dyslexic network (top right). c) Significantly lower values (p-value<.05) in eccentricity (increased centrality) were detected in the left hemisphere: middle-orbital frontal, superior motor, precentral,inferior parietal and lingual regions (t-values=-1.8,-2.7,-1.6,-1.6,-2.2); right hemisphere: rectus, opercular rolandic and mid and superior temporal regions (t-values=-2,-2.2,-2.9,-2.4). The colorbar is based on absolute t-values, highest in red).

In beta frequency band (13-30 Hz) the dysfluent dyslexic group showed significantly lower values of eccentricity in medial and superior left frontal regions, left fusiform gyrus, right rectus, right superior and inferior parietal and right inferior temporal regions (p-value <.05, t-values=-2.4,-2.2,-2.7,-2.5,-1.8,-1.7,-1.6), Figure 3.9.

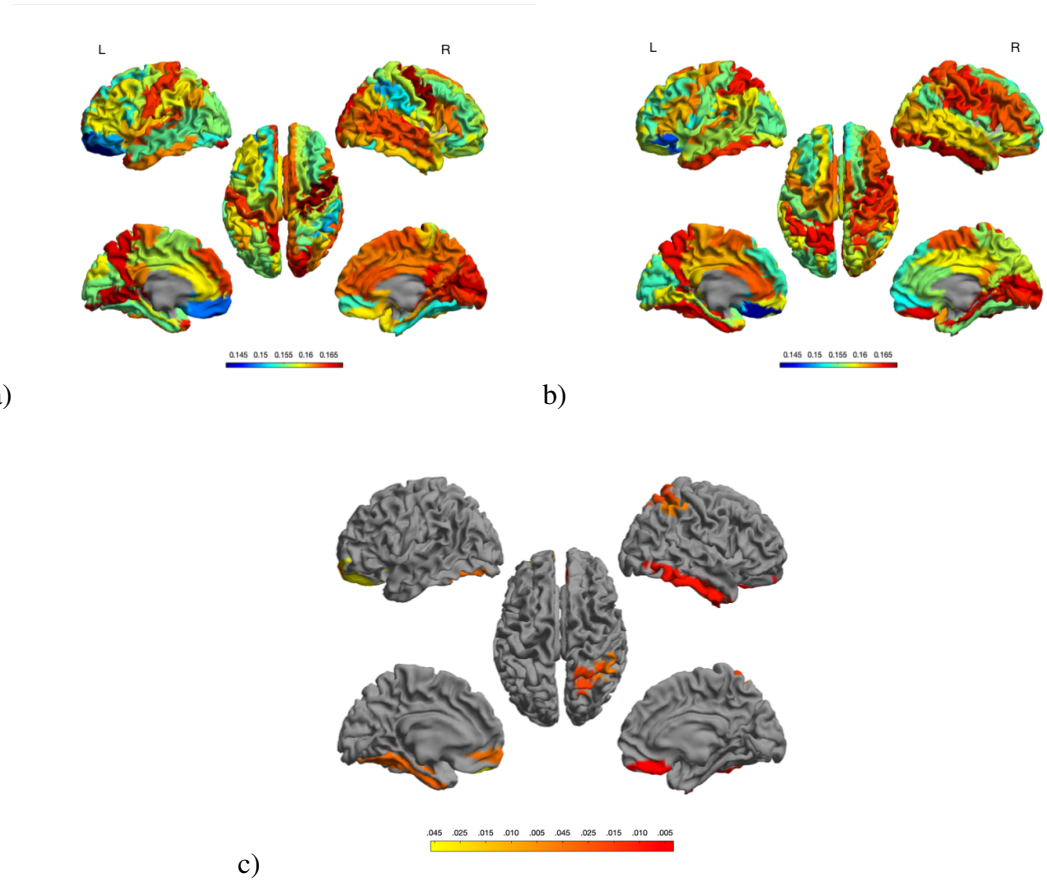


Fig. 3.9 Beta band (13-30 Hz) regional distributions of eccentricity in dysfluent, and fluent dyslexic readers and ROIs where significant difference was detected. Pictured here mean eccentricity values for all the nodes (78 ROIs) of the network are shown respectively for a) top left dysfluent dyslexic group and b) fluent dyslexic group. c) Significantly lower values of eccentricity were detected in, left hemisphere: frontal regions (superior and middle orbital) and fusiform gyrus (t-values=-2.4,-2.2,-2.7,-2.5); right hemisphere: superior and inferior parietal cortex and right inferior temporal regions (t-values= -1.8,-1.7,-1.6).

3.4 Discussion

The current study aimed to explore different patterns of RSFC and network topology within the dyslexic spectrum. The main research question was if a measure of fluency could be used as a valid classifier of RSFC and network topology, and help to better define the high heterogeneity of the disorder by using an MEG task-free paradigm.

At the cognitive level, the two groups significantly differ, in age and IQ (non-verbal), where the dysfluent group was much older and had lower intelligence scores. The nature of this sample did not allow to control for these factors since the group size was not large enough to divide the participants into age-matched groups. However, the TOWRE standardised scores used in the group stratification are age corrected, therefore age did not have a marginal effect in the behavioural contrast. The non significant effect in the comparison of the reading and spelling standardised scores indicates that there were no additional factors that influenced reading skills differences between the two groups.
Furthermore, no significant differences were detected in the comparison of overall power and mean PLI for delta, theta, alpha and beta frequency bands. These results are not surprising. Previous studies have demonstrated differences in mean power and connectivity measures (wPLI, PLI, coherence), for instance Arns et al. (2007); Dimitriadis et al. (2013); Fraga González et al. (2016a), but these compared typical versus dyslexic readers. Since all participants included in the present study were recently diagnosed for dyslexia, and therefore coming from the same population, the expectation was that the effects detected, if any, would be at the local level. Consequently, significant difference in power were detected in left inferior orbital and frontal cortices. More specifically, dysfluent dyslexic readers showed increased power in these areas compared to the fluent ones. The dysfluent group had an increased activation in frontal regions, which is in line with previous studies suggesting overactivation in dyslexic compared to typical readers (Pagnotta et al., 2015). An additional EEG study by Papagiannopoulou and Lagopoulos (2016), showed how an atypical frontal linguistic network in children with dyslexia, is represented by increase in theta power. Theta power synchronisation has typically been related to 'phasic change' during increasingly demanding tasks, (Papagiannopoulou and Lagopoulos, 2016). According to these results, there seems to be a specific 'tonic' resting state functional abnormality (or frontal increase in power) in children with dyslexia (Papagiannopoulou and Lagopoulos, 2016). In this case, findings of increased power in frontal regions in the dysfluent dyslexic group, compared to the fluent one, overlap with findings in the literature. It is still quite unclear where the overactivation in lower frequency bands might originate from. Promising insights came from animal studies, showing how neurobiological mechanisms increasing lower frequency peak oscillations (5-15 Hz), might be due to GABAergic inhibition (Papagiannopoulou and Lagopoulos, 2016; Xiao et al., 2012). Future studies, including a typical readers group and task-positive condition, involving increasing response demands, could be useful to confirm or deny this hypothesis.
Interestingly, no significant effects were detected in measures of functional connectivity, which can be explained in two ways. First, the PLI analysis did not show any significant effects in this task-free condition, because there is no difference in RSFC between more severe (dysfluent) and moderately

impaired readers (fluent). If this were true, no effects would have been found in the MST analysis, since the MST is the backbone of the PLI connectivity matrix (Stam et al., 2014; Tewarie et al., 2014b). The second, and more plausible, reason is that differences in PLI were not detected between dysfluent and fluent dyslexics because of potential biases in estimating functional connectivity. Fraga González et al. (2016a), in a EEG study comparing typical and atypical readers showed the same dissociation, where PLI analysis failed to reveal differences in connectivity strength, while the MST analysis did not. This indicates that MST is a robust and unbiased method to compare functional networks, (Stam et al., 2014).

Global metrics of the MST suggested differences in dysfluent and fluent tree configurations. Specifically, in the alpha band fluent group showed significantly higher values of diameter (longest distance between two nodes) and lower degree (number of nodes). This is in line with previous findings from Fraga González et al. (2016a), who found a trend of increased diameter for children with dyslexia compared to controls. As mentioned previously, according to the small-world network theory, the longer the diameter is, the more the topology is line-like, whereas an ideal and efficient network has a short diameter and a star-like topology (Fraga González et al., 2016a; Stam, 2014). Similarly, longer path length in dyslexics was found in DTI study of Chinese typical and atypical readers (Liu et al., 2015). Higher degree suggest a trend towards overloaded local processing, with a less integrated long-range communication and optimal balance between synchronisation and integration (Liu et al., 2015; Sporns and Zwi, 2004), which was the case for the dysfluent group in this study.

The present findings, of higher mean diameter and lower degree in the fluent group, suggest that within dyslexic spectrum the tree topology varies significantly between fluent and dysfluent readers. Current results overlap with previous findings (Fraga González et al., 2016a; Liu et al., 2015) suggesting that the diameter increases with fluency level. On the other hand the higher degree detected in the dysfluent group suggests less optimal long-range network integration and the tendency of information transfer within neighbouring areas or clusters, as also observed in Liu et al. (2015).

Furthermore, significant differences were found in beta band for tree hierarchy (Th). A measure of efficiency of a tree or network is given by the Tree hierarchy (Th). In graph theory efficient communication between all nodes, requires small diameter (star-like topology). It has been proposed that an optimal configuration of MST should be given by the balance between diameter 'reduction' (leaf number) and overload prevention (high BC) which is fundamental for an optimal network performance, given by the Th (Boersma et al., 2013). Tree hierarchy (Th) is, thus, a hierarchical metric that quantifies the trade-off between large scale integration and the MST overload (Tewarie et al., 2014a). It has been shown that Th changes with age and it contributes to different tree configurations between boys and girls (Boersma et al., 2013). Th also correlates with impaired cognition in multiple sclerosis patients (Stam et al., 2014). For the current study, the higher Th in fluent dyslexics partially overlaps with previous findings were increased Th was observed in controls compared to dyslexics (Fraga González et al., 2016a).On the other hand, mean Th was significantly decreased in the dysfluent group of dyslexic readers, which was also marginally older than the fluent one. Therefore, age might

have played a potential role in the different MST Th configuration, irrespective of the dysfluent/fluent division, as shown by (Boersma et al., 2013).
It is important to note here that the current findings overlap with the dyslexic group findings from previous studies, which did not differentiate between fluency sub-types. To rephrase, the variations between fluent and dysfluent dyslexic sub-groups in this study, overlap with the MST configuration seen previously in dyslexics compared to controls. Based on current findings, it appears that the fluent dyslexics' MST global configuration resembles the one in typical readers from previous studies. This highlights the idea that developmental dyslexia should be investigated in a developmental trajectory perspective, where the high heterogeneity within the spectrum deserves more attention when comparing dyslexics and peer readers.

Finally, significant differences were detected at the local MST level in alpha and beta frequency bands. For the alpha band (8-13 Hz), dysfluent group had increased values of degree, betweenness centrality and decreased values of eccentricity, mostly in left middle-orbital frontal, parietal and precentral areas involving some regions of the DMN such as, cingulum and insula. This is in line with Fraga González et al. (2018b)'s findings who found similar pattern in adult dyslexics. The increased values of BC, degree and lower eccentricity indicate that hubs in the left frontal and some regions of the DMN areas are more loaded compared to the fluent group.
In terms of oscillations, alpha activity has been linked to attentional processing and task-irrelevant inhibition activations, working memory maintenance in frontal to parietal stream (Jensen and Mazaheri, 2010; Popov et al., 2018a). Moreover, in a graph theory study Dimitriadis et al. (2013) showed significant correlations between pseudoword scores and reduced global and local network efficiency in the alpha band. The present findings suggest an over activated alpha oscillatory network in dysfluent dyslexics in attentional and DMN regions.

In beta band (13-30 Hz), the dysfluent group had increased values of degree, BC and lower eccentricity in left orbital and medial frontal cortices, precentral gyrus and fusiform gyrus, precuneus and superior temporal lobe. Moreover, significant decrease in degree was observed in left mid occipital, precentral and lingual regions and in the right superior and inferior parietal and supramarginal areas. Here the results indicate an overload of centrality or hubness, in some of the reading network regions (precentral gyrus, fusiform word area and superior temporal cortex) as well as in regions of the DMN, such as the precuneus. Beta band has been previously linked to inter-regional connectivity, maintenance of the status quo and cognitive states (Engel and Fries, 2010) which agrees with current wide spread effect of the MST metrics across both hemispheres. Additionally, in a graph theory study Vourkas et al. (2011) found a linear association between lower gamma (20-30 Hz) global efficiency and local efficiency and decoding ability in dyslexics compared to typical readers. Similar to current findings, in one of the older EEG studies investigating differences in power in beta among dyslexic subtypes, Milne et al. (2003) found that the ratio of anterior-to-posterior beta power differentiated dysphonetic from dyseidetic dyslexics.
Overall, results in beta band suggest general increase in network load of a spread network that partially

overlaps with some of the regions of the reading network but also involves regions of the DMN and parietal cortices in the dysfluent dyslexic readers.

3.5 Conclusion

The present study provides evidence for investigating different patterns of MEG task-free functional networks in sub-types of developmental dyslexia. In particular, the MST analysis showed how dysfluent dyslexic young readers present a more centralised and therefore less integrated functional network compared to less severe (fluent) dyslexics. In conclusion, this study provides confirmation of association between different functional network patterns and indices of severity in dyslexia. Following chapters will exploit the neural correlates of task-based activity in typical and atypical readers, in relation to the first stages of reading processing - the visual word recognition.

Chapter 4

"Super Mario": a novel MEG paradigm to investigate visuo-orthographic processing in reading

Chapter summary

As illustrated in the previous chapter, fluency can be a sensitive 'stratifier' of RSFC in the dyslexic spectrum. This means that single-word visual recognition is not only one of the fundamental stages in reading acquisition, but it also helps to define differences in related functional networks at rest. The following chapter will describe the MEG task ("Super Mario"), designed to investigate visuo orthographic processing in developmental dyslexia, or neural mechanisms involved in the first stages of visual word recognition. It starts with the introduction to neurophysiological correlates of letter identification (N170), the associated cortical regions and related connectivity studies. Following this, the rationale and design of the paradigm used here and in Chapter 5 are described. It concludes with the description of methods and results of the pilot study carried out to validate the experiment. Overall, this chapter aims to represent a thorough introduction, from both theoretical and methodological point of view, to the experimental study described in Chapter 5.

4.1 Introduction

4.1.1 Visual word recognition

Evolutionarily speaking reading is a recently acquired skill, that requires highly specialised neural system to recognise words independent of letter CaSe or font. Despite this, proficient readers can easily discriminate fine details, such as the difference between 'b' and 'd', or 'eight' and 'sight' (Cohen and Dehaene, 2004; Dehaene et al., 2005). Visual word recognition is, thus, an elaborate cognitive process that starts with visual encoding of the position and shape of letters, subsequently coded into graphemes and orthographic strings, to further activate lexical and phonological processes, in order to access their meaning (Bentin et al., 1999).

The automatic word recognition has been demonstrated as the inability of humans to ignore words, even when instructed to (for instance during a Stroop task), (Schadler and Thissen, 1981). It has been shown that automatic word recognition has a specific neurophysiological signature or event related potential (ERP). In an EEG study, Bentin et al. (1999) showed a negative prominent peak at 170 ms in occipito-temporal left sites, as a response to words, pseudowords and strings of consonants. In particular, the left N170 was larger for orthographic than non-orthographic stimuli, see Figure 4.1. Similarly, N170 specialization has often been confirmed in recognition of other objects, usually in the right hemisphere, that require fine visual expertise, such as faces, cars or birds (Kanwisher and Yovel, 2006; Rossion et al., 2002). Furthermore left lateralization of the N170 finding, has been reproduced across different languages, alphabets and orthographies (Shirahama et al., 2004; Tarkiainen et al., 1999). This visual expertise is specific for letter-like stimuli, but it is also an automatic recognition process, since it is present when the subjects are not consciously attending to stimulus (Sánchez-Vincitore et al., 2017). One of the theories that has been advanced to explain the left-lateralised N170 specificity is the phonological mapping hypothesis by Maurer et al. (2007). In relation to visual recognition to print, the left lateralisation is the result of automatic connections between phonological associated regions and occipito-temporal regions in the left hemisphere (Maurer et al., 2007). Or simply, the pairing of 'printed' letters and language during the literacy acquisition is a consequence of a linguistically driven response from visual association areas (Maurer et al., 2007; Sánchez-Vincitore et al., 2017). Moreover, the development of reading as a skill or, in this case, the repetitive pairing of the letter-string class of stimuli with the activation of language specific areas in temporal and frontal cortices might drive the specialisation and tuning of left occipito-temporal extrastriate visual region, highly responsive for word-like stimuli, also known as Visual Word Form Area (VWFA) (Maurer et al., 2010; McCandliss et al., 2003).

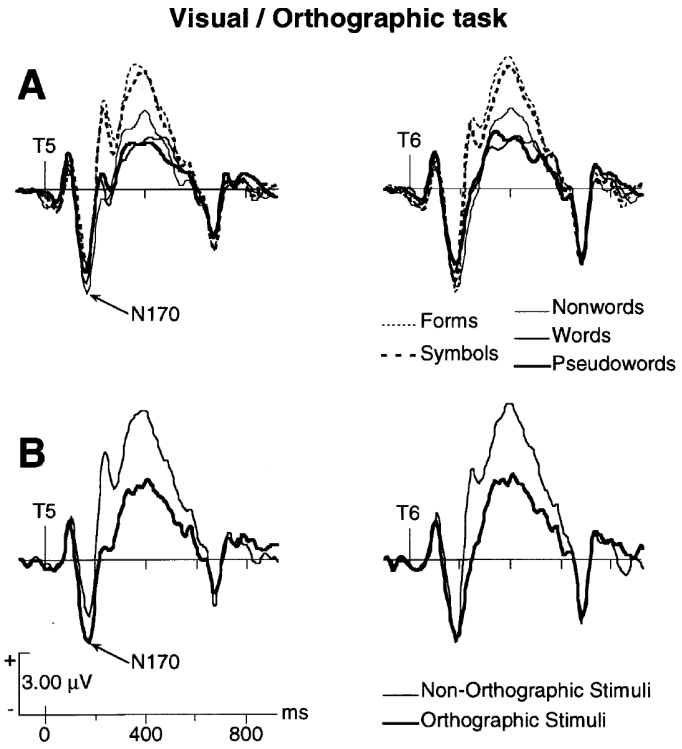


Fig. 4.1 First N170 ERP study. Bentin et al. (1999) that showed the N170 specificity of the response for visual and orthographic stimuli. In this study different word-like stimuli (words, pseudowords and nonwords), and non word-like stimuli (symbols, forms, alphanumeric symbols) were utilised to measure ERPs at the lateral posterior electrodes. N170 was the largest and strongest in response to word-like stimuli. *Adapted from Bentin et al. (1999).*

4.1.2 The Visual Word Form Area - VWFA

In the past two decades, the neuroimaging reading literature showed how a portion of the left occipito-temporal sulcus is selectively responsive to printed words (Dehaene et al., 2002; McCandliss et al., 2003; Price and Devlin, 2003). The 'brain letterbox', mainly known as the 'Visual Word Form Area' (VWFA) has been defined as the region that hosts modality specific, pre-lexical representations of visual words (Dehaene, 2009; Dehaene et al., 2002; McCandliss et al., 2003). Early evidence of the functional specialization of the left occipitotemporal sulcus came from, respectively, typical and brain lesions studies (Dehaene and Cohen, 2011). In particular, Cohen et al. (2000, 2002) studied differences in processing at the split-field reading task, in typical readers and split-brain patients with both fMRI and high density ERPs, showing how an area of the mid-portion of the left fusiform gyrus (aproximate Taliarch coordinates ±43 ±54 ±12) was activated whenever literate subjects were presented with strings of letters whilst the activation was absent in alexic patients, independent of the hemifield of presentation (Cohen et al., 2000). These findings have been furthermore confirmed in multiple studies across languages, orthographies and neuroimaging modalities (Bolger et al., 2005), for a complete metanalysis refer to Jobard et al. (2003).

The VWFA hypothesis opened a large debate in the neuroscience of reading about the functional specificity, for alphabetic processing, of this particular region within the ventral stream. The details of a still standing dispute on the matter can be found in Cohen and Dehaene (2004); Dehaene and Cohen (2011); Price and Devlin (2003). Hence, a major dispute in the study of visual word processing is whether printed words are perceived in a feedforward or serial manner, based on orthographic processing *only*, with other representations (lexical, phonological and semantic) being activated later on; or whether, the early visual processing is also subject to feedback information in an interactive mode, for a schematic summary see Figure 4.2, (Carreiras et al., 2014).
One way to distinguish between bottom up and top down processing, or better, to investigate if the cascade of events from the visual input to the semantics is hierarchical, or if higher level linguistic information (lexical, phonological or semantic) exerts any kind of top-down influence on early stages of visual word system, is to examine the temporal dynamics of information processing with high temporal resolution techniques, such as MEG and EEG (Carreiras et al., 2014).

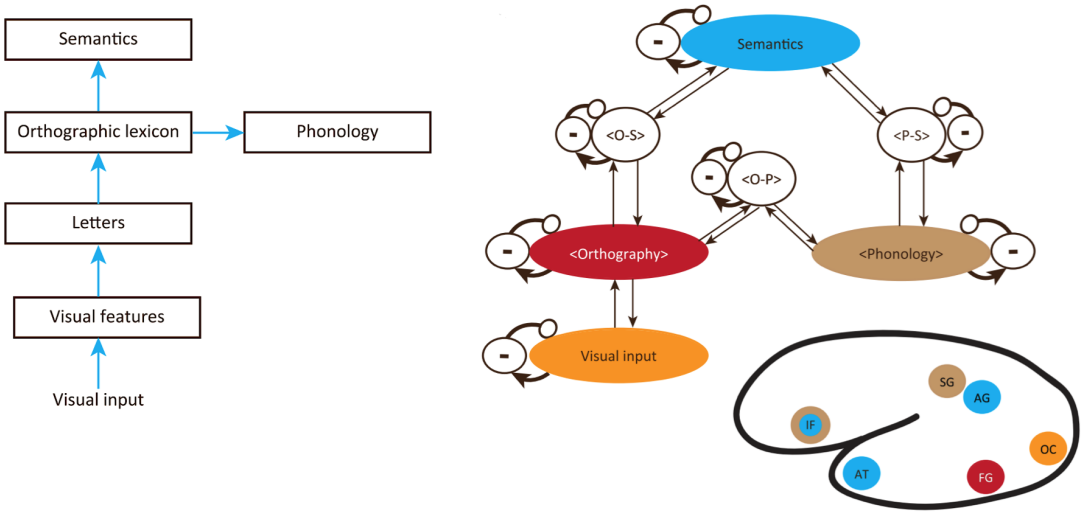


Fig. 4.2 Temporally modular and interactive connectionist models of reading. Schematic comparison between temporally modular or feedforward (left), and interactive feedback connectionist model (right) of processing during visual word recognition. The connectionist theory (right), pictures a more detailed model, including orthographic, phonological and semantic representations, and intermediate pools of neurons, denoted as <o-p>, <o-s>, <p-s>, and feedback/feedforward connections (arrows). According to this theory, the cascade of events from the visual input until the comprehension of the word, is characterised with intermediate serial processes that work rather in a parallel than serial fashion. To validate this hypothesis it is possible to study dynamic interactions between brain regions associated with different representations (colour coded), [IF=inferior frontal cortex; SG=supramarginal gyrus; AG=angular gyrus; AT=anterior temporal cortex; FG=fusiform gyrus with WVFA; OC=occipital cortex]. *Adapted from Carreiras et al. (2014) and based on initial theory of Seidenberg (1989).*

For instance, Pammer et al. (2004) used MEG to measure the spatiotemporal evolution of the first half second of the visual word recognition during a visual lexical decision task. By using a Synthetic Aperture Magnetometry (SAM) beamformer to estimate time series and measure power changes in 10-20 Hz frequency band they found that, when subjects were presented with words, there were two different phases in the occipito-temporal activation. First phase, from 0 to 200 ms, where an event related synchronisation (ERS) was localised predominately in the left hemisphere, in the lingual gyrus, cuneus and posterior fusiform gyrus (FG), and the second phase from 100 to 300 ms, in a form of event related desynchronisation (ERD), in the region of the VWFA (anterior fusiform gyrus) and posterior superior inferior frontal gyrus (IFG), (Pammer et al., 2004). In addition, when the stimuli presented were anagrams, VWFA was activated around 150-350 ms after stimulus onset and IFG at 75-275 ms. The authors concluded that there might be two different kinds of occipitotemporal activation, where the first one (0-200 ms) is localised in the posterior fusiform gyrus and it is pre-lexical, since the activation was present for both anagrams and words like stimuli, and a second one (100-300 ms), as ERD in the VWFA area. Interestingly, for this second component there was no difference in synchrony between words and anagrams, whereas it was present in the earliest stage with the activation of the posterior IFG. Pammer et al. (2004) suggested that the earlier activation of this area for anagrams compared to words, indicates an early incitement of feedback activity by a system that is required to make a decision on a lexical task, in order to facilitate the phonological processing in the grapheme-phoneme mechanism. This study is one of the first examples of neurophysiological correlates of the VWFA's pre-lexical functional specificity, that introduces an interactive early visual word system model, using MEG beamformer solution.

While *evoked* brain activity reflects event-related responses by averaging MEG activity across repeated trials, *induced* or frequency-domain analysis is based on time but not phase locked event related activity and might provide more information about dynamic changes in language-related processing (Eulitz et al., 2000).

To further investigate the early visual word processing components Cornelissen et al. (2009) employed a MEG task involving passive viewing of faces, consonants and words. In this case, a ROI analysis was applied to explore time frequency evoked (time and amplitude of ERFs) and induced activity in the left middle occipital gyrus (MOG), left IFG, left mid FG (VWFA). The authors confirmed the findings of Pammer et al. (2004), where the early IFG activation was left lateralised and specific for words. Moreover, in the time course of evoked responses there was a time overlap in the activation of left MOG, IFG and VWFA. In particular, the left IFG responded as quickly as the VWFA to words and only 14 ms after the activation in the left MOG. This evidence, seems to point away from the serial temporal processing, where an initial visual response to letter formed in MOG would sequentially lead to the abstract orthographic representation in the VWFA, and then in turn activate more language related areas, such as pars opercularis in the the inferior frontal gyrus (linked to phonological processing).

Other neuroimaging studies have shown early inferior frontal feedback activity onto the left ventral occipito-temporal cortex (VWFA). For instance, Woodhead et al. (2014) used MEG dynamic casual modeling (DCM) connectivity study to investigate the information flow between bilateral occipital cortex, ventral visual stream (VWFA) and inferior frontal gyrus. They observed stronger connections for words than false font stimuli, as early as 0-100 ms of stimulus onset, and feedback connections from left IFG to the vOT in the 1-200 ms time window. Further, in the 1-300 ms time window, stronger connectivity for words vs false fonts was detected in the feedforward swipe from left OCC to respectively, IFG and vOT (VWFA).
Altogether, these studies imply the presence of interaction between language areas and visual processing, in the first stages of word recognition. The 'visual word system' emerges as a distributed network of core regions that are activated within the first 300 ms of word-like stimuli onset. This would lead to the rejection of the serial hypothesis, and to advancing an interactive model where the activation of the VWFA does not mean exclusive 'functional specificity', but reflects rather the letter-string tuning habituation, where familiar words would elicit a faster and more automated response (Pammer et al., 2004).

4.1.3 The paradigm design - "Super Mario"

The first experimental study of this thesis has analysed differences in the RSFC within the dyslexic group based on fluency phenotypes. Fluency was measured with a single-word and nonword reading task (TOWRE), as a sensible psychometric measure of reading difficulties in dyslexia. A reasonable following step was to design a task that would tackle the first stages of visual word recognition, trying to understand if there are underlying mechanisms that could be classified as predictors of successful fluent reading acquisition. For that reason, and keeping in mind the target population in this work, there was a necessity to create:

- an interactive single-word task that would actively target the functional specificity of the visual word from system, keeping the participants engaged in the task, hence "Super Mario" as response cue [1].
- phonologically plausible stimuli, or strings of letters (pseudowords) that could be used as primers in the discrimination task, see Figure 4.3 for an illustrative example.
- proper manipulation of bi-gram frequency of the stimuli and pseudoword length, since it has been shown previously that word length has a significant effect on the activity in the occipital areas (Assadollahi and Pulvermüller, 2003).

[1] Originally, "Super Mario" was a Japanese video game created and designed by Shigeru Miyamoto and Takashi Tezuka in the late eighties by the Nintendo Ltd creative department. The attractive and captivating story line, where the main character Mario lives in a fantasy setting named the Mushroom Kingdom and has to fight against a lot of interesting creatures to ultimately save Princess Toadstool from her kidnappers, made it one of the most popular video games ever. Ever since, a lot of released versions have been produced by Nintendo, making it still very trendy. Source: Wikipedia (2019)

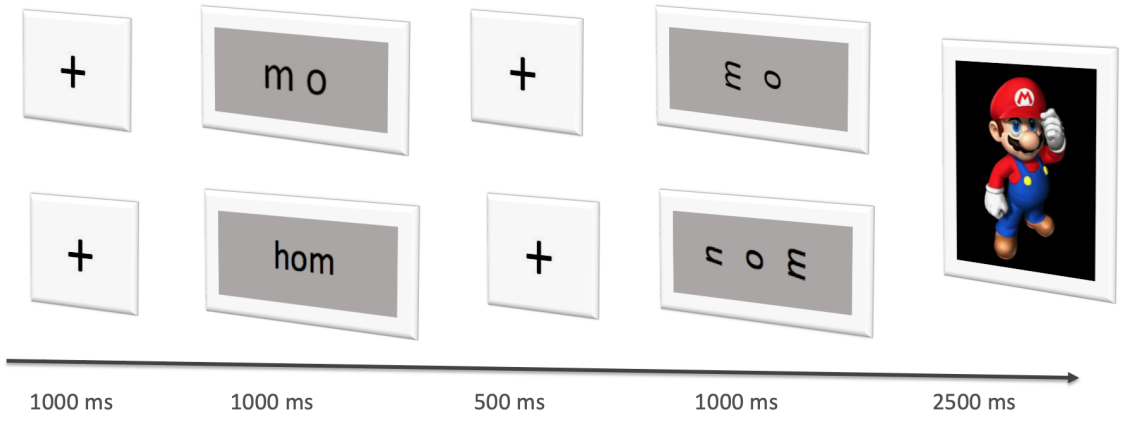


Fig. 4.3 "Super Mario", paradigm design. The sequence of the stimuli presented: the first fixation cross (1000 ms) was followed by the first stimulus presentation (S1), a phonologically plausible pseudoword presented for 1000 ms. S1 was then followed by the second fixation cross (500 ms), and a 'rotated' stimulus (S2), presented for 1000 ms. In each block (3, 4, 5 letters), in half of the trials S2 was the same string of letters just rotated, and in the other half (as shown in the second row) S2 was a different string of letters. Pictured here is one trial from the practise run (2-letter strings). Below is an example of a trial with 3-letters string of pseudowords (1st block). Participants were instructed to press the 'same' or 'different' key buttons when Super Mario appeared on the screen and were given up to 2,5 sec to make the decision. Each condition was composed by 60 trials (30 same and 30 different pairs of stimuli), presented in randomised order. Each block (condition) lasted around ∼5 minutes and participants were allowed self-paced breaks between the blocks. To make it child-friendly and to encourage participants to pay attention to the task, different feedback sounds for correct and incorrect trials were delivered. The correct response sounded like the original 'coin' sound in the authentic Nintendo game, therefore young participants were instructed to collect 'as many Super Mario coins as they could.' At the end of the task, individual performance or accuracy (number of coins collected) appeared on the screen.

Prior to employing the task to study differences in visuo-orthographic processing in typical and atypical young readers, this MEG paradigm was validated with a pilot study, described here.

4.1.4 Rationale of the current study

Given that there is a pattern of widespread cortical activation in the first half second of word recognition, as interaction between visual and language areas, a question that has been posed by neuroimaging of reading is how does this encoding network acquire its word specificity.
Previous studies have shown higher activation of the left vOT (VWFA area) and top-down influence of the left IFG for words, compared to pseudowords, anagrams, consonants and faces (Cornelissen et al., 2009; Pammer et al., 2004; Woodhead et al., 2014). Furthermore, Wheat et al. (2010) used a pseudohomophone (e.g. brein-BRAIN; or orthographic cues broin-BRAIN) priming MEG task to show early (< 100 ms) tuning of the inferior frontal and pre-central gyri, as evidence of prelexical access to phonological information during early visual word recognition. More interestingly, when they compared orthographic versus the phonological priming in the first $\sim$100 ms, the inferior frontal cluster composed by the posterior IFG (poIFG) and precentral gyrus (PCG) displayed greater induced 30-40 Hz activity for the shared phonology condition than the orthographic one (Wheat et al., 2010). The authors concluded that the early engagement of the left MOG-poIFG-PCG reflects the pre-lexical orthographic-phonological mapping in the visual word processing (Wheat et al., 2010). The role of visual priming has been extensively used in dyslexia literature to show how different linguistic mechanisms interact between each other. For instance, by manipulating the semantic, and the form overlap between morphologically paired words Quémart and Casalis (2015) to show that for dyslexic readers the priming effect was significant for in the morphological condition only, driven by orthographic properties. When using ERP study design measuring phonological and orthographic priming Savill and Thierry (2011), found group differences in N1 (amplitude) range, where orthographic modulations observed in typical readers were absent in the dyslexic group. These studies all point towards the idea that there might be perceptual difficulties at the word form level, that might impact reading in different ways.
Considering that previous MEG studies (Cornelissen et al., 2009; Kujala et al., 2007; Pammer et al., 2004; Wheat et al., 2010; Woodhead et al., 2014) were able to distinguish feedforward and feedback connectivity between occipito-temporal (visual) and inferior-frontal (linguistic) areas in both passive and active (lexical decision) word reading, an interesting question would be if the same network can be triggered by orthographic word-like stimuli only. Clearly to be able to address the dynamic features and potential differences in patterns of activation within the stages of visuo-orthographic processing only, a tailored approach is needed. The rationale behind studies described here (Chapter 4) and in Chapter 5, fits in this perspective, with the proposition of a tool validation that can help to better define and measure neural markers of visuo-orthographic processing.

The main goals of the current study are as follows:

1. Corroborate "Super Mario" as a valid paradigm for investigation of prelexical visuo-orthographic processing in a sample of skilled readers. This validation is considered a crucial step before applying the paradigm to better understand visuo-orthographic specialisation in (a)typical reading (Chapter 5).

2. Investigate if focusing on orthographic manipulation of non familiar words (pseudowords) would elicit the same neural responses described in tasks that used words and pseudowords contrast. In particular, since previous findings have shown how the VWFA is equally activated by pseudowords and real words (Ashby et al., 2009; Cohen et al., 2002), this study aims to understand if zooming onto pre-lexical processing only, would provide more insight on how this area develops its functional specialisation for printed words (Chapter 5).

3. The driving hypothesis for focusing only on pronounceable or phonologically plausible pseudowords was to limit the activation of more linguistic (lexical, semantic and phonological areas) and to try to emulate the emerging of the visual word form system expertise by using orthographically novel type of stimuli. Following this, the last aim is to examine if there are any top-down constraints on the visuo-orthographic processing here elicited as proposed by previous studies (Cornelissen et al., 2009; Kujala et al., 2007; Pammer et al., 2004; Wheat et al., 2010; Woodhead et al., 2014).

It has been mentioned previously that the overreaching goal of this thesis is to define better trajectories between atypical and typical reading development, but also, to try to define sub-types of developmental dyslexia. "Super Mario" was, therefore, designed to investigate visuo-orthographic processing, as the first stage of word analysis in reading. Ergo, the ultimate goal would be not only to describe the underlying neural mechanisms, but also, to pinpoint differences in visuo-orthographic specialisation in a/typical reading. Following this, the purpose of identifying neural correlates of (a)typical visuo-orthographic processing would be to further apply those in the definition of subcategories within the dyslexic continuum.

4.2 Methods

The MEG paradigm, hereafter "Super Mario", featured three blocks divided by pseudoword length (3 letters, 4 letters and 5 letters). Each block was composed of 60 stimuli (pseudowords) created using the online free ARC Nonword Database [2] by the Department of Cognitive Sciences (Macquarie University, Sydney, Australia). As shown in Figure 4.3, the second stimulus (S2), was always rotated by 180° and written in italic. In half of the trials S1 (cue stimulus) was the same pseudoword in S2 just rotated, and in the other half of the trials, was always rotated, but differing either by similar letter substitution ('b' for 'd' or 'n' for 'h') or by changing the letters order ('suz' for 'szu'). The participants were asked to make a discrimination (same or different?) and press the response button

[2] www.cogsci.mq.edu.au/research/resources/nwdb/nwdb.html

when they saw "Super Mario" (see Figure 4.3), after which they would hear a feedback sound for correct or incorrect answers.

4.2.1 Participants

Ten young to adult participants, recruited among research staff and their families kindly agreed to take part to the study. MEG and MRI data was recorded at the Aston Brain Centre (Aston University, Birmingham) over a period of six weeks. The general exclusion criteria described in Chapter 2 for imaging were applied: no history of psychiatric or learning difficulties (dyslexia), epilepsy or major recent surgeries, no metal implants in the body, normal or correct-to-normal vision. Before scanning, all participants provided written consent for participating in a MEG and MRI experiment. This protocol was approved by the Aston University Research Ethics Committee (REC), ethics code 1220.

4.2.2 MEG data acquisition and preprocessing

Ahead of the MEG session, participants did a practise run of five minutes, with two letters stimuli, to check if they understood "Super Mario"'s instructions and were playing the game properly.
As mentioned above, the task was divided in 3 conditions or blocks: 3 letters, 4 letters and 5 letters. Each condition was composed of 60 trials (180 in total), and lasted $\sim$5 minutes, after which there was always a break, where participants were asked to press a button when ready to continue. Each trial consisted of an initial fixation cross displayed for 1000 ms at the centre of the projector screen in the MSR, S1 as the first cue stimulus or prime pseudoword presented for 1000 ms, second fixation cross displayed for 500 ms, S2 as the second stimulus or the discriminatory cue displayed for 1000 ms, and finally the response cue target or "Super Mario" displayed for up to 2500 ms.
"Super Mario" paradigm was presented on a projector located in the MSR at the approximate distance of 86 cm from the seated participant, with a resolution of 1400 x 1050. To run the paradigm Neurobehavioral Presentation System (Neurobehavioral Systems, Albany, CA) software was used on an external Windows PC, which recorded the accuracy (hits, miss and incorrect responses) and reaction time (RT) for each participant, sent the triggers to the Elekta MEG recording PC, and delivered audio feedback to the participants attending the experiment through rubber earphones they worn, while performing the task in the MSR. The recordings lasted approximately between 12-17 minutes (based on the amount of time used during the breaks in between conditions and individual RTs. During the task, participants were seated comfortably in the upright MEG chair with the response pad resting on a cushion on their lap, and were instructed to use left and right index fingers to press the buttons when responding to the game, to minimise lateralisation due to the use of the same finger. Note that for this and further analysis the response period, when "Super Mario" cue occurred, was excluded from MEG analysis.Additional to "Super Mario" acquisition, for participants who did not have T1 MRI image stored on the Aston Brain Centre system, a structural scan was acquired for the MEG/MRI co-registration purposes.

Preprocessing

As described in detail in Chapter 2, MEG data were acquired with a whole-head-306 channel neuromagnetometer (Elekta-Neuromag, Finland), sampled at 1 kHZ, and preprocessed using the temporal space source separation (tsss) in MaxFilter software (Elekta Neuromag Oy, version 2.2.10). Again, to ensure the removal of artefacts/noise originating outside and inside/close to the MEG sphere, tSSS was applied with a sliding window of 30 seconds and a sub space correlation limit of 0.9 (Taulu and Simola, 2006; Taulu et al., 2005). Additionally, HPI head movement tracking was also recorded even though in this group of participants, movement compensation (mc) was not applied since participant's head movement was < 6 mm. For the purposes of sensor level analysis, channel position from the raw recordings was aligned to the mean or default sensor position.
Then, continuous tsss-ed MEG recordings were imported into Matlab® (MathWorks, 2016a) open source FieldTrip toolbox (Oostenveld et al., 2011), bandpass filetered 0.5-250 Hz and segmented into 3.5 seconds epochs (from -2.5 sec previous S2 onset to 1 sec afterwards). Only the correct trials, where participants were able to make the correct distinction between S1 and S2, were included in the analysis. On average, > 60% of correct trials for each condition, were included in the analysis (approximately 110-140/180 trials overall). Furthermore, each trial included in the analysis was visually inspected for artefacts and the noisy ones were excluded from further analysis.

4.2.3 MEG data analysis

In order to validate "Super Mario", first, time evoked sensor level analysis was carried out. Primarily to check if the stimuli would elicit classic print-related neurophysiological markers (N170) and, second to investigate difference between S1 and S2 elicited responses. Following this, source analysis using LCMV beamformer was performed.

Sensor level analysis

To further clean the data from movement, ocular, heart and muscular artefacts, independent component analysis (ICA) was utilised. The benefit of the ICA application within FieldTrip is that one can look at the time course of each component, and knowing the ECG topography for instance, spot the regular occurrence of eye or heart physiological contributions. Grand averages of evoked fields (100-300 ms), derived from planar gradiometers signal, were computed over subjects, for S1 and S2 in 1-30 Hz frequency bands. The comparison between S1 and S2 condition was accomplished with a two-tailed dependent t-test and controlled for multiple comparisons (MCP) by using non parametric cluster permutation analysis (number of permutations = 5000), implemented in Fieldtrip and described in Maris and Oostenveld (2007).

Source level analysis

As already described in the methods section (Chapter 2), to reconstruct the data in source space, the first step was to co-register, for each participant, individual MRI weighted T1 images with the MEG fiducials points and headshape, by using an in house Matlab script and MRILab Elekta software (version 1.7.25). Then, the resulting individual single-shell (5 mm) headmodels were nomalised in MNI space (Montreal Neurological Institute template) and used in further analyses. Next, Linearly Constrained Minimum Variance (LCMV) beamformer was applied to produce a 3D spatial distribution of power (1-30 Hz) of the neuronal sources of S1 and S2 in the 0-300 ms of stimulus onset. To compare the two conditions, S1 and S2 sources, grand average were computed over subjects and then compared, as above, with a two-tailed dependent t-test, controlled for MCP by using non parametric cluster permutation analysis (number of permutations = 5000), (Maris and Oostenveld, 2007).

4.3 Results

Ten young to adult volunteers (mean age, 24 years and 6 months; minimum 8 years old and maximum 53 years old) took part to the pilot study. For the purposes of the very first validation of the paradigm, it was important to include younger volunteers (less than 14 years old) to check if the design and length of the task would be feasible for younger children and target populations of the study described in Chapter 5. For all the volunteers, the accuracy level at "Super Mario" task was at the ceiling level (>90%), which indicated that for expert readers the timing of the stimuli presentation was appropriate in all three conditions (3, 4, 5 letters).

4.3.1 Sensor level outcome

At the group level, event-related fields (ERFs) cluster permutation t-testing did not show any significant results in the 100-300 ms time range, for lower frequency bands (1-30 Hz), with p-value > .05. For validation scope, during the design of the paradigm, individual t-tests between S1 and S2 were computed for a lower number of subjects (n=6), which did show above-chance-level difference in the event related average activity between S2 and S1.
As an additional sanity check, topographic and time distributions of the two conditions (S1 vs S2) and their difference (S2-S1) were visually explored by taking a look at the sensor-level activity in the magnetometers only. Figure 4.4 shows how the average signal of the S2 stimulus has a negatively larger amplitude, compared to the S1 (priming stimulus). Further, taking a closer look at group of sensors (magnetometers) that approximately overlap the VWFA (vOT sensors), the N170 ($\sim$140 ms) looks larger for the S2 stimuli. Figure 4.5 displays the topological distribution of the difference between S2 and S1, in steps of 10 ms from 0 to 600 ms after stimulus onset. Interestingly, the second graph on the right (100-200 ms) shows the canonical left lateralised N170 ERD topology, (Sánchez-Vincitore et al., 2017).

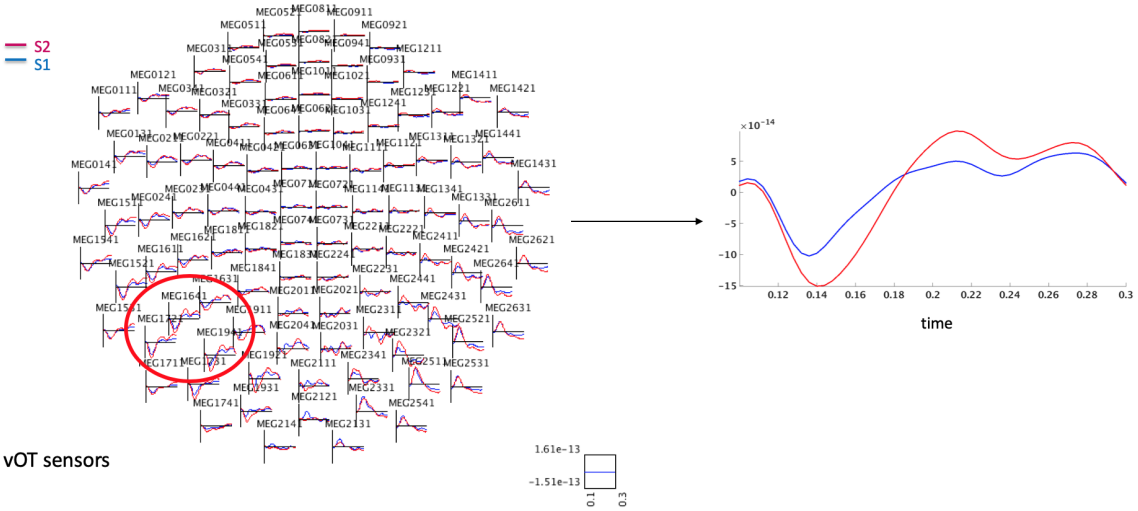


Fig. 4.4 Sensor level event-related grand averages for the cue (S1) and target stimuli (S2). S1 and S2 event-related grand averages, plotted at the sensor level (magnetometers). S1 (blue) and S2 (red) grand averages over subjects (n=10) are plotted here as a function of time (0-300 ms). On the right, a closer look at the amplitude of the ERP in the ventro-occipital channels (VWFA area), and their event related fields (ERFs) distribution.

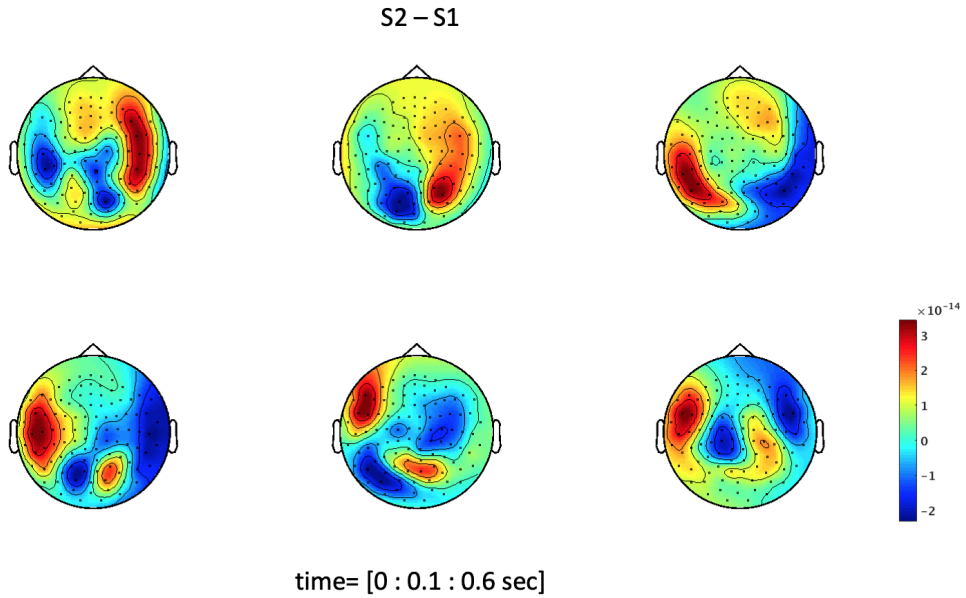


Fig. 4.5 Event related fields' (ERF) topology (pilot group).This figure shows the topological distribution of the S2-S1 grand average difference in the 0-600 stimulus onset time range, in steps of 100 ms each. The colorbar represents the amplitude of the difference.

4.3.2 Source analysis results

In addition to the advantages illustrated in the methods chapter, previous studies showed (Cornelissen et al., 2009; Kujala et al., 2007; Pammer et al., 2004) how analysing early visual word processing in source space, in lower frequency bands (1-30 Hz) is much more informative than the sensor-space analysis. Here, a LCMV beamformer was applied to 0-500 ms of activation in S1 and S2 conditions, that were then compared with cluster permutation (number of permutations=5000) t-testing implemented in FieldTrip, (Maris and Oostenveld, 2007). Here, the time window of interest was increased in respect to the time-evoked analysis to be able to look at the induced activity as well. The comparison between S1 and S2, showed significant differences (p-value< 0.05) in distributed negative clusters, illustrated in Figure 4.5. The main differences between S1 and S2 conditions, in the first 500 ms of stimulus onset, were detected in the middle occipital (MOG) cortex, left and right fusiform gyrus (VWFA area) and superior parietal cortex, overlapping with some of the previous findings (Pammer et al., 2004; Wheat et al., 2010). Contrary to the initial hypothesis and the assumptions of the reading literature there was no activation, either earlier (<200 ms) or later (from 200 ms to 500 ms), of the inferior frontal gyrus (IFG).

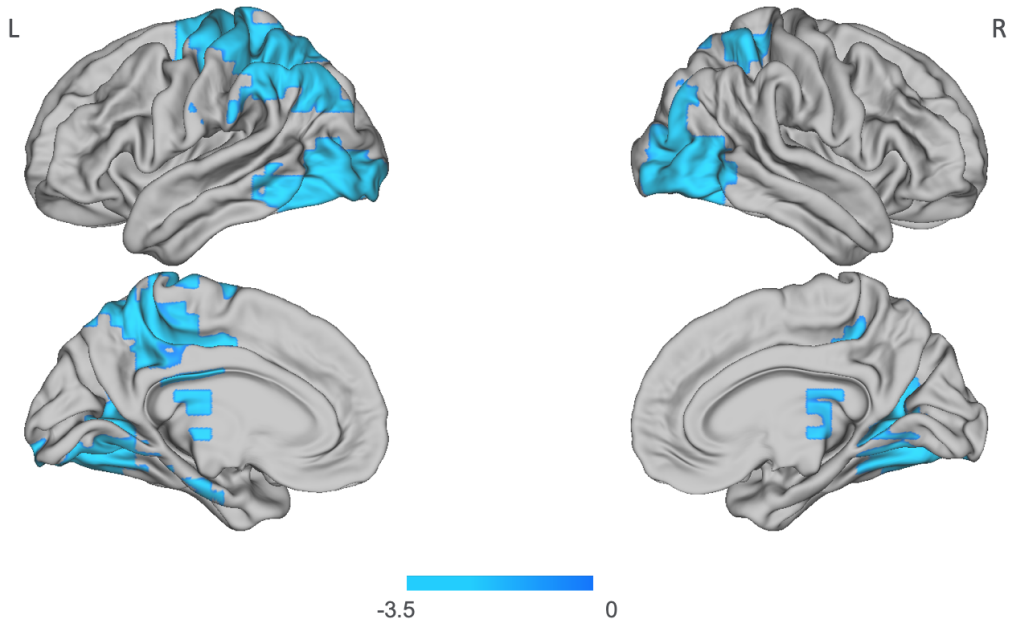


Fig. 4.6 "Super Mario" source level results pilot study. Brain maps of topological distributions of negative significant clusters (p-value=.007), in the 0-500 ms time range, using t-values. This shows significant differences in Event Related Desynchronisation (ERDs) in the S2 versus S1 comparison. Note that, contrary to the expectations, no feedback activity was observed from anterior ares, such as the left inferior frontal gyrus (IFG), suggesting that in this case the orthographic visual manipulation did not activate linguistic feedback, as observed previously in the literature.

4.4 Discussion

To reiterate, current study was designed to investigate if the newly designed task ("Super Mario") would:

- be a feasible paradigm to measure pre-lexical and orthographic manipulation of word-like stimuli in expert readers,
- elicit responses from the early visual word form system (vOT and VWFA), and if
- more linguistic areas, such as the IFG, would be equally activated in the discrimination of orthography of unfamiliar word-like stimuli, as proposed by the model of pre-lexical orthographic and phonological mapping.

Previous studies have mainly investigated visuo-orthographic processing in relation to target words as stimuli, either by passive or active (priming or discrimination tasks) paradigms, for instance Ashby et al. (2009); Pammer et al. (2004); Wheat et al. (2010). Inversely, the design of "Super Mario", using only pseudowords as stimuli, aimed to zoom in onto the pre-lexical processing, trying to simulate, as much as possible, the naturalistic 'learning phase during reading instruction', discussed in detail in the next chapter (Chapter 5). For that reason, in the effort to try to characterise better the underlying mechanisms of visuo-orthographic processing *only*, it was considered important to find a way to separate the visual word form information from the the linguistic one. At the behavioural level, all the participants performed at the ceiling level (> 90% accuracy), given a very short time of presentation (1000ms) and increasing difficulty (pseudoword length) which indicated that the task completion did not require complex and elaborate cognitive processing.

At the neural level, the outcome of this study primarily showed how manipulating the orthography of pseudowords leads to desynchornisation of neural activity in distributed sources of left and right cortex, as seen in Figure 4.6. More specifically, present findings confirmed the presence of an early visuo-orthographic processor composed by middle occipito-temporal cortex able to decipher letter-like stimuli, and inferior fusiform gyrus (VWFA), where orthographic abstract representation are stored (Cornelissen et al., 2009; Woodhead et al., 2014). In addition, the effect was spread onto the right visual form area. This is in line with the evidence of the VWFA's retinotopic letter-position sensitivity. In a high-resolution fMRI study Rauschecker et al. (2012) showed how changing the letters position elicited responses form bilateral vOT areas. This suggest that the left and right visual word processors work as 'unified' circuit to interface vision and language (Rauschecker et al., 2012). This obviously does not dispute the specialisation of the left vOT cortex and VWFA role in the visuo-ortographic processing, but rather builds upon the hypothesis of visual word form network that involves multiple areas depending on the stimulus configuration (in this case, rotated letters decreased the activation within the system permeable to coding letters position). Patterns of bi-lateral activation have been also observed during reading acquisition (Caffarra et al., 2017), which will be discussed in detail in Chapter 5.

Lastly, this study aimed to investigate if more linguistic areas (IFG) would play role in the "Super Mario"'s visuo-ortographic decision making. This was anticipated following models from the literature, that predict that the abstract representation of a word-like stimulus formed in the VWFA, is transferred to the phonological processing of the inferior forntal gyrus and, as proposed in some studies, how this neural interaction often occurs in parallel rather than in a serial fashion (Cornelissen et al., 2009; Wheat et al., 2010; Woodhead et al., 2014).
This appears not to be the case for "Super Mario". Instead, what this study showed is the presence of a top-down influence from the superior parietal cortex. This suggests an initial involvement in the first half-second (0-500 ms) of viso-orthographic processing of the attentional network, in the discrimination of the two conditions. Accordingly, when the visual word processor is exposed to unfamiliar stimuli (pseudowords) it would require more effort, or allocated attention (as feedback activity from the dorsal processing route), to be able to efficiently process the novel word-like information. This is not the first time that the role of attention has been proposed to be part of the visual automatic processing. For instance Yoncheva et al. (2010) showed how attention drove the N170 specialisation in the grapheme to phoneme conversion of artificial script. Moreover, bilateral occipital activation has been previously detected in the object and word like recognition in children learning to read (Caffarra et al., 2017). More generally, superior parietal cortices have been shown to have a pivotal role into modulating attention of attended and non-attended stimuli in the alpha band (Lobier et al., 2018). Thus, the dorsal attentional network seems to have a role in the discrimination of pseudowords's visual form manipulation, in a top-down fashion, in the first half second of information processing. This is an interesting finding that requires further investigation, perhaps with a more sophisticated analysis approach such as directed connectivity measures (Granger causality or Phase Slope Index). Lastly, based on cognitive theories previously illustrated (Chapter 1) it has been demonstrated how attention is a very important feature in reading. In facet, allocation of attention during the parsing of letters during the acquisition of fluency can be impaired in deficient reading (dyslexia), (Hari and Renvall, 2001; Hari et al., 1999; Lallier et al., 2010).

In summary, this pilot study results indicate that "Super Mario" is a feasible tool to measure visuo-ortographic processing of word-like stimuli. Specifically, present findings demonstrate how this novel type of task can be used to investigate neural correlates of pre-lexical processing, by probing specific and related (dorsal network) areas of the visual word system in the first half second of visual word-like discrimination.
To conclude, the outcome of this validation study confirmed that "Super Mario" is able to tackle visuo-ortographic processing and can be further applied to investigate the neural correlates of unfamiliar word recognition in typical and dyslexic readers (Chapter 5).

4.5 Conclusions

This pilot study was primarily carried out to validate a novel in house created paradigm, designed to measure automatised visuo-orthographic processing in typical and atypical young readers. The results showed that "Super Mario" can be applied to investigate the neural correlates of prelexical processing in dyslexic and typical young readers. The next chapter (chapter 5) will describe the empirical application of this MEG task to explore differences in early stages of visual word recognition in (a)typical reading.

Chapter 5

"Super Mario" in typical and atypical young readers

Chapter summary

This chapter will describe the outcome of "Super Mario" application as a tool to investigate visuo-orthographic processing in typical and atypical (dyslexic) young readers. It will start with the discussion of neural mechanisms involved in the development of visual word recognition, with particular focus on how neural correlates of early stages of the viso-orthographic processing can be applied to characterise dyslexic and typical reading. Following this, it will illustrate participants and methods used in this study. Finally, the results will be discussed.

5.1 Introduction

Ideally, to be able to fully comprehend how the brain of skilled readers is organised, neuroscientists should be able to study cortical changes prior, during and after reading acquisition (Schlaggar and McCandliss, 2007). In the cascade of events that lead from viewing print to reading efficiently, the first question would be how does the brain develop visual expertise for words?
Along with neuroimaging findings described previously, additional evidence from behavioural and eye-tracking studies (Grainger et al., 2003; Reichle et al., 2003) shows how the visual word recognition is characterised by the word superiority effect, where words and pseudowords elicit a perceptual facilitation, at very short fixation time (200-300 ms). To be able to further define these fast cognitive processes and to better understand the 'critical' time-course of these events, the use of single words neurophysiology (EEG and MEG) studies provides a very useful tool (Maurer and McCandliss, 2007). As introduced in the previous chapter, in the context of visual object fast specialisation framework, it has been widely acknowledged that the left N170 is the brain signature of rapid visual word recognition.
An important question in this perspective is if this perceptual specialisation emerges with literacy acquisition or represents a precursor of reading proficiency in general (Maurer et al., 2005b).
Developmental studies of reading offer, therefore, an unique opportunity to gain insights into the nature of the N170 specialisation and its left lateralization (Maurer et al., 2010). Moreoverbeing able to thoroughly depict neural mechanisms involved in the emergence of visual expertise for printed words, could help to identify predictors of probable reading deficit or delay, as in case of developmental dyslexia.
Within this framework, several studies have investigated the effects of early visual system exposure and its training to recognise 'novel' printed stimuli (words, pseudowords and symbols) in adults and young readers. For instance, Maurer et al. (2005b) used an implicit reading test to compare ERP mapping of words, pseudowords, symbol strings and pictures in 6 year-old kindergarten children and adults. Where skilled adult readers showed a fast (< 150 ms) left lateralised, N1 occipito-temporal response in the differentiation between symbols and letters, kindergarten children, with partial letter knowledge, did not exhibit this dissociation and the N1 effect (Maurer et al., 2005b). Contrarily, for non-skilled readers the pattern of activations (brain topography) was widely distributed on the cortical surface and the latencies of the ERPs were longer, reflecting most likely ongoing maturational changes. In addition, in children with high letter knowledge, right occipito-temporal familiarity effect was observed in the discrimination of words/symbols, indicating that an early exposure to literacy promotes an 'automatised visual processing' but very distant from the mature left-laterizied early visual system (Maurer et al., 2005b).
To further explore the right N170 effect and cortical changes that occur during the exposure to novel orthography, as it takes place during reading instruction, Maurer et al. (2010) used a artificial script training ERP study (participants were trained to memorise symbol-word pairs) in a group of adult skilled readers. The results showed a specific pre/post right-lateralised N170 effect for short term

learning of artificial orthography. Both of these studies converge on the hypothesis that evoked responses to newly learned scripts (Maurer et al., 2010) or during earning phase (Maurer et al., 2005b) reflect familiarity, or N170, that is not (yet) activating the automatized and efficient left lateralised early visual system. This was confirmed more recently by Sánchez-Vincitore et al. (2017) who used implicit (one back) and explicit (reading verification) tasks to show how the left lateralised N170 automaticity was specific for skilled readers but not for neoliterate ones. Furthermore, the new readers group (neoliterate) exhibited a prominent right-lateralised N170 engagement during the reading verification task.
Overall, ERP mapping studies suggest how the visual familiarity and the automaticity of the left-lateralised visual word system is characteristic of a mature and experienced reading network.
Following this line of work, an interesting research question would be to explore the neurophysiological correlates of visual word system in typical and atypical readers (dyslexic) in visuo-orthographic processing of pseudowords, using the "Super Mario" paradigm.

5.1.1 Visuo-orthographic processing in dyslexia

Before outlining the hypothesis of the current study, it is considered necessary to provide an overview of previous studies investigating visuo-orthographic processing in dyslexia. For a long time dyslexia has mainly been associated with deficits in the phonological processing and related brain areas (Ramus et al., 2003). Although it has been repeatedly shown that the reduced activation in superior temporal regions is linked to impaired phonological processing, considered the core deficit of dyslexia (Shaywitz and Shaywitz, 2005), different studies reported reduced activation in the inferior occipito-temporal areas, implying difficulties in the visual word recognition as well (McCandliss et al., 2003). Additionally, other neuroimaging studies have demonstrated how the dyslexic brain is not only characterised by reduced neural activation in the core regions of the reading network, but also presents increased activation, in other posterior and frontal regions. This phenomenon of over-activation has been linked to mechanisms of compensation (Shaywitz et al., 2002).
It can be appreciated that although dyslexia is a well defined reading deficit with persistent symptoms, it still remains quite challenging to weigh or measure the specific contribution of underlying cortical regions in the disruption of the functional reading network.
As proposed earlier, one way to reduce the variables of the problem, in the attempt to identify predictors of the reading deficit, would be to either focus on the early reading acquisition stages (pre-school age range) or, within the time-scale of reading processing, on (early) neural activation during the analysis of unfamiliar words, (Maurer et al., 2007). In this framework, N170 studies have been an extensively applied method to investigate visuo-orthographic processing in dyslexic readers.
One of the first evidence of impaired orthographic processing in dyslexics goes back to Helenius et al. (1999). Using an MEG study, the authors showed how the 'early' visual evoked activity dissociated between adult fluent and dyslexic readers only for the letter-string condition. In functional brain imaging (fMRI), with a big sample size (n=144) Shaywitz et al. (2002) found how children with

dyslexia exhibited a disrupted activity in posterior reading regions and how this correlated with their performance at the task. Assuming that the posterior reading network in dyslexic readers presents a different pattern of activation in the first stages of visual word-like recognition, it is important to understand if this is already present at early stages of reading instruction or it is the result of long-term unsuccessful reading (Maurer et al., 2007). The question would therefore be, if the different pattern of orthographic specialisation in the dyslexic population are cause or consequence of the reading impairment.

Several studies have tried to provide an answer. In a MEG study, Parviainen (2006) investigated the temporal sequence of neural activation in first-graders (7-8 years old) exposed to words embedded in different layers of noise, and showed similar but delayed activation patterns, compared to adult expert readers. Following this, in two ERP longitudinal studies Maurer et al. (2007, 2011) showed different developmental trajectories of the N170 tuning in typical and dyslexic readers. For the latter, the N170 specialisation occurred much later (fifth grade, 11 years old) than for unimpaired readers (second grade, 8 years old). To further investigate if impaired tuning of the N170 was specific to developmental dyslexia or more general feature of the reading instruction, Mahé et al. (2013) employed a lexical decision ERP mapping task to compare activations in dyslexic, poor and typical young readers. They showed how the impaired N170 automaticity was specific to dyslexic readers (Mahé et al., 2013). More recent studies have examined the specialisation of the occipito-temporal system in relation to fluency improvement (Fraga González et al., 2016b). In this study, fluency training was used to examine changes in N170 amplitude in eighteen (N=18) children with dyslexia. Specifically, Fraga González et al. (2016b) investigated if N170 would normalise in the dyslexic group after the training and if the same ERP signature could be used as a predictor of improvement or not. Interestingly, the authors observed how the N170 amplitude decreased in the post training phase and this pattern was only detectable in children who actually improved their performance after the training (described as 'improvers'). Taken together these findings, point towards the idea that the association between decrease of left N170 amplitude and fluency gain, mirrors an 'inverted U-shaped' dynamic visual learning process (Maurer et al., 2011). As also suggested earlier by Maurer et al. (2006) and Fraga González et al. (2014), children with dyslexia exhibit a stronger N170 (more activated VWFA), since they rely more on the orthographic processing, whereas trained and skilled readers exhibit a decreased N170 (Fraga González et al., 2016b).

In summary, N170 specialisation and the visuo-orthographic responses form the occipito-temporal system become, respectively, smaller and more lateralised as the reading acquisition progresses. It follows that N170 has the potential to be applied as a neural marker of reading acquisition.

5.1.2 Objectives of the current study

The current study aims to investigate neural correlates of visuo-orthographic processing in typical and atypical young readers (dyslexic) by applying the "Super Mario" paradigm, presented earlier, in chapter 4. In particular, the main goals of this chapter are to:

1. Examine "Super Mario" behavioural differences in performance (accuracy at the task) between dyslexic and typical children.

2. Explore interactions between dyslexic reading skills and performance at the task to confirm that "Super Mario" can be employed as a valid tool to define predictors of reading impairment, in developmental populations.

3. Study differences in neural correlates of visuo-orthographic specialisation (N170) in typical and atypical (dyslexic) young readers, at both sensor and source level. Finally, compare the current findings with the pilot study ones (Chapter 4).

In sum, based on the results in Chapter 4, the aim of the present study is to apply "Super Mario" to investigate neural correlates of visuo-orthographic processing in a group of typical and dyslexic readers. It would be of particular interest to probe if the same constellation of cortical areas, as seen in the pilot study (Chapter 4), is activated in this developmental population in, respectively, dyslexics and peer readers.

5.2 Methods

Details of participant's recruitment, neuroimaging exclusion and inclusion criteria, and data analysis have been previously described in the methods chapter (Chapter 2). The current project was approved by the Aston University Research Ethics Committee (REC), (approval number 1220). The study aimed to investigate differences in orthographic processing in impaired and non-impaired young readers (6 to 18 years old), by using a recently in-house developed MEG paradigm ("Super Mario"). In the following section employed materials and methods will be illustrated.

5.2.1 Participants

All participants and their guardians provided written and informed consent for participating in the study and screened for possible exclusion criteria before undergoing neuroimaging procedures (MEG and MRI). Each participant was rewarded with £20 Amazon voucher and offered a picture of their brain, derived from the T1 MRI image. Demographic information of the two groups and their performance can be found in Table 5.1.

Dyslexic group

As previously described in Chapter 2, the Aston Brain Centre also hosts the DDAU, where on a fortnightly basis, an educational psychologists assess cognitive and behavioural skills of children who struggle at school. This offers the opportunity to ABC researchers to approach families and invite them to take part to the ongoing neuroimaging projects. In this occasion, participants were first recruited upon their visit to the DDAU to take part to the ongoing longitudinal behavioural-genetics study (Chapter 6). Of those, 30% came back to the ABC for a second visit, to take part to the current neuroimaging study. As stated before, at the time of the recruitment and neuroimaging acquisition, researchers were not aware of the final outcome of the educational assessment to avoid potential biases in the analysis. Overall, nine young (mean age 11 years 10 months) dyslexic participants took part to the Super Mario study. One participant was further excluded because did not have the accuracy information at the time of the MEG acquisition.

Control group

For the control group, or typically developing readers, the recruitment was carried out via official online Aston Brain Centre's Twitter and Facebook pages, and the Aston University official bulletin (Aspects). More details about the means of recruitment and inclusion/exclusion criteria can be found in Chapter 2. Overall, twenty-one (N=21) typical young readers took part in this study. To create an age matched control sample for the dyslexia group, fifteen (n=15) participants were included (mean age 11 years and 7 months) in the data analysis. Specifically, the groups were matched based on mean age of the sub-group and not individually, Table 5.1.

5.2.2 MEG acquisition, preprocessing and data analysis

Again, details of general procedures of data acquisition, preprocessing and general analysis are illustrated in Chapter 2. For the MEG data acquisition, in a pseudo-randomised fashion, before or after Super Mario task all participants underwent a 6 minutes resting state scan. Additionally, for each participant, after MEG scanning, a T1 weighted MRI and DTI sequences were acquired. Before the MEG data acquisition participants had a practise run outside the MSR to make sure they understood the game's instruction properly. To make the instructions simpler, children were recommended to play "Super Mario" video game in the scanner 'trying to catch as many coins as possible'. Coins in this case, was the positive reinforcement (audio-feedback) heard every time they gave the right response. The task paradigm was the same as in the pilot study (Chapter 4). Composed by 3 blocks or conditions (3, 4 and 5 letters) of approximately 4-5 minutes each, ran in a self paced manner. To try to minimise potential sources of movement artefacts, participants were recommended to use the breaks in between the blocks to stretch, move, talk to parents or researchers to, then, restart playing the game, keeping as still as possible. Throughout, the same acquisition procedure, fully described in

the methods chapter (Chapters 2) and in the "Super Mario" pilot study (Chapter 4), was carried out here too.

Preprocessing

Accordingly, preprocessing procedure was the same as described in chapter 4 and introduced in chapter 2. In addition, for participants whose head movement exceeded 5 mm (6 out of 23 participants in total) movement compensation/correction, described in chapter 2, was carried out. Following this, continuous tsss-ed MEG data were imported into FieldTrip Matlab toolbox (Oostenveld et al., 2011) for further analysis. Generally, Fieldtrip presents a set of defined functions perform various types of sensor and source level analysis. [1]

Sensor level analysis

Event-related sensor level analysis was performed for each group (typical and dyslexics) to explore if there were any differences in the time specific elicited responses to the orthographic manipulation of the stimuli. As described in chapter 2 and 4, clean from muscular, heart or eye artefacts (ICA-ed) data segments of combined gradiometers, were used to compute grand averages of event related fields (ERFs) of respectively S1 and S2, from 0 to 300 ms after stimulus onset (0-300 ms), in the 1-30 Hz frequency bands.
Within each group, S1 and S2 ERF grand averages were compared via means of cluster permutation t-testing (number of permutations=5000), implemented in FieldTrip according to Maris and Oostenveld (2007). In brief, Fieldtrip based event related cluster based permutation t-testing is applied to gain inference about the existence or not of difference in processing either at the group or individual level (Maris, 2012). In this case, the null hypothesis was that S1 and S2 would be processed in the same way in the first 300 ms of stimulus onset (0-300 ms). However, the method offers the possibility to visually inspect the cluster topology or, rather, over which sensors the significant clusters were distributed, as shown in Figure 5.3 and Figure 5.5.

Source level analysis

Same as for the pilot study (chapter 4), to investigate further induced effects of the experimental modulation, source level analysis was carried out. As mentioned in Chapter 4, the benefits of studying non stimulus-locked or induced brain activity can provide more details about cognitive processes involved in language processing (Eulitz et al., 2000; Pulvermüller et al., 1997; Reply et al., 1999). The detailed procedure of source data reconstruction can be found in Chapter 2. In brief, individual T1 MRI images were used to build the forward solution, normalised and employed to reconstruct a 3D distribution of power (1-30 Hz) with the LCMV beamformer of S1 and S2 sources in the 0-1000 ms

[1] the preprocessing pipeline and time evoked sensor level analysis (Event Related Field) used in this thesis, is schematically described in Figure A.4 in Appendix A at the end of this thesis.

time range from stimulus onset. In this case, the time range was increased compared to the pilot study (0-500 ms), to be able to capture induced effects and take into account that print tuning of younger participants, especially dyslexics, is generally slower (time-delayed) compared to adults (Mahé et al., 2013). It also has been shown how the timings of both induced and evoked visual word recognition change from childhood to adulthood, as an effect of more automatised processing in experienced readers (Maurer et al., 2005b). For each group, first stimulus presented (S1) and second always-rotated stimulus presented (S2) source grand averages were compared over subjects, with two-tailed cluster permutation (number of permutations=5000) t-testing (Maris and Oostenveld, 2007).

5.3 Results

5.3.1 Behavioural results

As already mentioned in this thesis age is a very important factor to be taken into account when studying neural correlates of reading acquisition (see for instance Maurer et al. (2006)), or developmental mechanisms in general. To check if there were any-age related effects, prior to MEG data analysis, independent samples t-tests was performed to compare mean age and accuracy at the task between dyslexics (N=8, one participant was excluded from the analysis since lacking this information) and typical readers (N=15), using SPSS 24 software (BM Corp. Released 2017. IBM SPSS Statistics for Mac, Version 24.0. Armonk, NY: IBM Corp). As shown in Table 5.1, the two groups did not differ significantly (p-value > .05) in the distribution of their performance or any other variables investigated.

	Typical Mean (SD)	Dyslexic Mean (SD)	p-value
N	15	8	
Sex ratio (m:f)	(7:8)	(6:3)	
Age	11.75 (2.45)	11.10 (3.38)	.82
Hits	152.80 (17.9)	138.5 (32.5)	.051
Incorrects	23.27 (15.5)	35.38 (25.9)	.113
Misses	3.73 (6.2)	8.5 (13.9)	.062

Table 5.1 Demographic description of participants and their performance at the "Super Mario" MEG paradigm. Although no significant differences were detected (p-value > .05) it can be appreciated that the typical readers group had the tendency to perform better at the task. The accuracy of the typical readers was around 84.8%, whereas the dyslexic ones was around 76.9%.

Table 5.1 shows how control(Typical) group's accuracy was around 84.8% whereas the dyslexic one was around 76.9%. For schematic purposes and to gain a better sense of the data, Figure 5.1 shows the proportion of correct (hits), incorrect and misses or no-responses, in the dyslexic (DD) and typical readers (CTRL). It can be observed that although there was no statistically significant difference between the mean accuracy between the two groups, dyslexic readers did less well at the task, with higher variability of the distribution of errors.

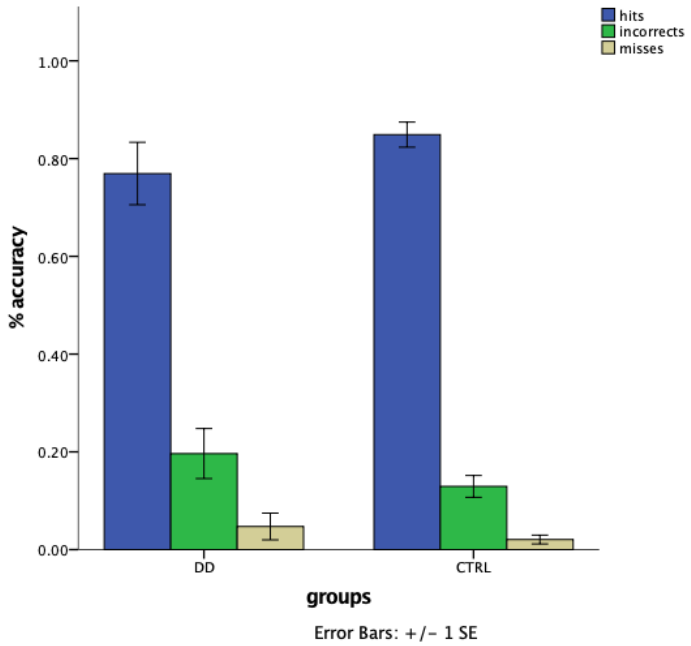


Fig. 5.1 "Super Mario" performance in dyslexic and typical readers. Histogram plot of "Super Mario" performance in dyslexic and typical readers. Although not significantly (p-value > .05) different accuracy distributions of hits (correct trials), misses and incorrect ones (incorrects) pictured here show the tendency of the dyslexic group (DD) to perform worse compared to the control one (CTRL). Error bars show the +/- 1 standard error of the mean.

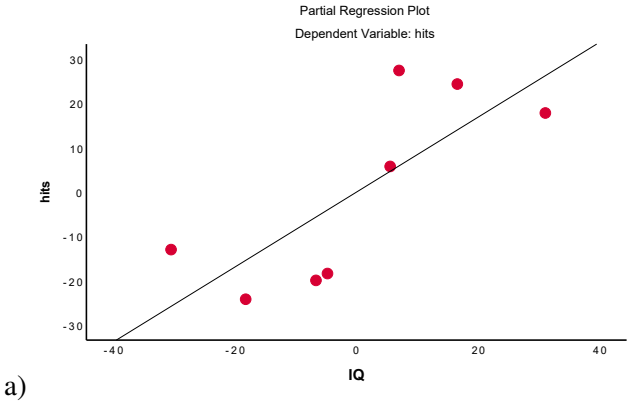
Correlations between "Super Mario" and behavioural measures

To be able to correctly evaluate "Super Mario" as a novel developmental paradigm of visual specialisation in orthographic analysis, and to establish this task in the overall context of this thesis investigating the role of fluency as a classifier of heterogeneity in dyslexia; it was very important to check the presence of linear associations between "Super Mario" level of performance and psychometric measures of reading of individual participants. For this reason non-parametric Spearman partial correlation (controlling for age) between reading, spelling and fluency (TOWRE) standardised scores, described in Chapter 2, and task accuracy was performed with SPSS software. One participant was excluded from the analysis since at the time of the MEG recording, accuracy was not yet computed. The results of partial correlation analysis for eight (N=8) dyslexic participants are illustrated in Table 5.2.

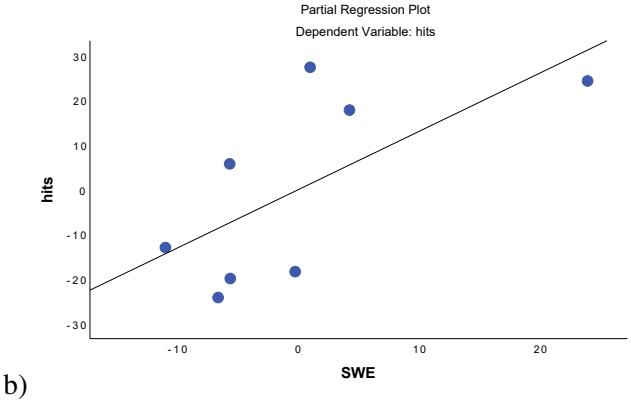
	Hits		Incorrects		Misses	
N=8	r_s	p-value	r_s	p-value	r_s	p-value
IQ	**0.864**	.012	**-0.849**	.016	-0.715	.071
PDE	0.57	.181	-0.693	.084	-0.213	.647
SWE	**0.816**	.025	**-0.762**	.046	-0.372	.412
TOWRE	**0.818**	.025	**-0.87**	.011	-0.341	.454
Reading	**0.756**	.049	**-0.837**	.019	-0.478	.278
Spelling	0.507	.245	-0.597	.157	-0.292	.525

Table 5.2 Correlations between cognitive measures and Super Mario accuracy in dyslexic readers. Partial non-parametric correlations between cognitive profile and Super Mario accuracy. In this case the Spearman r_s was carried out first and then the correlations were regressed out controlling for age. Critical values of Spearman r_s are expressed in bold (p-value<.05). In particular, non-verbal IQ significantly correlated (r_s = 0.864, p-value<.02) with the number of correct trials, and consequently negatively correlated with the number of the incorrect ones (r_s=-0.849, p-value <.02). Sight word efficiency (SWE) or single word reading fluency measure, positively correlated with the number of correct trials (hits) (r_s=0.816, p-value<.03) and in opposite direction with the number of incorrect trials (incorrects), (r_s=-0.762, p-value<.05). In addition, positive and negative r_s were detected in the correlation between the total score at the fluency test (TOWRE) and, respectively, the number of hits and incorrects (r_s=0.756, -0.837, p-values<.05)

When controlling for age, significant positive correlations were found between the number of correct trials (hits) and IQ, sight word efficiency (SWE), Total word efficiency (TOWRE) and reading standard scores [r_s= 0.864;0.816;0.818,0.756, (p-values = .012; .025; .025; .049)]. Opposite trend was detected between the number of incorrect trials and IQ, SWE, TOWRE and Reading standardised scores. Thus, significant negative correlations were detected between IQ and the number of incorrect trials [r_s= -0.849, (p-value= .016)]; SWE and the number of incorrect trials [r_s=-0.762, (p-value =.046)]; TOWRE and the number of incorrect trials [r_s=-0.87, (p-value = .011)] and reading standard scores and and the number of incorrect trials (r_s=-0.837; p-value=.019). Figure 5.2 shows schematic scatter plots of positive correlations between IQ, fluency and reading standardised scores and number of correct trials (hits).



a)



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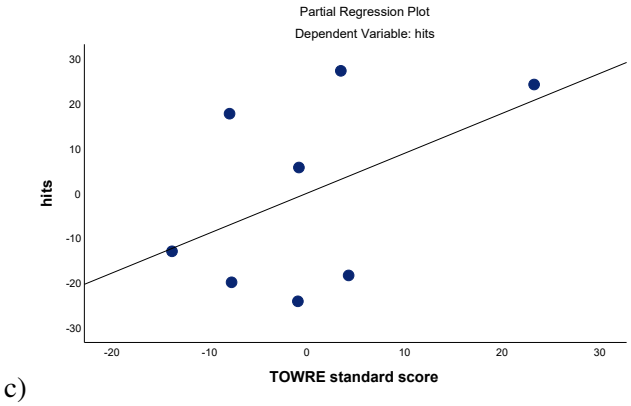


c)

Fig. 5.2 Residual plots of the correlation between dyslexic's group IQ, fluency (SWE and TOWRE) and "Super Mario" accuracy. Partial regression plots of the linear significant associations between a) non-verbal IQ b) Sight Word Efficiency (SWE) and c) Test of Word Reading standardised scores (TOWRE standardised scores) and the proportion of correct trials at the Super Mario game (hits). This is a graphic representation of the results in Table 5.2. Overall, single-word fluency measures are positively correlated with the performance at the task, which indicated that "Super Mario" is a sensitive measure for the visuo-orthographic processing and can be applied as a valid tool to define fluency traits within the dyslexic continuum.

In summary, although within a small sample size (N=8) a positive correlation was found between "Super Mario" accuracy measures and IQ and standardised scores of fluency (SWE and TOWRE) and reading, when controlling for age. Specifically, higher accuracy at the game (number of correct trials) positively correlated with the SWE, which is a measure of single word reading. Interestingly, there was no association detected between the level of performance and the Phonemic Decoding Efficiency, a sub-test that measures the number of pronounceable non-words. In addition, the same standardised scores, correlated negatively with the number of incorrect responses, mirroring the results of the interaction with the number of correct responses.

5.3.2 MEG results

Although, the main focus of current analysis was to examine the spatiotemporal distribution of source-level neural activation in stimulus one (S1) and in the second rotated stimulus (S2), sensor-level analysis was first performed to gain a better sense of the data and to be consistent with the same analysis pipeline applied in the pilot study (Chapter 4).

5.3.3 Sensor level

The same procedure described in the previous chapter (chapter 4) was utilised here. Event Related Field (ERF) analysis was performed within each group to investigate if time-related neural activation differed in the processing of S1 and S2. Cluster based dependent samples t-testing, described in chapter 4 and implement in Fieldtrip according to Maris and Oostenveld (2007), was performed between grand averages of S1 and S2 in the time range of 0-300 ms stimulus onset. To correct for multiple comparison biases, 5000 permutations were applied.

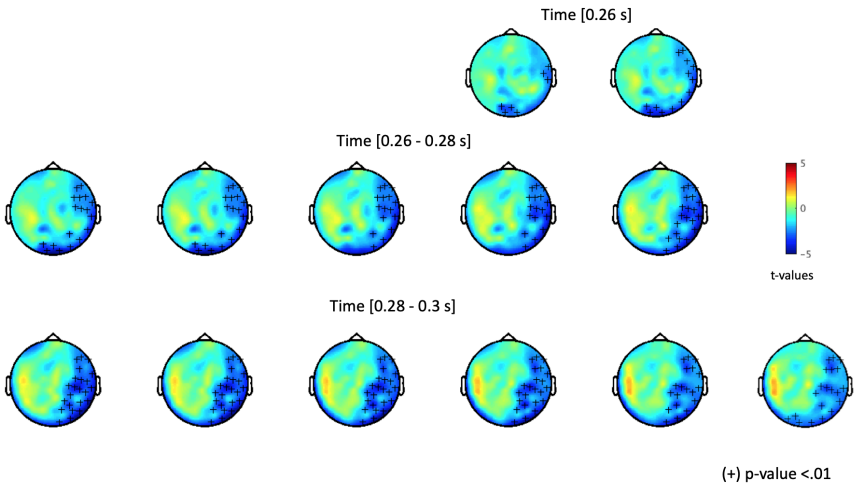
Typical readers

For the control (15 typical readers) group cluster based permutation t-testing (number of permutations = 5000) resulted in three negative significant clusters (p-value <.05). Table 5.3 shows the p-value of each cluster and the respective time latency. The sensor distribution of the significant clusters are illustrated in Figure 5.3.

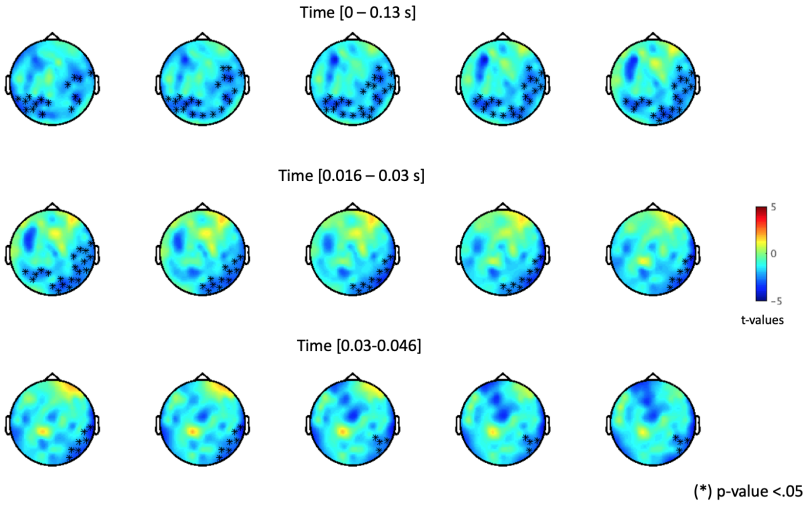
Significant clusters		
Sign	p-value	time
Negative	.0059	0.26 - 0.3
Negative	.019	0 - 0.05
Negative	.035	0.073 - 0.11

Table 5.3 Typical readers sensor level significant clusters p-values and time range. Sensor level analysis:Significant clusters in the typical readers group. Summary table showing significant negative clusters and their respective p-values and time latency.

Another way to depict the distribution of the effect, or the difference in time evoked activity between S2 and S1 or event related desynchronization (ERD) is shown in Figure 5.4.



a)



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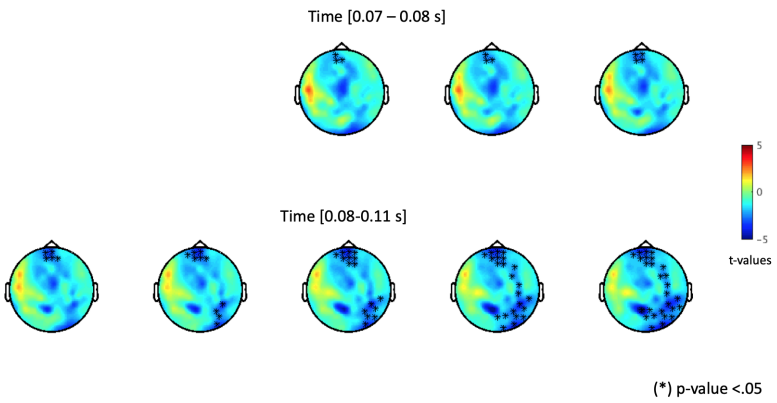


c)

Fig. 5.3 Typical readers: sensor level topology of significant clusters. This figure shows the topological distribution of 3 significant negative clusters over sensors illustrated in Table 5.3 a) Cluster 1 ['+' stands for p-value < .01, time range (260-300 ms) ; b) Cluster 2 ['*' stands for p-value < .05, time range (0-50 ms)] c) Cluster 3 [p-value < .04, time range (73-110 ms)]. These plots show the topology of the significant clusters illustrated in Table 5.3117

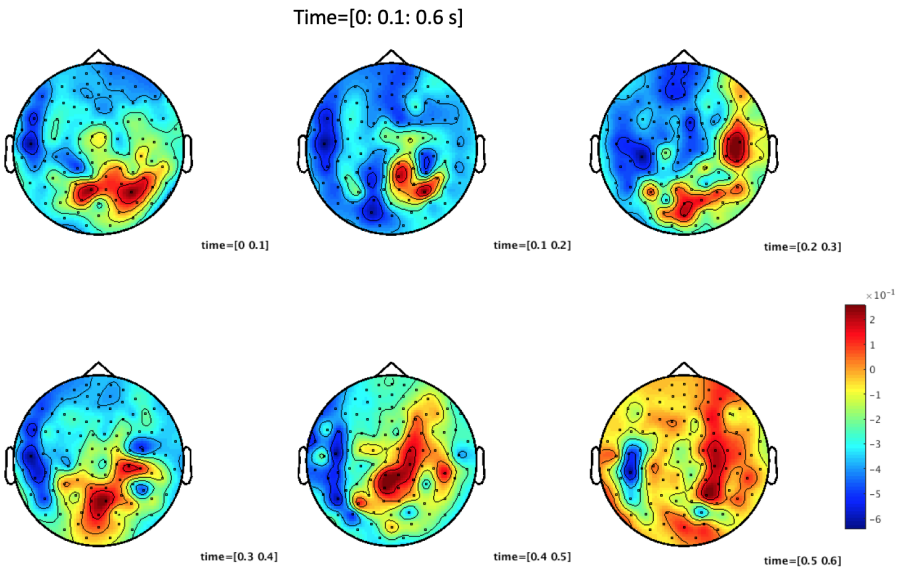


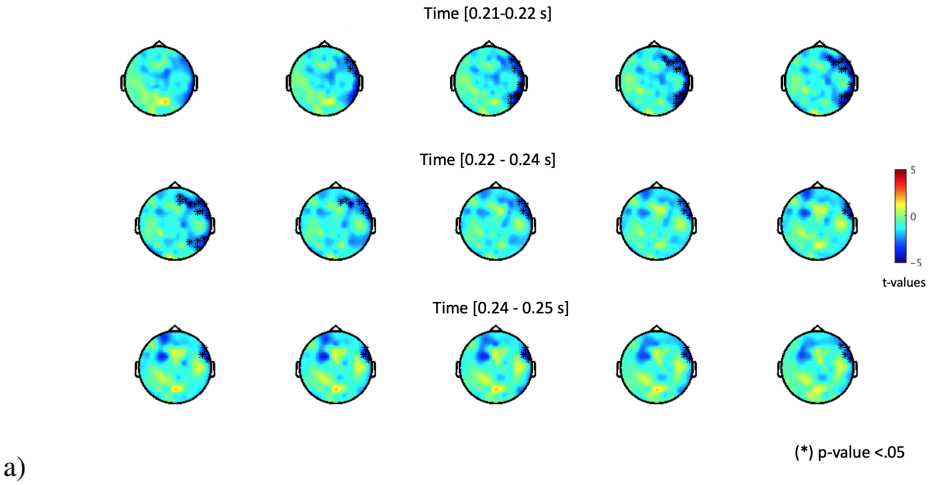
Fig. 5.4 Typical readers: Event Related Desynchronisation (ERD) of S1-S2. This figure shows the topology of the S2-S1 event related desynchonisation (ERD) in the 0 to 600 ms of stimulus onset, where red areas are the ones where the difference was the highest. The colorbar shows the amplitude of the difference.

Dyslexic readers

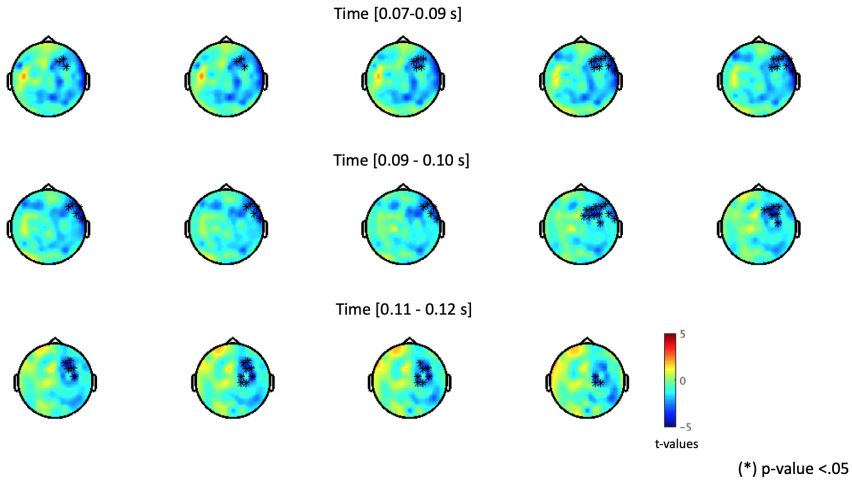
Same as before, dependent samples t-tests were carried our via cluster permutation testing to compare ERFs between S1 and S2 in the 0-300 ms time range in nine (N=8) dyslexic participants. As a result of the analysis, corrected for MCP via 5000 permutation, 3 negative clusters were significant (p-value $< .05$). Table 5.4 illustrates a schema of the results.

Significant clusters		
Sign	p-value	time in ms
Negative	.035	0.21-0.25
Negative	.042	0.07 -0.12
Negative	.047	0.28-0.3

Table 5.4 Sensor level analysis:Significant clusters in the dyslexic readers.Summary table of significant negative clusters with respective p-values and time range of interest.



a)



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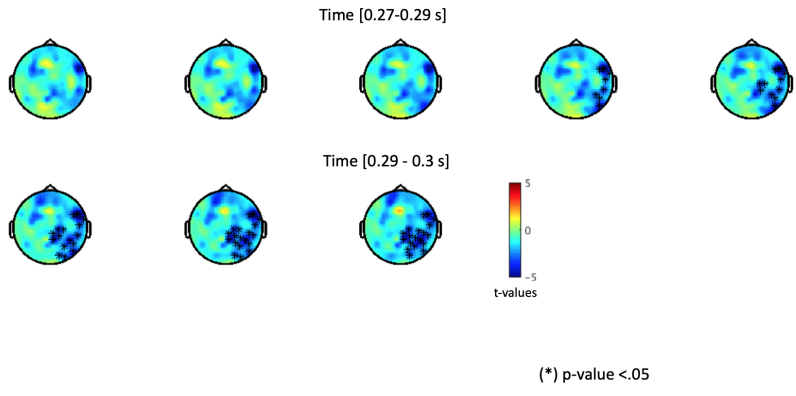


c)

Fig. 5.5 Dyslexic group: Sensor level topology of significant clusters. This figure shows the topological distribution of 3 significant negative clusters over sensors illustrated in Table 5.4 a) Cluster 1 ['*'stands for p-value < .05 (p-value = .035), time range (210-250 ms)]; b) Cluster 2 [p-value < .05 (p-value = .042), time range (7-120 ms)]; c) Cluster 3 [p-value < .05 (p-value = .047), time range (270-300 ms)]

Same as for the control group, Figure 5.6 portrays the ERD of the S1 vs S2 comparison.

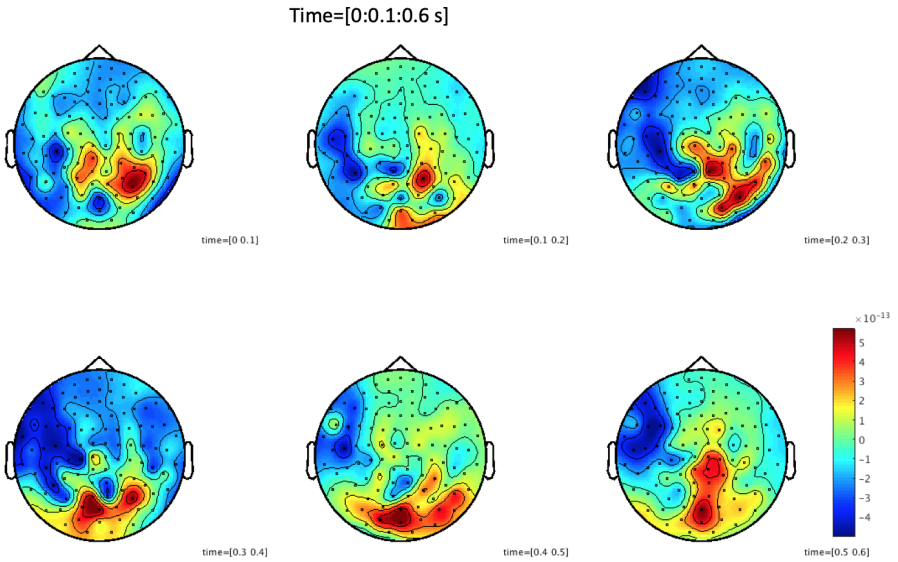


Fig. 5.6 Dyslexic group: Event Related Desynchornisation (ERD) of S1-S2. This figure shows the topology of the S2-S1 ERD in the 0 to 600 ms of stimulus onset for the dyslexic group (N=8). The colorbar shows the amplitude of the S2-S1 difference.

At the sensor level, both groups investigated in this study, typical and atypical (dyslexic) young readers showed significant event related desynchronization (ERDs) patterns in both very early (< 100 ms) and later (200-300 ms) stages of S1 vs S2 discrimination. The topology of the significant clusters points towards a bilateral recruitment of posterior areas for the stimuli discrimination, and a larger difference between S1 and S2 evoked time series in the right occipital areas for both groups in the time range of 200-300 ms.

5.3.4 Source level results

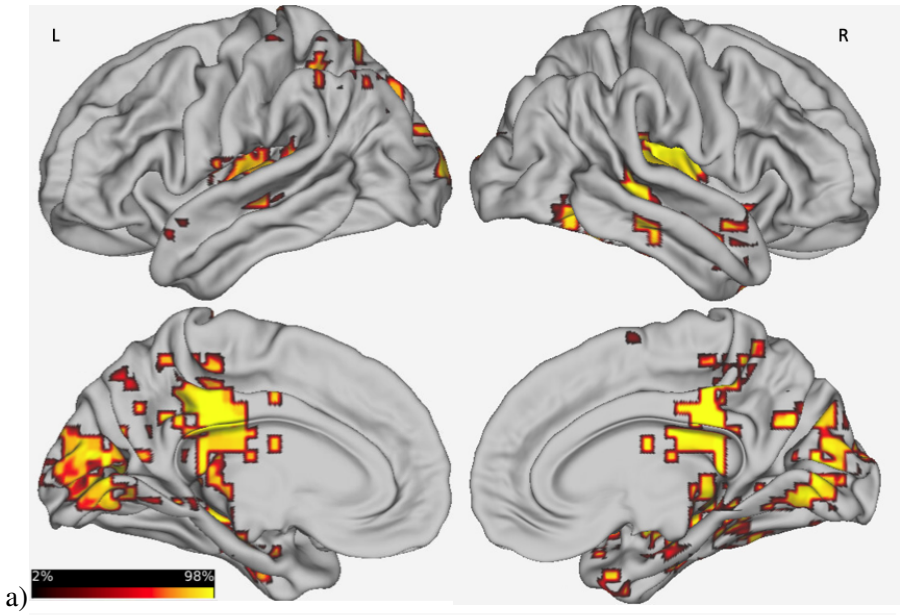
Between the two groups only the typical readers one, showed significant differences in the comparison of source activity S1 vs S2, via cluster based permutation (number of permutations=5000) t-testing. Specifically, for the control group after detection of significant effects in the wider (1-30 Hz) frequency range, the same analysis was performed again for each frequency band (delta, theta, alpha and beta) separately, to examine frequency specific effects. This time the cluster alpha threshold for significance was lowered to $\alpha = .025$ to correct for potential MCP due to the repetition of the same contrast (S1 vs S2) over four frequency bands.
The analysis revealed that there was a significantly positive increase of activity in the comparison S1 vs S2 in the theta band (4-8 Hz). The source images of the significant clusters are shown in Figure 5.7.

Based on the Talairach atlas coordinates (Lancaster et al., 2009), the significant clusters were spatially distributed over left cortex in the occipital, inferior frontal gyrus, and left limbic cortex and cingulate gyrus. In the right lobe the main effects were distributed over right occipital gyrus, right temporal lobe/superior temporal gyrus, and right insula and cingulate gyrus. Table 5.5 shows the signficant t-values (p-value <.05) and the related coordinates. Marginal effects were observed over right and left lingual gyri and both limbic lobes.

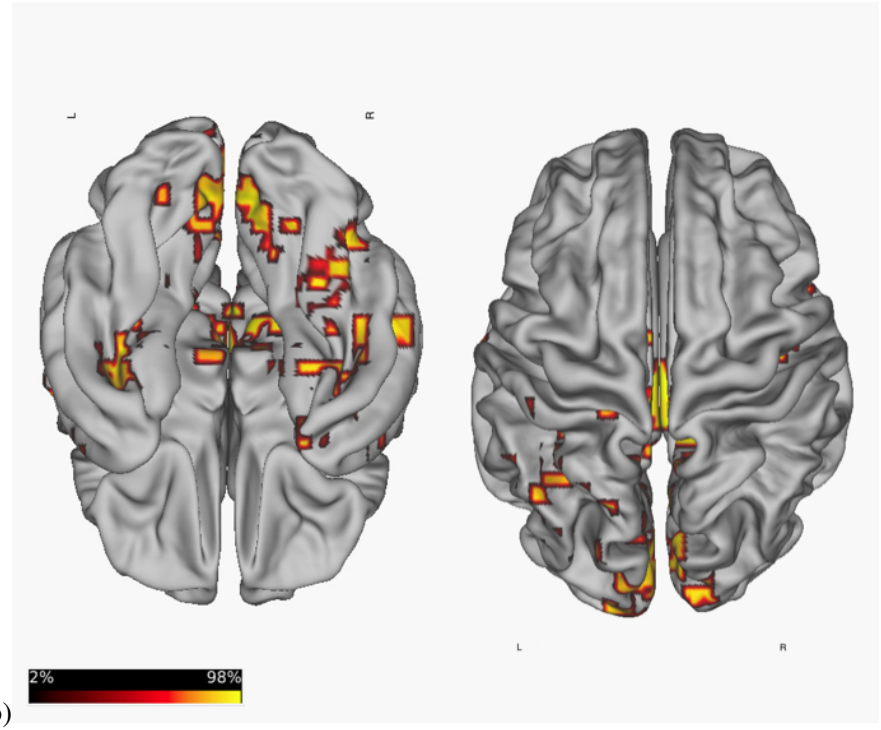
These results suggest a bilateral event related synchronisation or, increase in power in theta band, in the comparison between S1 and S2 in specific regions associated previously to orthographic visual processing such as left occipital cortex and inferior frontal gyrus (Pammer et al., 2004), but also in correspondent contralateral areas. This would indicate that a distributed network of sources localised over both hemispheres and linked to a wider object-recognition network, are recruited during the discrimination of S1 vs S2.

		Coordinates		
	t-values	x	y	z
Left hemisphere				
Occipital Cortex	3.1	-21	-62	54
IFG	2.52	-47	-28	-1
Limbic cortex/cingulate	3.7	-2	-29	34
Right hemisphere				
Occpital gyrus	3.1	3	-75	1
Superior temporal gyrus	3.2	45	-27	-1
Insula	3.2	34	-20	15
Cingulate gyrus	3.18	3	-28	33

Table 5.5 Typical group source level results:Talairach atlas coordinates of significant clusters in typical readers source activation during the S1 vs S2 "Super Mario" discrimination. Talairach atlas coordinates of significant clusters in typical group in the source activation during the S1 vs S2 "Super Mario" discrimination. Summary table of the topological distribution of the significant clusters interpolated to the Talairach atlas. The difference is represented in t-values and divided by hemispheres. The coordinates were reconstructed using a software developed by Lancaster et al. (2009).



a)



b)

Fig. 5.7 Typical readers source level results: topology of the significant clusters.This figure shows the source images of significant positive clusters (p-value < .02) listed in Table 5.5 interpolated onto a template MNI brain, in the 0-1000 ms time range. The units are t-values expressed in percentage of change (stronger in yellow). a) shows the mid sagittal sections of the left and right slices. b) left the axial (posterior lobes in the front), and on the right the dorsal view (posterior lobes in the back).

5.4 Discussion

The study described in the current chapter was achieved to test "Super Mario"'s applicability into detecting differences in the pre-lexical visual processing in typical and atypical young readers, matched by age.

The first aim was to explore differences between typical and atypical readers in the accuracy at the task. Although no significant differences were detected between dyslexics and peer readers, the control (typical readers) group performed generally better (84.8%) compared to the dyslexic one (76.9%). This indicated that "Super Mario" was not too complex to decipher for impaired readers, even when the pseudowords got longer (last block of the task had 5 letters-long pseudowords). From the cognitive and developmental point of view, current findings seem to suggest that visual manipulation of novel orthographic stimuli, it is not marginally impaired in 11 years-old dyslexics compared to peer readers. Obviously, the dyslexic group size (N=8) does not allow to generalise to the whole dyslexic population but this agrees with the hypothesis present in the literature, where it has been suggested that the formal reading instruction affects the visual system and its interaction with the language one (Reis et al., 2001). In other words, both groups in this study have underwent formal literacy instruction, being in year 6-7 of schooling, and therefore their ability to respond to unfamiliar words is increased due to them being at the end or way passed the reading acquisition phase, as also shown by studies of ex-illiterates and illiterates people (Kolinsky et al., 1990). In this study, first and second-graders and ex-illiterates performed better at the visual search study (to detect a part within a figure) than illiterates, suggesting that reading instruction does have an influence on the visual post-perceptual analysis (Kolinsky et al., 1990).

More interestingly, within the dyslexic sample and when controlling for age, the positive correlations between the accuracy at "Super Mario" task and, non verbal IQ, fluency and reading standardised scores indicate that the paradigm is a sensible tool to detect different patterns within the dyslexic group. This is in line with previous studies, where it has been found how the performance (accuracy) at different types of tasks (word and object recognition, letter speech sound-integration) was correlated with reading skills (Caffarra et al., 2017; Žarić et al., 2014). Overall, these interactions suggest, that from the cognitive point of view Super Mario can be considered a sensible tool to detect changes in developmental trajectories (based on psychometric reading measures) within the dyslexic continuum.

The last aim of "Super Mario" study was to compare the current outcome against the pilot study one and examine differences in visuo-orthographic specialisation between dyslexic and peer readers. Same as for the pilot study, both sensor and source level analyses were performed. At sensor level, contrarily to the pilot's study results, both groups exhibited significant ERD over bilateral posterior areas in early (<100 ms) and later (200-300 ms) evoked time series. These results agree partially with the U shaped N170 specialisation proposal, where an increased negativity around ∼200 ms of stimulus onset would mean more neural recruitment, within a less experienced reading network, for the unfamiliar word-like stimuli (Maurer et al., 2007). This seems to be the case here, with the ERD detected over bilateral posterior sensors detected ∼73-110 ms in controls (Figure 5.3 b) and around

$\sim$280 ms in the dyslexic group (Figure 5.5 c). Here, the evoked responses in the dyslexic group to the S1 vs S2 discrimination were delayed compared to the peer readers. This can be traced back to the slower allocation of the attentional resources in dyslexic readers (Visser et al., 2004). As well as to delayed neural responses already observed in dyslexic compared to typical readers (Salmelin et al., 1996). "Super Mario" sensor-level results, with bilaterally distributed ERD that occurs earlier for proficient readers than dyslexics, point towards the idea that differences in specialisation of the fast visual word recognition between dyslexic and typical readers explored in previous studies (Fraga González et al., 2014; Maurer et al., 2005b, 2007) are rather specific to using paradigms that *always* included words as a comparison condition. In a bigger perspective, sensor-level "Super Mario" results indicate that the visual orthographic processing of novel unfamiliar stimuli in dyslexics and typical readers at age 11, has a very similar topology, with the impaired readers having slightly delayed evoked responses. The idea of a 'slower' viso-orthographic processor in dyslexics has already been shown in the MEG literature (Salmelin and Helenius, 2004; Salmelin et al., 1996). This is confirmed by the lack of significant difference in performance at the task, between the two groups, where the dyslexic participants did not perform significantly worse than controls.

At the source level, significant differences were detected in the control group only. The effect was specific to the theta band (4-8 Hz) and largely resembled the sources activated in the pilot study (see Figure 4.6 and Figure 5.7). In the current outcome, the effect was distributed over the left occipital cortex, inferior frontal gyrus and limbic cortex. In the right hemisphere significant negative sources (clusters) were detected in the occipital, superior temporal, insula and cingulate cortices. This pattern of distributed activity overlaps with the one found in adult typical readers in the pilot study.

Furthermore, the sources detected in the control group, specific to theta induced activity in the visual word form areas but also in different regions of the right hemisphere might indicate two different aspects. First of all, theta band has been associated with processing of novel stimuli and working memory (Nokia et al., 2012). The topological distribution of induced activity in the 0-1000 ms of S1 vs S2 analysis would suggest that the initial ERD detected at the sensor level propagates into two directions, one in visual orthographic-processor specific areas (occipital and inferior frontal gyrus) and the other one into more object recognition and attentional network, involving right superior temporal and limbic areas. Similar findings were observed in (Pammer et al., 2004) in a task presenting anagrams and words where induced bilaterally distributed desynchornisation was observed in 300-500 ms after stimulus onset. Moreover, patterns of bi-lateral distribution of neural activation were found in previous studies investigating changes in visual categorisation of words, non-words and objects in relation to the reading instruction (Caffarra et al., 2017).

In the context of prelexical visuo-orthographic analysis the results observed here and in Chapter 4, concur with the recent proposition of the left VOT (ventro-ccipito temporal cortex) as a hub of dichotomous lexical categorisation based on the word-likeness of orthographic strings (Gagl et al., 2016). The lexical categorisation model (LCM) proposes that the word-likeness (how similar or dissimilar a non-word or a pseudoword can be to the known lexicon) of different stimuli occurs

in the posterior left VOT cortex and before the lexical decision has been made (Gagl et al., 2016). Additionally, the activation of the left IFG, that failed to be elicited in the adult group, suggest a linguistic top-down influence in the visuo-orthographic decision making. This is in line with previous fMRI findings investigating letter identification and orthographic decision in young readers (9-13 years old) (Liebig et al., 2017).

Clearly, present source level results require further investigation into the specific involvement of the detected areas in the current sample. Future studies should, therefore, focus on specific ROIs to be able to weigh contribution of each neural parcel and ultimately measure the direction of the information flow (feedback-feedforward) or directed functional connectivity, for an example see Žarić et al. (2017). Furthermore, the failure to detect significant differences in the dyslexic sample at the source level opens the discussion to possible methodological pitfalls. The group size of the dyslexic sample (N=8) might have had an effect on the power of detecting source-level effects in this particular group. Further investigations and a bigger sample size will shed light on the matter.
On the other hand, sensor level analysis, offered an interesting within group variance study opportunity, where it has been noted that in this dyslexic sample, single word reading level, non verbal IQ and reading skills are predictive of viso-orthographic processing of pseudowords. It has been therefore concluded that "Super Mario" has the potential of being a valid tool to measure neural correlates of prelexical orthographic analysis, in relation to individual fluency and reading skills.

This falls into the general outline of this thesis where it has been highlighted how developmental dyslexia is a heterogeneous disorder that needs more fine-grained methods to be fully comprehended.

5.5 Conclusion

To conclude the study illustrated in this chapter offers an example of a novel MEG paradigm application that probes orthographic processing in a prelexical discrimination task. Current findings suggest that the early visual word-like stimuli recognition has the same pattern of event related activation (ERD) in both typical and atypical readers, where the response is time delayed compared to peer ones. Overall, this would suggest that the previously proposed predictors (N170) of impaired fast visual specialisation in dyslexics would hold true only when lexical information is taken into account.

Chapter 6

Resting state functional connectivity and network topology in dyslexia related genotype: a MEG case-study

Chapter summary

This chapter will illustrate a neuroimaging-genetics case study investigating interaction between dyslexia-related genotype expression and MEG resting state functional connectivity (RSFC), in a group of children with dyslexia. In particular, the relationship between RSFC profile, network topology and expression of a specific gene (PCSK6), in a group of children diagnosed for dyslexia will be laid out. This chapter offers a unique example of tailored MEG-genetics study to an atypical (dyslexic) population, exploring associations between gene expression, MEG connectivity and brain networks. The outcome of the current chapter is an unique example of how functional networks in dyslexia can be defined by a related genotype.

6.1 Introduction

Developmental dyslexia embodies a perfect example of a heterogeneous disorder that requires a multi-disciplinary approach to better understand the core deficits and the underlying mechanisms of diverse reading phenotypes. In the last two decades, while reading research has been devoted to building an integrative way for studying the reading disability, including neuropsychology, brain anatomy, neuroimaging (MEG and MRI) and behavioural techniques, behavioural genetics was striving into finding common variants that could explain better the aetiology of the disorder. Notwithstanding the advances in molecular genetics into defining the candidate genes and endophenotypes of developmental dyslexia, mentioned previously (Chapter 1), the exact function of these contributors at the cellular level mechanism linked to observed neural differences still remains unclear (Paracchini et al., 2016). Here, the focus will be on investigating resting state functional connectivity and network topology of the PCSK6 expression in a sample of children with dyslexia.

6.1.1 PCSK6, handedness and dyslexia

Since the beginning of brain anatomy studies and the discovery of the left (hemisphere) localised speech and language functions, it was proposed that developmental dyslexia was due to abnormal lateralisation, which led to think that left handedness would be more frequent among dyslexic individuals than the proficient readers (Paracchini et al., 2016). Advances in neuroimaging have suggested that the picture looks more complicated than that. For instance, Hervé et al. (2013) investigated how hemispheric specialisation (HS) of different cognitive functions (attention, motor, language etc.) is a dynamic process resulting from decreased *inter*-hemispheric and increased *intra*-hemispheric connectivity. The authors showed how brain asymmetries are more heritable than handedness, thus excluding single-gene transmission of this 'trait' (Hervé et al., 2013). This means that the vast majority of left-handers have a left-hemisphere language dominance and most of the dyslexic individuals are right-handed (Hervé et al., 2013; Paracchini et al., 2016).

It seems that looking for direct relationship between single-gene expression, categorical phenotypes (left or right handed?, dyslexic or not?), is not the best way to define possible associations between gene and behaviour. For instance, despite the high amount of Genome Wide Association studies (GWAS) a handedness associated gene has not been reported yet (Paracchini et al., 2016). It has been proposed, instead, that the most sensible way for investigating genetic influences in developmental disorders would be to move towards fine-grained phenotypic measures of known functional genetic networks, capable of discerning differences within the heterogeneity of the disorder itself (Newbury et al., 2014).

In this perspective, promising insights came from the studies of cilia[1], handedness and dyslexia. For example in a GWAS, of a small cohort of participants (number of participants = 744) diagnosed with dyslexia, Scerri et al. (2011) found a significant association between the expression of a particular

[1] for an extensive explanation of the ciliogenesis please refer to Paracchini et al. (2016)

gene (PCSK6) and a measure of a relative hand skill (PegQ). The PegQ, described in chapter 2, is a quantitative measure that highly correlates with the hand preference and has a right shifted normal distribution (Annett and Kilshaw, 1983; Newbury et al., 2014). PCSK6 regulates the protein NODAL pathway, that has a key role in left/right asymmetry in the embryonic development (Brandler et al., 2013). More precisely, cilia are organelles that have a pivotal play in the neuronal cell migration and contribute to the formation of the cerebral cortex, by leading the early patterns of embryo development influenced by the activation of the NODAL pathway. Anomalies in this process, called ciliopathies or cilia malfunction disorders, are a class of pathologies that can result in abnormal body left-right asymmetries, such as *situs invertus* and absent corpus callosum and vermis (Paracchini et al., 2016).

Moreover, other dyslexia gene candidates such as DCDC2, DYX1C1 and ROBO1 have been found to play a role in cilia biology, (for detailed reviews refer to Newbury et al. (2014); Paracchini et al. (2016)). The specific biological models are beyond the purposes of this thesis, however studying biological pathways of genotypes implicated in dyslexia and their association with left/right phenotypic asymmetries might be relevant into establishing functional brain connectivity differences, (Newbury et al., 2014).

6.1.2 Current study

The pathways between genes and behaviour are paved with several levels of complexity. Especially in developmental disorders there are very few direct links between single-gene and its phenotype. Therefore, studying intermediate phenotypes or *endophenotypes* could help to better understand the routes from gene to behaviour. A lower or endophenotypic level of analysis means to investigate neural correlates of genetic influences of specific behavioural traits (Knopik et al., 2017).

This chapter portrays a MEG-genetics study investigating RSFC and network topology (endophenotype) in relation to PCSK6 expression in a sub-sample of children with dyslexia. In particular, patterns of MEG functional connectivity and minimum spanning tree (MST) will be compared between two groups of children with dyslexia, matched by age and reading scores, and stratified into groups of carriers and non carriers (PCSK6+ and PCSK6-) of the allele previously associated with expression of relative handedness (PegQ) in individuals with reading disability (Brandler et al., 2013). The current study is part of longitudinal investigation and long term collaboration between Prof. Joel Talcott (Aston University) and Dr. Silvia Paracchini from St Andrews University (Glasgow), who have been leading large national and international cohort behavioural-genetics investigations in developmental dyslexia, for an example see Brandler et al. (2013); Scerri et al. (2011, 2017). As mentioned previously, the sub-sample of participants here investigated is not representative of participants who participated in the above behavioural genetics study. Traditionally, GWAS need a big sample size ($\sim$1000 participants) to reach population significance, which for neuroimaging studies is very costly in terms of time and resources. Nevertheless, the main focus of the present study was to examine potential differences in RSFC and network topology (MST) in matched, by age and reading scores, dyslexic sub-groups that only differed by the genetic component.

6.2 Methods

6.2.1 Participants

As for all the previous neuroimaging studies described in this thesis, participants and their families were recruited upon their referral at the DDAU and invited to take part, primarily in the behavioural genetics study, and secondarily, in the neuroimaging one. After obtaining, written and informed consent from both parents and their children, behavioural measures were collected. Participants were rewarded with a 10£ Amazon voucher. Following this, families were approached again to see if they wanted to participate to the neuroimaging study, and if so, a second visit to the ABC was scheduled. Overall, fourteen children (N=14) diagnosed with dyslexia were included in this MEG-genetics study: seven (N=7) PCSK6 carriers, hereafter referred as PCSK6+ group, and seven (N=7) non carriers, referred as PCSK6- group, see Table 6.1. It is worthwhile mentioning here that more children were recruited to participate into the RSFC study, see Chapter 3, but only 14 participants had genetic information at the time of this analysis. The genotyping of the saliva samples is carried out by the geneticists at the University of St Andrews, and runs in batches of approximately 90 samples. Therefore, it occurs quite often that there is a time gap between collecting neuroimaging data from children and retrieving their genotype information [2].

6.2.2 Behavioural measures

For the behavioural data collection, children were invited to spend some extra time, after their educational assessment, with one of the ABC researchers, where a sample of saliva was collected with the Genotek kit, fluency measures (TOWRE - single word and non word reading), Annette handedness questionnaire (Annett, 1985) and PegQ board game data were collected. All of these are fully described in Chapter 2. In addition, parental behavioural questionnaires (BPM and ASEBA) measuring psychiatric (anxiety and depression) and ADHD symptoms were administered, and questionnaire about language family history was collected as well. As mentioned in Chapter 2, behavioural measures (BPM and ASEBA) were not included in the analysis, since a part of a larger study investigating behavioural and cognitive correlates of children assessed for dyslexia and PCSK6. Lastly, parental consent was obtained to be able to eventually access children's reading and spelling standardised scores, performed by the educational psychologist during the assessment. The behavioural data collection was carried out at the end of the educational assessment and lasted for approximately 15 minutes.

[2] next batch of genetic information is due in Spring 2019

PegQ

As illustrated in Figure 2.2 (Chapter 2), PegQ is a relative hand skill peg-board task, used to measure the time taken by the participant to move a row of 10 pegs from one location to another with the left (L) and right (R) hand separately (Annett and Kilshaw, 1983; Brandler et al., 2013). Here, each hand was tested three times, alternating the L/R order and from 'each' run the average time to complete the task was extracted and PegQ calculated:

$$PegQ = [2(L-R)/(L+R)] \tag{6.1}$$

It is known form previous studies (Annett, 2008; Brandler et al., 2013; Scerri et al., 2011) that this measure has a normally distributed values, with a positive mean, where a positive PegQ index means superior relative right-hand skill and a negative PegQ indicates superior relative left-hand skill, (Brandler et al., 2013). Additionally, it has been shown how hand preference estimates (based on hand preference questionnaires) correlated strongly with PegQ index (Brandler et al., 2013).

6.2.3 MEG data acquisition, preprocessing and analysis

For this particular study, the MEG data was acquired between 2015 and 2016 by previous in-post ABC researchers. The MEG resting state protocol here utilised had the same duration as described in chapter 3 (6 minutes) with alternating eyes-closed/eyes-open epochs, with the only difference that here, the researchers gave verbal instruction to the participants on when to close their eyes (via the MEG intercom) whereas in the RSFC MEG study described in Chapter 3, participants heard an acoustic cue. The data were preprocessed and analysed the same way as described in Chapter 3. For the pipeline analysis, see Figure 3.1. In short, 78 resting state time series were reconstructed in source space, using an atlas (AAL) based beamformer, in four frequency bands (delta, theta, alpha and beta) of interest (Hillebrand et al., 2012). These 78 virtual electrodes (VE) time series then were imported in the BrainWave software Version: 0.9.152.4.1 [3] developed by Stam et al. (2007). Subsequently, for each frequency bands of interest (alpha, beta, theta and delta) PLI estimates were computed. Following, functional connectivity for all 78 ROIs of the atlas was estimated by using phase lag index (PLI), (Stam et al., 2007). The matrices of PLI were then compared between groups via permutation t-testing, (Nichols and Holmes, 2001). Following this and based on the PLI results, for the significant frequency bands, topographies of the functional network were reconstructed with the minimum spanning tree (MST), described in Chapter 2 (Table 3.1) and in Tewarie et al. (2015). The advantage of the MST application is not only in the un-biased comparison of different types of networks, it is also in the vast range of derivative metrics or indices that can describe different proprieties of the 'trees'. Regional MST indices of degree, eccentricity and betweennes centrality (BC) were calculated via permutation paired t-testing (Nichols and Holmes, 2001) in delta (1-4 Hz) and theta (4-8 Hz) frequency bands.

[3] http://home.kpn.nl/stam7883/brainwave.html

6.3 Results

First, demographic, cognitive and phenotypic measures between the two groups will be described. Then, the connectivity estimates (PLI) results will be outlined, followed by comparison of the MST metrics.

6.3.1 Reading and phenotypic measures

As mentioned before, the peculiarity of the current study is that, given the small sample size (N=14), PCSK6+ and PCSK6- participants were matched by age. Moreover, as described in Table 6.1, the two groups did not significantly differ on reading, fluency (TOWRE) and spelling standardised scores. Finally, PegQ index was not significantly different between two groups either, even if the prevalence of left-handers was higher in the PCSK6+ group and hence, PCSK6+ group was less right-hand side lateralised (0.081) than the PCSK6- one (0.152).

	PCSK6$^+$ Mean (SD)	PCSK6$^-$ Mean (SD)	p-value
N	7	7	
Sex ratio (m:f)	(5:2)	(5:2)	
Age	148.43 (39)	154 (34)	.87
L:R	4:3	2:5	.80
TOWRESS	83.43 (14)	83.29 (13)	.73
Reading	85.29 (16)	91.29 (22)	.54
Spelling	85.86 (14)	80.14 (11)	.56
PEG Q	0.081 (.124)	0.152 (.091)	.34

Table 6.1 Demographic description of PCSK6$^+$ and PCSK6$^-$ groups of dyslexic readers. The two groups did not differ marginally on any of the cognitive reading standardised scores, as well as on the PegQ index, although the carrier group had a less right-handed lateralised index.

6.3.2 Connectivity results - PLI

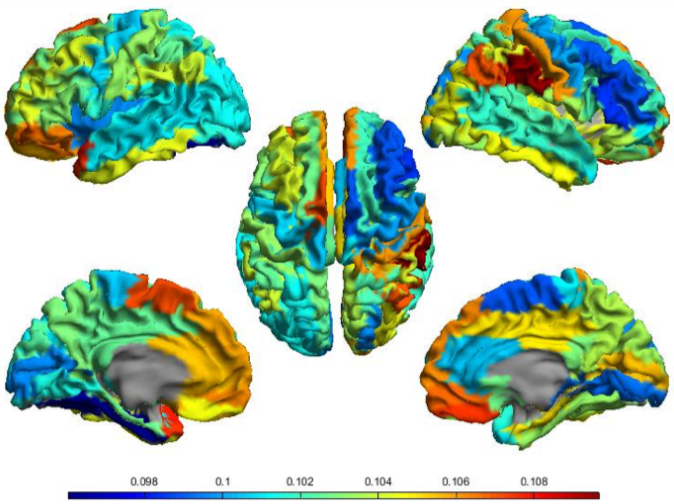
MEG power estimates did not show any significant difference in the comparison between PCSK6$^+$ and PCSK6$^-$, same applied for overall mean PLI of all four frequency bands of interest (1-4 Hz, 4-8 Hz, 8-13 Hz, 13-30 Hz). In terms of connectivity measures, significant (p-value<.025) results were detected respectively in delta (1-4 Hz) and theta (4-8 Hz) frequency bands.

Delta

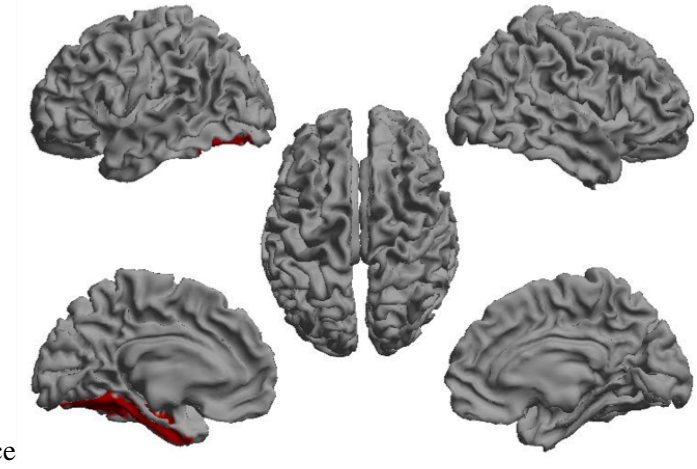
In the PCSK6^{+} versus PCSK6^{-} comparison of regional values of functional connectivity (PLI), by means of permutation t-testing (number of iterations = 1000) showed significantly lower values (p-value<.025, t-value= -4.3) in the left fusiform gyrus, Figure 6.1. In other words, PCSK6^{+} exhibited lower connectivity (PLI) in the left fusiform gyrus, compared to non carrier group (PCSK6^{-}).

Theta

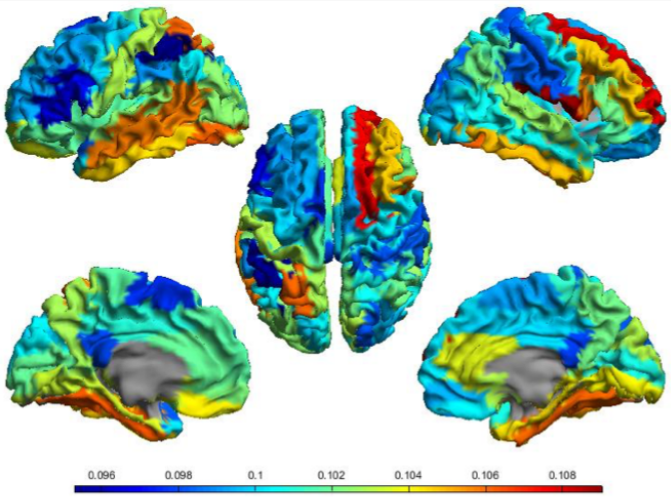
In theta (4-8 Hz) frequency band, permutation t-testing (number of iterations=1000; p-value<.025) showed how children part of the PCSK6^{+} group had increased PLI values in the left superior frontal cortex (blue region in Figure 6.2 b) and significantly lower connectivity values in the left inferior parietal region (red region in Figure 6.2 b), (p-value <.025, with t-values = 5.8 and -4.3).



a) PCSK6^{+}

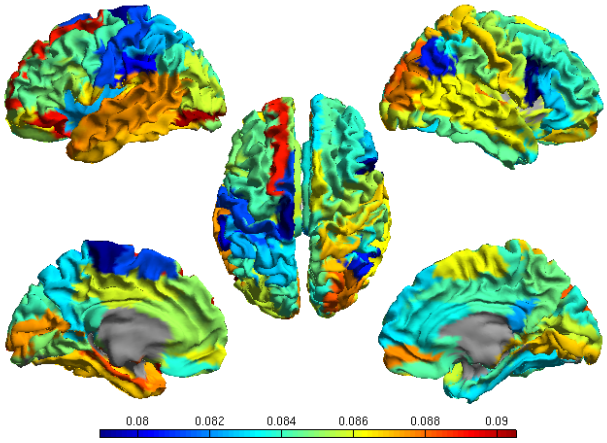


b) difference

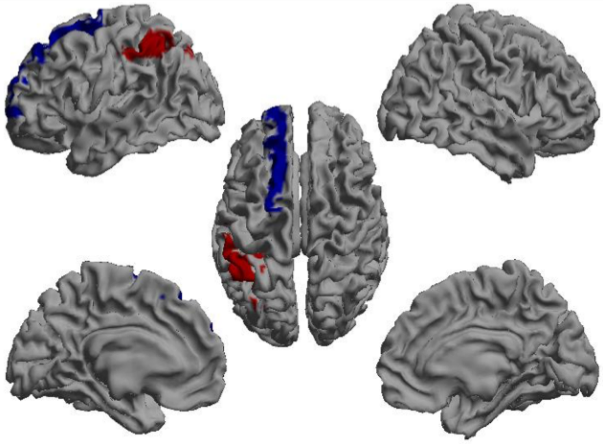


c) PCSK6^{-}

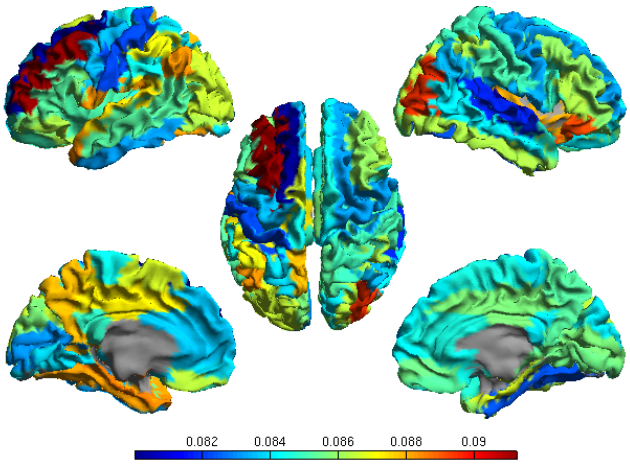
Fig. 6.1 Connectivity (PLI) results in delta (1-4 Hz) frequency band in the comparison between PCSK6+ and PCSK6- dyslexic groups. The mean PLI values (ranging from 0 to 1) is expressed across the whole 78 ROIs of the atlas. b) region coloured in dark red is the left fusiform gyrus (VWFA area) where significant negative differences (t-value=-4.3; p-value<.025) between carriers and non-carriers was detected. c) mean PLI distribution for each ROI in the PCSK6^{-} group.



a) PCSK6^{+}



b) difference



c) PCSK6^{-}

Fig. 6.2 Connectivity (PLI) results in delta (1-4 Hz) frequency band in the comparison between PCSK6+ and PCSK6- dyslexic groups. Theta band (4-8 Hz) PLI outcome a) PCSK6^{+} mean distribution of PLI values for each of the 78 ROIs in the 4-8 Hz frequency band. b) significant differences in connectivity (t-values = 5.8;-4.3; p-value <.025) in the left frontal superior and inferior parietal cortices. PCSK6^{+} group exhibited increased connectivity (PLI) in the left superior frontal cortex (blue) and decreased values of PLI in the left inferior parietal (dark red) c) distribution of connectivity (PLI) in PCSK6^{-} group.

6.3.3 MST results

To explore the topological proprieties of the functional network estimated with PLI, minimum spanning tree (MST) analysis was carried out for the PLI-significant frequency bands (delta and theta). As previously discussed in Chapters 3, MST is an unique type of sub-graph designed to overcome some of the biases in the computation of the brain network analyses (Stam, 2014; Tewarie et al., 2015). It represents a sub-network of highest or strongest weights of the connectivity functional network, in this case the of the PLI connectivity matrix (Van Mieghem and Magdalena, 2005). For the comparison of MST proprieties in PCSK6^{+} and PCSK6^{-} contrast, both global and local metrics were employed.

Global measures

There are a number of MST estimates that can be used to describe the global proprieties of the tree, described in Chapter 3 (see Table 3.1 for detailed description). Here, the focus was on diameter, leaf number (fraction) and tree hierarchy. For differences in local network topology, measures of degree, eccentricity and betweenness centrality were investigated. To recapitulate, diameter is a measure of the largest distance between any two nodes of the tree, leaf fraction is fraction of nodes with degree = 1 in the tree, and tree hierarchy is a measure of trade-off between large scale integration in the MST and the overload of central nodes, (Fraga González et al., 2018b). For the global measures, one-way ANOVA was performed using SPSS to compare the two groups. Significant global MST descriptives differences (p-value=.018) were detected in the theta frequency band (4-8 Hz), suggesting that the PCSK6^{+} group has a less integrated tree compared to the non-carrier group, Table 6.2. In MST metrics leaf fraction represents the number of nodes with degree=1 (leaves) in the morphology of the tree. Generally, if a MST has a more 'star-like' (more integrated) tree distribution, the diameter will decrease at the increase of the leaf number (Fraga González et al., 2016a; Stam et al., 2014).

		PCSK6+ Mean (SD)	PCSK6- Mean (SD)	p-value
Theta	Diameter	0.192 (.038)	0.200 (.010)	.528
	Leaf fraction	0.531 (.007)	0.543 (.009)	**.018**
	Tree Hierarchy	0.365 (.015)	0.371 (.019)	.575
Delta	Diameter	0.200 (.0151)	0.198 (.011)	.762
	Leaf fraction	0.541 (.0191)	0.542 (.010)	.868
	Tree Hierarchy	0.376 (.0154)	0.38 (.013)	.676

Table 6.2 Summary table of MST (whole brain) global measures in theta and delta frequency bands.Global MST descriptives in theta and delta frequency bands of PCSK6$^+$ and PCSK6$^-$ groups. Significant differences (in bold) were detected in the theta band (p-value =.018) where the non-carrier group had significantly higher values of leaf fraction (L) indicating a more integrated (star-like) MST network.

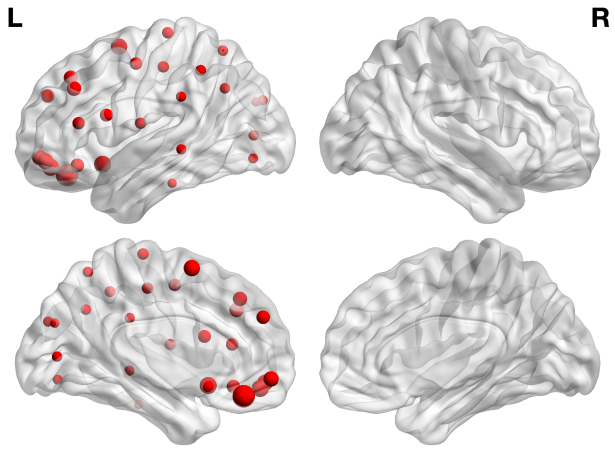
Local Minimum Spanning Trees

Detailed description of local MST descriptives can be found in Chapter 3. As a reminder, the degree of a node is number of edges connected to it, betweenness centrality (BC) represents the measure of 'hubness' or a load of a region within the network (for instance the node with the highest BC, will be the one that is crossed with the shortest paths between two nodes, hence the idea of aggregate cluster of nodes or hubness), eccentricity of node measures the longest path between that particular node and any other node of the tree and it is low if that ROI is central in the network (Fraga González et al., 2018b; Stam et al., 2014). These measures are used to identify the crucial nodes in the tree, and describe their network proprieties in terms of number of neighbours, network load and centrality in the pathways of cortical communication (Fraga González et al., 2016a; Stam et al., 2014).

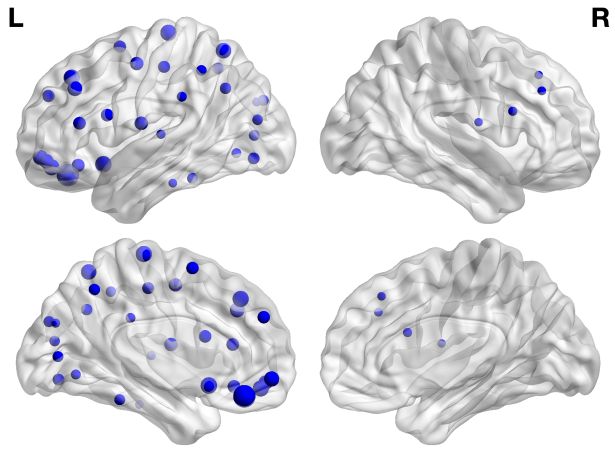
In the delta (1-4 Hz) frequency band permutation t-testing showed how the PCSK6$^+$ group had significantly lower values in local or regional degree and eccentricity (Figure 6.3 and Figure 6.4). In the theta (4-8 Hz) frequency band, PCSK6$^+$ had significantly lower values of betweenness centrality (BC). Specifically, in delta band significant lower degree values were found in the left superior parietal cortex (p-value<.025, t-value= -4.5), as shown in Figure 6.3.

For the eccentricity measures, PCSK6$^+$ exhibited significantly lower values (p-value<.05, t-value=-2.5) in the left middle cingulum (Figure 6.4) indicating that this ROI has a more central role (the lower the eccentricity is, the more central the node in the network is) in the carrier network compared to the PCSK6$^-$ group.

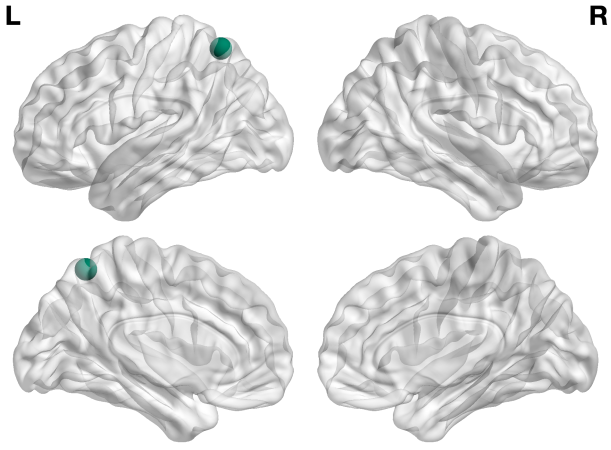
Finally, in the theta band significant differences in betweenenss centrality (BC) were detected, for the PCSK6$^+$ in the left precuneus (p-value<.025, t-value=-5.9) compared to the PCSK6$^-$ group. This suggests that left precuneus plays a very different role in the two trees, while it is an important edge or bridge within the PCSK6$^-$ MST, it has a marginal role in the carrier network, see Figure 6.5.



a) PCSK6^{+}



b) PCSK6^{-}



c) difference

Fig. 6.3 MST delta (1-4 Hz) frequency band degree topology and difference in PCSK6+ and PCSK6- networks. Delta (1-4 Hz) frequency band degree results. a) PCSK6^{+} strongest degree values. Here, for each group above-mean (> 50% strongest connections) degree values are pictured to show different network topology. b) PCSK6^{-} strongest degree values in the network. Generally PCSK6^{-} group (below) has a more distributed values of MST degree c) Significant difference was detected in the left superior temporal cortex, where the PCSK6^{+} showed a less connected node compared to the PCSK6^{-} MST. The size of the green node is based on t-value.

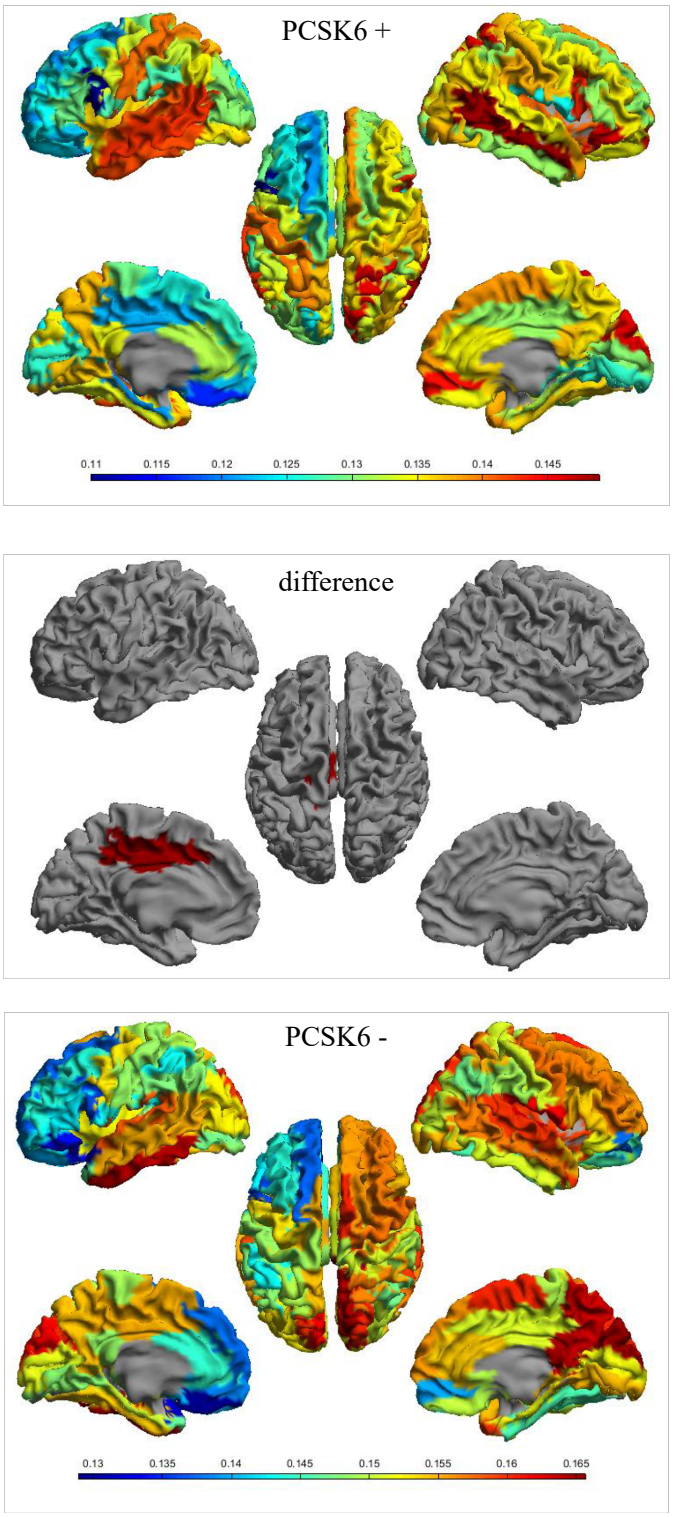
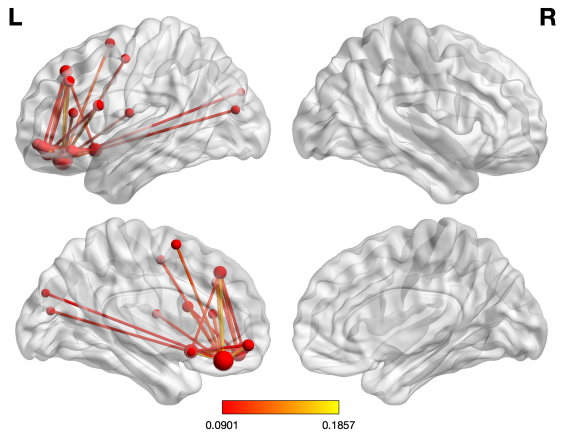
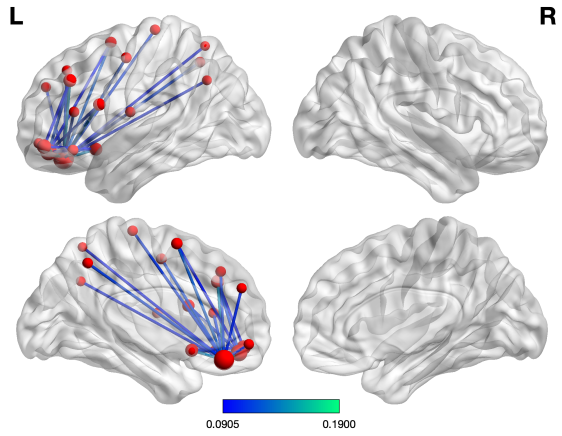


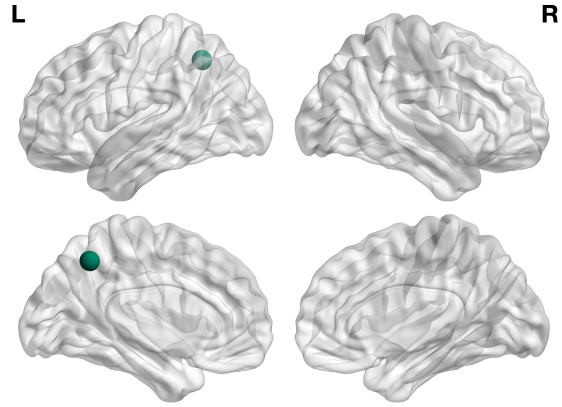
Fig. 6.4 MST delta (1-4 Hz) eccentricity topologies in the PCSK6^{+} and PCSK6^{-} trees. At the top, mean distribution of eccentricity in the PCSK6^{+} group. Middle: significantly lower values of eccentricity (p-value<.05, t-value=-2.5) were found in the left cingulum (in dark red), suggesting that this region is more central in the PCSK6^{+} tree. Bottom: local values of eccentricity in the PCSK6^{-} tree.



a) PCSK6^{+}



b) PCSK6^{-}



c) difference

Fig. 6.5 MST betwenness centrality (BC) measures of PCSK6^{+} and PCSK6^{-} groups in theta (4-8 Hz) band. Local highest (>50% above-average) betwennness centrality (BC) values in the theta band (4-8 Hz) in the a) PCSK6^{+} and b) PCSK6^{-} MSTs. c) significant differences were detected in the left precuneus (p-value<.025, t-value=-5.9), suggesting that this region is a central hub in PCSK6^{+} tree. The links between the nodes reflect the BC values of the respective networks, derived from the BC connectivity matrix. Conventionally the nodes with the largest/highest values of BC are considered to have a central role in the information transmission within the network.

6.4 Discussion

To the best of author's knowledge, this is the first MEG-genetics study investigating intermediate phenotypes of RSFC and network topology associated with PCSK6 expression in a sub-sample of children with dyslexia.
The outcome of the current pilot study suggests that different patterns of RSFC and network topology could be defined in a small sample size of children with dyslexia, divided by their genotype information. It is important to acknowledge that the findings described here are still of preliminary nature and a bigger sample size will either confirm or contradict the present results. However, the age and reading-level matched sample, has offered an unique opportunity to explore subtle differences in task-free connectivity and MST, specific to the expression of PCSK6 *within* the dyslexic spectrum.

Overall, present results suggest that the lower frequencies (delta and theta) long range functional connectivity profile significantly varies between the two genetic sub-groups of dyslexic readers. In particular, PCSK6^{+} children exhibited lower connectivity in regions of the reading network (left inferior fusiform/visual word form area and inferior parietal cortex) and higher connectivity in the left frontal superior cortex. This is in line with the RSFC literature, showing reduced activation in the vOT areas in dyslexic young readers (Finn et al., 2014). Additionally, reduced connectivity between phonology related regions (inferior-parietal and the fusiform gyrus) has been observed in young readers, in a RSFC fMRI study (Koyama et al., 2011). It appears that here, similarly to the findings in the (dys)fluent stratification illustrated in Chapter 3, the carrier group (PCSK6^{+}) shows more dyslexia-like endophenotype of functional connectivity compared to the non-carrier (PCSK6^{-}) group. The increase in connectivity in lower frequency bands (delta and theta) in the left frontal areas, has been previously showed in a task-free MEG study in younger adult dyslexics compared to typical readers, and linked to probable compensatory effects in impaired young readers (Ozernov-Palchik and Gaab, 2016; Pagnotta et al., 2015). Additionally, in task based studies, it has been demonstrated how children with dyslexia have an increased slow activity (delta and theta) in the frontal and right temporal regions of the brain (Arns et al., 2007).
Compared to the results in Chapter 3, where significant differences in MST were present in alpha (8-13 Hz) and beta (13-30 Hz) frequency bands, the present results seem to relate more to potential influences of the genotype expression rather than to the dyslexic connectivity phenotype. It is quite difficult to speculate given the small sample size, and no previous MEG-genetics studies, but potentially the slow (or lower) frequency resting state network dynamics differences detected in the present study might reflect genetic (PCSK6) endophenotypes. A future study with a larger sample, including a typical or control group, with no diagnosis of dyslexia, could corroborate this hypothesis.

As mentioned previously, the MST has been widely applied to investigate networks in other developmental disorders, such as ADHD, (Janssen et al., 2017) and in clinical populations, such as epilepsy, schizophrenia and TBI (Stam, 2014). Following the functional connectivity estimation (PLI), MST graph analysis was applied to measure differences in network topology between the two genetic sub-groups in this sample of children with dyslexia. In the global tree morphology

configuration, based on the leaf fraction metrics, the non carrier group (PCSK6$^-$) showed a more centralised ('star-like') tree compared to the carrier one (PCSK6$^+$). In particular, according to graph theory, PCSK6$^-$ group had a more integrated network compared to the PCSK6$^+$ (Sporns, 2012; Stam et al., 2014). In the local or regional MST metrics, the carrier group showed significantly lower values of degree and eccentricity in the delta frequency band (1-4 Hz) and significantly lower values of BC in the theta band (4-8 Hz). In particular in the comparison of regional values of number of neighbours connected to a node (degree), the results demonstrated how PCSK6$^+$ group had significantly lower number of neighbours, or was less connected, in the left superior parietal cortex. This is in line with previous fMRI studies investigating RSFC and task-induced connectivity in the reading brain, which showed decreased connectivity in the executive regions of effortful processing such as the superior parietal cortex (Koyama et al., 2010) and the involvement of this area in the allocation of attention during a lexical and phonological decision tasks in young children (Liebig et al., 2017). Based on the current findings, in the PCSK6$^+$ group this area seems to be less connected and thus less integrated in the large scale network, in the delta frequency band, when compared to the non-carrier group.

Furthermore, the PCSK6$^+$ group displayed significantly lower eccentricity values in the left cingulum, when compared to the non-carrier group. To summarise, eccentricity of a node is defined as a longest path from this node to any other node in the tree. As degree and BC is a measure of centrality of a node, in particular, the lower the eccentricity of a node, the more central that region in the tree is (Stam et al., 2014; Tewarie et al., 2015). Therefore, in the carrier group the left cingulum has a central role compared to the non-carrier network. This partially overlaps with previous findings by Scerri et al. (2012) that investigated the effects of candidate genes for dyslexia and specific language impairment in a DTI study and found that the volume of the posterior part of corpus callosum and cingulum were highly correlated with the expression in the dyslexia-related loci. The authors suggested how this fibre tract connects both language and general cognitive cortical areas (parietal-occipital-temporal cortices), (Scerri et al., 2012). On the other hand, the cingulum has been linked to the disrupted connectivity in neuroanatomical models of dyslexia (Cui et al., 2016; Vanderauwera et al., 2017). In this case, the cingulum had higher central role in the network of the children who carried the dyslexia-related genotype. In the current analysis, in delta frequency band (1-4 Hz) and hence in the long-range connectivity topological distribution, the left cingulum had a more loaded (central) role in the PCSK6$^+$ tree, when compared to the non-carrier one. According to this outcome, this region plays an important role in the structural architecture of the reading network and seems to have also a more central role in children who expressed the PCSK6 genotype in the delta band MST.
Further analyses, in particular a multimodal approach comparing functional and structural connectivity, are needed to fully investigate the specific role of the cingulum in the resting state functional network and in the relation of the PCSK6 expression.

Lastly, in the measure of local hubness (BC) in the theta band, the PCSK6$^+$ group had significantly lower values in left precuneus, when compared to the non-carrier group. This region has been previously linked to dyslexia, in particular in a task positive and task free fMRI study, Schurz et al.

(2015) showed increased connectivity between left inferior parietal (IPL) cortex and left precuneus in dyslexics compared to peer readers. This suggests an over-activation of the reading left network with regions of the DMN, such as the precuneus. Current results, indicate that in the PCSK6^{+} group the left precuneus was less integrated or centralised when compared to the non carriers.

The present MST results showed how the carrier group PCSK6^{+} had decreased values of centrality (degree, BC and eccentricity) compared to the non carrier group (PCSK6^{-}). In the the graph theory interpretation, the less centralised network means more integrated whole brain network, which leads to less loaded central nodes (Tewarie et al., 2014b). This has been justified as a lack of specialised sub-networks, such as the left reading network (Fraga González et al., 2018b). Further investigations and a bigger sample size are necessary to confirm this hypothesis in case of the PCSK6 endophenotypes. In addition, it has been previously described how PCSK6 has an influential role in the early stages of left/right asymmetries development (Paracchini et al., 2016), suggesting that its expression could have an effect on correlated cortical areas such as planum temporale and corpus callosum (Scerri et al., 2011).

The present study offers a pilot observation on possible ways to better investigate functional connectivity and network topology of dyslexia-related genotype(s). Indeed investigating functional connectome with an unbiased method, such as the MST, provides an ideal way to compare different types of networks or 'trees', for instance the structural (DTI) and functional (MEG) ones. Although of high interest to this study, this hypothesis has not yet been explored in the human brain (there is some evidence from knock-out mice studies, see Tam and Loebel (2007)). Following this, an example study would be to investigate lateralisation (asymmetries) neural pathways in relation to genetic molecular functional networks (biological pathways) and behavioural phenotypes (handedness and reading profiles).

Finally, caution must be applied when implying causative effects between genes-brain-behaviour. Although, identifying endophenotypes (cortical phenotypes) of specific genes is very important, it should not be ignored that the ultimate goal, from the behavioural genetics perspective, is to understand better differences in behavioural phenotypes. This means that it is always necessary to quantify the extent to which gene expression is influencing the behaviour and to which the neural expression (Knopik et al., 2017). There is still a long road ahead before defining causative, correlative or consequential effects between gene, brain and language skills (Bishop, 2013).

6.5 Conclusions

To conclude, this chapter represents a pilot study, investigating MEG RSFC as a correlate (endophenotype) of a dyslexia-associated genotype (PCSK6). Although preliminary, the outcome of this study offers an example of a network based investigation of neurobiological pathways within developmental dyslexia. Future studies investigating the neurobiology of dyslexia associated genotypes should

explore this methodology further into comparing, for instance, structural and functional connectomes to classify intermediate phenotypes of the reading impairment.

Chapter 7

Diffusion Tensor Imaging (DTI) study in developmental dyslexia - the left arcuate fasciculus in typical and atypical readers

Chapter summary

This chapter describes the results of the diffusion tensor imaging (DTI) study, which compares indices of white matter diffusivity in typical and atypical young readers, in both whole brain and in one of the core regions of the reading network, the arcuate fasciculus (AF). Second, within the dyslexic spectrum, it explores the link between reading measures and white matter integrity, or fractional anisotropy (FA) in the left arcuate fasciculus (AF). To conclude, implications of the results, perspective studies and methodological challenges are discussed.

7.1 Introduction

In the past decade, the deterministic single deficit cognitive model, has been replaced by a probabilistic and multifacotrial approach into understanding the etiology of developmental disorders (Pennington, 2006). Since then, dyslexia can be thought of as a neurobiological disorder, originating from genetically-driven cortical, sub-cortical, and white matter abnormalities (Ozernov-Palchick et al., 2016; Steinbrink et al., 2008). Overall, the extensive neuroimaging literature on the subject (for instance see Pugh et al. (2000); Richlan et al. (2009); Shaywitz et al. (2002)), suggests that disruption of functional connectivity between regions of the reading network is at the basis of the reading dysfunction. For instance, atypical neural connectivity, such as decreased white matter integrity in the arcuate fasciculus, a white matter tract connecting dorsal posterior and anterior regions, has been observed for family risk for dyslexia children as early as infancy (Langer et al., 2017; Ozernov-Palchick et al., 2016). In this regard, it has been advanced that the structural deficit of the arcuate fasciculus, as an underlying structural or anatomical counterpart of functional dysfunction, prevents an efficient distal communication between regions of the reading network (Vandermosten et al., 2012b). Previous chapters have broadly explored neurophysiological mechanisms involved in typical and atypical reading, ranging from event-related activity to resting state functional connectivity networks. Current chapter aims to investigate changes in white matter architecture in typical and dyslexic readers. Specifically, this study examines potential differences in the measure of diffusivity (fractional anisotropy) in first, WM connectome as a whole, and in one of the core regions linked to language processing, the left arcuate fasciculus (AF).

7.1.1 The importance of DTI studies in dyslexia

The advances in the MRI imaging techniques and the development of diffusion imaging, have provided the opportunity to measure morphological proprieties of white matter structure in the brain. Details of this MRI method are described in Chapter 2. In short, DTI is a 3-D tractography technique that quantifies the structural integrity and direction of the white matter bundles, by estimating the diffusion of water molecules along the myelinated axons (Basser et al., 1994). This phenomenon of molecules running in parallel with the tracts orientation is called *anisotropic* diffusion, and can be applied to virtually reconstruct 3D images of the WM structures (Catani et al., 2005; Vandermosten et al., 2012b). The most applied metrics in diffusion tensor imaging has been, so far, the fractional anisotropy (FA). The FA index, is given by the the degree of myelineation of the axons, and is generally used to measure white matter integrity (Wakana et al., 2007).

In a detailed review of eleven tractography studies, Vandermosten et al. (2012b) showed how some specific WM regions are associated with dyslexia. In particular, strong correlations between group differences (typical and dyslexic readers) and reading skills were found in the dorsal WM regions, which are the left arcuate fasciculus (AF) and corona radiata (CR); and in ventral tracts such as left inferior frontal occipital fasciulus (IFOF) and left inferior longitudinal fasciculus (ILF). As nicely

illustrated in Figure 7.1, the main functional regions of the reading network pictured on the right, are respectively connected via, the dorsal white matter stream, composed by arcuate fasciculus and corona radiata, and the ventral one, constituted by the inferior frontal (IFOF) and longitudinal (ILF) fascicoli (Vandermosten et al., 2012b). It has been suggested that the dorsal pathway (AF and CR) is the anatomical link of the phonological fronto-temporo-parietal circuit, presumably more activated during reading acquisition; whereas the ventral pathway (IFOF and ILF) provides the anatomical scaffolding of the automatised and, therefore, mature left ventral occipito-temporal visual word processor, (Pugh et al., 2001; Richlan et al., 2009; Shaywitz et al., 2002; Vandermosten et al., 2015).

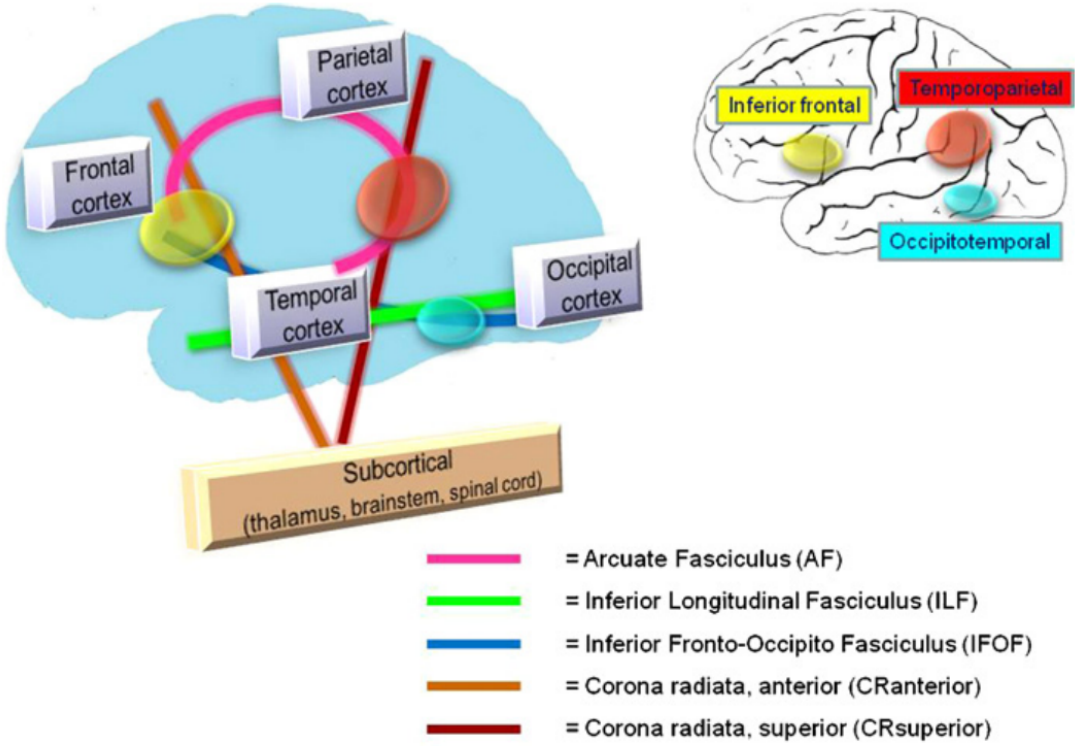


Fig. 7.1 Main white matter (WM) tracts in the reading network. This figure shows the schematic description of the main white matter (WM) tracts (on the left) connecting the main cortical hubs of the reading network (on the right). The dorsal WM pathway is composed by the left arcuate fasciculus (AF) and corona radiata (CR), this association tract has been suggested to subserve the dorsal phonological route. The ventral WM pathway, is composed by the inferior longitudinal fasciculus (ILF) and inferior fronto-occipital fasciculus (IFOF) and it has been suggested to be the structural association pathway of the ventral orthographic route. *Adapted from Vandermosten et al. (2012b)*

Following this, the assumption would be that pre-reading left dorsal WM temporo-parietal anomalies would reflect the phonological deficit, whereas ventral WM differences would reflect a secondary deficit in building the orthographic representation, due to failure of the proper reading acquisition. However, replication studies confirming this hypothesis are lacking in this area (Vandermosten et al., 2015).
To better define specific role of the dorsal and ventral WM pathways, some studies examined the role of these as correlates and predictors of the reading impairment. For instance, Myers et al. (2014) identified arcuate fasciculus and corona radiata as key clusters in the prediction of the reading impairment when controlling for other important contributors such as family history, cognitive capacity, environment and pre-literacy ability. Furthermore, Vandermosten et al. (2015) studied pre-readers at risk for dyslexia and showed that WM anomalies can be used as predictors of later reading deficit and that the ventral pathway, especially the IFOF, plays a key role too. On the other hand, lesion studies in adults, and school-aged dyslexics reported WM anomalies in the dorsal stream, especially in the left arcuate fasciculus (Rauschecker et al., 2009; Vandermosten et al., 2012a).

Arcuate Fasciculus

Before describing the main hypothesis of the current study, it is important to describe why and how the arcuate fasciculus is both evolutionarily and structurally an important region of interest (ROI) for the application of tractography imaging into investigating biomarkers of the reading development.

First language studies of anatomical models described how a lesion of the 'arcuate' tract connecting Broca's speech and Wernicke's comprehension areas led to conduction aphasia. For example, a connecting pathway between posterior frontal and superior temporal lobe was already described by Dèjerine as 'Burdoch's arcuate fasciculus' (Burdoch being the medical doctor who described it earlier (Catani et al., 2005; Déjerine, 1895)). This was further explored by clinical and post-mortem studies, until the advent of diffusion studies in the late eighties (1985) which allowed a 3-D reconstruction of white matter tractography (Catani et al., 2005). Although, DTI is a relatively young neuroimaging technique, it has been extensively used to measure structural connectivity in the perisylvian areas (Catani et al., 2005). Figure 7.2 shows one of the first 3-D reconstructions of direct and indirect tracts of the arcuate fasciculus (AF). The AF is part of the longitudinal fasciculus, that directly connects Broca's with Wernicke's anatomical regions and plays a key role in both development and functionality of the reading system. In particular it has been linked to word identification (Hoeft et al., 2011), reading fluency (Nagy et al., 2004), phonological awareness (Yeatman et al., 2011) and measures of composite reading ability (Gullick and Booth, 2014). Therefore, of particular interest for the studies of this thesis is the link between fluency level and brain connectivity, as in this case, the diffusivity of the AF and measures of reading in dyslexic readers.

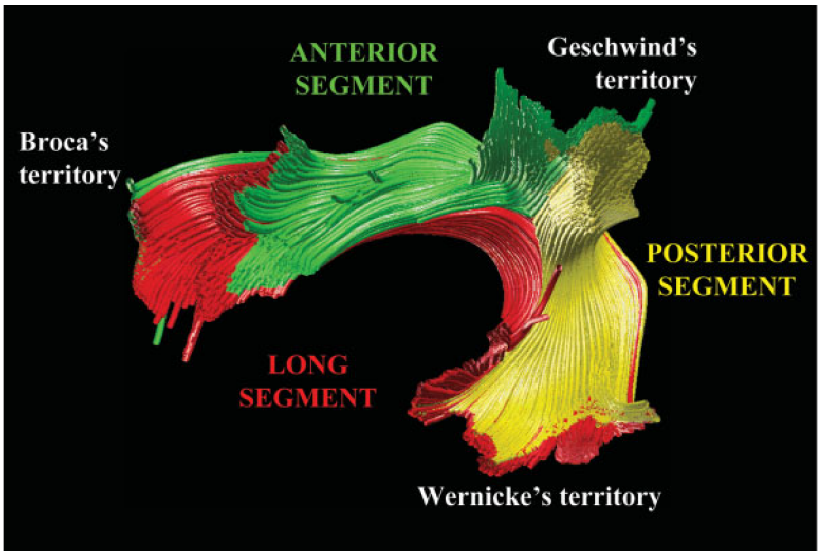


Fig. 7.2 First 3-D white matter reconstruction of the arcuate fasciculus. Example of one of the first 3-D white matter reconstruction studies of the arcuate fasciculus. Here pictured are the three sub-tracts or segments of the arcuate fasciculus (AF): AF direct (red), AF anterior (green) and AF posterior (yellow). Each of the subtracts connects different regions of the language network. *Adapted from Catani et al. (2005)*

As previously mentioned, the idea of dyslexia being a disconnection syndrome, with disrupted connections between main regions of the reading network (angular gyrus and inferior frontal, extrastriate occipital, and temporal areas) and the advances in the diffusion imaging has led neurosicentists to investigate if the dis-connectivity in the reading network can be explained by differences in the underlying WM tracts, such as the arcuate fasciculus, as the main connecting tract of temporo-parietal regions with frontal cortices (Klingberg et al., 2000). Accordingly, several studies have investigated the relationship between connectivity in the left temporo-parietal WM tracts and the reading ability, showing how lower FA values in these areas predicted or correlated lower reading performance (Beaulieu et al., 2005; Klingberg et al., 2000). Consequently, the question was if lower FA in these WM tracts could be used as a predictor of developmental dyslexia.
Some studies have advanced the hypothesis that the dorsal stream, mainly the arcuate fasciculus and corona radiata, is a predictor of later reading impairment (Myers et al., 2014). More recently, Wang et al. (2017) investigated the maturational (from pre-school to school age) changes in children with and without familiar risk for dyslexia. The authors compared WM maturation both longitudinally and cross-sectionally and saw how children at risk showed reduced FA in the arcuate fasciculus in both longitudinal and cross sectional contrasts (Wang et al., 2017). This means that reduced FA together with familial risk, were both strong predictors of reading abilities, as well as changes in the FA were positively associated with the reading development, where faster changes in FA correlated with increased reading skills in 'good readers', (Wang et al., 2017). Overall, these results seem to

suggest that WM changes, especially in the dorsal tract, play a pivotal role in the maturation of the efficient reading network. Moreover, the association with risk factors and psychometric measures implies that changes in FA might be used as dynamic predictors of (a)typical reading acquisition.

7.1.2 Present study

The current study aims to investigate whether differences already reported in the WM architecture, in particular in the direct tract of the left arcuate fasciculus, would be replicated in the comparison of typical and a-typical young readers.

So far, it has been described how decreased FA in the left temporoparietal regions is distinctive in individuals with dyslexia (Beaulieu et al., 2005; Dougherty et al., 2005), especially in the pre-reading stage of development (Wang et al., 2017). Another interesting longitudinal study (Gullick and Booth, 2015) has shown how specific arcuate fasciculus subsection, the direct segment of the AF, strongly correlated with standardised scores of reading skills. In particular lower FA in the direct AF at time 1, uniquely and exclusively predicted lower reading ability at time 2 (Gullick and Booth, 2015). The authors concluded how the connectivity in the direct segment of the AF has a predictive role in changes of reading ability over time, in younger and older children (Gullick and Booth, 2015).

Hence, the goal of the current study was to check if differences in FA in the left AF, defined as predictors of developmental dyslexia, could be detected in an older group of dyslexic and non-impaired readers. Given that decreased FA in the left AF has been observed in longitudinal studies but also in adults and pre-school dyslexics (Vandermosten et al., 2012b, 2015), the research question, in this case, was whether different outcome would be detected in comparing older dyslexics (mean age 11 years 2 months) children with age-matched typical readers. Furthermore, within the dyslexic group correlations between FA estimates and standard scores at reading batteries (reading, spelling and fluency) were exploited.

7.2 Methods

As mentioned before, the current study [1] is part of a collaboration between researchers from the Aston Brain Centre and Prof. Maaike Vandermosten from KU Leuven University (Belgium). For this purpose, the data analysis pipeline and the study design are very similar to the one described in Vandermosten et al. (2012a).
Therefore, from the methodological point of view, one the first questions this study wanted to answer was if the same analysis pipeline could be applied to different type of data, acquired with different MRI machine and coming from a different type of population. While here the participants are children, in Vandermosten et al. (2012a) were adults.

[1] This PhD has been funded by the European International Training Network (ITN) called ChildBrain, whose overreaching goal is to increase the knowledge and expertise of neural mechanisms of (a)typical brain development. As part of this training program different still-ongoing collaborative projects have been initiated by the early stage researchers (PhD students) and principal investigators from diverse European Universities, part of the ITN.

7.2.1 Participants

The study design and protocol was approved by Aston University Research Ethics Committee (ethics numbers 1220 and 408). As before, the recruitment of the dyslexic sample was carried out during the first visit at the DDAU within the Aston Brain Centre, for more details see Chapter 2. Overall, twelve (N=12) dyslexic participants were recruited for this study. Additionally, 6 out of 12 dyslexic participants took part to the Super Mario study, described in Chapter 5; and all 12 took part to the MEG RSFC study described in Chapter 3. For the dyslexic group the data was acquired over a period of two years (2016-2018). For the typical or control group, overall eighteen typical young readers were recruited with the same modalities described in chapters 5 and 2, by advertising the study on University monthly bulletin (Aspects) and social media (Facebook and Twitter). In the control group, one participant was excluded because of age (17 years old) and another one was excluded during the preprocessing phase because of too much motion artefact. Thus, the typical readers or control group included 16 participants overall (N=16). For this group the data was acquired over a four month period (June 2018 - September 2018). Some of the typical group participants (N=13) also took part in the Super Mario study, described in Chapter 5.
All the participants and their caregivers provided written consent to take part to the study and the usual MRI/MEG exclusion criteria for neuroimaging, described in chapter 2, were applied. Additionally, all the participants were rewarded with £20 Amazon vouchers for their participation and offered individual pictures of their brain after the experiment was carried out.

7.2.2 Data acquisition and analysis

All the data was acquired at the Aston Brain Centre Day Clinic, Aston University (Birmingham) using a Magnetom Trio a Tim Siemens system 3T with a 32-channels head coil. Data acquisition specifics were as follows: matrix size = 122x100; field of view =240; repetition time= 8800; echo time= 89; saggital slices = 68; slice thickness = 2 mm; voxel size= 2 x 2 x 2; B-values (b1=0; B2=1000). The acquisition time was around 10 minutes, prior to the DTI sequence weighted T1 and localizer images were acquired. Additionally, the diffusion gradient along the 64 - directions with b-value of 1000 was used.

Preprocessing and data analysis

Raw data was saved, anonymised and transferred onto an external hard drive to be copied to an offline site. As described in Vandermosten et al. (2012a), all images were checked for possible artefacts to be further transferred to DTI-Explorer, a Matlab based toolbox with a GUI interface for DTI preprocessing and data analysis (Leemans et al., 2009). Within DTI-explorer, DTI images were corrected for eddy current and motion induced artefacts using the b-matrix, the results of the correction were then overlaid over individual T1 and visually inspected. Following this, whole brain (WB) tracotgraphy was calculated using voxel size of [2 2 2], fractional anisotropy threshold of 0.2 to

seed and end tracking, angle threshold of 40 °, and fibre length range of 50–500 mm.
Further analyses and exctraction of the direct AF was performed using the TrackVis software developed by Wang and Wedeen (2007). Figure 7.3 shows a reconstructed and T1 overlaid whole brain connectome of a 14 year old participant part of the typical readers group.

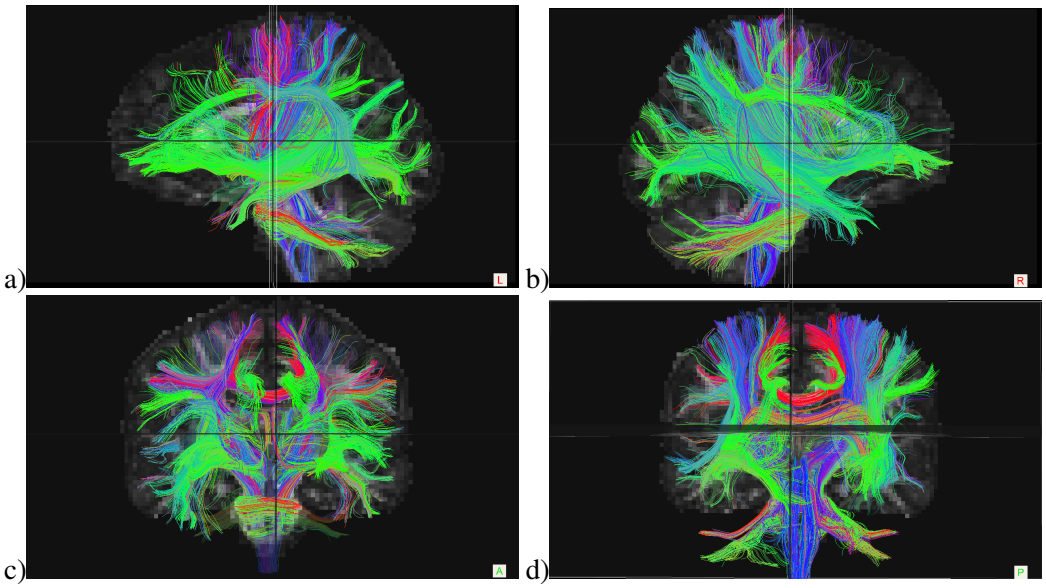


Fig. 7.3 Whole brain white matter connectome reconstructed and overlaid on individual T1. a) left orientation b) right orientation c) anterior d) posterior. It is interesting to note here, how the left arcuate region is much more pronounced compared to the right one. This pattern has already been seen in previous studies, (Vandermosten et al., 2012a).

Tract segmentation was performed in native space to avoid potential biases induced by the normalisation (Vandermosten et al., 2012a). Since delineating manually single tracts is rather time consuming, for the preliminary proof of method replicability and because of the high number of crossing fibres in the inferior tracts, for the purposes of this study it was decided to focus only on the extraction of the direct segment of the AF, as the strongest WM correlate of the reading disability (Vandermosten et al., 2012a). As fully described in Vandermosten et al. (2012a), to delineate the direct tract of the AF, ROIs were defined according to the Wakana protocol (Wakana et al., 2007). Note that in Wakana et al. (2007)'s paper the direct AF is referred to as 'SLF' and 'SLFt'. Following the tract delineation, mean FA and number of tracts (NoT) were extracted from each subject for group comparison. Figure 7.4 shows an example of the delineated direct AF of the same 14 years old subject in Figure 7.3.

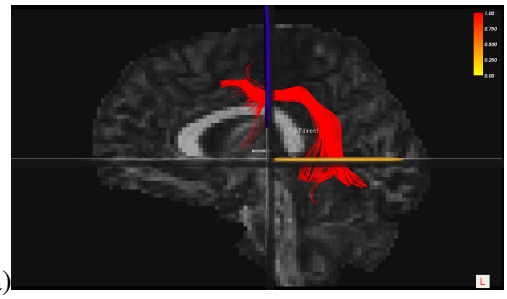
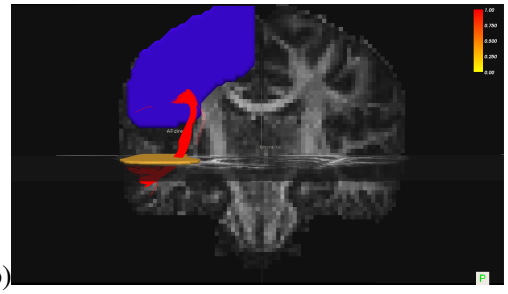
a)  b) 

Fig. 7.4 Single subject's Arcuate Fasciculus (AF) reconstructed in native (T1) space. For one subject the same as in the previous figure (Figure 7.3) AF segmented and overlaid over the subjects T1. a) left orientation b) posterior. Also visible the origins in the Broca (blue) Wernicke (orange) areas.

7.3 Results

Overall, all the comparisons between typical and dyslexic readers were carried out using SPSS software (version 25) with independent samples t-tests with significance set up at the p-value<.05.

7.3.1 Group level outcome

Demographic information and mean differences between typical and dyslexic group are described in Table 7.1. No significant differences were detected in the comparison of age, whole brain (WB) number of tracts (NoT), WB FA and arcuate fasciculus (AF) anisotropy index (FA), (p-value > .05).

	Typicals Mean (SD)	Dyslexics Mean (SD)	p-value
N	16	12	
Sex ratio (m:f)	(9:7)	(9:3)	
Age months	135.88 (38.9)	128.33 (34.3)	.648
Arcuate Fasciculus			
NoT	157 (63)	159 (115)	.127
mean FA	0.508 (.025)	0.502 (.032)	.081
Whole Brain			
NoT	9891.31 (3405)	11175.83 (4090)	.316
mean FA	0.49 (.020)	0.50 (.021)	.979

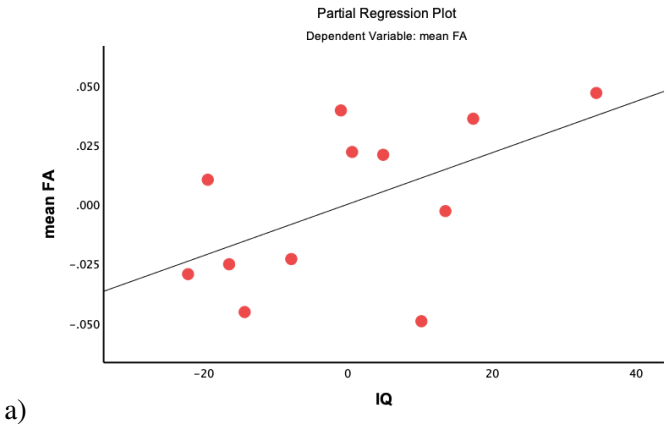
Table 7.1 Summary table of demographic and DTI estimates.Summary table of mean values and standard deviation (SD) of age, number of tracts (NoT) and mean FA for both the arcuate fasciculus and the whole brain connectome. No significant results (p-value>.05) were detected in the independent samples t-tests between typical(control) and dyslexic readers for age, number of tracts (NoT) in respectively arcuate fasciculus (AF) and the whole brain, and mean fractional anisotropy values (mean FA) of both. Hence, equal variance of distribution was assumed over groups.

7.3.2 Dyslexia and correlations with reading skills

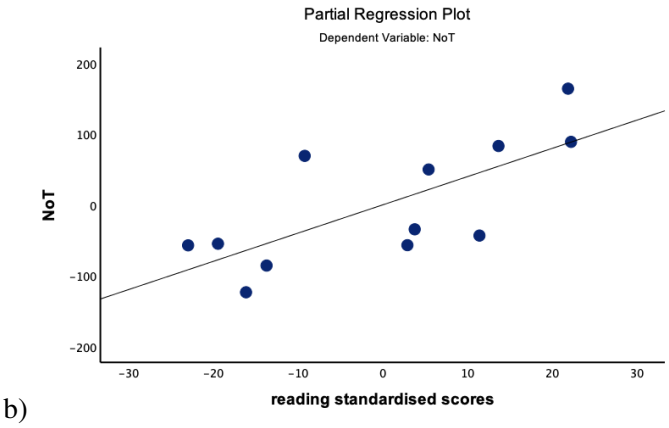
To investigate further if there were any associations between values of FA in the AF and measures of reading skills, a partial correlation (corrected for age) was carried out between mean FA of the direct AF and non-verbal IQ, fluency (TOWRESS), reading and spelling standard scores using SPSS. As summarised in Table 7.2, positive correlations were found between number of tracts (NoT) in the AF and reading, and mean FA and IQ. Figure 7.5 depicts graphically the interactions between IQ and FA, reading and spelling and the NoT.

	IQ		Fluency		Reading		Spelling	
N=12	*r*	p-value	*r*	p-value	*r*	p-value	*r*	p-value
Arcuate Fasciculus								
FA	0.546	.082	0.321	.335	0.36	.276	0.299	.371
NoT	0.47	.144	0.372	.26	**0.725**	.012	0.598	.052

Table 7.2 Dyslexic group:correlations between reading standardised scores and DTI measures in the arcuate fasciculus. This table shows partial correlation coefficients *r* (corrected for age) between psychometric standard scores of fluency (TOWRESS), reading and spelling, and number of tracts extracted for the arcuate fasciculus and the mean FA in that tract. Significant positive correlation was detected between the number of tracts (NoT) in the arcuate fasciculus and reading scores (r=0.725, p-value=.012).



a)



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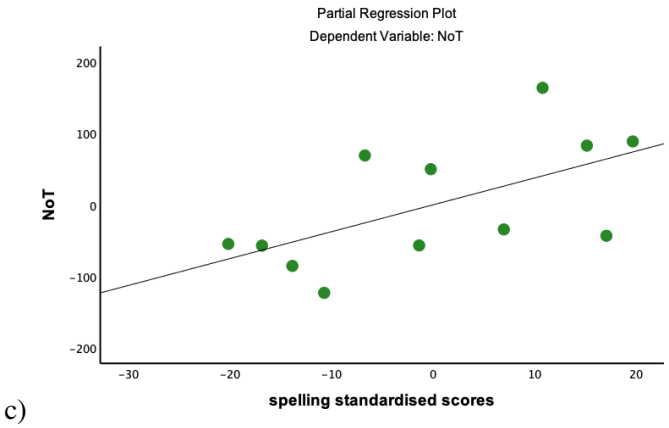

c)

Fig. 7.5 Arcuate fasciculus FA in relation to psychometric profiles in dyslexia:residual plots.Partial correlations (corrected for age) between cognitive measures, FA and NoT in the left AF. Residual plots: a) Correlation between the mean FA in the arcuate fasciculus and non-verbal IQ of the dyslexic group (r=0.546, p-value=.082). b) interactions between the number of tracts (NoT) of the left AF and the standardised reading scores of the dyslexic group (r=0.725, p-value=.012). c) relationship between the number of tracts in the direct AF and the spelling standardised scores (r=0.598, p-value=.052). All the partial correlations were age-corrected, see table 7.2

7.4 Discussion

The present study was carried out as a replication study of a method that has been previously applied by Vandermosten et al. (2012a) to investigate the structural connectivity in adult dyslexic and typical readers. Therefore, the first aim was to apply the same DTI pipeline to the paediatric population described here. Provided that the pipeline was feasible for the current data, the second aim was to explore white matter diffusivity, in age-matched groups of typical and atypical (dyslexic) young readers, in one of the core regions of the structural reading connectome, the left arcuate fasciculus (AF). In particular, the focus was on the direct segment of the left AF, repeatedly linked to different aspects of the reading impairment (Klingberg et al., 2000; Myers et al., 2014; Vandermosten et al., 2012b; Yeatman et al., 2011, 2014). The third and final goal of the study described in this chapter was to investigate correlations between the integrity of the left AF and standardised reading scores within the dyslexic group.

Overall, for all the participants the manual segmentation of the direct tract of the left AF, was successfully accomplished. Second, typical and dyslexic readers did not differ in the diffusivity measures (FA) of the left AF. These results seem to suggest that the dorsal WM tract, although it plays an important role in the prediction of the reading delay in pre-readers (Vanderauwera et al., 2017; Vandermosten et al., 2015), may not be the best biomarker of differences in the structural connectivity in older dyslexics and peer readers. In particular, most of the previous findings connecting AF integrity to reading skills, have used longitudinal type of studies (Gullick and Booth, 2014; Hoeft et al., 2011; Vanderauwera et al., 2017; Wang et al., 2017; Yeatman et al., 2011). This suggests that when comparing the FA in the arcuate fasciculus, it is important to take into account the developmental trajectories and individual differences in the WM changes.

For instance, in a study investigating the relationship between development of WM and reading in children (7-15 years old), Yeatman et al. (2012) showed how children with stronger reading skills (above-average) went from initial low FA to higher values in the following three years, whereas children with weaker reading abilities (below average) had higher FA at first, that declined over time. It follows that there seems to be a double dissociation between good and poor readers in changes of the FA in the left arcuate and inferior longitudinal fasciculus (ILF) over time (Yeatman et al., 2012). It appears that in the current study, the lack of significant differences in the FA of the left AF between dyslexic and typical readers, might be due to the the critical age ($\sim$ 11 years old) of interest where in both dyslexic and typical readers the FA developing curve is at the same level. In fact, according to the dual process model (Yeatman et al., 2012), the development of the FA in the left WM tracts (AF and ILF) is linear between 7 and 15 years for both good and poor readers but the specific rate of development, not only covaries with reading skills, but it is different for the two groups. Specifically, the developing trajectories of FA curves of both good and poor readers, cross at around 12 years of age (see Yeatman et al. (2012) and in Figure B.1). Future longitudinal studies should explore further this 'U' shaped developmental trajectory in the WM reading architecture in relation to developing reading skills, and try to pinpoint the critical periods where anisotropic differences between dyslexic

and typical readers change the least and the most.

Third, in the dyslexic group significant positive correlation was found between the number of tracts (NoT) of the direct AF and the reading standardised scores (r=0.725, p-value=.012), when correcting for age. It has been previously suggested that developmental variation in FA is reflected by changes in 'axonal packing density' partially due to the number of tracts within a specific ROI (Huber et al., 2018b). In addition, previous studies have associated changes in axonal proprieties (number, density, calibre of axons) to axial diffusivity (Vandermosten et al., 2012b). Moreover, not statistically significant (p-value >.05) but higher correlation coefficients, were found between mean FA and non-verbal IQ (r= 0.546, p-value=.082), NoT and spelling standardised scores (r=0.598, p-value=.052), as shown in Table 7.2 and Figure 7.5. This confirms findings present in the literature, showing positive associations between reading skills and FA in the left dorsal and ventral WM network in preschoolers, older children and adults (Gullick and Booth, 2015; Hoeft et al., 2011; Klingberg et al., 2000; Yeatman et al., 2011). Interestingly, mean FA had the lowest correlation coefficient (r=0.321) with fluency scores, indicating that the the association previously shown between single word reading and AF, could be significant only in younger age range (Cui et al., 2016). It is important to reiterate that the correlations here conducted were corrected for age, to take into account maturational changes, so the stringent criteria might have smoothened the effect.

One way to explore further present findings would be to compare reading skills with inferior longitudinal fasciculi and investigate if, for instance, reading and spelling scores would correlate with increased or decreased FA in regions that are supposed to structurally sustain more automatised processes and the visuo-orthographic cortical stream, activated in older readers (Vandermosten et al., 2012a). Put differently, one of the reasons why in this case the diffusivity of the AF did not show significant correlations between reading skills and FA in the arcuate fasciculus could be that, at this stage of the reading development (10-11 years old), the left ventral (mainly ILF and IFOF) WM tracts is the main structural region that provides connectivity between cortical regions of the automatised reading processor (Vandermosten et al., 2015).

The reason why the ventral stream has not been approached in this analysis is because, the manual delineation and extraction (used here) of the ILF and IFOF is not ideal, since anatomically the inferior tracts present a lot of crossing fibres, where naturally the FA would be very low or null (Vandermosten et al., 2012b). One way to overcome this methodological challenge would be to study the WM structural connectivity by applying an AAL approach, where the FA is computed between preselected ROIs of the dorsal and ventral streams. Currently, to the best of authors knowledge child or developmental normative atlases (AALs) are not yet present in the literature, besides neonatal ones (Deshpande et al., 2015).

On these grounds, the results of the current study suggest that dyslexic and typical readers do not present differences in the WM connectivity in the arcuate fasciculus, when analysed at 10-11 years of age. Previous studies have demonstrated how this particular region has an important role in the development of reading skills and can be used as a predictor of future reading impairment

(Huber et al., 2018a; Vanderauwera et al., 2017). The absence of significant effects in this case, can be justified by both the relatively small sample size and the 'older' age range in comparison to previous studies, where according to the white matter developmental model, the AF has reached its developmental maturation (Yeatman et al., 2012). Moreover, the heterogeneity of the FA maps of the left AF, especially in developmental populations, can explain the lack of significant group differences (Beaulieu et al., 2005). Nevertheless, positive anisotropic-reading skills correlation has been detected between both NoT and FA, confirming previous findings and suggesting that the left AF can be used as an anatomical correlate of different reading phenotypes within the dyslexic spectrum.

7.5 Conclusion

This study aimed to investigate potential differences in structural connectivity in one of the core regions of the structural reading connectome, the direct left arcuate fasciculus. Although no differences between typical and atypical young readers were detected, positive correlations between structural connectivity and reading skills in dyslexics suggest that this region is associated with atypical reading. Lastly, the preliminary findings here described, intend to offer a critical overview of the potential challenges into defining anatomical based functional specificity of a particular region, especially in a developmental perspective.

Chapter 8

General discussion

Chapter summary

The present PhD project was funded by and is part of the European International Training Network (ITN) named ChildBrain [1], a consortium of different European universities with the overall objective to advance the understanding of neurodevelopmental cognitive disorders by using brain research. Within this framework, the studies described in this thesis, aimed to promote the knowledge of the neural underpinnings of developmental dyslexia. In particular, by adopting a multimodal cognitive, genetic and neuroimaging (MEG and DTI) approach this work focussed on investigating, not only differences between impaired and typical young readers, but also diverse phenotypes within the dyslexic continuum. This chapter will present a summary of the research carried out in this thesis and discuss the main outcomes of this work. Lastly, it will illustrate perspective research and potential future challenges in developmental neuroimaging.

[1] http://www.childbrain.eu/

8.1 Aims and findings

Developmental dyslexia is described as a failure in acquisition of fluent and accurate reading, despite proper level of formal instruction and adequate intellectual skills. It affects 5 to 17% of school children and, although the cognitive and behavioural models have proposed that the core deficit is in the phonological processing, the neurobiological causes of the reading disability still need to be fully explained.

The advent of brain imaging has brought new insights about the neural mechanisms involved in the reading processing. Neuroimaging studies of reading have been able to pinpoint not only the reading network, as a mosaic of distinct brain regions going form visual to language specific cortical areas, but also patterns of dis-communication among these have been repeatedly linked to dyslexia (Pugh et al., 2000). Additionally in the past two decades advances in genetic studies have proposed biological common pathways in the aetiology of the reading disorder (Eicher and Gruen, 2013). Hence, dyslexia presents itself as a complex developmental impairment whose aetiology has still to be fully explained by quantifying the contribution of each of the proposed neurobiological components.

Thus, a vast range of cognitive, behavioural, neural and genetic theories have proposed that the underlying mechanisms of the dysfluent (dyslexic) reading are very diverse and can be explained by multiple factors such as impaired phonological and sensory processing, neural disconnection between regions of the reading network, genetic risk factors that predispose some individuals to develop the deficit compared to others and so on.

The general idea is that developmental dyslexia is a multivariate deficit that presents itself with a variety of associated symptoms, where the driving factor is the deficit in accurate and fluent reading. Although the reading deficiency has a stable clinical definition, the specific underlying deficits can vary across age, languages, orthographies and cultures. Therefore the main challenge of cognitive neurosciences investigating the reading delay has been to design a comprehensive method or approach, capable of tackling the heterogeneity of the dyslexic spectrum.

So far, functional and structural neuroimaging studies have been able to fully describe the functional and structural reading networks and identify decreased connectivity (under-activation) in dyslexics compared to typical readers. Although this is valuable knowledge, it is not very useful when designing educational interventions specific for dyslexic readers, and it also does not play an essential role for identifying the potential neural predictors of a later reading impairment, given the high inter-individual variability among poor readers.

More recent theories of dyslexia have proposed that a comprehensive neurobiological framework is essential to be able to study the etiology of the disorder. For instance, Ozernov-Palchick et al. (2016) proposed that a multifactorial model of dyslexia, that integrates Pennington (2006)'s multiple deficit model (MDM), and more recent integrative MDM by van Bergen et al. (2014), which step away from the deterministic and single deficit focused theories, to a more probabilistic and multifaceted ones. According to this model, experimental evidence from neuroimaging, genetic, and behavioural is

incorporated to illustrate the independent significance of each of the components for dyslexia but also their reciprocal relationship (Ozernov-Palchick et al., 2016). In this framework, the interplay of these components on the 'developmental axis' could be informative of the locations of the brain alterations, the severity of the deficits, and the connectivity strength between brain structures that support reading (Ozernov-Palchick et al., 2016).
Similarly, the present PhD's objective was to expand on the current neuroimaging findings by offering a thorough investigation of brain networks underpinning complex symptoms of dyslexia. For this reason a multimodal cognitive, genetic, structural (DTI) and functional (MEG) neuroimaging approach was employed. Respectively, the *intra*-dyslexic variability was defined by examining the MEG RSFC and network topology in relation to cognitive and genetic factors. Following this, visuo-orthographic processing was investigated in typical and atypical reading to delineate temporal differences in the first stages of reading processing, the pre-lexical orthographic decision making. Following this, structural connectivity, by means of fractional anisotropy, was explored in typical and atypical readers. In each of the experimental chapters a correlational cognitive-neuroimaging (MEG and DTI) approach was utilised to distinguish neural correlates of different dyslexic sub-types.

In sum, the aims of this PhD thesis were to:

- Investigate the MEG RSFC and network topology in relation to genetic and reading correlates (fluency) within the dyslexic spectrum (Chapters 6 and 3).
- Explore differences in the oscillatory dynamics of the viso-orthographic processing in typical and atypical young readers by applying a novel MEG paradigm (Chapters 4 and 5).
- Study differences in the structural connectivity in typical and atypical readers, and the correlation between white matter integration and reading skills in dyslexic readers (Chapter 7).

The RSFC and network topology study described in Chapter 3 provides a strong advocacy for investigating task-free neurophysiological connectivity to better describe sub-types in developmental dyslexia. Studying the temporal dynamics of oscillatory communication in the dyslexic brain at rest has helped to define specific interactions between the reading network regions and their synchronisation and integration within the functional connectome. Moreover, using a single-word reading (fluency) measure to study differences in MEG RSFC, has provided a valid method to define different neurophysiological phenotypes based on the severity of the reading impairment (dysfluent/fluent dyslexic readers). The MEG atlas based analysis allowed portrayal of local and global whole-brain connectivity proprieties in different frequency bands and set the platform to perform network analysis. Thus, increased activation in the left frontal areas of the more severe dyslexic readers, compared to the fluent ones, has shown how in the dysfluent cases compensatory mechanisms in the frontal regions may occur. Furthermore, the increase of the 'neural load' in specific reading related and DMN regions in the dysfluent tree showed how, in the 8-30 Hz frequency range, more impaired (dysfluent) dyslexic

readers have a less integrated functional network.
It has been shown here how distinct oscillatory patterns define configurations in network topology among children with dyslexia. This indicates the importance of discriminating different dyslexic neurofunctional sub-types based on fluency phenotypes. In other words, to know how task-free functional network configuration changes linked to reading skills, helps to characterise neural mechanisms that could predict future reading impairment in children at risk for dyslexia. Moreover, by describing the functional network with the MST, this study has offered a good example on how unbiased graph theory metrics could be further applied with trees obtained with different neuroimaging techniques. Graph theory, and hence MST, is a powerful model to represent connectivity. It has been proven by now, that network science applied to brain connectivity using metrics of integration, segregation, and small-worldness provides a meta-analytical platform to depict functional and structural connectivity deriving from different types of neuroimaging methods (e.g. dMRI, fMRI, and M/EEG) For instance, the MST could be used to delineate other types of networks, such as the structural (DTI) one and then used to compare functional (MEG) and structural (DTI) MSTs.

Equally, the results of the RSFC and MST study described in Chapter 6, demonstrate how the MEG AAL based and network (MST) approach is an efficient and fine-grained method able to better identify intermediate phenotypes in the dyslexic spectrum. Although with a small sample size, this PCSK6 pilot study sets a good precedent of how combining genetics with MEG source space analysis is a powerful tool to describe the neurophysiological correlates of dyslexia-associated genotypes. The outcome of the present study shows how long range connectivity (in theta and delta frequency bands) changes in relation to the genetic expression and how the integration and synchronisation of the functional connectome (MST configuration) differs in relation to the genetic profile. This is the first MEG-genetics study that investigated the PCSK6-associated functional network in developmental dyslexia. This outcome suggest that MEG-genetics studies are worthwhile exploring into defining neurofunctional endophenotypes in developmental disorders with genetic influence. Being this the very first investigation of the PCSK6 influence on brain connectivity, it would be important for future research to carry out a replication study with a bigger sample size.
The studies described in Chapter 3 and 6 constitute archetypes of application of the AAL based MEG approach to investigate functional connectivity and network topology *within* the dyslexic spectrum. The reason behind studying MEG task-free connectivity, was to provide a robust way for describing whole-brain synchronisation and integration. This method could be effectively applied to classify neural predictors of the reading impairment in preschoolers and, most of all, could be equally adopted across languages and orthographies. To the best of author's knowledge, this is the first instance of a MEG source space task-free connectivity and brain network study in developmental dyslexia. Furthermore, the application of the MST in two different sub-groups (fluent/dysfluent and PCSK6+/PCSK6-) of young impaired readers makes this method a very promising tool to investigate temporal dynamic changes in the MEG RSFC in developmental disorders. Additionally, these results have taken a step

forward into defining the high variability in dyslexia by specifying topological changes in connectivity distribution linked to cognitive (reading skills) and genetic factors.
Future studies investigating both neuro-genetic functional and structural connectivity should consider the application on the MST as an un-biased method to compare different tree topographies in relation to the biological pathways between gene-brain-behaviour.

Traditionally, dyslexia has been associated with impaired phonological decoding (Shaywitz and Shaywitz, 2005). Relatively recent studies have demonstrated how deficits in visual word recognition are also strong predictors of the reading impairment, especially in the initial stages of the reading acquisition (Fraga González et al., 2014). To better understand the neurophysiological underpinning of the visuo-orthographic processing, a novel MEG-paradigm ("Super Mario") was developed to specifically target the pre-lexical stages of visuo-word recognition. Chapter 4 describes the validation of the paradigm in adult experienced readers. In this study source-level results showed how a widespread network of occipito-temporal and superior parietal areas is involved in the visuo-orthographic processing of unfamiliar pseudowords. These results suggested that in the pre-lexical recognition task, attentional areas (superior parietal) seem to have a top-down influence on the visuo-ortographic decision making. Following this, in chapter 5 the same paradigm was utilised to investigate potential sensor and source level differences between young dyslexic and peer readers. Here, the focus was on both the behavioural performance (accuracy at the task) and neural correlates of the visuo-orthographic processing (N170). Although no differences at the overall accuracy between dyslexic and typical readers were detected, the performance at the task, in the dyslexic sample, correlated with the participant's individual reading skills. This outcome confirmed that relative reading skills in dyslexia correlate with the accuracy in visuo-orthographic processing.
Furthermore sensor level MEG results revealed a bilateral event related desynchronisation (ERD) in early and later stages of visual word recognition, in both dyslexic and peer readers. Ergo, the behavioural and the sensor-level MEG outcome provided important insights about developmental trajectories of the pre-lexical reading stage, suggesting that by the age of eleven years old, both typical and atypical readers are able to discern unfamiliar words (pseudowords) by the first 300 ms of stimulus onset.
This has relevant significance for the cross-sectional developmental neuroimaging studies, that should take into account age-related changes in the reading neurofunctional profiles. It has been already proposed in this thesis how, in the case of the pre-lexical processing, impaired readers have an 'U' shaped learning curve (Maurer et al., 2005a) and how the reading deficit can be linked to a delayed reading acquisition (Parviainen, 2006). Based on the current results, it would appear that at the age of 11 years old, when the formal reading instruction has been completed, the prelexical processing shares the same neural mechanisms in impaired and non-impaired readers.
Additionally, the MEG source-level analysis showed how in typical 11 years-old readers, the visuo-orthographic processing activated distributed bi-lateral sources of the occipital, inferior frontal and

limbic cortices and was specific to long-range connectivity in the theta frequency band (4-8 Hz). Although in a small sample size, these results indicate that the pre-lexical word recognition relies on long-range neural communication between the viso-orthographic, attentional and memory related cortical regions. A perspective research question would be to apply the same MEG paradigm and analysis to a younger (a)typical reading population and see how these pattern change at different stages of reading acquisition.
The source-level outcome of both Chapter 4 and 5, has important implications into defining the specificity of the 'visual-word processor' and consequently of the VWFA area role in the ventral reading stream (McCandliss et al., 2003). The present results suggest that the specificity of the vOT cortex in the visual word recognition is characteristic for familiar or lexical word recognition, whereas the pre-lexical pure visuo-orthographic processing (of pseudowords) relies on a distributed dorsal (attentional) and ventral networks. This as well is relevant to future developmental studies, investigating neural predictors of the reading delay that should consider differences in the pre-lexical visuo-orthographic processing, before defining the specialisation of distinct cortical regions in the lexical word recognition.

The third objective of this thesis was to replicate a DTI study previously carried out in adult dyslexics and peer readers (Vandermosten et al., 2012a), in a group of typical and atypical young readers. Specifically, the aim was to examine differences in fractional anisotropy (FA) in one of the core regions of the structural reading network, the left arcuate fasciculus in dyslexic and typical young readers. In addition, interactions between reading skills and FA within the dyslexic spectrum were explored. Albeit no significant differences in the FA of the left arcuate fasciculus between typical and atypical young readers were detected, interesting outcome came from the anisotropic-reading correlational approach. In particular positive association between higher reading skills in the dyslexic sample and increased value of axon diffusivity were found in the dyslexic group. These results highlight the importance of examining the cognitive correlates when measuring variance in structural connectivity estimates, in relation to high inter-individual variability detected.
Particular mention of the interactions detected within the dyslexic readers in this thesis, between non-verbal IQ and "Super-Mario" accuracy (Chapter 5), and IQ and FA and number of tracts in Chapter 7, is required. This interesting finding of positive correlations between cognitive attainment and some of the measures (neuroimaging and non) used in this thesis confirms the hypothesis of un-coupled cognitive and reading development. In fact, the mere definition of developmental dyslexia excludes cognitive impairment. Ferrer et al. (2010) proposed how dyslexic readers might have a disruption in the interconnection between IQ and reading over time. Interestingly, the positive interactions detected in this thesis between IQ and accuracy at the task (Super Mario) and DTI connectivity confirm this hypothesis. Therefore, children with dyslexia that have higher IQ would perform better at the task and have a different diffusivity profile, which could be a mere effect of the cognitive attainment and hence, un-related to the poor reading skills. This is pertinent to future neuroimaging studies of dyslexia,

which should take into account this detected disassociation and how it might change over time in poor readers.

Finally, the overall outcome of the work described in this thesis puts the emphasis on the need to redefine the tools used for studying the aetiology of dyslexia. While previous neuroimaging research has successfully identified functional and structural cortical networks involved in the reading deficit, in the developmental framework comparing typical and atypical young readers seems not to provide additional enlightenment about differences in neural mechanisms involved. Instead, looking at developmental dyslexia as a wide spectrum that can be 'stratified', for instance on cognitive or genetic information, has offered valuable understanding of the cortical and subcortical underpinning mechanisms in moderate to severe reading impairment in children.
On that account, the aspiration for the next generation neuroimaging studies should be to try to adopt the mulitmodal neuro-genetic-behavioural correlational approach into defining (a)typicalities in trajectories of the cognitive development.

8.2 Future perspectives and challenges

Dyslexia, is only one of the many deficits part of learning difficulties in child development. Although the prevalence of developmental disorders, such as attention deficit hyperactivity disorder (ADHD), dyscalculia (math problems), dyslexia and specific language impairment (SLI), ranges between 3% and 8% in school-age children (Norbury et al., 2016), it has been recently highlighted how 30% of UK children do struggle at school (Astle et al., 2018). According to the UK Department for Education, in 2017, 30% of children failed to meet the targets in reading or maths at age 11 (Astle et al., 2018).
So far, the understanding of the underlying causes and neural mechanisms came from studying children with a specific deficit (i.e. pure dyslexia or dyscalculia), or coming from distinct clinical samples with official neuropsychological diagnosis (i.e. ADHD). This has been an useful practise into defining differences between typical and impaired cognitive skills, but comes with the risk of 'restraining' the high variability of the learning slopes (typical and atypical), to the mere comparison of specific cases versus controls. The work described in this thesis shows how it is important to consider the heterogeneity within the learning difficulties, by identifying for example different phenotypes of dyslexia. Dyslexia is indeed an impairment that has been defined as a continuum that presents itself with high rates of comorbidity and different severe to mildly impaired reading processes (Lyon et al., 2003; Rose, 2009). It follows that, when studying such disorders, it is crucial to keep in mind that both typical and atypical reading trajectories do change over time (Wise Younger et al., 2017). For these reasons, and in lack of the possibility to perform longitudinal studies, it is of paramount importance to adopt a multimodal approach that includes psychometric, genetic and neuroimaging modalities. Here it has been shown how a basic constituent of reading (single word fluency) can guide the stratification or classification of brain networks in dyslexia. Furthermore, building upon traditional psychometric measures to create experimental conditions ("Super Mario") able to tackle

stages of visual word recognition, such as the orthographic processing, has the potential to be used as an alternative to rather long and expensive educational attainment screening procedures. The use of MEG, in this case, offers an elegant way of measuring brain network changes in task-free and reading-positive conditions, by providing a detailed picture of spatial and temporal dynamics of some of the aspects of reading (RSFC and visuo-orthographic processing). Moreover, the application of brain network analysis offers a methodological common platform where it is possible to integrate structural (DTI) and functional connectivity (MEG) information by building a multimodal neural archetype of the developing brain.

It has been illustrated how reading is a evolutionarily recent cognitive skill and how its acquisition takes advantages of, or is parasitic to, preexisting (more ancient) brain networks (Dehaene-Lambertz et al., 2018). This hypothesis is confirmed by the work described in this thesis, in the MEG studies of task based and task-free connectivity. For instance the RSFC and brain network analysis have depicted changes in the alpha and theta frequency bands, suggesting that differences among dyslexic readers are in the long range connectivity. On the other hand, task based source analysis showed how young readers rely on long range communication in the theta frequency band during visuo-orthographic processing. Modulations in alpha and theta oscillations have been previously linked to other cognitive processes such as encoding of new information in working memory and modulation of visuospatial attention (Lobier et al., 2018; Schwaiger et al., 2001). This demonstrates how MEG, with its time-scale millisecond precision and advanced source reconstruction, can be applied to describe frequency based neural synchronisation, and to identify large-scale brain network integration, during cognitive processing and at rest. Additionally, the DTI study described in this thesis has shown how long range dorsal white matter connectivity is linked to cognitive performance (reading skills) in children with dyslexia. Based on previous studies (Brookes et al., 2011) and the results illustrated here, networks studied with different modalities (MEG and DTI) suggest the presence of inter-dependencies. This evidence highlights the advantages of multimodal neuroimaging and the need for future research to design methodologies that can reconcile information coming from different networks. For instance, recent advances in multi-modal analysis have proposed how using a multilayer brain network approach can be an efficient way to compare not only, MEG networks in different frequency bands, but also across modalities such as MEG and DTI (Mandke et al., 2017).

The perspective for future child development investigations should, therefore, be the application of a multi-faceted approach that incorporates psychometric, genetic and brain network studies, with the ultimate goal to integrate these different aspects into defining typical and atypical trajectories in cognitive development.

Finally, it is worthwhile discussing methodological considerations in developmental neuroimaging. Notwithstanding the recent advances in the estimation of functional and structural connectivity at rest and during task-positive paradigms, with the use of source modelling for the MEG and ROI or seed based analysis for the DTI, as well as the promising advent of network analysis mentioned above, a

lot of challenges are currently faced when collecting and analysing child-brain data.
First, it is known that the motion artefacts are much higher when performing neuroimaging in children, compared to adults. In most of the developmental studies, at least 25% of the acquired data is not usable for further analysis, due to too much motion artefact. Future movement compensation algorithms should take into account developmental neuroimaging data when developing artefact correction techniques. On the other hand, the use of infant brain size MEG scanners could reduce the distance between the sensors and the head, increase the signal to noise ratio and, hence, the spatial accuracy in the source localisation. For instance, child-size MEG scanners have been successfully used in paediatric hospitals (at bed side distance) to accurately map the seizure onsets for children who suffer from epilepsy or other neurological disorders and require surgical intervention, such as baby-MEG in Boston's Children Hospital).
Second, the use of adult size atlases in both DTI and MEG connectivity studies comes with a pitfall of a lower spatial precision when localising sources in the brain. Currently there are no normative child-brain atlases applicable for source MEG analyses. Human brain connectome and large-scale studies should try to develop standardised age-appropriate atlases that would guide a more accurate source localisation. The child atlases could be further applied to compare connectomes derived from different neuroimaging techniques without the trouble of transforming structural or functional data in common spaces. Following this, the application of network and graph theory analysis would increase its replicability. For instance, with the nodes of the DTI and MEG networks spatially overlapping this method could provide a common basis where to compare structural and functional connectivity estimates.
Ultimately, although the ideal and most robust developmental studies are the longitudinal ones, since they allow to investigate individual and group-level developing trajectories, oftentimes the time and resources of specific projects are limited. For that reason, open science data repositories are a very promising tool that would enable neuroscientists across the world to have the same opportunities to share and reproduce neuroimaging studies.

8.3 Conclusion

In conclusion, this PhD thesis offers a demonstrative journey into the design and application of a multimodal (cognitive, genetic, structural and functional neuroimaging) approach for the investigation of neurobiological correlates of typical and atypical reading development. Additionally, it aims to highlight the importance of characterising the high heterogeneity of dyslexia by defining different reading phenotypes within the spectrum. Specifically, by examining functional and structural cortical architecture, it aims to offer a illustrative model for the investigating of the neural mechanisms of developmental dyslexia in relation to individual reading skills and genetic influences.

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Appendix A

Supplementary material of Chapter 3

List of “Super Mario” stimuli

Condition 1

1.	pog	31.	dif
2.	dat	32.	pni
3.	mip	33.	kin
4.	ral	34.	xlo
5.	nas	35.	bap
6.	mib	36.	nic
7.	faw	37.	xiv
8.	lig	38.	vih
9.	pty	39.	som
10.	rla	40.	nig
11.	kvo	41.	pob
12.	bos	42.	tnb
13.	tim	43.	jin
14.	vbi	44.	cum
15.	gno	45.	btp
16.	klo	46.	zca
17.	sce	47.	jav
18.	cin	48.	mpl
19.	hom	49.	vop
20.	giv	50.	fum
21.	lti	51.	cid
22.	prt	52.	rot
23.	cki	53.	fos
24.	tod	54.	von
25.	fip	55.	sou
26.	nop	56.	zen
27.	blt	57.	bon
28.	ris	58.	gip
29.	som	59.	evo
30.	tir	60.	lin

Fig. A.1 Super Mario list of stimuli condition 1

Condition 2

61.	shum	91.	zyst
62.	bice	92.	muns
63.	nade	93.	yuns
64.	teap	94.	zupt
65.	derl	95.	vaud
66.	marl	96.	deps
67.	berk	97.	saxt
68.	mest	98.	shix
69.	weaf	99.	gops
70.	prot	100.	tunx
71.	runk	101.	pyxt
72.	mact	102.	nard
73.	phet	103.	yeaz
74.	klup	104.	fyde
75.	skad	105.	yest
76.	vups	106.	juid
77.	dwyx	107.	veys
78.	jops	108.	monx
79.	nyld	109.	sixt
80.	voft	110.	soid
81.	lynd	111.	hukt
82.	zopt	112.	veud
83.	jocs	113.	gost
84.	nows	114.	gans
85.	fied	115.	nuks
86.	nund	116.	goxt
87.	lynz	117.	wuks
88.	ound	118.	durd
89.	fekt	119.	durz
90.	bics	120.	joid

Fig. A.2 Super Mario list of stimuli condition 2

Condition 3

121.	stree	151.	scrax
122.	barch	152.	loide
123.	glack	153.	glift
124.	loast	154.	porst
125.	blork	155.	twerz
126.	keast	156.	bloed
127.	churt	157.	gleft
128.	glamp	158.	quyst
129.	prait	159.	blaus
130.	flact	160.	turze
131.	creft	161.	tunse
132.	flimp	162.	gwous
133.	ziced	163.	shest
134.	smict	164.	crext
135.	frupt	165.	lirld
136.	gwaws	166.	flirs
137.	skeft	167.	smoze
138.	yimpt	168.	coaze
139.	phups	169.	chups
140.	slykt	170.	nepps
141.	dwooz	171.	wanth
142.	vipte	172.	thewd
143.	whoct	173.	nonns
144.	rirde	174.	pread
145.	binth	175.	wrupt
146.	doizz	176.	fonse
147.	yanth	177.	sheaz
148.	comps	178.	jouze
149.	linth	179.	frens
150.	higns	180.	sonse

Fig. A.3 Super Mario list of stimuli condition 3

Detailed description of Fieldtrip pre-processing pipeline

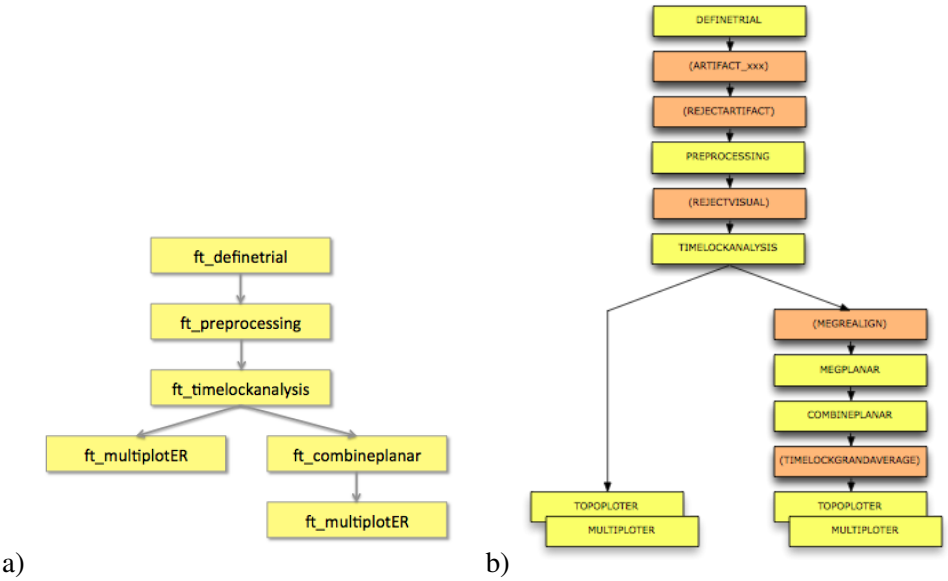

Fig. A.4 FieldTrip preprocessing and Event Related Fields (ERFs) averaging pipeline: a) is the set of functions used to perform trigger-based trial selection (*ft_definetrial*), preprocessing (*ft_preprocessing*), avergaging evoked activity over specific time windows(*ft_timelockanakysis*), combining two gradients at each sensor in single positive values (*ft_combineplanar*), and lastly, plotting the topology of the ERFs distributed over time with *ft_multiplot* b) Same procedure as in B.4 a) but with additional steps in between, with the possibility of computing MEG planar gradients as well.

Appendix B

Supplementary material of Chapter 7

Double dissociation model by Yeatman et al. (2012)

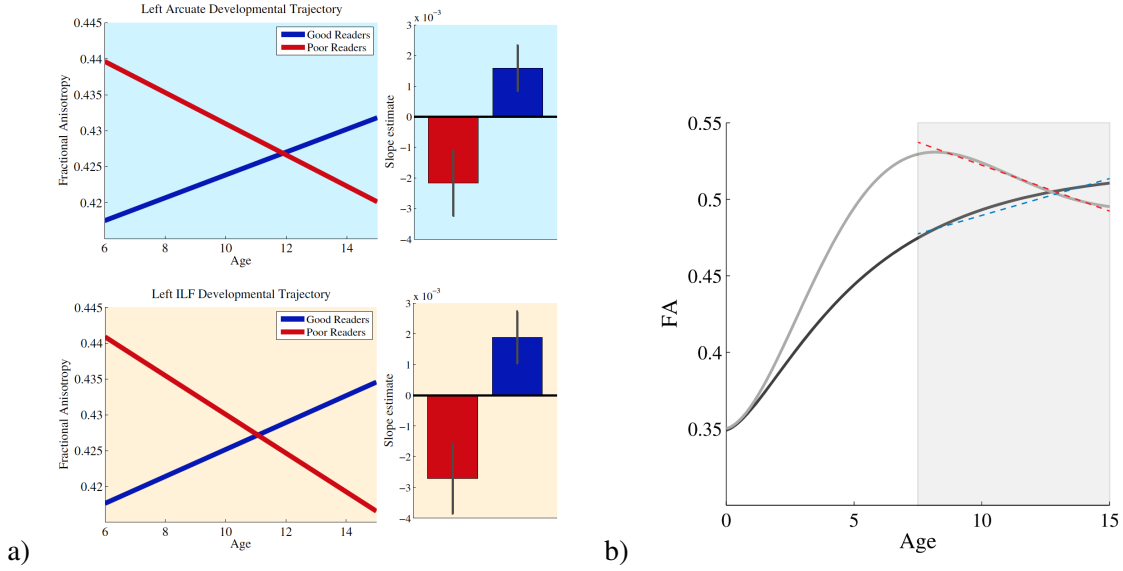

Fig. B.1 These two figures illustrate Yeatman et al. (2012) dual-process model. a) changes in white matter development between 7 and 15 years old, in the left arcuate fasciculus and inferior longitudinal tract. In particular good and poor readers have opposite patterns. On the right, changes in the developmental slopes, between the two groups. b) same as above, modelled according the homeostatic equations that lead the development over time. Black-curve represents good readers and the grey one, the poor ones (below-average). The dotted lines are the rate of the FA development. For the purposes of the Chapter 7, it is important to highlight how the developing curves of both groups, cross at approximately 11 years of age. (Adapted from *Yeatman et al. (2012)*).