Developing multidimensional metrics for evaluating paediatric neurodevelopmental disorders

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Healthy brain functioning depends on efficient communication of information between brain regions, forming complex networks. By quantifying synchronisation between brain regions, a functionally connected brain network can be articulated. In neurodevelopmental disorders, where diagnosis is based on measures of behaviour and tasks, a measure of the underlying biological mechanisms holds promise as a potential clinical tool. Graph theory provides a tool for investigating the neural correlates of neuropsychiatric disorders, where there is disruption of efficient communication within and between brain networks. This research aimed to use recent conceptualisation of graph theory, along with measures of behaviour and cognitive functioning, to increase understanding of the neurobiological risk factors of atypical development. Using magnetoencephalography to investigate frequency-specific temporal dynamics at rest, the research aimed to identify potential biological markers derived from sensor-level whole-brain functional connectivity. Whilst graph theory has proved valuable for insight into network efficiency, its application is hampered by two limitations. First, its measures have hardly been validated in MEG studies, and second, graph measures have been shown to depend on methodological assumptions that restrict direct network comparisons. The first experimental study (Chapter 3) addressed the first limitation by examining the reproducibility of graph-based functional connectivity and network parameters in healthy adult volunteers. Subsequent chapters addressed the second limitation through adapted minimum spanning tree (a network analysis approach that allows for unbiased group comparisons) along with graph network tools that had been shown in Chapter 3 to be highly reproducible. Network topologies were modelled in healthy development (Chapter 4), and atypical neurodevelopment (Chapters 5 and 6). The results provided support to the proposition that measures of network organisation, derived from sensor-space MEG data, offer insights helping to unravel the biological basis of typical brain maturation and neurodevelopmental conditions, with the possibility of future clinical utility.

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Abbreviations and specialist terminology

ABC	Aston brain centre
ACRS	Abbreviated Conner's' Rating Scale
ADHD	Attention-deficit/hyperactivity disorder
ASEBA	Achenbach System of Empirically Based Assessment
ASR/	Adult Self-Report for adults aged 18 to 35 years
ATT	Attention behaviour functioning
BCa	Bootstrap confidence interval
BD	Block design
BPM	Brief problem monitor
C	Clustering coefficient
CAMHS	Child and Adolescent Mental Health Services
CBCL/6-18	Child Behaviour Checklist for children aged 6 to 18 years
CI	Confidence Interval
CPRS	Conners' Parent Rating Scale-Revised
CTRS	Conners' Teacher Rating Scale-Revised
C_w	Clustering coefficient in a weighted graph
C_w-s	clustering coefficient in a surrogate graph
d	Cohen's d effect size
DDAU	Dyslexia and Developmental Assessment Unit
DLPFC	Dorsolateral prefrontal cortex
DSM-IV	Diagnostic and Statistical Manual of Mental Disorders 4th-edition
DTI	Diffusion tensor imaging
EC	Eyes-closed
EEC	Electroencephalography
EO	Eyes-open
EOG	Electroculogram
EXT	Externalised behaviour functioning
FC	Functional connectivity
FFT	Fast Fourier Transform
fMRI	Functional magnetic resonance imaging
FSIQ	Full scale intelligence quotient
GCP	Good Clinical Practice
GTA	Graph theoretical analysis
HPI	Head position indicator
ICC	Intraclass correlation coefficient
INT	Internalised behaviour functioning
IQ	Intelligence quotient
L	Characteristic path length
L_w	Characteristic path length in a weighted graph
L_w-s	Characteristic path length in a surrogate graph
MEG	Magnetoencephalography
MRI	Matrix reasoning
MRI	Magnetic resonance imaging
MST	Minimum spanning tree
NHS	National Health Service
NICE	National Institute for Health and Clinical Excellence
PLI	Phase lag index
PRI	Perceptual Reasoning
PSI	Processing Speed
REC	Research Ethics Committee

RS	Resting-state
SE	Standard error
SSS	Signal Space Separation
SQUIDS	Superconducting quantum interference device
SI	Similarities
SL	synchronisation likelihood
TBR	Theta/beta ratio
TDC	Typically developing controls
TRF	Teacher's Report Form
TRT	Test-retest reliability
tSSS	Temporal Signal Space Separation
VCI	Vocabulary
VCI	Verbal Comprehension
WASI	Wechsler Abbreviated Scale of Intelligence
WISC-IV[uk]	Wechsler Intelligence Scale for Children-Fourth UK Edition
YSR	Youth Self-Report
λ/lambda	Normalised characteristic path length
γ/gamma	Normalised clustering coefficient
σ/sigma	Small-world index/small-worldness

1. Introduction to neurodevelopment and brain function

1.1. Introduction

The neurobiological underpinnings of neurodevelopmental disorders remain largely elusive, despite much scientific research attention (Konrad & Eickhoff, 2010). Early neuroimaging studies sought to localise brain abnormalities in discrete brain regions (Castellanos et al., 1994; Hynd et al., 1991; Pineda et al., 2002; Semrud-Clikeman et al., 1994). However, there is a growing consensus that correlation patterns in distributed regions (i.e. functional connectivity) during spontaneous neural fluctuations (rest-state) may characterise abnormalities in brain function (Boersma et al., 2011; Brookes et al., 2011; van den Heuvel, Stam, Boersma, & Hulshoff Pol, 2008). Network analysis provides a sophisticated approach to characterise and visually represent the organisation of functional brain networks. Using this approach, studies such as those by Babiloni et al. (2002), Bonavita et al. (2011), Bos et al. (2014), and Hardmeier et al. (2012), have reported altered abnormalities in network topology in various clinical populations. In addition, the reported alterations often highly correlate with symptom severity, and measures of behavioural and cognitive functioning. Network analysis therefore offers a computationally powerful and biologically significant tool to investigate resting-state network organisation in neurodevelopmental conditions, typically those characterised by behaviour and cognitive problems, proving a means of gaining important insights into underlying neurobiological pathophysiological mechanisms.

This introductory chapter begins with a description of the biological underpinnings of neurodevelopmental disorders. This then follows a general discussion of the two disorders investigated in this thesis, namely attention-deficit/hyperactivity disorder (ADHD), and dyslexia. In addition, there is a critical examination of current neuroimaging practices, their strengths, and limitations. Lastly, a preliminary description of the general aims of the thesis and brief outlines of each chapter are presented.

1.1.1. Neurodevelopmental disorders

The changes in cognitive and behavioural patterns observed in infancy demonstrate that the specific development of the brain is very important (Fatemi et al., 2009; Rice & Barone, 2000). Hence, the observed lack of an age-appropriate behavioural function illustrates an

alteration in early brain development (Casey, Giegg, & Thomas, 2000; Hoff, van den Heuvel, Bender, Kerbergen, & De Varies, 2013; Kolb & Gibb, 2011; Kolb, Mychasiuk, & Gibb, 2014), thought to occur during neuronal migration (Richlan, Kronbichler, & Wimmer, 2013) and typically denoted by macroscopic structural changes and myelination (Paus et al., 1999). These processes have critical time windows, beginning *in utero* and in the child's first years of life (Kolb et al., 2014). During this time critical and important molecular processes occur, which if rendered inefficient or disrupted, potentially result in subsequent atypical brain organisation (Rice & Barone, 2000).

Neuroimaging studies have revealed that children with neurodevelopmental disorders experience either accelerated (Heather et al., 2012) or reduced (Eckert et al., 2003; Krain & Castellanos, 2006; Seidman et al., 2011) brain growth during their childhood. Abnormal brain structures reflect delayed or pathologically prolonged myelination (Rice & Barone, 2000). In a healthy infant brain, increasing myelination is reflected in white matter volume increases (Giedd et al., 1999). This enables the rapid transfer of information in neural systems and has been implicated in higher order behavioural and cognitive functioning (Deoni, Dean, O'Muircheartaigh, Dirk, & Jerskey, 2012; Deoni et al., 2011; Giedd et al., 1999). Neuroimaging data in studies of neurodevelopmental disorders has revealed disrupted myelination and changes in axons (Herbert et al., 2004; Zikopoulous & Barbas, 2010). Several accounts have been put forward to explain these disruptions to brain anatomy and myelination. These include environmental variables, such as prenatal viral infections (Libbey, sweeten, McMahon, & Fujinami, 2005), and activities of genes associated with myelination e.g. myelin proteolipid protein (Plpl) (Fatemi et al., 2009).

The rapid myelination and macroscopic anatomical changes that take place in infancy make this period a time of considerable vulnerabilit*y* to various distinctive susceptibilities (Perry, 2008; Deoni et al., 2011). Neurodevelopmental disorders are believed to be the outcome of abnormality in or inefficiency of these processes (Ashtari et al., 2005; Szpir, 2006). Neurodevelopmental disorders include conditions such as autistic spectrum disorders, ADHD, learning disabilities, and foetal alcohol syndrome.

These disorders are estimated to affect approximately 3-4 % of all children in England (Department for Work & Pensions, 2012) and are associated with a multitude of symptoms, which are often persist into adulthood (Asherson, Kuntsi, & Taylor, 2005). Developmental disorders often constrain the child's ability to develop along typical trajectories (Reynolds & Goldstein, 1999; Szpir, 2006). The high rate of symptom overlap in many neurodevelopmental disorders means that these children are at a high risk of meeting the

diagnostic criteria of one or more neurodevelopmental conditions (Jensen et al., 2001; Kronenberger & Dunn, 2003).

Further still, studies show that children with developmental disorders have higher rates of mental illness (Jensen et al., 2001; Magnuson & Constantino, 2012), drug addiction (Biederman, 2005; Cunha et al., 2013), and are more likely to commit crimes as adults (Macdonald, 2010; Tannock, 1998). As a result, the adverse effects associated with documented impairments in developmental disorders have enormous social, emotional, mental, and financial implications for families and society in general (Department for Work & Pensions, 2012; Macdonald, 2010). Behavioural and cognitive deficits associated with these conditions include increased levels of arousal, impulsiveness, difficulties with attention, hyperactivity, intellectual disability, aggression, inhibition, sensory abnormalities, anxiety, and limited social abilities (Germano, Gagliano, & Curatolo, 2010; Purvis, & Tannock, 2000).

1.1.2. Attention deficit/hyperactivity disorder (ADHD): a complex phenotype

This thesis focuses on ADHD, the most common, and probably the most controversial neurodevelopmental condition of childhood (Acosta, Arcos-Burgos & Muenke, 2004; APA, 2000; Konrad & Eickhoff, 2010; Wallis, 2010). ADHD is characterised by persistent age-inappropriate levels of inattention and/or hyperactivity-impulsivity that are more frequent than is usually expected in typically age-matched peers (Franzen et al., 2013). Although it was once suggested that many children outgrow its symptoms (Faraone, Biederman, & Mick, 2006), an increasing number of studies have revealed that ADHD symptoms persist into adulthood (Barkley, 2002; Hulme & Snowling, 2009; Mannuzza et al., 1993; Simon et al., 2009).

ADHD affects approximately 8% of all school-age children worldwide (Farone, Sergeant, Gillberg, & Biederman, 2003). Furthermore, this condition affects up to 50% of the child psychiatric population (Cantwell, 1996). ADHD is associated with psychological skill deficits that put those affected at risk of further impairments in social functioning (Frazier et al., 2007; Wang et al, 2013), academic achievement (Klein Wendling, Huettner, Ruder, & Peper 2006; Simon et al., 2009), and restricted overall quality of life (Mannuzz et a., 1993).

A diagnosis of ADHD is based on developmentally age-inappropriate symptoms of inattention, and/or impulsivity and motor restlessness. As a result, three ADHD subtypes exist, namely predominantly inattentive subtype (ADHD-I), hyperactive/impulsive subtype (ADHD-H), and ADHD combined subtype (ADHD-C). It should be noted that the validity and

usefulness of the three subtypes is highly contested (Baeyen, Roeyers, & Walle, 2006; King & Young, 1982; Milich, Balentine, & Lynam, 2001).

To warrant a diagnosis of ADHD, age-inappropriate symptoms must be present before the age of seven, pervasive in more than one setting of the child's life, and associated with psychological, social, and educational impairments (APA, 2000). ADHD symptoms are typically managed with stimulant drugs, including Concerta, Medikinet, and Equasym. Most ADHD medications contain the active ingredient methylphenidate, a dopamine agonist that blocks dopamine transporters, and improves dopamine brain function (Swanson & Volkow, 2009; Wang et al., 2013; Volkow et al., 2001). Note, however, that despite being one of the most extensively studied disorders of childhood (Wallis, 2010), ADHD remains a highly controversial condition. Debates centre on discrepancies regarding the hallmark for diagnosis, formal guidelines for assessment, the causes of ADHD, its treatment, and on the accuracy of prevalence rates (Band & Scheres, 2005; Foy & Earls, 2005; Greenhill, 1998).

Earlier clinical work in ADHD mainly focused on neuropsychological abilities (See Barkley, 1997, for a review). Dominant theories emphasized the role of executive functions, associated with one's ability to inhibit and control impulses. Using a battery of neuropsychological tasks, researchers assessed various executive dysfunctions including mental/cognitive flexibility, planning, and working memory. As a result, several developmental impairments were proposed as fundamental problems underlying ADHD. The dominant cognitive theory of the core impairments underlying the symptoms involved an executive function deficit (Pennington & Ozonoff, 1996; Tannock, 1998; Wilding, 2005). This theory gained support from data revealing difficulties with self-managing demands, making ADHD a cognitive disorder, with developmental impairments of executive functions.

However, although the model of executive function impairment is still widely acknowledged, a large body of work has demonstrated that impaired executive functions characterise other neurodevelopmental and psychiatric conditions (Band & Scheres, 2005; Cunha et al., 2013; Wilding, 2005). Another limitation of this theory concerns the commonly applied neuropsychological assessments used to measure the ability to engage in mental and executive functions. According to Band and Scheres (2005), these measures load highly on several latent cognitive components, and as a result do not represent pure measures of mental function. Instead, Band and Scheres (2005) proposed that to gain further insight into individual differences between those with ADHD and controls, researchers have to delineate the different components of cognition. Interestingly, data from Avisar and Shalev (2011),

Lemiere et al. (2010), and Manly et al. (2001), who used this approach, contradicted earlier reports of impaired sustained attention in ADHD. Explaining this result, Manly et al. (2001) hypothesized that observed individual differences between those with ADHD and controls depend solely on whether the measured variable is speed or accuracy.

Elsewhere, data from other studies suggested that response inhibition was the primary deficit in ADHD (Barkley, 1997; Desman, Petermann, & Hampel, 2008), and that this was independent of cognitive task demands (Wodka et al., 2007). Working memory and set shifting have also been implicated in ADHD (Rohlf et al., 2012). The most consistently replicated feature of ADHD is intra-individual/within-subject variability in task reaction time (Castellanos & Tannock, 2002; Klein et al., 2006; MacDonald, Nyberg, & Bäckman, 2006). According to this theory, behaviour problems observed in children with ADHD are more characteristic of inconsistency rather than incompetence.

Note however that none of the above listed dysfunctions is unique to ADHD. Whether it is sustained attention (Avisar & Shalev, 2011), working memory (Rohlf et al., 2012), inhibition (Barkley, 1997; Desman et al., 2008), or within-subject variability (Castellanos & Tannock, 2002), it appears that poor performance on tasks designed to assess vigilance does not necessarily imply core ADHD specific cognitive problems. This has meant that despite the identification of several cognitive deficit theories, none of these has had a substantial effect on explaining the etiology of ADHD, meaning that the exact set of causes underlying ADHD remain ambiguous (Avisar & Shalev, 2011).

Recent years have witnessed a shift in focus from neuropsychological dysfunction (Pennington & Ozonoff, 1996) to a focus on the role of brain anomalies (Castellanos & Acosta, 2004; Castellanos et al., 2008). Symptom similarity between children with ADHD and patients with neurological disorders prompted Mates (1980) to hypothesise that ADHD was a brain disorder, mainly affecting the prefrontal cortex (Seidman, 2006). Mates (1980) explained that documented lesions in both animals and humans were often associated with restless, reckless, and disruptive behaviour. As a result, of this association, the search for possible biological brain-markers in relation to ADHD has been prominent, as this would further the understanding of the condition and eradicate the reliance on subjective practices in identifying those with ADHD. In addition, if it is identified a brain-marker has the potential to allow early diagnosis in those at risk as well as helping provide appropriate treatment (Wallis, 2010).

Developments in various imaging techniques have enabled researchers to investigate this possibility. Building on findings from cognitive models, neuroimaging studies have consistently reported reduced activity of the prefrontal cortex (Bedard et al., 2014), ventral striatal (Scheres, Milham, Knutson, & Castellanos, 2007) and anterior cingulate (Bush et al., 1999) during neuropsychological tasks designed to test vigilance and mental flexibility.

1.1.2.1. The ADHD brain

Interest in the role of the brain, particularly the prefrontal cortex, in relation to ADHD is very high. The prefrontal cortex has been widely researched, due to its involvement in the therapeutic actions of psychostimulants (Schmeichel, Zemlan, & Berridge, 2013). Studies showing that pharmacological interventions lead to the activation of dopaminergic and the noradrenergic neurotransmission (Schmeichel et al., 2013), have provided crucial support to the idea that ADHD is the result of brain anomalies. Data from Berridge et al. (2006) and Volkow et al. (2001) revealed that Methylphenidate, a commonly prescribed drug intervention, reduced the level of open dopamine receptors, significantly increasing extracellular dopamine levels. These neurotransmission effects on cortical dopamine have been hypothesised as improving prefrontal cortex-dependent cognitive functions such as attention and working memory (Berridge et al., 2006; Engert & Pruessner, 2008; Schmeichel et al., 2013).

Further still, substantial evidence from both animal and human studies has suggested that prefrontal cortices, specifically the dorsolateral prefrontal cortex (DLPFC), are critical for executive functions and attention (Forbes, Poore, Krueger, Barbey, Solomon, & Grafma, 2014; Fuster, 1997). Interestingly, the DLPFC is the slowest region to develop (Giedd & Rapoport, 2010). In lesion patients, DLPFC damage has been associated with impaired executive function, deficits in verbal, and spatial knowledge (Barbey, Koenigs, & Grafman, 2013), and higher personality expressions of neuroticism and conscientiousness (Forbes et al., 2014).

Whilst such findings provide crucial insights into the potential neural correlates of abilities and of cognitive and behavioural problems, such associations are too broad and in many cases not unique to a specific disorder. This is why despite remarkable progress in understanding the involvement of the DLPFC in executive functions, and in behavioural competencies in ADHD, efforts to establish the exact relationship between these factors has mainly yielded ambiguous results (Avisar & Shalev, 2011). Inconsistency in these studies on ADHD probably arises from two key factors.

The first issue hindering the identification of a clear link between underlying neuropsychological deficits and brain abnormalities in ADHD relates to the use of neuropsychological assessments. Many have questioned the appropriateness of task-based neuroimaging studies, often applied to assess cognitive functions in clinical psychology (Band & Scheres, 2005; Wilding, 2005). This is because the core behaviour problems experienced in ADHD, such as impulsivity and inattention behaviour, make it highly challenging to follow instructions and remain motivated in the face of cognitive stress (Wodka et al., 2007). This means that individuals with ADHD are disadvantaged on many neuropsychological tests, and as a result, poor performance and corresponding brain abnormalities may reflect group differences and not genuine neural correlates.

The second issue hindering the identification of a clear link between underlying neuropsychological deficits and brain abnormalities relates to the fact that ADHD is a highly comorbid disorder (Biederman et al., 1993; Geller et al., 2004; Germano, et al., 2010; Wahlstedt, Thorell, & Bohlin, 2009). As a result, documented symptoms are often not heterogeneous (Avisar & Shalev, 2011; Band & Scheres, 2005; Wahlstedt & Bohlin, 2010). Of all neurodevelopmental disorders, ADHD co-occurs most commonly with dyslexia (Kronenberger & Dunn, 2003). This disability is characterised by difficulties in reading, despite normal intelligence. The phonological processing module of dyslexia suggests that those with the condition have specific difficulties in single word reading, fluency and comprehension (Siedman, 2006). Genetic and environmental factors play key roles in the aetiology of these developmental disorders. Generally, the standard protocol for characterising and diagnosing both conditions is based on behavioural and cognitive measures. However, emerging data from neuroimaging studies has shown potential in differentiating between children with ADHD and dyslexia (Barry et al., 2009; Clarke et al., 2002). As will be discussed later, the research for this thesis aimed to overcome these two key limitations.

1.2.3. Disrupted brain network architecture of resting-state neural fluctuations

While the neuroimaging field was previously dominated by convergent studies seeking to identify localised abnormalities in distinct brain regions based on task performance, there is now a growing consensus that brain activity is not restricted to task-driven activations. Several studies have demonstrated that the brain remains active, even during spontaneous/ awake or passive mind wandering (Bonavita et al., 2011; Laufs, Krakow, Sterzer, Eger, Beyerle, & Salek-Haddadi, 2003; Mazoyer et al., 2001; Raichle et al., 2001). The scientific enthusiasm for investigating spontaneous neural fluctuations at rest ('resting-state') has

primarily been attributed to the discovery of a group of brain regions that remain active during passive states (Raichle et al., 2001). These regions form what is known as the default mode network (DMN) comprising the medial prefrontal cortex, the precuneus cortices/posterior cingulate and the mediolateral inferior parietal cortices (Konrad & Eickoff, 2010).

The advantage of resting-state paradigms over task-based research designs is that the demands of task performance, such as sustained attention, motivation, and training, are avoided (Fox & Greicius, 2010). According to Smith and Smith (2004), such factors affect the performance of children with developmental disorders, consequently masking their true abilities. In healthy participants, regions of the default mode network are strongly interconnected and typically remain active during wakeful rest compared to task performance (Fox & Raichle, 2007; Greicius & Menon, 2004). The opposite trend has been observed in several psychiatric populations.

In light of these findings, recent years have witnessed a shift in focus. This is from the idea of the brain as an entity split into discrete brain regions, to viewing the brain as a complex network of functionally connected systems (Douw et al., 2008), that can be studied both during wakeful and rest periods (Stam Jones, Nolte, Breakspear, & Schelten, 2007a). In viewing the brain as a complex network system, researchers have relied on the knowledge that within the nervous system, neural population couplings result in systems of locally specialised (segregated) clusters and globally integrated networks. According to the 'brain network system' approach, normal brain functioning (e.g. perceptions, cognition, and emotions) requires the integration of functionally specialised but widely distributed brain areas (Bullmore, & Sporns, 2009; Douw et al., 2011; Stam & Reijneveld, 2007). The network approach therefore offers the best current option for evaluating brain function in both typical and clinical populations.

Convergent data from neuroimaging studies has identified functional alterations in neural networks in ADHD, especially the frontostriatal pathways (Ashtari et al., 2005). Interestingly, in ADHD, the development of white matter pathways, connecting prefrontal and parieto-occipital areas with the striatum and the cerebellum, have been shown to be abnormal (Liston, Cohen, Teslovich, Levenson, & Casey, 2011; Silk Vance, Rinehart, Bradshaw, & Cunningham, 2009a).

Developmentally, the processes occurring during the early years of life result in network organisations that ensure optimal information processing and subsequent function (Fair et

al., 2007; Perry, 2008). During development, in addition to migration, neurons also differentiate and connect with other neurons, to form specialised communities (Huttenlocher. 1979). In typical development, behaviour and cognitive functioning maturity coincide with the development of network organisation (Chen, Mui, Gross, & Beaulieu, 2013b; Reijneveld, Ponten, & Berendse, 2007). Researchers have proposed that cognition, whether typical or impaired, can only be fully understood with a knowledge of the brain's spatial organisation (i.e. its topology) (Bos et al., 2014; de Haan, Mott, van Straaten, Scheltens, & Stam, 2012; Tewarie et al., 2014). Investigating disrupted network topology/architecture of the resting brain, in disorders characterised by disabilities in cognition, intellectual ability and behavioural immaturity, is therefore of high relevance.

1.2.4. Graph theoretical analysis

Graph theory has provided a sophisticated computational tool to characterise and visually represent network organisation of brain fluctuations (Schwarz & McGonigle, 2011; Sporns, Chialvo, Kaiser, Hilgetag, 2004; Tewarie et al., 2014). Often credited to the work of Euler, graph theory is a branch of mathematics concerned with the study of abstract network structures. Using mathematical representations, a graph visually articulates a complex brain as a set of regions (nodes) linked together, with the fewest possible connections (edges) (See Figure 1.1) (Bullmore & Sporns, 2009; Stam et al., 2007a).

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Figure 1. 1 Graphical representation of complex large-scale networks

A representation of the brain modelled as a network consisting of distributed regions (nodes) and their connections (edges). Figure adapted from (Bassett, http://online.kitp.ucsb.edu/online/brain-m11/bassett/pdf/Bassett_Brain11_KITP.pdf, http://web.med.unsw.edu.au/bcw08).

Studies applying graph theory have gained insight into how the properties of brain network topology are able to ensure efficient organisation in a typical brain. According to researchers such as Bullmore and Sporns (2009), and Fair et al. (2008), cognitive functions are supported by rapid integration of information processing across segregated brain regions. The mechanisms of segregation of local brain regions and integration of spatially discrete regions (Bullmore & Sporns, 2009) are respectively assessed using two dominant network metrics. These are known as the clustering coefficient (a measure of the degree of node-neighbourhood connectedness), and the characteristic path length (a measure of how well a network is connected) (Stam et al., 2014). Clustering coefficient reflects local network connectivity (i.e. densely connected local neighbouring clusters) while characteristic path length reflects global network connectivity.

The optimal organisation of high local clustering and short path lengths form what has now come to be described as the small-world network and has been demonstrated in many biological systems (Watts & Strogatz, 1998; Bullmore & Sporns, 2009; Bullmore & Sporns, 2012). This topology is considered optimal for functioning because the high number of short distance connections is coupled with fewer more specialised long-range connections in a cost effective manner (Bullmore & Sporns, 2009; Stam & Reijneveld, 2007; Stam & van Straaten, 2012). Stam (2004), who revealed that functional brain networks in healthy adults were characterised by small-world properties, reported the first application of graph theory. Studies have since revealed that during typical development processes network topology shifts from a random network topology, that is characterised by larger numbers of paths with equal probability of connecting any two nodes in a network, to a more small-world organisation (Boomsma, & de Geus, 2008; Fair et al., 2009; Smit, Stam, Posthuma, Boersma et al., 2011; Wu et al., 2012).

Changes in network topology are sensitive to genetic factors (Smit, 2013), cognitive abilities (van den Heuvel, Stam, Kahn, & Pol, 2009), gender (Gong et al., 2009), and sleep (Verweij et al., 2014). Clinically relevant, studies have revealed that the small-world topology is disrupted/disconnected by disease, damage, or atypical development. This is known as the 'disconnection hypothesis' (Breakspear et al., 2003; Friston, 1998). Abnormal or altered network topology has been reported in schizophrenia (Friston, 1998; Micheloyannis, 2012), Alzheimer's disease (Tijms et al., 2013), autism (Peters et al., 2013), severe reading difficulties (Vourkas et al., 2011), late-life depression (Ajilore, Lamar, Leow, Zhang, Yang, & Kumar, 2014), and fragile X syndrome (van der Molen, Stam, & van der Molen, 2014). In addition, disrupted small-world architecture in psychiatric conditions is often associated with behaviour problems, cognitive deficits, and symptom severity (Stam et al., 2014). As a result,

disturbances in functional networks, specifically of small-world organisation, have been proposed as the underlying pathophysiological mechanisms of documented clinical problems in psychiatry (Schwarz & McGonigle, 2011).

1.2.4.1. Possible biological mechanism underlying graph network topology

As stated earlier, brain networks with a small-world topology ensure optimal information processing based on an optimal balance between segregation and integration. Neuroimaging studies have revealed that the relationships between local and global structures illustrate individual differences in underlying physiological mechanisms (Jann et al., 2012), such as white matter tracts (Vaessen et al., 2011; Kim et al., 2011). The strongest support for the hypothesis that graph-based measures of network integration/path length reflect structural white matter abnormalities with altered distant fibre bundles has mainly come from diffusion tensor imaging (DTI) studies. With DTI, researchers have been able to quantify white matter structure through fibre tractography. Combining DTI and graph theory, Vaessen et al. (2011) revealed associations between white matter network abnormalities and deficits in cognitive function in epilepsy. This study also revealed that patients with severe cognitive impairments had significantly lower clustering and higher path lengths compared to typical controls and patients with little or no cognitive impairment. Using similar methodological procedures, Kim et al. (2011), showed that among cannabis users, individuals with less optimal global network organisation scored significantly higher on schizotypal and impulsive personality characteristics. Interestingly the study conducted by Vaessen et al. (2011) revealed that in epilepsy white matter organization disruption and not white matter volume correlated with symptom severity.

The above studies suggest that altered network organisation reflects physiological processes that increase vulnerability to cognitive and behaviour problems. More importantly, Vaessen et al. (2011) allude to the idea that functional connectivity (i.e. the temporal correlation between the sub-components of different brain regions) is sensitive to cognitive ability, regardless of anatomical basis (i.e. white matter volume).

1.2.4.2. Limitations of graph theory

Application of classical graph theoretical network analysis has increased insight into functional network organisation, in modelling development, mental state (i.e. rest vs. cognitive engagement), and disease-related changes at both local and global time-scales.

However, its application relies on assumptions that have been shown to display bias between subject network comparisons (van Wijk, Stam, & Daffertshofer, 2010). These include the network size (i.e. the number of nodes in networks), the sparsity of the network (i.e. the percentage of existing links/connections) and average degree (i.e. the number of links per node) (Boersma et al., 2013b; Tewarie et al., 2014; van Dellen et al., 2013; vanWijk et al., 2010). These factors often vary between individuals. For instance densities have been shown to change with development (Gong et al., 2009; Otte et al., 2015), which according to Otte et al. reflects reduced white matter integrity. (See Chapter 2 for a detailed discussion of other limitations of graph theory). As a result, these limitations make network comparison challenging. In fact Stam et al. (2014) argues that not controlling for these factors explains why some studies applying graph theoretical analysis for those with Alzheimer's' disease report pathological increases of clustering coefficient and path length while others reveal the opposite trends as reported by Tijms et al. (2013).

To minimise dependence on the percentage of available connections in conventional graph measures, researchers normalize graph metrics using surrogate/random networks (see Chapter 2, section 2.7.1., for a detailed account of normalisation). In so doing, the dependency of network measures (predominantly clustering and path length), on edge weighting, and on global functional coupling is minimised (van Dellen et al., 2014). However, normalisation does not fully eliminate the problem, and may in fact introduce other biases (See Tewarie et al., 2014; van Wijk et al., 2010, for a review).

1.2.5. Minimum spanning tree

An alternative approach of visually articulating and comparing brain networks of different groups is to construct a subgraph that connects all nodes in the original weighted (i.e. fully connected) graph without forming any circles or loops and is independent of average functional coupling strength (van Dellen et al., 2013). Such a subgraph is known as a spanning tree. The minimum spanning tree (MST) is the least total weight of possible spanning trees in the original graph (Boersma et al., 2013). As a subset of the strongest connections in the original network, an MST forms the 'critical backbone' of information processing of a weighted graph (Tewarie et al., 2014; van Dellen et al., 2013), which enables direct network comparisons between groups.

As is the case for graph-based network topology, minimum spanning tree analysis results in two extreme topologies, namely a path and a star (Stam et al., 2014, see Chapter 2, section 2.7.2., for a thorough discussion). These topologies are characterised using several metrics.

Note, however, that although MST comprises the strongest connections in the original graph (Otte et al., 2015), most of its metrics are highly correlated; and would therefore benefit from fewer but more independent measures (Stam et al., 2014). Currently evidence is lacking regarding the most robust measures. For this reason, the selection of the MST measures in chapters 4, 5, and 6 were informed by the other only high temporal resolution study (Boersma et al., 2013b) to apply MST to investigate age-related changes in networks. These authors showed that MST metrics, diameter (i.e. largest path between any two nodes of the tree), leaf number (i.e. the number of tree nodes forming exactly one connection to another tree), eccentricity (i.e. the longest distance between node i and any other tree node in the MST), and hierarchy (a measure of the optimal balance between integration and overload of central nodes), were sensitive to developmental changes in network organisation. These measures can be used to characterise network organisation in the context of the two extreme tree topologies (Stam et al., 2014).

As a result, studies exploring changes in functional networks will focus on these measures. According to Stam et al. (2014), the leaf number is an especially important measure because it determines the extent to which a tree is more chainlike or more star-like. For Otte et al. (2015), the lower the eccentricity, the more central a node is, in a tree. Hence, increased eccentricity means increased shortest path length between nodes, which suggests a less integrated and efficient topology. It has been proposed that higher clustering and longer path length possibly reflect larger diameter, eccentricity, and lower leaf number (Stam et al., 2014). The researchers hypothesise that it is likely that a more regular network (i.e. high clustering and high shortest path length) corresponds to more chain-like trees, while networks that are more random correspond to more star-like trees. MST measures have been shown to be able to capture longitudinal age-related network changes (Boersma et al., 2013b) and pathology (Tewarie et al., 2014; van Dellen et al., 2013).

1.2.5.1 Possible biological mechanisms underlying MST network topology

Can changes in minimum spanning tree be interpreted in the context of neural structure and function? According to Wu et al. (2006), minimum spanning trees represent 'super highways' and 'peripheral roads' in neural networks (Stam et al., 2014). Support for the argument that minimum spanning tree represents the most important or core shortest paths in neural networks (Olde Dubbelink et al., 2014; Otte et al., 2015; Tewarie et al., 2014), comes from recent reports of associations between MST metrics and symptom severity in clinical populations. MST metrics are associated with network alterations in shortest path length, which Otte et al. attributed to changes in white matter integrity. Hence, in

neurodevelopmental disorders where abnormal myelination has been reported, it makes sense to use MST to understand network topology. Equally, in healthy participants, slow myelination in older age may underlie changes in network topology that may in turn explain documented cognitive decline.

It is important to note however, that despite being, bias-free, minimum spanning tree network analysis is a relatively new approach and not much is known about what its measures imply in relation to conventional graph analysis (Stam et al., 2014). In addition, because of discarding weighted connections that form loops/circles in the original graphs, it is challenging to assess local network efficiency such as clustering, using MST metrics (Stam et al., 2014; Tewarie et al., 2014). This means that minimum spanning tree network analysis applied on its own would not improve understanding of local network topology. This, and the fact that more is known about the link between conventional graph theoretical measures and cognitive functioning, as well as behavioural functioning, is the main reason why the studies in Chapters 4, 5, and 6 applied both conventional normalized graph-based measures and minimum spanning tree metrics to characterise network architecture.

In this thesis, brain activity was investigated using the Magnetoencephalography (MEG) technique. Due to its excellent temporal resolution, this technique offers direct measurement of neural activity with millisecond time precision (Stam & Reijneveld, 2007), hence providing a technique well suited for investigating neural fluctuations in real time. (Nolte & Marzetti, 2014). According to Boersma et al. (2013a) recorded brain oscillations are understood to derive from synchronisation of neural networks in the brain underlying sensors. MEG is used routinely in mapping brain functions associated with motor, visual, and auditory cognitive functioning. Compared to EEG, another non-invasive direct technique to measure ongoing brain activity and with excellent time resolution (Nolte & Marzetti, 2014), MEG has better spatial resolution and considerably reduced participant preparation time, making it suitable for paediatric and clinical neuroimaging.

1.2.6. Aims and questions addressed by this thesis

In sum, this thesis attempts to answer four questions. First, are conventional graph theoretical network measures stable/reproducible across repeated testing processes (Chapter 3)? Second, are graph and minimum spanning tree measures sensitive to changes in network organisation in relation to development in typical individuals (Chapter 4)? Third, if they are sensitive to changes during typical development, could they be applied to analyse brain network topology in ADHD (Chapter 5)? Fourth, as previously discussed, comorbidity

with dyslexia strongly suggests shared neurodevelopmental-related influences. Hence, the work reported in Chapter 6 investigated whether ADHD and dyslexia are characterised by co-shared abnormalities in functional networks. Alongside these core questions, elements of the study also assess the relationship between functional network organisation and cognitive and behavioural problems.

It is argued that the addition of a passive measure of brain function alongside current standard methods for assessing ADHD and dyslexia has the potential to:

1. Enhance diagnosis by providing a more objective measure for assessment alongside current measures;
2. Improve the evaluation of severity in regard to the behavioural and cognitive phenotypes;
3. Provide possible biological markers, which could be used for identifying those at risk, monitoring progress, and exploring the implications of pharmacological intervention;
4. Facilitate an understanding of the underlying pathophysiological mechanisms of documented problems.

1.2.6.1. Brief summaries of thesis main studies

Using cross-sectional analysis, this thesis seeks to explore whether it is possible to meaningfully interpret network measures obtained from resting-state data, to understand network changes in relation to development, and to understand substantive disruptions indicative of network changes in atypical neurodevelopment, specifically ADHD and dyslexia, the two neurodevelopmental disorders that occur more frequently than is expected by chance during childhood.

Chapter 2: General methodology

This chapter provides a summary and description of the tools applied in this thesis including measures of cognitive ability, of behavioural problems, and those of rest-state brain function. This chapter also provides a justification for the application of two different network analyses.

Chapter 3 (Experimental study 1): Reproducibility of graph network measures of functional brain networks

Increased application of graph theory in clinical populations has proved a computationally powerful and biologically meaningful tool for characterising disease states as well as providing novel insights into underlying biological processes. However, despite increased application, reproducibility of graph metrics has received very little attention and until recently, remained to be evaluated in MEG resting-state studies. A necessary task, in ensuring the continued application of graph metrics of brain functional network organisation in clinical populations, is therefore to demonstrate that such measures have sufficiently good reproducibility on repeated testing. Demonstrating satisfactory reproducibility of these measures would help validate their clinical potential to assist understanding of underlying pathophysiological mechanisms. The aim of this study was therefore to examine the test-retest reproducibility of functional connectivity and network parameters using a repeated testing approach. Note that the metric reproducibility of MST measures was not assessed in this study. Unlike graph-based measures, MSTs are mathematically quantified to reflect the more important network properties, and as a result can withstand connectivity noise and potential dependence on network size (Tewarie et al., 2014).

Chapter 4 (Experimental study 2)

There is a growing consensus that cognition is associated with functional organisation of brain networks. Given that disruption of brain organisation is understood to underlie developmental disorders such as ADHD and dyslexia, it is crucial to gain insight into how typical brain organisation develops in typical participants. To achieve this, this chapter estimated whole-brain functional brain connectivity across development, first in children and adults and later across a broad age-range. Then using graph theoretical analysis and minimum spanning tree, organisation of functional networks was mapped and compared across different age groups.

Chapter 5 (Experimental study 3)

It is becoming increasingly clear that a key feature of neurodevelopmental conditions is the profound alterations in the pattern of local and/or global functional connections in complex brain neural systems (Chen et al., 2013b). Neurobiological correlates of ADHD remain largely elusive. Data from various studies has suggested that ADHD is associated with altered function of spatially distinct brain networks. However, with a focus typically on pre-

defined regions, the disruption of whole-brain functional network organisation remains poorly understood. Using graph theory and MST, this study seeks to characterise complex brain network organisation in a clinical medicated sample of children and teenagers with ADHD and age matched with typically developing controls. This study also explores behaviour and brain correlations as well as brain and cognitive correlations to highlight the effect of disruption on normal brain organisation.

Chapter 6 (Experimental study 4)

Among neurodevelopmental disorders, ADHD co-occurs most frequently than expected by chance with dyslexia, sometimes resulting in ambiguity when behaviourally diagnosed (Gualtieri & Johnson, 2005). The study presented in Chapter 6 sets out to investigate whether resting-state MEG functional brain network measures could distinguish between the two neurodevelopmental disorders using conventional graph theory and MST to characterise underlying functional brain networks.

Chapter 7 (final discussion)

This general discussion reframes the significance of studies carried out for this thesis, discussing whether hypotheses were confirmed or rejected. This leads on to a critical review of how general knowledge in the field has changed by the addition of data from studies reported in Chapters 3, 4, 5, and 6. Lastly, interpretation of results leads on to an outline contextualising findings in relation to the general neurodevelopment field.

2. General Methods

2.1. Chapter summary

This chapter provides a description of the methods employed and described in Chapters 3, 4, 5 and 6. These involved experiments with typical adults, typically developing control, and children with diagnoses of attention-deficit/hyperactivity disorder (ADHD) and those with developmental dyslexia. Measures used and described include psychometric assessments, behaviour measures, and the magnetoencephalography (MEG) imaging technique.

2.2. Ethical considerations

For the study with healthy adults (REC # 452), typically developing children (REC # 408), and children with developmental dyslexia (REC # 375) all protocols were reviewed and approved by the Research Ethics Committee (REC) at Aston University. In addition, a protocol and risk assessment, sanctioned by the National Health Service (NHS) and approved by the National Research Ethics Service (NRES) Committee East Midlands-Nottingham-1 (REC #: 12/EM/0282) was implemented for the study involving children with ADHD.

All participants (aged over 18) provided their written informed consent or assent (aged under 18) prior to participating in study procedures. Participants were advised on their rights to withdraw from the research at any stage and assured that their decision would be respected. Participants were also given an oral debriefing after each test procedure, to provide assurance as to their not feeling distress or discomfort. The researchers involved in data collection had previously undergone training in administering first aid at work, obtained a 'Criminal Records Bureau Enhanced Disclosure' for working with children and vulnerable adults as well as undertaking the NHS Good Clinical Practice (GCP) training course.

All information collected from the assessments was kept in a coded form and transferred to a password-protected computer system, in line with the University and NHS Codes of Confidentiality.

2.3. Participant recruitment

2.3.1. Healthy adult volunteers

Typical participants with no history of neurological problems were recruited into the study from the student and staff population at Aston University over a period of two years. This was done through advertisements in the university newsletter, and the psychology undergraduate 'Research Participation Scheme (SONA) website. See Chapter 3 for the inclusion and exclusion criteria.

2.3.2. Control group (i.e. typically developing children)

Presumed typically developing children were recruited into the study over a period of two years through advertisements in the Aston University newsletter and the Think-Tank Birmingham Science Museum. All children attended mainstream schools in and around the Birmingham area in the West Midlands. Children received an Amazon voucher for their participation. Families were reimbursed for travel expenses. See Chapter 4 for the inclusion and exclusion criteria.

2.3.3. Children with a diagnosis of ADHD

Children fulfilling Diagnostic and Statistical Manual of Mental Disorders (DSM-IV-TR) (American Psychiatric Association, 2000) criteria for ADHD were identified through specialised clinics for Child and Adolescent Mental Health Services (CAMHS) based at the Worcestershire Health and Care NHS Trust covering the neighbouring county of Worcestershire (See Figure 2.1 for the identification and referral pipeline). It is important to emphasize that unless otherwise stated, all references to the DSM in this thesis refer to the DSM-IV-TR (APA, 2000) and not the DSM-V (APA, 2013). This is because the DSM-IV-TR was the most widely accepted manual used by clinicians and researchers at the time of recruitment. ADHD diagnosis criteria followed clinical guidelines set out by the National Institute for Health and Clinical Excellence (NICE, 2008). According to the NICE guidelines, a diagnosis is established following a comprehensive clinical and psychosocial assessment of the child. This should involve a discussion about the child's behaviour and symptoms in different settings, a semi-structured interview with the child's parents regarding the child's full developmental and mental functioning history, and finally assessing the child's behaviour symptom profiles using Conners' rating scales (ACRS) (Conners, 1985) with the parent

and/or teacher. On the Conners' Parent Rating Scale-Revised (Conners, Sitarenios, Parker, & Epstein, 1998), subscale scores > 1.5 (standard deviation) above the mean score of a child's normed age and gender are used to identify those with age-inappropriate behavioural problems (Silk, Vance, Rinehart, Bradshaw, & Cunninghton, 2009a).

Neurobiological risk factors in ADHD

Referral flow chart

Initial child assessment by Paediatrician

No

Yes

Child outside of (7-17 years) age-range — No — Child aged between 7 and 17 years age range

Yes

Primary diagnosis of a major psychiatric disorder e.g. autism — No — Primary clinical diagnosis of ADHD

Yes

History of substance abuse — No substance abuse history

Yes

Paediatrician provides overview of the study to eligible families and consent to those interested

Inclusion criteria

Families consenting to be contacted by Aston researchers receive a full information pack containing details of study, age appropriate information sheets and instruction video of the MRI protocol

Follow-up phone call no earlier than a week to discuss potential concerns and arrange for consenting families to travel to Aston University

Figure 2. 1 Identification and subsequent referral of children with ADHD by clinicians to the Aston Brain Centre

An illustration of the referral guidelines outlining the inclusion and exclusion criteria followed by clinicians.

2.3.4. Children with developmental dyslexia

Children with developmental dyslexia were an opportunistic sample recruited by the research associate (Dr. Gascoyne) from the Dyslexia and Developmental Assessment Unit (DDAU) at the Aston Brain Centre. The children were part of a separate cross-sectional study investigating genetic links associated with literacy difficulties, predominantly dyslexia. Diagnosis was confirmed by educational psychologists providing a range of cognitive and literacy assessments with the purpose of determining eligibility for special education. Following the assessment, families were approached by the research associate to explore

the prospect of participating in the study. It was strongly emphasised that declining to participate would have no implication for the family's current or future relationship with the clinic. Those interested were offered a scheduled visit to the centre. Consent, withdrawal, data protection, and confidentiality issues were discussed with the families prior to their visit. See Chapter 6 for inclusion and exclusion criteria.

2.3.4.1. Contraindications for the Magnetoencephalography (MEG) imaging technique

Exclusion questionnaires were used to identify and confirm whether potential participants had any permanent metallic foreign bodies or illness, current or previous that would put them at risk in relation to the technique. None of the exclusion criteria outlined in the initial screening forms was met by any prospective participant.

2.4. Cognitive measures

To provide a better understanding of differences in brain functioning between clinical and non-clinical groups, it is useful to examine the relationship between brain measures and measures of behaviour and cognitive functioning.

Cognitive abilities were assessed using subtests of age-appropriate Wechsler Intelligence Scales. The Wechsler Intelligence Scale for Children 4th UK (WISC-IV[UK]; Wechsler, 2003) was administered for children aged between 6 years 0 months and 16 years. For those over 16 years, the Wechsler Abbreviated Scale of Intelligence (WASI; Wechsler, 1999) was administered. The Wechsler scales are normed tests of general intellectual functioning, routinely administered to identify: (1) those with significantly high intellectual abilities and (2) those with functional and cognitive impairments, in both clinical and community samples.

In this thesis, cognitive abilities were assessed in children by using a battery of two or four subtests assessing both verbal and non-verbal skills. Verbal ability was assessed using the similarities (SI; a measure of verbal reasoning), and vocabulary (VC; a measure of word and verbal formation knowledge) subtests. Non-verbal performance was assessed using the matrix reasoning (MR; a measure of fluid reasoning) and/or block design (BD; a measure of the ability to analyse and create abstract visual representations) subtests. SI and MR were used as core measures, particularly when a child was not willing or able to complete the entire battery of the tests. When administered, WISC intelligence tests result in three types

of scores namely, raw, scaled, and full scale intelligence quotient (IQ). Full scale IQ was not an option in studies presented in this thesis as only sub-tests were administered. According to Flanagan and Kaufman (2009), by itself, the raw score is meaningless because it cannot be referenced to the general population.

To interpret one's performance on a sub-test, in the context of the general population, particularly relative to age-matched peers, raw scores are often converted to standard scores. For studies in this these, scaled scores (mean = 10 and standard deviation = 3) were reported as the standard scores. All four subtests have been found to correlate highly with the Verbal Comprehension Index (VCI) and Perceptual Reasoning Index (PRI) domains in both full IQ (Strauss, Sherman, & Spreen, 2006; Wechsler, 2004) and FSIQ-4 measures (Axelrod, 2007). Note however, that as part of their psychological and cognitive functioning assessment, children with developmental dyslexia would have completed an age-appropriate full scale IQ test, (FSIQ) administered by an educational psychologist using the WISC-IV[UK] (Wechsler, 2003). Hence, to avoid repetitive testing, their SI, VC, BD, and MR subtest scores were obtained from their FSIQ, for comparison.

2.5. Behavioural functioning measures

Psychosocial adaptive and maladaptive behaviours were assessed using the Achenbach System of Empirically Based Assessment (ASEBA; www.aseba.org) measures. Developed in the 1960s by Achenbach, the ASEBA scales are empirically based questionnaires that assess a spectrum of competencies, problems, and adaptive functioning in children, adolescents and adults, in community (Frigerio et al., 2009) and clinically based populations (McClendon et al ., 2011). The validity of the ASEBA scales across diverse societies is well established, as is the association with DSM-oriented diagnostic categories (DSM-IV-TR). (See, Achenbach & Rescorla, 2003; Ivanova et al., 2014, for a review).

The scales describe behaviour within the past six months. Items on these questionnaires are scored: 2 'very true', 1 'somewhat true', and 0 'not true' of the subject. Based on a subject's behavioural profile (derived from raw, T score and percentile scores), it is possible to determine the extent to which one's behaviour deviates from normal functioning (i.e. whether they fall into normal, borderline or clinical range).

For the experimental research described in Chapters 4, 5, and 6, age-appropriate ASEBA scales were administered to assess behavioural functioning. For typically developing controls and those with ADHD, the Child Behavior Checklist for ages 6 to 18 years (CBCL/6-

18; Achenbach, 1991) was completed by a guardian (usually the mother), on behalf of the child, whilst the parents/guardians of children with developmental dyslexia completed the Brief Problem Monitor for ages 6-18 years (BPM-P/6-18; Achenbach, McConaughy, Ivanova, & Rescorla, 2011). In typical adults, the Adult Self-Report for ages 18-59 (ASR/18-59; Achenbach & Rescorla, 2003) was administered (See Chapter 4 for a detailed discussion of the ASR scale)

Whilst the ASEBA scales generate several behavioural problems, the focus in this thesis was on the attention and the two key behavioural problem domains, identified through the internalising and externalising scales. The internalised behaviour functioning scores are associated with inward problems (i.e. towards the self), while the internalised scores relate to outward problems (i.e. towards others). On the ASEBA questionnaires, the internalising scale summarises scores from anxiety/depression, withdrawn/depressed, and somatic complaints subscales, while the externalising problems scale is associated with aggressive behaviour and rule breaking problems (Achenbach, 1991; Achenbach & Rescorla, 2003). When assessed, a T-score ≥ 63 is considered to be within the clinically significant range for possible functional impairments (Achenbach, 1991; Geller et al., 2004).

2.6. Magnetoencephalography (MEG)

Figure 2. 2 The Elekta Neuromag Triux Magnetoencephalography system

The first attempts to measure magnetic fields outside the human scalp were conducted in 1968 by Cohen, who applied a copper wire induction coil to detect the very weak magnetic fields, in a magnetically shielded room (Vrba & Robinson, 2001). Following several developments, modern MEG systems are now able to register magnetic fields generated by electrical activity in the brain during neuronal firing, using Superconducting Quantum Interference Devices (SQUIDS) (Vrba & Robinson, 2001; Johnson, Schwindt, & Weisend, 2013). These are located close to the scalp and are cooled by liquid helium, which at the extremely low temperatures ensures low impedance and the subsequent detection of magnetic currents. The Elekta-Neuromag TRIUK whole head MEG system (See Figure 2.2), used in the research for this thesis, comprises 306 sensors arranged in groups of three, namely two orthogonal planar gradiometers and one magnetometer. Together these result in 204 gradiometers and 102 magnetometer sensors that are highly sensitive, with the ability to acquire measurements from both deep and superficial source locations. Whilst gradiometers detect the difference in magnetic fields using two counter-wound coils, magnetometer sensors register magnetic fields using a single pick-up coil (Henson, Mouchlianitis, & Friston, 2009). Unless otherwise stated, the MEG signals used for all experimental studies in this thesis refer to those acquired from the magnetometer sensors. The consequence of only considering magnetometers was that this strategy significantly reduced both the time and computational memory requirements imposed by considering all 306 sensors in estimating network synchronisation and topological measures. This strategy has previously been adopted by studies investigating sensor-level functional activity using the 306-channel Vecktorview MEG system. For instance, Deuker et al. (2009) only considered planar gradiometers to examine functional network parameters while Jin et al. (2011) focused on magnetometers. Despite focusing on different types of sensors, the studies reported similar reproducibility patterns of functional network. However, as will be discussed in Chapter 7, filtering out data from gradiometers is likely to have consequences on estimated synchronisation.

2.6.1. Suppression of interferences and artifacts from MEG recordings

The MEG system is equipped with an option for interference suppression known as internal active shielding (IAS), which was used in all studies reported in this thesis. IAS inside the magnetically shielded room allows signals from within the helmet sphere to be subtracted from those inside the room. As a result, signals/artefacts originating from outside the helmet are automatically suppressed. In addition, the system also utilised the online movement tracking tool, known as continuous MaxMove. This helped track participants' head movements during data acquisition, which was later compensated for in off-line processing.

Additional off-line processing was carried out using the MaxFilter software.

2.6.2. Maxfilter

Maxfilter is a vital pre-processing step for Elekta data acquired using maxshield (MaxfilterTM user's guide, version 2.2, 2010), to ensure the removal of artefacts/noise originating from outside of the sensor array. The Maxfilter program implements both the Signal Space Separation' (SSS) and the temporal extension of the SSS method (tSSS) which separate magnetic signals coming from within the sensor array/the brain from those originating outside of the sensor array/the brain (Taulu & Kajola, 2005). tSSS is capable of suppressing interference coming from both outside the brain and from very close to the sphere/inside the sensors (e.g. heartbeats and/or eye-blinks) (Taulu & Simola, 2006; Vrba & Robinson, 2001). Hence, in the research for this thesis head movement correction and tSSS were carried out on all continuous MEG recordings. A combination of both has been shown to effectively remove any disturbances associated with head movement and artefacts (Nenonen et al., 2010), which is particularly important for MEG data acquired from children, whose smaller head sizes may allow for more movement while inside the MEG helmet (Wehner, Hamalainen, Mody, & Ahlfors, 2008).

2.6.3. Acquiring MEG resting-state data

Resting-state (RS) is defined as an unconstrained passive condition, free from active tasks (Jin, Jeong, Seol, Kwon, & Chung, 2013). For this thesis, MEG recordings were acquired while participants sat in a reclining chair with their head inside the MEG helmet during eyes-closed (EC) and eyes-open (EO) resting conditions. During the EC rest, participants were instructed to sit quietly and not think of anything in particular whilst during EO they were instructed to look at a white cross mark projected centrally on a black screen and refrain from too much blinking and movement.

2.6.4. Estimating functional connectivity from MEG resting-state data

Measuring the electrical potential generated by a single neuron along the scalp is impossible because the potential is too small to be picked up by this technique. Because of this, MEG recordings index neural activity of large synchronised neural populations on the order of sub-millisecond temporal resolution (Miller, 2013). Synchronisation of brain areas is crucial for network communication and cognition (Gross et al., 2006). The high temporal resolution of

MEG provides a well-suited technique for the study of such synchronisation in resting-state networks (RSNs). The brain, like other complex networks, strives to ensure a balance between local specialisation and global integration. An optimal balance between these two has been shown to facilitate dynamic information flow in the brain, often regarded as essential for cognition (Douw et al., 2011).

To determine if brain units are functionally connected (coupled/communicating), statistical interdependences/correlations between brain regions or sensor/electrode pairs must be computed (See David, Cosmelli, & Friston, 2004; Murias, Pievani, de Haan, & Wu, 2011; Tsiaras et al., 2011, for detailed reviews). Statistical interdependences are derived from measures/estimates of functional connectivity between spatially distinct but functionally connected neural units. In other words, correlations between different spatial regions/sensors provide estimates of functional connectedness. Based on measures of functional connectivity, several networks have been identified in neuroscience. Of these, the most widely studied and described network is the Default Mode Network (DMN).

Initially identified in a meta-analysis, the DMN encompasses brain regions that are typically more active during rest than during active task performance (Raichle et al., 2001). Due to its high spatial resolution, functional magnetic resonance imaging (fMRI) brain imaging technique was initially favoured as a more appropriate method of identifying resting state networks such as the DMN. This technique measures changes in blood oxygenation or blood flow as a proxy indicator of neural activity. However, the popularity of fMRI in measuring spatially distinct temporal interdependencies between blood-oxygenation-level-dependent (BOLD) signals is confounded by its inability to capture electrophysiological neural activity with real time precision (i.e. low temporal resolution) (Brookes et al., 2011). This is the primary reason why MEG has gained considerable attention in recent years as a very sensitive (temporally) functional brain imaging technique (Miller, 2013). As stated earlier, unlike the fMRI imaging tool, which measures indirect neural activity, MEG registers magnetic fields generated by genuine electrical signals within neuronal populations (Hamalainen, Hari, Ilmoniemi, Knuutila, & Lounasmaa, 1993) in real time. In addition, several researchers (Brookes et al., 2001; de Pasquale et al., 2010; van Dellen et al., 2013) have been able to successfully reproduce fMRI RSNs using the MEG brain imaging technique.

Statistical interdependences analysed in the later chapters presented in this thesis were estimated from sensor-level data. Note however that sensor level MEG data are vulnerable to the effects of volume conduction (Hillebrand & Stam, 2014) as well as the mixing of signals originating from spatially distinct neural units. Both concerns potentially result in

erroneous estimates that could underestimate or overestimate functional connectivity (Cohen, 2014; Hillebrand & Stam, 2014). Although these issues can be solved through the projection of signals to source-level, some such as Brookes, Woolrich, and Barnes (2012), Hillebrand and Stam (2014), and Hillebrand et al. (2012), stress that this is not a straightforward solution. Given that the voxels in source space are not independent, leakage between them is highly possible, which according to Brookes et al. (2012) can consequently lead to erroneous connectivity estimates.

A more direct solution to respond to the effects of these issues is the use of functional connectivity estimates that are insensitive to spurious interactions (Hillebrand & Stam, 2014). Volume conduction and spurious correlations in pairwise sensors result from two sensors detecting magnetic flux from the same source. This occurs with zero phase lags or radius (Cohen, 2014). Hence, to eliminate the effects of volume conduction, it is essential to discard zero-phase-lag correlations. Several phase-based measures have been developed to avoid distributions that centre around zero-phase-lag (See Cohen, 2014; David et al, 2004; Niso et al., 2013, for a review). Such phase measures are based on distributed phase differences between two sensors (or electrodes in EEG). These measures require fewer assumptions, and as such are suitable for exploratory analysis where fewer analytical hypotheses are needed (Cohen, 2014).

In the research for this thesis, the phase lag index (Stam, Nolte, & Daffertshofer, 2007b) a linear and non-linear measure (Vourkas et al., 2011) was chosen to estimate functional connectivity. This is because not only is it insensitive to confounds associated with recording magnetic fields from the scalp, but it has also been shown to be sensitive to real changes in synchronisation in several clinical populations (Stam et al., 2007b). Studies comparing phase lag index to measures such as synchronisation likelihood have found that this measure is less affected by volume conduction and performs better than previous functional connectivity estimates (Hillebrand & Stam, 2014).

2.7 Network analysis of functional brain connectivity

The notion that a network can be represented as a graph consisting of spatially discrete units linked together by connections has been around since the 18th century. However, its application in neuroscience is in its early stages. Graph theoretical analysis is a branch of mathematics in which complex network systems are visually represented as a graph.
A graph in this context consists of sets of discrete nodes/vertices connected by lines/edges (See Figure 2.3 below).

Figure 2. 3 An illustration of the concepts of graph theory
A mathematical representation of a complex network system as a graph consisting of sets of vertices/nodes (circles) connected together by paths/edges.

Graph theoretical analysis has helped researchers understand the global and local characteristics of networks (Rubinov & Sporns, 2010; Stam & Reijneveld, 2007). Graph theory is often credited to Euler's research, in which he demonstrated that it was not possible to walk around the city of Konigsberg, crossing each of the seven bridges exactly once, and return to the starting point (Figure 2.4). This was known as the '*Konigsberg Bridge problem'*. By reconstructing the city and representing it as a graph consisting of a set of discrete elements/regions (i.e. node/vertex) connected together by links (i.e. edges), Euler showed that to cross the seven bridges without retracing one's steps, every region (vertex/node) had to have an even degree (i.e. links/connections coming in and out of a region/node). However Figure 2.4, shows that the graph had four nodes of odd degrees, (i.e. a = 5, b = 3, c = 3 and d = 3), making it 4 vertices of odd degrees. Hence, the degrees in the '*Konigsberg Bridge probl*em' were not drawable; and therefore it was not possible to cross each bridge once and return to the starting point.

Figure 2. 4 The 'Konigsberg Bridge Problem'
Representation of the 18th century city of Konigsberg showing A) seven bridges (i.e. 1-7) and the river between the landmasses. Figure B) shows Euler's graphical representation illustrated as a collection of four nodes (circles a-d) linked together by connections/edges (grey lines).

2.7.1. Modern network science

The field of graph theory has provided a powerful tool for understanding network behaviour in biological and social systems (Telesford, Burdette, & Laurienti, 2013). Following Watts and Strogatz's (1998) publication, it was recognised that all dynamically complex and efficient networks share certain common properties. Until this time, not much was known about the behaviour of highly dynamic systems. These inherent common properties form a pattern of network organisation known as the so-called 'small-world' (Watts & Strogatz, 1998). Several studies have shown that the brain can be conceived of as a small world network (SWN; Frantzidis et al., 2014; Supekar, Musen, & Menon, 2009; Wang, Zuo, & He, 2010). These networks have highly clustered short (local) connections and fewer long (global) connections or shorter path lengths (Watts & Strogatz, 1998). Dense clustering/wiring at the local level ensures efficient information processing in specialised structures, whilst short path lengths between nodes ensure more efficient global information processing. It is therefore possible to apply graph theory analysis to characterise and visually represent functional network. One is also able to determine whether organisation in functional connections resembles that of a small-world topology. It is understood that the more efficient functional brain connections are the more the brain networks will resemble a small-world organisation (Figure 2.5B).

To determine network efficiency the studies reported in this thesis used two fundamental measures: clustering coefficient (C) and path length (L) as measures of the local and global connections. It was predicted that younger children and those with developmental disorders would show connectivity patterns that deviated from a SWN. C is related to the spatial

property of a node in a network neighbourhood (Stam, 2012). Based on the measures of C and L, the SWN lies between two extremes, namely regular and random networks (Figure 2.5).

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Figure 2. 5 Graph metric network topologies
Figure 2.5 A) and C): illustrate the two extreme levels of organisation namely regular and random respectively; and B), the small world organisation. A small-world network is characterised by low L and high C, ensuring optimal information transfer within complex networks (Watts & Strogatz, 1998).

Computationally, clustering coefficient as a measure of functional segregation within a network, representing local connectedness (Rubinov & Sporns 2010) or the brain's ability to carry out specialised processing in closely interconnected clusters/modules (e.g. brain regions or sets of sensors). In modern network science, a node (vertex) forms a neighbourhood consisting of other nodes that are closely connected to it by an edge (Figure 2.3). The clustering coefficient of a node (small circles) is the degree to which other nodes in the neighbourhood are also connected to each other (Watts & Strogatz, 1998). For example in Figure. 2.3 the clustering coefficient of any node (small circles) is the degree to which all other nodes in a neighbourhood are connected to each other. The average clustering of a network therefore represents a measure of how well a network is able to efficiently process information in specialized structures (Cohen, 2014; Liang et al., 2012).

Path length (L) on the other hand represents the global connectedness by quantifying the degree to which a brain network is able to efficiently transfer information between discrete specialised brain areas (Rubinov & Sporns, 2010). The average of the shortest distances from one node to another node is known as the characteristic path length (Watts & Strogatz, 1998). The average path length of a node $_i$ in a network therefore denotes the minimum number of edges linking two nodes (Liang et al., 2012; Niu et al., 2013). A low path length value suggests that very few pathways are required to get from one node to any other node in a network (Stam & Reijneveld, 2007).

It has been suggested that in a connected brain, discrete elements functionally segregate to facilitate specialised functions like vision and language, while higher functions depend on the integration of information from local clusters (Douw et al., 2011; Koyama et al., 2011). Hence, it is highly likely that disrupted local clustering and global inefficiency are most likely to be linked to the deficits reported in a given clinical population. It is becoming increasingly clear that a key feature of developmental disorders is an alteration of the normal pattern in functional brain networks, specifically disrupted clustering (local structures) and/or path lengths (global network structures) (Ahmadlou, Adeli, & Adeli, 2012; Bos et al., 2014; Itahashi et al., 2014). Several imaging studies employing graph theory have shown abnormalities in resting-state brain networks in children with autism, fragile X, reading difficulties and ADHD (Ahmadlou et al., 2012; Tsiaras et al., 2011; van der Molen, Stam, & van der Molen, 2014). For example, network visualisation in children with ADHD, revealed a significant decrease in path length and higher clustering coefficient in those with a diagnosis, compared with controls (Ahmadlou et al., 2012). This is consistent with the notion of disrupted or delayed organisation of brain networks. Such results suggest that graph theoretical network metrics are suited for investigation potential biomarkers for developmental conditions.

It should however be noted that several researchers have stated that the application of graph theory relies on assumptions that may impede network comparisons between subjects. Given that individual networks vary in size (i.e. number of nodes), and density (i.e. number of connections in a network; Olde Dubbelink et al., 2013; van der Molen et al., 2014) of the connected network, a normalisation approach is required (van Dellen et al., 2013) to ensure the application of metrics that are independent of global coupling strength (Boersma et al., 2012). In this thesis normalisation was computed using the BrainWave software. Briefly, clustering coefficient (C_W) and path length (L_W) were compared to metrics derived from 50 surrogate networks (i.e. C_w-s and L_W-s respectively). These were based on Erdos-Renyi's random graph model that involves randomly re-shuffling the edge weights in real networks (see Erdos & Renyi, 1960, for a review).

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Figure 2. 6 Representation of graph theoretical analysis computation pipeline
A represents MEG time series for magnetometer sensors in frequency-filtered bands. B represents the 102 x 102 (Chapter 3) or 97 X 95 (Chapters 4, 5, & 6 association matrix of cross-correlations between nodes (magnetometer sensors) estimated using phase lag Index. C represents a weighted network with magnetometers represented as vertices/nodes and PLI values as path lengths/edge weights or connections. From this C_W, and L_W, were computed and later normalized to generate normalised C_W (gamma), and L_W (lambda).

Subsequently, the surrogate networks have the same number of nodes, edges, and degree that are similar to as the original networks (Liang et al., 2012). Although random networks do not represent real complex networks (van der Molen et al., 2014), they provide baseline network models for comparison (Bullmore & Sporns, 2009). For this reason, the main findings within each study primarily address network changes corresponding to normalised metrics. The normalised clustering coefficient (gamma) and path length (lambda) were defined as the ratio of C_W/C_W-s and L_W/L_W-s respectively (Figure 2.6). The SWN index was then represented by the ratio of gamma and lambda. Computationally, a network was defined as having small world properties if gamma (C_W/C_W-s) > 1 and lambda (L_W/L_W-s) ≈ 1 (Humphries & Gurney, 2008).

However, while graph theoretical analysis of brain network provides good insight into potential neural markers of brain functions, its application relies heavily on network size, i.e. number of connections (nodes) and average degree (average number of connections at each individual node within a network) (Hillebrand & Stam, 2014), that is not entirely resolved by normalisation (see van Wijk, Stam, & Daffertshofer 2010) for a detailed discussion). These variables have been shown to differ between people, as a result of either development, or pathology (Gong et al., 2009).

2.7.2. Minimum spanning tree

Minimum spanning tree analysis provides a more computationally sound solution to the problem of network comparison (Stam et al., 2014). In a connected, weighted and undirected graph *G*, a spanning tree is a subgraph of *G* containing all *G*'s vertices connected together without circles/loops (Boersma et al., 2013; Olde Dubbelink et al., 2013; van Steen, 2010). A minimum spanning tree is therefore a spanning tree of minimum total weight among several spanning tress. For a thorough review of minimum spanning tree methods, see Jackson and Read (2010), and Mares (2008). Given that, this type of network analysis connects all nodes without cycles its networks will have an identical number of connections. Hence, unlike classical graph theory, minimum spanning tree analysis offers a bias-free characterisation of the network topology, both within and across studies, as only important connections are taken into account (van Diessen, Otte, Braun, Stam, & Jansen, 2014).

In this thesis, for each participant's weighted graph *G*, minimum spanning tree was computed using formulas implemented in BrainWave, using Kruskal's algorithm (Kruskal, 1956). Constructed trees consisted of 97 nodes and 98 edges. Briefly, to run the Kruskal algorithm on a connected graph of weighted edges, the algorithm first sorts all edge weights (connectivity values) in ascending order, and then constructs a tree, starting with the smallest/minimum edge/weight between two nodes (in this study that would be the smallest phase lag Index value) (Figure 2.7). This is repeated until all edges are connected in a subgraph (edges = n-1) without cycles/loops. During tree construction, if the addition of an edge results in a cycle or loop, then that edge is excluded (see Jackson & Read, 2010; Kruskal, 1956; Mores, 2008, for a detailed review of this algorithm).

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Figure 2. 7 Constructing a minimum spanning tree using Kruskal's algorithm
Figure 2.7 is an illustration of A) connected and undirected graph, and B) a minimum spanning tree/ sub-graph that connects all nodes/vertices without loops, using Kruskal's algorithm.

Similarly to networks derived from graph theoretical analysis, the topology of a network derived from minimum spanning tree analysis must ensure efficient communication between all vertices (Olde Dubbelink et al., 2013). Minimum spanning tree networks are on a continuum between two extreme network topologies: namely the path-like/decentralised and star-like/centralised topology (Figure 2.8). Similar to graph representation, spanning trees sub-graph representations have leaf nodes and edges between nodes (Stam et al., 2014). The nodes in the path-like or decentralised configuration are connected on a single line path to two other nodes, except the two nodes (leaves) at either end of the tree, that have only link or degree = 1 (i.e. black circles in Figure 2.8 topology A) (Olde Dubbelink et al., 2013; Stam et al., 2014). In contrast, the star-like or centralised topology represents a configuration in which only one central node exists, on to which all other nodes are connected with one link (Olde Dubbelink et al., 2013; Stam et al., 2014). Changes in tree topology are therefore interpreted as either an alteration towards a more line-like topology (i.e. less integrated) or a more star-like topology (more integrated) (Stam et al., 2014).

Figure 2. 8 The two extreme minimum spanning tree topologies
Figure A) is a line-like configuration with few tree leaves and a long diameter. With the exception of the nodes, two at either end (black circles); all nodes in a path shape are connected to two other nodes. Those with one link are known as leaf nodes (Stam et al., 2014). Figure B) is a star-like topology with several leaves and a moderate diameter. Topology alters with increasing leaf number, and as such, there exist moderate tree configurations besides the two extremes.

The two extreme topologies are characterised using several metrics. Note however that, as explained in Chapter 1, many of the minimum spanning tree measures are highly correlated, and hence can be substituted by fewer more independent measures (Stam et al., 2014). For studies reported in this thesis, dynamic patterns of minimum spanning trees were assessed

using four metrics whose formulas are implemented in the BrainWave software. These measures were selected because they have previously (Boersma et al., 2013) been shown to be sensitive to age-related changes in functional networks in typically developing children. These included:

1. Diameter is a measure of the longest shortest path or distance between any two nodes of an MST (Boersma et al., 2013; Stam et al., 2014; Tewarie et al., 2014). It has been proposed that a small diameter is a key feature for ensuring efficient communication between nodes of a tree (Tewarie et al., 2014). This corresponds to a star-like topology. This has a lower bound of 2, and an upper bound of M = N − 1. M corresponds to the number of paths in a tree (Otte et al., 2015). In a star-like or centralized network configuration with a diameter of 2, there is a risk that the central node might become overloaded because it has a betweenness centrality (i.e. ratio of number of shortest paths passing through a particular and total number of paths in the tree) of 1 (Boersma et al., 2013; Olde Dubbelink et al., 2013). Hence, for optimal efficiency, tree topology should strive for an equilibrium/balance between a reduced diameter and avoidance of overload. This trade-off is captured by the tree minimum spanning tree hierarchy (T_H) measure (Boersma et al., 2013).

2. Tree hierarchy provides a measure of the optimal balance between integration and overload of central nodes (Otte et al., 2015; Tewarie et al., 2014). According to van Dellen et al., 2013, this metric assess whether tree topology has an efficient organisation to ensure optimal transfer of information between nodes in the least possible paths without overloading the central node. Such a topology has a star-like structure. This is calculated using the formula (Boersma et al., 2012)

Equation 1 Computing network hierarchical organisation

$$T_H = \frac{L}{2MBC_{max}}$$

where L is leaf number, M the number of vertices and the BC_{max} is the maximum value of betweenness centrality (BC). The BC of a reference node is defined as the fraction of all the shortest paths between any two node pairs passing through the reference node, divided by the total number of paths between the two node pairs) (Olde Dubbelink et al., 2013). To determine the balance between a small diameter and overloading of central nodes (i.e. nodes with a high value of BC) the denominator MBC_{max} is multiplied by 2 to guarantee that T_H ranges between 0 and 1 (Boersma et al., 2013; Tewarie et al., 2014). As a result, an optimal tree topology will combine short distances and prevent overloading of central nodes. The optimal tree

configuration is a combination of short distances and decreased overload of central nodes (Otte et al., 2015), corresponding to values around 0.5 (Tewarie et al., 2014).

3. Eccentricity is a measure of the centrality of a node in a tree (Stam et al., 2014), quantifying the longest distance between node i and any other tree node (Olde Dubbelink et al., 2013; Stam et al., 2014; van Dellen et al., 2013). This measure will be low if node *i* is central in the minimum spanning tree (Boersma et al., 2013; Stam et al., 2014), suggesting a more star-like topology.

4. Leaf number is defined *as* the number of nodes (leaves) on the tree. It is used to assess sub-graph topology and network efficiency. Leaf number has a low bound of 2 and an upper bound of n-1 (n being the number of vertices) (Otte et al., 2015). As a result, for any given tree, the leaf number corresponds to number of leaves, divided by the possible maximum number of leaves, in line with the size of the tree (Boersma et al., 2013). According to Stam et al. (2014), a star-like topology corresponds more to shorter diameter, low eccentricity, higher leaf number, and BC. When leaf number increases, diameter decreases (van Dellen et al., 2013).

Recently several studies were able to demonstrate the sensitivity of minimum spanning tree measures in neurodevelopment and pathology (Boersma et al., 2013; Demuri & Fraschini, 2013; Otte et al., 2015; Schoen; Chang, Lee, Bob, & Mashour, 2011; van Diessen et al., 2014). However, as a network computation tool, this type of network analysis is a relatively new approach and not much is known about the functional role of its measures or according to van Dellen et al. (2013), what they tell us about disrupted electrodynamic networks.

Secondly, minimum spanning tree measures are primarily concerned with global organisation (van Dellen et al., 2013) within a network, and not local structures. This means that it remains challenging to probe for local/short range connection disruptions as underlying markers of under or atypical development, as has been demonstrated in several imaging studies (Ahmadlou et al., 2012; Tsiaras et al., 2011; van der Molen et al., 2014) using graph theoretical analysis. For these reasons, the research presented in this thesis applied both classical graph theoretical measures and minimum spanning to provide a new structure for investigating large-scale functional organisation.

2.7.2.1. Network analysis: functional connectivity and graph-based computations

All network-related analysis was computed with the BrainWave v0.9.76 package, a freely available open-source software package developed by Stam (http://home.kpn.nl/stam7883/brainwave.html), for multivariate analysis application with EEG/MEG and RS time-series. This application offers a platform for Fast Fourier Transform (FFT)/power analysis, estimating functional connections (using measures such as Coherence, Imaginary Coherence, Phase Coherence, Phase Lag Index, and Synchronisation Likelihood), spectral analysis (with measures including eigenvector centrality, eigenratio spectral gap, and algebraic connectivity) and network analysis using both graph theoretical analysis and minimum spanning tree. Figure 2.9 illustrates the general procedures involved in computation of graph theory and minimum spanning tree network parameters. The formulas of measures used in this thesis are implemented in the BrainWave software. Network parameters were computed based on single epochs rather than averages over the four epochs (See Tewarie et al., 2014 for the rationale for this approach).

Figure 2. 9 Illustration of graph-based networks and minimum spanning tree sub-graphs
Schematic illustration of network analysis using B) classical graph theory, and C) minimum spanning tree, based on weighted connectivity metrics derived from functional connectivity estimates in A). The association matrix in B) represents all weights in the original matrix, while MST captures a sub-section of connections in the original matrix. From these a full connected graph G and loop-less subgraph G are generated respectively. In B), classical network parameters are subsequently assessed while in C), Kruskal's algorithm is applied to contrast trees with no circles for subsequent computation of minimum spanning tree measures.

3. Investigation of the reproducibility of functional connectivity and graph-based network metrics in healthy adults

3.1. Chapter Summary

The application of graph theoretical analysis (GTA) in functional neuroimaging data has provided a useful tool for characterising the organisation of brain networks. To validate the clinical potential of graph theory in order to improve the understanding of the aetiology of pathology and its diagnosis, however, requires an assessment of the reproducibility of graph theoretical measures. The primary aim of this study was to evaluate the reproducibility of functional networks derived from resting-state MEG time-series as recorded in typical adult volunteers in a repeated measures design. Results revealed significant differences in functional connectivity and graph measures in relation to resting-state, frequency band, and metric order. Results in the current study suggest that reproducibility of eyes-open, high frequency beta band, and small-world index functional networks are comparatively low. Network measures during eyes-closed showed good-to-excellent reproducibility. This is important for continued investigation of alterations in resting-state functional network. This element of the study provided a template for selecting satisfactorily robust processing approaches for further investigation of functional brain networks as potential clinical biomarkers, as described in subsequent chapters in this thesis. Given that network measures have been reported as markers of cognitive impairment, personality traits, intelligence, and typical development, the good reproducibility revealed in the current study is encouraging for future studies exploring the effects of pathology on the organisation of brain networks.

3.2. Introduction

It is now generally agreed that cognitive functions rely on an optimal balance between local specialisation and global integration of the information processes in the brain (Dimitriadis, Laskaris, Simos, Micheloyyannis, & Fletcher, 2013; Douw et al., 2011; Stam, Nolte & Daffertshofer, 2007b; van den Heuvel, Stam, Kahn, & Pol, 2009). This agreement stems from an understanding of the brain as a complex system of highly dynamic and interacting networks. The increased application of graph theoretical analysis to characterise brain architecture in neuroimaging time-series has offered a tool to systematically explore the underlying dynamics of functional networks (Braun et al., 2012; Wang et al., 2011). As a result, there is increasing interest in the implication of graph measures in relation to both atypical and atypical brain function (Jin, Seol, Kim, & Chung, 2011). In quantifying the local and global properties of network topology, graph theory has been fundamental in identifying both widely and locally distributed network alterations associated with psychopathology.

Graph metrics in functional networks are derived from various neuroimaging time-series by estimating the relationship/correlation between spatially distinct neural units (Deuker et al., 2009). The correlations (estimated using various measures e.g. coherence, phase lag index) can be between brain regions of interest (ROIs)/voxels for source space (e.g. in functional magnetic resonance imaging: fMRI) computations or they can be between sensors/electrodes in magnetoencephalography/electroencephalography (M/EEG), for sensor-space computations. Computed correlations are used to generate a functional connectivity/association matrix. A threshold can be applied to a matrix to generate a binary adjacency matrix of edge values set to 0 (i.e. connectivity values < applied threshold) or edge values of 1 (i.e. connectivity values > than applied threshold) (Schwarz & McGonigle, 2011). Alternatively, no threshold is applied and all the original information is retained in a correlation matrix. The former approach results in an unweighted matrix while the latter is a weighted matrix, both of which can be represented as a graph (Deuker et al., 2009).

Various graph theoretical measures have been used to characterise network architecture. These include clustering coefficient, path length, small world index, modularity and degree (See Bassett, Brown, Deshpande, Carlson, & Grafton, 2011; Boccaletti, Latora, Moreno, Chavez, & Hwang, 2006; He & Evans, 2010; Stam, de Haan, & Daffertshofer, 2009, for a review). Graph metrics have been shown to reflect pathology (Rombouts, Barkhof, Goekoop, Stam, & Scheltens, 2005), age-related changes in typical development (Boersma et al., 2011; Micheloyannis et al., 2009; Wu, Sato, Qi, Kawashima, & Fukuda, 2013), the effects of pharmacological intervention (Kelly et al., 2009), changes in spontaneous brain states

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(Braun et al., 2012; Itahashi et al., 2014), and task performance differences between individuals (Deuker et al., 2009). For example, clustering coefficient and modularity have been shown to be reduced in autism (Peters et al., 2013; Rudie et al., 2013), while increased path length has been reported in patients with fragile X disease (van der Molen, Stam, van der Molen, 2014), and patients with Alzheimer's disease (Stam, Jones, Nolte, Breakspear, & Scheltens, 2007a). In tandem with reports of altered resting-state functional connections, research has revelled associations with behavioural and cognitive functioning, suggesting that network topological properties (local and global) may underlie documented impairments in social, cognitive, and behavioural domains in pathology (Itahashi et al., 2014). This implies that brain functional network measures may function as useful biomarkers for investigating brain abnormalities (Vaessen et al., 2010), atypical development, ageing, and longitudinal monitoring of disorder progression.

There is now a general consensus among researchers that the continued application of graph theoretical analysis first requires reproducibility assessment, on repeated testing of the same individuals (Braun et al., 2012; Deuker et al., 2009; Jin et al., 2011; Niu et al., 2013; Telesford, Burdette, & Laurienti, 2013). The aim of this study was to evaluate the inter-session reproducibility of functional connectivity and graph-based network measures in typical adults. Prior to this, this chapter first reviewed those high temporal resolution studies that have previously evaluated reproducibility of functional connectivity and network variables.

3.2.1. Reproducibility of resting-state brain functional connectivity and network measures

Given that resting-state spontaneous fluctuations in brain activity are unconstrained, one could argue that such intrinsic brain activity is challenging to reproduce on repeated testing (Jin et al., 2011). As a result of such fluctuations graph measures derived from resting-state data may not be very robust. What is more, according to Damoiseaux et al. (2006) and more recently Diaz et al. (2013), there exist approximately ten patterns/dimensions of resting-states. Diaz et al (2013) explains that these patterns relate to various cognitive phenotypes. In addition, it also appears that graph-based network parameters can be altered by factors that can be hard to measure in participants. For instance, Verweij et al. (2014) recently revealed altered graph theoretical parameters (i.e. decreased local integration) in the prefrontal cortical areas were less efficient following sleep deprivation. Hence, a necessary first step in validating the application of graph metrics of functional network topology in

clinical populations is to demonstrate that such measures are sufficiently reproducible in test-retest investigations involving the same individuals. There is evidence to consider that resting-state functional connectivity might be highly reproducible across repeated testing. Data reported by Smit, Stam, Posthuma, Boomsma, and de Geus (2008) revealed that network features of small-world networks are highly heritable.

Over the years, several studies have demonstrated sufficiently good reproducibility for graph metrics derived from task-based paradigms (Atri et al., 2011; Caceres, Hall, Zelaya, Williams, & Mehta, 2009; Clement & Belleville, 2009; Putcha et al., 2011; Teleford et al., 2010; Wei, Yoo, Dickey, Zou, Guttmann, & Panych, 2004). However, despite the increased application of graph theoretical parameters in resting-state investigations there have been very few attempts in electrophysiology M/EEG studies to quantify the test-retest reproducibility of network measures during resting-states. Given that these techniques have high temporal resolution, they make it possible to study network interactions that are cognitively meaningful, because measured activity is at real time resolution.

To the best of the author's knowledge, to date only three high temporal resolution studies are reported to have investigated the reproducibility of network metric at rest. Of these two were derived from resting-state MEG data (Deuker et al., 2009; Jin et al., 2011) and one using resting-state EEG (Hardmeier et al., 2014a). Collectively, these studies and several fMRI studies (Braun et al., 2012; Liang et al., 2012; Liao et al., 2013; Schwarz & McGonigle, 2011; Wang et al., 2011; Weber et al., 2013), have generally reported poor-to-moderate reproducibility.

A consistent finding from all the above studies is that the reproducibility of graph-based network metrics is highly dependent on several pre-processing strategies. These include the type of resting paradigm (i.e. eyes-closed vs. eyes-open), measure used to estimate functional connectivity, the frequency range (slow vs. fast oscillations), and type of network (i.e. weighted or unweighted), (See Table 1 for a summary of reproducibility results using M/EEG studies)

3.2.2. Previous studies investigating graph metric reproducibility of brain functional networks

The first study to evaluate the inter-session reproducibility of functional networks was conducted by Deuker et al. (2009). Using MEG, the researchers assessed graph metric

reproducibility for global network parameters (See Table 3.1 for a full summary of the graph metrics investigated) that were estimated using mutual information in sensor-level eyes-open rest and during an n-back memory task. Deuker et al. (2009) reported good test-retest reproducibility for first-order network parameters (i.e. measures computed from one property e.g. clustering coefficient), low frequencies, and task-based networks at a 6-8 week test-retest interval. In contrast, the test-retest reproducibility of eyes-open rest, high frequency beta and gamma band, and second-order network parameters (i.e. measures computed from more than one property e.g. normalised clustering coefficient and small-world index) was considerably lower.

Although very informative, this study has limitations that constrain the generalisability of the reported results. First, the study by Deuker et al. (2009) primarily focused on task-based functional networks, and as a result mainly reported on reproducibility estimates associated with n-back task performance. Second, functional network measures were derived binary unweighted networks, meaning that a threshold was applied. The application of thresholds to association matrices in network science is not fully understood, and several researchers remain sceptical about its application (Rubinov & Sporns, 2010; van den Heuvel, Stam, Boersma, & Hulshoff Pol, 2008; Wang et al., 2011). Given that there is no standard protocol for selecting an appropriate threshold, researchers use a range of arbitrary values to transform association matrices into adjacency binary matrices (a_{ij}) (Achard & Bullmore, 2007; Braun et al., 2012: Hardmeier et al., 2014a; Liang et al., 2012; Schwarz & McGonigle, 2011; Weber et al., 2013). For some, a binary threshold (r_c) can be set as a single cut-off threshold value that is applied to all networks as $-1 < r_c < 1$ (Achard, Salvador, Whitcher, Suckling, & Bullmore, 2006; Schwarz & McGonigle, 2011). For others, a threshold is set for each network, depending on different topological properties (Braun et al., 2012; Liao et al., 2013; Telesford et al., 2013). The former, results in similar graphs for each subject, while in the latter approach, the graphs generated differ from each other (Schwarz & McGonigle 2011; Wang et al., 2011). A recent study by Braun et al. (2012) showed that reproducibility varies depending on whether they were computed from weighted or unweighted networks. This suggests that the results reported by Deuker et al. (2009) may not apply to those where weighted graphs are studies

A final limitation of the MEG study by Deuker et al. (2009) is that resting-state was only recorded during eyes-open rest. It has been proposed that topological network organisation is distinguishable based on whether one's eyes are open or closed (Jin et al., 2011; Jin, Jeong, Lee, Jeon, & Chung, 2014; Jin, Jeong, Seol, Kwon, & Chung, 2013; Xu et al., 2014). What's more, Deuker et al. (2009) too acknowledged this and concluded by hypothesising

that functional network metrics derived from eyes-closed could yield higher reproducibility. The researchers explained that the lower reproducibility of metrics derived from eyes-open functional networks could be related to the signal-to-noise ratio in the eyes-open state being negatively affected by alpha suppression and eye movements during data acquisition.

Also using mutual information as a measure of functional connectivity, the second and only other MEG study conducted by Jin et al. (2011) found that reproducibility was fair-to-moderate for weighted nodal centrality measures at a two week test-retest interval in sensor-level data. In addition, reproducibility was lower in eyes-closed and gamma frequency band compared to eyes-open rest. Similar to Deuker et al. (2009), data from Jin et al. (2011) found that network reproducibility was influenced by resting condition, the choice of graph metric, and frequency band. Both studies reported lower reproducibility of gamma band networks. A major limitation of the study by Jin et al. was that the researchers only focused on nodal centrality measures.

The most recent study to investigate reproducibility of network metric was conducted by Hardmeier et al. (2014a). Using eyes-closed resting EEG time series, the researchers investigated the long-term (at one and two years) graph metric reproducibility of weighted networks using a measure of functional connectivity that are insensitive to the effects of volume conduction. Generally, reproducibility was low for high frequency beta and the small-world index.

Table 3. 1 Summary of previous M/EEG studies investigating the reproducibility of funtional connectivity and graph measures

Study	Modality	RS paradigm	FC measure	Findings
Deuker et al. 2009	MEG	Eyes-open (EO) rest and n-back task	Mutual information (MI) (binary/unweighted networks)	• Good test-retest(TRT) for n-back task, first-order measures and low frequencies • Lesser TRT for EO rest, higher frequency beta and gamma, and second-order measures
Jin et al. 2011	MEG	Eyes-open and eyes-closed (EC) rest	Mutual information (weighted networks)	• Fair-to-moderate TRT for nodal centrality measures and EO • Lesser TRT for EC, and higher frequency gamma
Hardmeier et al. 2014a	EEG	Eyes-closed rest	Phase lag index (PLI) and weighted phase lag index (wPLI) (weighted networks)	• High long-term TRT for global wPLI and corresponding graph measures • Low-to-moderate TRT for PLI and graph measures • Lesser TRT for beta and small-world index

A summary of M/EEG studies investigating graph metric reproducibility of functional brain networks.

Unlike research in the M/EEG community, several fMRI studies have been conducted to evaluate the reproducibility of functional networks across repeated testing. Using fMRI, Liang et al. (2012) found slight differences in topological parameters derived from weighted and binary networks. Pearson correlated metrics, in the absence of global signal regression, were highly reproducible. In addition, frequency-dependent analysis revealed higher reproducibility for higher frequency (slow 4:0.027-0.073 Hz) networks compared to lower frequency (slow 5:0.01-0.027 Hz) networks. Also using fMRI, Wang et al. (2011), investigated both the short-term (< 1 hour) and long-term (> 5 months) reproducibility of global and local network metrics of functional networks derived from resting-state. The

researchers found that metrics in weighted networks yielded higher test-retest reproducibility compared to binarised networks. Nodal degree network parametric was least affected by the approach. These results are consistent with findings reported by Schwarz and McGonigle (2011), who also investigated network reproducibility using resting-state fMRI.

The final resting-state fMRI study discussed was conducted by Braun et al. (2012). The researchers investigated the influence of frequency-range and global regression pre-processing on graph metric reproducibility. The researchers reported a higher reproducibility of broader frequency band (0.008-0.15 Hz) networks compared to standard frequency bands (0.04-0.08). In addition, unlike the MEG data reported by Braun et al. (2012), and Deuker et al. (2009) reported a higher reproducibility of second-order measures compared to first order measures. The researchers discussed the likelihood that the higher reproducibility of second-order network properties was attributable to the fact that these measures reflect properties of brain function that could be more reliably explored using fMRI. However, this has not been replicated by other researchers. For a review of more fMRI studies investigating graph metric reproducibility, see Telesford et al. (2013).

Overall, fMRI studies graph metric reproducibility suggest that global signal regression is associated with higher reproducible network parameters (Telesford et al., 2013), and that the application of partial correlations to investigate network parameters result in lower reproducibility compared to using Pearson's correlation coefficient, because variance is reduced. In contrast, due to contrasting methodological considerations in the M/EEG studies, it is challenging to draw clear concrete conclusions.

The current study

In the general framework of this thesis, this chapter functioned as a preliminary study to investigate graph metric reproducibility of functional weighted networks.. This study aimed to inform the choice of processing steps and graph measures acceptable for use with typically developing participants and atypical paediatric populations. As discussed earlier (See section 3.2.2), the first study (Deuker et al., 2009) mainly focused on global metrics associated with task performance in binary networks. The second study (Jin et al., 2011), concentrated on nodal centrality measures in local weighted networks during eyes-closed and eyes-open states. The most recent (Hardmeier et al., 2014a) mainly focused on long-term measures in global weighted network parameters during eyes-closed. All studies were estimated at a sensor-level.

Due to low spatial resolution, it is likely that MEG signals picked up at sensors may originate from the same source (de Pasquale et al., 2012). Consequently, this may result in spurious connectivity estimates between nearby sensors (Stam et al., 2009). The exclusion of zero-lags often associated with volume conduction provides a solution to this issue (Stam et al., 2007b). As a result, sensor space computations benefit from applying a measure of functional connectivity that is insensitive to zero-lags, something that a measure such as mutual information, as employed by Deuker et al. (2009) and Jin et al. (2011) is not able to do. While networks reported in Hardmeier et al. (2014a) were computed using such a measure, this study only reported on the reproducibility of eyes-closed only and did not consider the reproducibility of network metrics in the gamma band.

Hence the current study aimed to evaluate the reproducibility of functional network organisation parameters by overcoming the limitations of previous studies. This involved an investigation of graph metric reproducibility for global weighted functional brain networks derived from both eyes-closed and eyes-open resting data based on a measure of functional connectivity that is insensitive to the effects of volume conduction.

<u>Hypotheses</u>

1. It was predicted that functional networks derived from eyes-closed rest would reveal higher reproducibility compared to eyes-open rest.
2. Generally higher reproducibility would be observed in low frequency (theta and alpha) bands compared to high frequency beta and gamma. According to Bassett and Bullmore (2006), Deuker et al. (2009), and Honey, Kotter, Breakspear, & Sporns (2007), low frequency oscillation bands are more anatomically constrained. Hence, it was hypothesised that these systems were more likely to be robust over repeated measurements compared to dynamic systems in high frequency bands.
3. Finally, it was also predicted that second-order measures (e.g. normalised characteristic path length and small-world index) would yield lower reproducibility compared to first-order graph metrics. This is because first order measures are derived from one property, and thus avoid the added variance introduced in second-order metrics that are quantified from more than one property (Deuker et al., 2009).

3.3. Materials and Methods

3.3.1. Participants

The Aston University Ethics Committee reviewed and approved the study protocol (REC Reference: 452). All participants gave their written informed consent prior to the experiment. Testing sessions were two to six weeks apart. The sample was recruited from the student and staff population at Aston University (See Table 2 for demographic characteristics). All participants had normal or corrected to normal vision. Initially thirty adults were recruited into the study. However, ten subjects were excluded from further analysis, for the following reasons. Six failed to complete the two recordings, one was receiving medication for a major mental illness at the time of the second scan, one had a diagnosis of developmental dyslexia (self-reported on a behaviour problems questionnaire), and excessive artefactual MEG signals in two participants meant it was not possible to attain clean epochs. The final sample consisted of twenty adults eligible to participate in the study and with no major psychiatric or neurological disorders.

3.3.2. MEG data acquisition

Magnetic fields were recorded inside a magnetically shielded room using a 306-channel whole head MEG system (Vector View, Elekta Neuromag Oy, Helsinki, Finland). The sampling rate was 1 kHz and data were recorded at a 0.1 Hz high pass filter and a 300 Hz low pass filter. Prior to MEG data acquisition, five head position indicator coils were attached to the head of each participant: three on the forehead and one behind each of the ears. Subsequently, anatomical landmarks and further head points (~400) across the entire head were digitised using a 'Polhemus sensor' (Henson, Mouchlianitis, & Friston, 2009). Head position was tracked continuously during data acquisition, and corrected to a standard position in Maxfilter (Elekta Neuromag). Only data from magnetometer sensors are reported in this thesis.

Resting-state MEG was recorded on two repeated occasions while participants were seated comfortably in a reclining chair. During the eyes-open resting-state, participants were instructed to relax, and keep their gaze fixed on a white cross mark projected centrally on a black screen. During eyes-closed, participants were asked to relax but remain awake. Eye (excessive blinking) and body movements were discouraged. Each resting paradigm lasted approximately two minutes. All MEG recordings were scheduled in the morning at the same

time, to take into account subject circadian rhythms, as advised by Deuker et al. (2009).

3.3.3. Data pre-processing

Noise and artefact suppression of MEG data was achieved with the temporal signal-space separation (tSSS: Taulu & Hari, 2009), with movement compensation implemented in the MaxFilter software (Elekta Neuromag). Following this, the data were inspected visually for several artefacts, including eye-blinks, muscle activity, excess movement, and for saturated channels, using the Graph software (Elekta Neuromag). This enabled a comparison of raw and MaxFiltered data alongside each other, helping to determine the performance of tSSS in magnetoencephalographic artefact suppression. Following the application of tSSS and the visual inspection of data, segmentation was undertaken with the SplitScreen software (Van Dyck, Elekta Neuromag). Care was taken to ensure that selected epochs contained no residual artefacts, resulting from eye-blinks, excessive muscle activity, slow eye movements, or system and technical related issues such as trapped flux 'pop-corn noise'. Eight non-overlapping artefact-free epochs (four eyes-closed and four eyes-open) of 4096 samples (4.096 seconds) were selected at the beginning of each continuous MEG resting-state condition when participants were most alert (Jin, Jeong, Lee, Jeon, & Chung, 2014). Data from Hardmeier et al. (2014b) previously found that short four second epochs yielded higher reproducibility compared to long epochs of the same duration. Finally, for each participant, 8 epochs of 4 seconds were converted into plain ASCII files and imported in the BrainWave software package (Stam: http://home.kpn.nl/stam7883/brainwave.html). All subsequent analysis including band-pass filtering, FFT power analysis, estimation of functional connectivity, and graph theoretical measures were computed using formulas implemented in BrainWave. The package has been used successfully in several studies looking at network analysis of time series derived primarily from resting-state MEG and EEG data (Hardmeier et al., 2012; van der Molen et al., 2014). Note that all results refer to average phase lag index matrices. No regional analysis was conducted, as it is not clear whether graph-based measures in small networks carry meaningful information (van Wijk, Stam, & Daffertshofer, 2010).

Estimating FC using PLI

The phase lag index (PLI) (Stam et al., 2007b), was used as the measure of functional connectivity/coupling strength in band-filtered time-series, corresponding to standard frequency ranges theta (4-8 Hz), lower or alpha1 (8-10 Hz), upper or alpha2 (10-13), beta

(13-30 Hz) and gamma (30-45 Hz). The phase lag index is a measure of the asymmetry of the distribution of phase differences between two instantaneous signals (Stam et al., 2007b). Spurious connectivity arises from two electrodes/sensors measuring activity from the same source. When this occurs, the instantaneous phases of the two time-series phase lag around zero or π (mod π) (Hardmeier et al., 2014a). The higher the asymmetry, the more likely that one signal is leading or lagging compared to another signal. Consequently, correlation interdependencies estimated using PLI are less likely to be affected by the influence of common sources or volume conduction. In other words, phase lag index was developed as a measure that is insensitive to volume conduction specifically because it avoids zero phase lags, which in turn avoid spurious connectivity due to volume conduction (as discussed in Cohen, 2014; Niso et al., 2013 and Stam et al., 2007b). Comparing coherence and imaginary coherence to phase lag index Stam et al. (2007b) showed that the two former measures were strongly influenced by spurious correlations due to common sources. In addition, the phase lag index measure has been shown to perform well in estimating real changes in synchronisation, and is often used to estimate coupling information in both clinical and non-clinical populations (See Peraza, Asghar, Green, Halliday, 2012; van der Molen et al., 2014; van Straaten et al., 2014). When computed, phase lag index values range between $0 \leq$ phase lag index ≤ 1. A zero value represents no coupling or coupling with a phase difference centred around zero or π, while a value of one represents phase differences of non-spurious interactions, generated from an asymmetric distribution that is different from zero (mod π). (The computational review of phase lag index is beyond the scope of this thesis. See Stam et al., 2007 for a detailed discussion of phase lag index computation). Instantaneous phase synchronisation between the time series was based on the Hilbert transformation.

Global network construction

Graph measures were computed from individual network nodes (magnetometer sensors) and average network measures per epoch for each participant in the frequency bands of interest for the two resting conditions. To overcome the limitations associated with using arbitrary thresholds, all original weights in the connectivity matrix were retained. Constructed graphs consisted of a set of 102 nodes (i.e. 102 magnetometer sensors) connected by weighted edge (i.e. phase lag index values, where w_{ij} undirected weights represented connections between any two nodes $_i$ and $_j$) (Stam et al., 2009). For each network, weighted clustering coefficient (C_W), weighted characteristic path length (L_W), normalized C_W (gamma: γ), normalized L_W (lambda: λ), and the small-world index (sigma: σ) were computed according to formulas implemented in BrainWave. For the formulas and mathematical

definitions of these measures see Hardmeier et al. (2014), Humphries and Gurney (2008). Latora and Marchiori (2001), Nui et al. (2013), Rubinov and Sporns (2010), Stam et al. (2009) van der Molen et al. (2014), and Watts and Strogatz (1998).

Together these measures represent network parameters of functional integration (L_W, & lambda), measures of functional segregation (C_W, & gamma, see Figure 3.1 for an illustration), and network efficiency (the small-world index). Calculation of graph metrics can be based either on a single graph property or on more than one graph property (Jin et al., 2011). As explained earlier, metrics computed using the former are known as first-order metrics while the latter are second-order metrics. Hence, first order metrics in this study are global PLI, C_W, and L_W while second second-order metrics are normalised clustering, normalised characteristic path length, and the small-world index.

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Figure 3. 1 Visual illustration of the core graph theoretical measures
The core graph-based network measures i.e. measures of integration and segregation. The two big circles represent clusters with more within module connections (local) than between-module (global) connections. A high number of triangles in a network suggest high segregation in a network, which is assessed using clustering coefficient. Network weights (grey, blue, and green links) reflect global efficiency, assessed using path length. Figure adapted from Rubinov and Sporns (2010).

Weighted clustering coefficient (C_W) is a measure of functional segregation that represents local connectedness (Rubinov & Sporns, 2010). It relates to the brain's ability for specialised processing in closely interconnected clusters/sub-graphs (e.g. brain regions or sets of sensors). Hence a network with a high average clustering coefficient represents a network of densely connected local clusters. By definition, C_W of a node (small circles) is the degree to which other nodes in a neighbourhood are also connected to each other (Watts & Strogatz, 1998). In Figure 3.1, C_W of any node (small circles), such as the ones linked together with blue lines, would be the degree to which all other nodes with blue edges are

also connected to each other in that neighbourhood. Mean C_W is therefore the global estimate of local network connectivity (Hardmeier et al., 2014; Liang et al., 2012).

Weighted short path length (L_W) represents global connectedness (Hardmeier et al., 2014). The metric quantifies the degree of the brain's ability to rapidly and efficiently integrate highly specialised information in spatially discrete brain regions (Rubinov & Sporns, 2010). In other words, this metric L_{ij} is concerned with estimating how efficiently a network is connected (Liang et al., 2012; Nui et al., 2013). In Figure 3.1, the route marked by green edges is an example of the most efficient route connecting nodes in the two modules (i.e. sub-graphs). L_W can be thought of as routes by which information flows. The average of the shortest distances from one node to another node is known as the characteristic path length (Watts and Strogatz, 1998). In a network, (See Figure 3.1) any path will consist of two or more edges with differing weights. Hence, a network with low characteristic path length would be characterised by system of short distance connections between nodes (Supekar, Musen, & Menon, 2009). The average of all path lengths is the global measure of a network's functional integration.

As explained in Chapter 2, individual differences in network size, and edge weights (van der Molen et al., 2014) mean that computed networks often vary, making network comparison between subjects challenging. A graph theoretical approach-based solution to this problem is to compute graph measures that are independent of network size. This process is known as normalisation. In the current study, normalised measures were calculated using formulas in BrainWave. Below is a description of the computation.

<u>Normalisation</u>

In BrainWave, surrogate/random networks were constructed, based on Erdos and Renyi's random graph model (See Erdos & Renyi, 1960, for a review). This involves a re-shuffling of the original network, where size is not affected but the structure is affected. (Hardmeier et al., 2014). From the surrogate network, new measures of C_W (i.e. C_W-s) and L_W (L_W-s) are computed. (See Figure 2.6, Chapter 2 for an illustration of this process.) Computationally, normalised C_W (gamma) is defined as the ratio of C_W in a real network and C_W in a comparable surrogate network (C_W-s), while normalised L_W (lambda) is defined as the ratio of L_W in a real network and L_W in a comparable random network (L_W-s).

A network is said to have small world properties if normalised gamma (i.e. C_W/C_W-s) > 1 and lambda (i.e. L_W/L_W-s) ≈ 1 (Humphries & Gurney, 2008). As explained in Chapter 2, section

2.7.1., compared to a random network, a network with a small-world topology has higher clustering (gamma > 1) and approximately low path lengths (lambda ≈ 1) (Liang et al., 2012). A network can have small world properties and not have an overall small-world configuration. To formally assess if network topology has a small-world configuration, also known as sigma, the ratio of gamma and lambda must be > 1 (Humphries & Gurney, 2008). Graph measures of the four epochs per subject in each frequency band of interest during eyes-open and eyes-closed rest were averaged for all further computations.

Reproducibility of functional network parameters

To determine reproducibility of functional connectivity and graph-based network measures, the current study and those reviewed thus far use the intraclass correlation coefficient (ICC) (Shrout & Fleiss, 1979) as a tool for determining reproducibility in repeat study designs. It quantifies the degree of between-subject variability compared to total variability. ICC is typically used to compute inter-rater reproducibility estimates, i.e. how consistent raters are, relative to one another, as they measure a given outcome. When estimating reproducibility on repeated measurements (i.e. session 1 and session 2), ICC computation assumes that if a measure is estimating a given phenomenon in the same group of people, then its performance on repeated testing should be reproducible. It is computed using different models that focus on the source of variability in population samples, raters and estimated measurements (See McGraw & Wong, 1996; Shrout & Fleiss, 1979 for a review) . ICC is similar to the Pearson correlation, because both measures try to establish whether variables are linear in their relationship to one another. However, Pearson correlation cannot determine how consistent measures are relative to one another, as far as agreement in the measurement is concerned, which is, however, possible with intraclass correlation computation.

For each resting condition, frequency band, and metric, average test-retest estimates were computed across the two recordings using the intraclass correlation coefficient (ICC: Short & Fleiss, 1979). ICC has widely been used to assess test-retest graph metric reproducibility in neuroimaging studies (Deuker et al., 2009; Jin et al., 2011; Niu et al., 2013). In this study, ICC values for absolute agreement measurements were computed using a two-way random effect model (2,1), which assess how consistent the two raters are, relative to each other on average, from person to person. ICC values were calculated using the equation shown as Equation 3. 1 below (Shrout & Fleiss, 1979):

Equation 3.2 ICC computation

$$ICC\,(2,\,1) = ((MS_s - Ms_e)\,/\,(MS_s + (k\,\text{-}1)MS_e))$$

where k represents number of repeated measurements (i.e. two test recordings), while the MS_s and MS_e represent between-subject and within-subject variance respectively. Mean and variance values were calculated for each metric. In line with previous studies, test-retest estimates were categorised as poor if ICC values were < 0.4, moderate if ICC was 0.4 - 0.6, good if ICC was 0.6 to 0.75, and excellent if iCC was 0.75 to 1 (Hardmeier et al., 2014a; Liao et al., 2013; Niu et al., 2013; Sampat et al., 2006; Telesford et al., 2013). ICC values > 0.4 are generally considered to be sufficiently reproducable (Faria et al 2012). See Figure 3.3 for an illustration of the ICC computation pipeline.

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Figure 3. 2 Intraclass correlation computations across MEG data recordings
An overview of ICC computation (based on Deuker et al., 2009) of functional networks. No threshold was applied, hence computed graph metrics were based on weighted phase lag index matrices estimated during both recording sessions. Functional connectivity and graph metric reproducibility was assessed using the ICC intraclass correlation coefficient, computed for each metric between the two sessions.

3.4. Results

Table 3. 2 Demographic characteristics

	Typical adults (M ± SD)
N	20
Age (years)	30±7
Gender (F:M)	13:7
Handedness (R:L)	15:5
Years of education	15±5
Days between scans	2-6 weeks

Demographic characteristics of participants presented as mean ± standard deviation. Key: M = mean; SD = standard deviation; N = number of participants; F: M = females: males; R: L = right: left.

3.4.1. Coupling strength between sessions in eyes-closed and eyes-open resting conditions

Figure 3.3 displays mean coupling strength (i.e. average phase lag index) at each frequency band for eyes-closed and eyes-open resting conditions. Mixed ANOVA models (drawn from SPSS version 21), were used to investigate average phase lag index differences between the two sessions at each frequency band, with frequency as within-subjects factor and test session (1st and 2nd), as a between-subjects factor. These were computed separately for eyes-closed (Figure 3.3A) and eyes-open (Figure 3.3B) resting networks. With the exception of the theta band in the eyes-closed resting-state, results revealed that global coupling strength in both resting conditions decreased as the frequency bands increased. Results were significant at $p > .05$, and effect size estimates were reported as partial eta-squared values (η_p^2).

Eyes-closed resting-state:

For the eyes-closed condition (Figure 3.3A), Mauchly's test indicated that the assumption of sphericity had been violated ($X^2(9) = 81.09$, $p < .01$,) therefore degrees of freedom were corrected using Huynh-Feldt estimates of sphericity ($\varepsilon = .61$). A significant main effect of frequency band, $F(2.45, 93.05) = 339.24$, $p < .01$, (η_p^2) = .90, but not for session, $F(1, 38) = .71$, $p > .05$, (η_p^2) = .02 was observed. No significant interaction between frequency band and session was found $F(2.45, 93.05) = .19$, $p > .05$), (η_p^2) = .01.

For eyes-open rest (Figure 3.3B), Mauchly's test similarly indicated that the assumption of sphericity had been violated ($X^2(9)$ = 24.28, p< .05, therefore, degrees of freedom were corrected using Huynh-Feldt estimates of sphericity (ε = .90). A significant main effect of frequency band, $F(3.60, 136.68)$ = 920.51, p < .01, (η_p^2) = .96, but not for session $F(1, 38)$ = 1.64, p > .05, (η_p^2) = .04 was observed. No significant interaction between frequency band and session was observed $F(3.60, 136.68)$ = .91, p > .05) (η_p^2) = .02.

Together the results reported above found that average coupling strength varied as a function of frequency band, but not session, in both resting conditions. In addition, from the visual representation of these results, presented below, it is clear that coupling strength remained much the same across testing sessions.

Figure 3. 3 Average phase lag index in different frequency bands across the two repeated MEG recordings

*Figure 3.3 represents average coupling information across five frequency bands in A) eyes-closed rest and B) eyes-open resting during the first and second recording sessions. Error bars are ± 2 SE, standard error. Note, * p < .05. Statistically, average coupling strength varied as a function of frequency, range p < .05, but not session, for both resting conditions.*

Figure 3.3 reveals that coupling strength generally decreased as frequency bands increased. According to Jin et al. (2011) this trend suggests that coupling information in low frequency bands is potentially stronger compared to higher frequency bands. The observation of increased functional coupling during the eyes-closed condition for lower and upper alpha bands is not consistent with the results reported by Jin et al. (2011). However, this observation is in line with prior literature (Xu et al., 2014), suggesting that neuronal activity is modulated by visual attention, often demonstrated when participants close and open their eyes. During eye-open, desynchronization of alpha band rhythm occurs, while when eyes are closed, alpha band rhythm is generated. The main observation from the current study was that reproducibility of network functional networks are greatly influenced by processing choices. This is crucial as it helped in identifying the most robust processing choices of functional networks in subsequent chapters.

3.4.2. Formal assessment of functional brain network parameters test-retest reproducibility

The reproducibility of FC global network parameters was evaluated using the intraclass correlation coefficient (ICC) (Shrout& Fleiss, 1979). The results are presented in Table 3.3 and Figure 3.4. Generally, metric reproducibility varied from moderate to excellent reproducibility, depending on resting condition, frequency band, and type of graph measure. Unless otherwise stated, results are reported as means ± SD. In accordance with previous studies (Braun et al., 2012; Deuker et al., 2009; and Zhang et al., 2011), negative ICC estimates were set to zero (i.e. not reproducible). It has been argued that negative ICC estimates occur when within-participant variance is greater than between-participant variance (Deuker et al., 2009), a situation that researchers consider unusual (Jin et al., 2011). Nevertheless for all statistical purposes ICC values with negative estimates were set to zero. However, negative ICCs are displayed in Table 3.3 and Figure 3.4 for mere visual articulation. In Table 3.3, results show that test-retest reproducibility of functional connectivity and graph measures ranged from 0 to 0.98 ($M = 0.41$, $SD = 0.33$). The most robust measure was mean global phase lag index, in the gamma frequency band network of eyes-closed condition: for a single measure the $ICC = .96$ (95% $CI = 090$-0.98; $F = 47.2$, $p < 0.01$), and for average $ICC = .98$ (95% $CI = .95$, $.99$). Mean global phase lag index was also highly robust in the eyes-open condition: for a single measure, $ICC = .93$ (95% $CI = .83$-$.97$; $F = 26.3$, $p< 0.01$) and for average $ICC = .96$ (95% $CI = .91$-$.99$). Generally, eyes-closed condition, functional connectivity (phase lag index) low frequencies, and graph-based first-order measures showed sufficiently good reproducibility (Figure 3.4). In contrast eyes-open,

second-order graph measures and high frequency beta showed lesser test-retest reproducibility.

Table 3. 3 ICC values for eyes-closed (EC) and eyes-open (EO) rest across the five frequency bands

		Phase lag index	C_w	L_w	Normalized C_w	Normalized L_w	Small-world index
EC	Theta	.74* (.32, .90)	.79** (.46, .92)	.72* (.27, .89)	.78** (.43, .91)	.67* (.14, .87)	-.12 (-2.11, .57)
	Alpha1	.51 (-.29, .81)	.54 (-.20, .82)	.64 (.06, .86)	.55 (-.19, .82)	.43 (-.51, .78)	-.02 (-1.81, .61)
	Alpha 2	.79** (.45, .92)	.79** (.45, .92)	.81** (.52, 93)	.47 (-.40, .79)	.50 (-.33, .80)	.34 (-.77, .74)
	Beta	.26 (-.99, .71)	.09 (-1.48, .65)	.42 (-.54, .77)	.01 (-1.73, .62)	-1.21 (-5.79, .18)	.33 (-.78, .74)
	Gamma	.98** (.95, .99)	.97** (.94, .99)	.96** (.90, .98)	.12 (-1.39, .66)	.36 (-0.70, .75)	-.42 (-3.05, .46)
EO	Theta	-.41 (-3.02, .47)	-.31 (-2.71, .50)	-.23 (-2.46, .53)	.51 (-.30, .81)	-.03 (-1.84, .61)	.17 (-1.26, .68)
	Alpha1	-.22 (-2.42, .54)	-.04 (-1.87, .60)	-.06 (-1.92, .60)	-.04 (-1.88, .66)	.51 (-.28, .81)	-.45 (-3.13, .45)
	Alpha 2	-.05 (-1.89, .60)	.12 (-1.40, .66)	.01 (-1.72, .62)	.50 (-.32, .81)	.71* (.26, .89)	.51 (-.28, .81)
	Beta	.41 (-.57, .77)	.26 (-1.00, .71)	.53 (-.24, .82)	-.28 (-2.62, .51)	-.93 (-4.76, .28)	.47 (-.40, .79)
	Gamma	.96** (.91, .99)	.95** (.87, .98)	.93** (.81, .97)	.32 (-.81, .74)	.48 (-.38, .80)	.22 (-1.10, .70)

Mean global ICC estimates for global network metrics across frequency bands in EC, eyes-closed, and EO, eyes-open resting conditions. Mean ICC values are mean and 95% confidence intervals estimated from bootstrapping. When averaged together, ICC estimates of the six metrics and global PLI ranged from 0 to 0.98 (M= 0.346 ± 0.325 (SD)). ICC estimates with negative values were set to zero. ICC < 0.4 indicates low reproducibility, 0.4-0.6 fair, 0.6-0.75 good, and >0.75 excellent reproducibility. * indicates ICC estimates that meet the criterion for sufficiently good reproducibility and ** those with excellent reliability. ICC values > 0.4 are generally considered to be sufficiently reproducible (Faria et al., 2012).

Figure 3. 4 Mean ICC values in resting-state conditions across the five frequency bands

Figure 3.4 represents mean ICC estimates across frequency bands in the eyes-closed (top figure) and eyes-open (bottom figure) resting conditions. Generally, graph measures, particularly in the eyes-closed condition, show sufficient good (> 0.5) to excellent reproducibility (> 0.75). Second-order metrics (i.e. normalised clustering, normalised path length, and small world index) yielded lower ICC scores. As explained earlier (Section 3.3.6), negative ICCs are considered to be theoretically unusual by most and normally excluded from formal ICC calculations. These values are plotted in Figure 3.4, for visual a visual illustration purpose only.

Results in Table 3.3 and Figure 3.4 reveals that test-retest reproducibility varied across different graph measures. As stated earlier, first-order measures represent metrics that were computed from a single graph property (i.e. phase lag index, clustering coefficient and

characteristic path length), while second-order metrics represent graph measures derived from more than one property (i.e. normalised clustering, normalised path length and the small-world index. The former are second-order because they are ratios of measures computed in the original and surrogate networks. Results in Table 3.3 and Figure 3.4 reveal a very interesting pattern. It appears that functional connectivity and graph-based network measures were those most affected by rest-state dependent properties. To elaborate, during eyes-closed, beta band and small-world index tended to be the least reproducible compared to first-order measures and other frequency bands. During eyes-open, by contrast, first-order measures and low frequency theta, and alpha1 tended to show lower reproducibility compared to higher frequency beta and gamma and second-order measures. As a result of this observation, all further computations were conducted separately for the two resting conditions.

First-order vs. second order test-retest reproducibility during eyes-closed and eyes-open rest

Eyes-closed rest: Using a 1-way repeated ANOVA, results for the eyes-closed rest condition revealed a significant effect of metric order (i.e. whether the graph metric was computed as a first or second-order metric) (F(1.05, 4.19) = 9.13, $p < .05$, (η_p^2) = .70 (Sphericity not assumed). Comparisons found that the ICC scores for global functional connectivity F(1, 4) = 8.18, $p = .046$, and first-order metrics F(1, 4) = 10.40, $p = .032$, were significantly higher than second-order metrics.

Eyes-open rest: Note however that the main effect of order-metric within the eyes-open condition was not significant (F(1.00, 4.02) = .01, $p > .05$, (η_p^2) = .002 (sphericity not assumed). Taken together for both resting conditions, the test-retest reproducibility of functional connectivity and graph measures was highly dependent on the order of the measure. The results suggested that these effects were more pronounced for the eyes-closed resting condition, in which comparisons revealed that second-order measures were comparatively less reproducible than functional connectivity, clustering coefficient, and path length.

Frequency band-dependent test-retest reproducibility estimates in eyes-closed and eyes-open rest

Eyes-closed: The choice of frequency band did influence reproducibility (See Figure 3.4). A repeated ANOVA for eyes-closed revealed a significant main effect of frequency band

(F(3.37, 16.84) = 4.30, p < .05, (η_p^2) = .46 (sphericity not assumed). Although comparisons revealed no significant differences between the ICC scores for different frequencies, a trend for higher reproducibility of gamma band measures compared to beta was observed F(1, 5) = 4.47, p = .09, (η_p^2) = .47.

Eyes-open: Similarly a repeated ANOVA of frequency bands within the eyes-open revealed a significant main effect of frequency band (F(4, 20) = 3.45, p < .05, (η_p^2) = .41 (sphericity assumed). Comparisons revealed that network measures within the gamma band, were significantly higher than those for theta (F(1, 5) = 6.73, p = .049, (η_p^2) = .57), lower alpha (F(1, 5) = 9.54, p = .027, (η_p^2) = .66) and beta band (F(1, 5) = 7.51, p = .041, (η_p^2) = .60), but not for alpha2 (F(1, 5) = 1.72, p = .25, (η_p^2) = .26). In summary, the results for both resting conditions found that the test-retest reproducibility of network measures varied across frequency bands. The extent of these differences was more pronounced for the eyes-open condition than for eyes-closed. In the former state, the ICC scores for measures in gamma was significantly higher than all other frequencies except for alpha2 band.

3.5. Discussion

The aim of this study was to investigate the reproducibility of functional connectivity and graph-based measures derived from weighted phase lag index functional connectivity matrices of resting-state MEG data. This is necessary because low reproducibility potentially undermines the clinical relevance and statistical power of network measures in applied neuroscience.

As discussed earlier, to the best of the author's knowledge, three non-invasive high temporal resolution studies have previously investigated the reproducibility of network measures. Using MEG and mutual information as a measure of functional connectivity both Deuker et al. (2009) and Jin et al. (2011) explored test-retest measures. Investigating global measures, Deuker et al. reported higher reproducibility for the n-back task memory task, first-order measures and low frequencies. In contrast, eyes-open rest, second-order measures and high frequency beta and gamma revealed comparably low ICC scores. The study by Jin et al. reported moderate ICC scores for of eyes-open for centrality measures. Generally, the reproducibility for eyes-closed and higher frequency gamma was lower by comparison. Most recently, using EEG and phase lag index as a measure of functional connectivity, Hardmeier

et al. (2014a) reported lower reproducibility for beta band and small-world index during eyes-closed rest.

In the current study, generally small-world index, and eyes-open rest yielded lower ICC scores compared to other network measures and eyes-closed rest respectively. Overall, though, with the exception of beta band and the small-world index, many network measures, particularly first-order measures during eyes-closed, yielded moderate-to-excellent ICC scores. These results supported all proposed hypotheses during eyes-closed but not eyes-open rest. Unless otherwise noted, due to poor, and at times negative ICC scores (See Figure 3.4) of network measures during eyes-open, the discussion below mainly focused on reproducibility of network measures derived from eyes-closed rest data

The higher reproducibility of measures during eyes-closed rest compared to eyes-open contradicts the findings reported by Jin et al. (2011), the only high temporal resolution study to have considered both eyes-open and eyes-closed conditions. In addition, during eyes-closed rest, second-order measures revealed lower ICC scores, compared to first-order network measures. This observation supports findings reported by Deuker et al. (2009), Hardmeier et al. (2014), and Jin et al. (2011). Interestingly, for first-order measures, results in Table 4 and Figure 3.4 revealed that the highest inter-session test-retest reproducibility was for the gamma band, a frequency that previous studies have shown to be the least reproducible. Finally, in the current study, beta band and second-order measures, particularly the small-world index, yielded poor-to-moderate ICC scores for eyes-closed rest. This is consistent with Deuker et al. and Hardmeier et al. These results are explained in detail below.

Reproducibility of functional coupling and graph-based network measures

With the exception of beta band and small-world index, network graph metrics were sufficiently reproducible on two repeated occasions. Results reported in the current study suggested that underlying mechanisms in networks during eyes-closed rest reconfigure with little variance compared to those during eyes-open state. Marx et al (2003) and Xu et al. (2014) have previously explained that underlying functional network configurations often correspond to the external environment, typically modulated by direction of attention. Hence the observation of higher reproducibility during eyes-closed is most likely to be associated with this type of rest being experimentally a more controlled state compared to eyes-open rest. Furthermore, it has previously been proposed that the low ICC scores for network measures during the eyes-open condition could be the result of the signal-to-noise ratio

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being negatively affected by alpha suppression and by eye movements such as blinking during eyes-open rest (Deuker et al., 2009).

Metric-order based reproducibility

Within eyes-closed, second-order measures yielded showed lower IC scores in higher frequency beta and gamma bands. It has been proposed that the low reproducibility of second-order metrics is most likely to be explained by increased 'variance' associated with being computed from more than one graph metric property (Deuker et al., 2009). Moreover, with the exception of the beta band, the reproducibility of the small-world index, a measure computed from four properties, was lower than that for normalised measures, which are computed from two network properties. Increased variance is therefore a plausible explanation.

Frequency-dependent test-retest reproducibility

Considering all the network measures explored, low frequency oscillations generally revealed higher reproducibility. Previous studies have reported network topology differences in low and fast neural oscillations (Deuker et al., 2009; Jin et al., 2011; Liang et al., 2012), which are often associated with cognition. Explaining the higher reproducibility of low frequencies, Bassett and Bullmore (2006), and Honey et al. (2007) proposed that low frequency band networks are more anatomically constrained. From a physiological function standpoint, this implies that network measures in dynamically less reconfiguring systems will be more robust across repeated measurements (Deuker et al., 2009). In addition, de Pasquale et al. (2010) proposed that fast oscillations in brain networks are less stable and more sensitive to network reorganisation due to their involvement in mental activities. It has been proposed that dynamically reconfiguring systems in beta and gamma frequencies help sustain higher cognitive functions and perceptions (Deuker et al. 2009). These arguments are in line with those of Engel and Fries (2010), who proposed that fast rhythms represent states of increased arousal. It therefore appears that compared to low frequencies, the underlying mechanisms within beta band networks are highly susceptible to re-configurations.

It is not clear why the network reproducibility of network measures in the beta band in both the current study and a previous task-based study (Deuker et al., 2009) was significantly lower. In considering the potential functional role of different frequencies, beta band oscillations remain to be understood (Engel & Fries, 2010). The authors proposed that

coupling in beta band is involved in maintaining current states, both sensory-motor and cognitive. Alongside this, studies have shown that correlations between regions/nodes of default mode networks (Brookes et al., 2011; de Pasquale et al., 2012) and motor networks (Brookes et al., 2011) are in fact strongest in the beta band. Elsewhere, Bassett et al. (2009) has argued that beta band activity and not gamma band activity is important for the co-ordinating properties of functional connections. This is supported by results in Figure 3.4 of the current study that showed that beta oscillation for long-distance functional connections (i.e. first-order path-length) was generally higher compared to other network measures within this frequency.

Comparing results in the current study with previous sensor-level literature

The discrepancy in results between the current study (with regards to the reproducibility of the different resting-states, and high frequency gamma) and studies by Deuker et al. (2009), Hardmeier et al. (2014), Jin et al. (2011), may be associated with methodological differences, including the type of resting state investigated, the frequencies considered, measure used to estimate functional couplings, and network properties explored.

First, both Deuker et al. (2009) and Jin et al. (2011) used mutual information to estimate functional connectivity, while the current study applied the phase lag index to estimate network functional connectivity. It is possible that the two measures of functional connectivity quantify different aspects of coupling information (Hardmeier et al., 2014a). A recent review exploring the effects of processing approaches on network reproducibility, mostly in fMRI data, found that network topologies varied depending on the measure applied to estimate functional connectivity (Telesford et al., 2013).

Second, Deuker et al. (2009) investigated the reproducibility of global metrics, derived from binary networks. Although they are often used in the fMRI community, the generation of unweighted binary graphs, as discussed earlier, is not fully understood (Stam and Reijneveld, 2007; Schwarz and McGonigle, 2011). The lack of standardized thresholds often prompts researchers to use arbitrary values to generate edges of 0 and 1 values. This may explain why a comparison of graph metric reproducibility derived from both binary/unweighted and weighted networks (Wang et al., 2011) showed higher reproducibility for metrics computed from weighted networks. This is most likely to be due to weighted networks being better representations of 'real networks' because they retain all the original information in the correlation matrices, compared to unweighted binary networks (Jin et al., 2011; Schwarz & McGonigle, 2011; Stam & Reijneveld, 2007).

Third, although the study by Jin et al (2011) explored graph metric reproducibility of weighted networks, like the current study, Jin et al. focused on nodal centrality measures and not global network metrics. Currently there is no study that has attempted to investigate the reproducibility of nodal centrality measures. As such, it is not clear which measures (i.e. nodal centrality or global measures) are more robust. Hence, the differences in results possibly arise from differences in topological properties evaluated in the two studies. For future purposes, the reproducibility of the two types would need to be investigated.

Finally, the low ICC scores for beta band in eyes-closed rest are consistent with Hardmeier et al. (2014). However, higher reproducibility of gamma, especially for first-order metrics, contradicts Deuker et al. A more likely explanation of higher gamma band reproducibility probably lies with the methodological inconsistencies discussed above. Although the study by Hardmeier et al. (2014), which applied a similar measure of functional connectivity, could shed light on this argument, the researchers did not investigate gamma band reproducibility. The authors explained that this frequency is highly sensitive to muscle artefacts. While this is a possibility (See Muthukumaraswamy, 2013 for a review), and according to Whitham et al. (2007, 2008) may at times also spread into the beta band, Muthukumaraswamy (2013), explains that 'steady-like' paradigms (much like the ones explored in the current study), are not as affected by muscle artefact harmonics in high frequencies, as the task-based paradigms. Therefor while the results contradict those reported by Jin et al. and Deuker et al, it is maintained that these differences are related to methodological approaches.

Methodological considerations

The results reported in the current study were all computed at sensor-level. This limits the certainty with which one is able to identify the sources of the spontaneous functional activity associated with different resting conditions and frequency bands. In addition, whilst the intraclass correlation coefficient is the standard statistical method for assessing test-retest reproducibility in network science, according to Telesford et al. (2013), this measure is dependent on population homogeneity. Hence, it is possible that outliers in functional network data from some participants may have affected overall observed inter-session reproducibility. Future studies would benefit from comparing performance of the intraclass correlation coefficient with other test-retest reproducibility statistics that are not dependent on the distribution such as the coefficient of variation (CoV), and Bland-Altman plots.

Another limitation concerns the use of phase lag index to estimate functional coupling. This measure is insensitive to the effects of volume conduction that arise from excluding

instantaneous zero-lag contributions. However, zero phase lag connectivity does exist in the brain, and may reflect either volume conduction, or true phase lag brain connectivity (Chawla, Friston, & Lumer, 2001). As a result, there is a possibility that using phase lag index to estimate functional coupling has the effect of potentially excluding real brain connectivity.

Finally, participants' resting-states, both before and during test sessions, were not taken into consideration. According to both Damoiseaux et al. (2006), and Diaz et al. (2013) resting-state is characterised by several dimensions (e.g. sleepiness, comfort, and somatic awareness), that are associated with cognition. Hence, it possible that a change in some of these dimensions in some or all individuals during the second recording session e.g. sleep deprivation, may have affected overall reproducibility of networks measures. Future studies will benefit from documenting participants' resting-state profiles (i.e. thoughts and feelings) before and after experiments, using self-report scales such as the Amsterdam Resting-State Questionnaire (ARSQ), a newly developed scale (Diaz et al., 2013, 2014).

3.6 Conclusion

In conclusion, this study was the first MEG study to investigate the reproducibility of functional connectivity and graph measures, based on phase lag index weighted matrices derived from resting-state MEG data. Results revealed that at the sensor-level, estimated reproducibility is influenced by resting-state, frequency band, and graph metric that is being investigated. The results reported offered support for the continued application of eyes-closed resting-state graph-based network analysis, compared to eyes-open rest. Nearly all metrics (except small-world index, and beta band) evaluated in the eyes-closed condition, out-performed those in the eyes-open condition. This study therefore provided a quantified template, albeit with some acknowledged limitations, for the validation of graph theoretical network parameters, helping support their application as potential clinical biomarkers in subsequent chapters of this thesis, and applied neuroscience in general.

4. Developmental changes in the resting-state functional organisation of large-scale networks at the whole-brain level: a cross-sectional study

4.1. Chapter summary

Efficient information processing within and between specialised, but spatially distributed functional brain regions underpins the successful development of higher cognitive functions (Douw et al., 2011). In the present study, adopting a cross-sectional sampling approach, functional brain organisation was characterised using network analysis; and this was compared between children (aged 7-13 years) and adults (aged 20-35 years), as well as across a broad age range (7-57 years).

A comparison of network properties revealed that whilst children and adults showed small-world organisation at the global level, there were differences in network organisation properties. The effects of developmental changes in functional large-scale networks were found primarily in the beta and gamma bands. First, children's brains showed over-connectivity of whole-brain functional coupling and normalised characteristic path length. Second, leaf number and hierarchical organisation were significantly decreased in adults compared to children, within the gamma band.

Overall, the results from graph theoretical analysis and minimum spanning tree analysis suggest a shift during development from a random network organisation towards a more structured, hierarchical, and line-like network organisation in higher frequency beta and gamma oscillatory activity, that are indicative, with the development of more efficient integrated information processing in spatially distributed networks. Given that higher oscillatory frequencies appear to reveal age-related changes in functional network topology, they potentially hold promise as biological markers of progressive functional refinement and network integration through the process of development.

4.2. Introduction

4.2.1. Brain development during childhood and adolescence

Neuroimaging studies of the developing brain (Paus, Keshavan, & Giedd, 2008; Sowell, Thompson, Tessner, & Toga, 2001; Whitford et al., 2007) have been fundamental in understanding the maturation of the structure and function of the brain during childhood and adolescence. These studies have provided evidence to suggest that during childhood and adolescence changes in both the structure and function of brain regions are vital for the normal development of intellectual abilities, and for physical and behavioural maturation (Paus et al., 1999; Nagy, Westerberg, & Klingberg, 2005; Raz et al., 2005; Uhlhaas, Haenschel, Nikolic, & Singer, 2009). Majority of the studies investigating human brain maturation have mainly focused on structure-related changes occurring from childhood through to later life. Documented age-related changes include an overall brain volume reduction, changes in individual brain region volumes, an increase in total white matter volume, and a regional reduction in grey matter (Giedd et al., 1999; Paus et al., 1999; Sowell et al., 2003). It is generally agreed that human brain maturation-related changes in grey and white matter reflect two fundamental maturational processes: namely, myelination of axons and synaptic pruning (Huttenlocher, 1979; Paus et al., 2000, 2008; Sowell et al., 2001).

Developmental changes in grey and white matter volumes serve different functions (Paus et al., 2008). Decreased grey matter tissue is understood to result from natural elimination of excess synapses and neuropil (Purves & Lichtman, 1980), while increases in white matter volume are believed to index the ongoing myelination of spatial regional connections in the cortex (van Baal, Boosma, & de Geus, 2001). Myelin increases the speed of neuronal signalling. The general conclusion has been that changes in white and grey matter correspond with cellular maturation transitions from synaptic over-production to pruning at the neuronal level. (van Baal et al., 2001; Thatcher, 1994). Increases in white matter volume throughout childhood and adolescence underlie the greater connectivity reported in these groups (Giedd & Rapoport, 2010). Myelination and dendritic arborisation are additive and increase levels of white matter; synaptic pruning and apoptosis are subtractive and reduce levels of grey matter.

It is, however, important to highlight the fact that functional magnetic resonance imaging (fMRI) studies, such as those discussed above, have largely focused on developmental changes within structural brain regions. The results in such studies do not provide

information about how brain regions interact. This limitation has meant that the underlying neural dynamics of brain function remain largely unknown (Finn et al., 2014).

The high temporal resolution of magnetoencephalography (MEG) and electroencephalography (EEG) are currently well suited to methodologies for investigating the neural underpinnings of brain interactions. The discovery of strong correlations in brain regions during resting-states or task-free states (i.e. rest) (Raichle et al., 2001) has provided a powerful tool for investigating functional brain relationships through development, particularly in very young children and in older participants, as this minimises reliance upon active compliance, and task training demands (Fair et al., 2009).

Researchers have reported positive associations between cognition and efficient functional network organisation using resting-state paradigms (Douw et al., 2011). Recently, concepts from graph theory have been used to characterise functional networks derived from resting-state M/EEG (Bassett & Bullmore, 2006; Douw et al., 2011). Graph theoretical studies of brain development typically demonstrate a shift from a random organisational pattern towards a more structured network (i.e. more small world-like) as the human brain matures (Boersma et al., 2011; Fair et al., 2009; Smit et al., 2012; Wu et al., 2012). Altered or abnormal functional organisation has been linked to atypical development (Ahmadlou et al., 2012; Bos et al., 2014). As a result, it has been proposed that changes in the functional organisation of brain networks, such as a decrease in short-range connections, might more accurately reflect changes in cognitive abilities through the process of development (de Haan et al., 2012).

Disruption to the typical brain organisation has been proposed as underlying several developmental conditions, including attention deficit/hyperactivity disorder (Giedd & Rapoport, 2010) and developmental dyslexia (Finn et al., 2014): the two conditions investigated and reported on in chapters 5, and 6. Hence, to gain an insight into how the normal brain organisation develops, it is crucial to estimate functional brain connectivity and to characterise whole-brain organisation through the process of development. Brain network investigation of neurobiological changes occurring during development is fundamental to developing an improved understanding of atypical neural development and the emergence of psychopathology.

Surprisingly, studies of functional connectivity networks derived from resting-state functional magnetic resonance imaging (fMRI) data (Fair et al., 2008; Meunier, Achard, Morcom, & Bullmore, 2009; Supekar, Musen, & Menon, 2009) found no significant differences (between

children, typically 7 to 9 years, and adults ~ 19 and 31 years) in age-related changes in clustering, path length, or small-worldness. According to Boersma et al. (2009), structural imaging modalities measure brain activity at slow time scales, and as such are only able to capture gross changes in underlying structural networks, excluding more subtle developmental neuronal mechanisms that influence functional networks that are understood to be more sensitive to high resolution techniques.

As discussed earlier the first high temporal resolution imaging study was conducted by Micheloyannis et al. (2009), who investigated patterns of functional networks using resting-state EEG. Using synchronization likelihood (SL) to map functional connectivity and later to characterise network organisation, in children (8-12 years) and adults (21-26 years), the authors reported significantly higher synchronisation, and local clustering in children's brains in high frequencies in the beta and gamma band. The second study was conducted by Boersma et al. (2011). This longitudinal resting-state functional connectivity EEG study explored age-related network changes in children, first at age 5 and again at 7 years. The authors reported significantly decreased synchronisation and increased characteristic path length across all frequency bands, as well as increased clustering in the alpha band from 5 to 7 years. The results suggest that interaction within functional network undergoes changes that parallel development. The authors discuss the view that these changes suggest a formation of more mature network topologies that represents the normative trajectory of development from 5 to 7 years. It has been proposed that such changes are associated with decreases in grey matter volume, and reflect a pruning of cell bodies and synaptic connections (Huttenlocher, 1979; Paus et al., 2008; van Baal et al, 2001). These studies show a general trend towards decreased local clustering/segregation (Otte et al., 2015) and increased average shortest long-range connections/integration with development (Fair et al., 2009) typically in comparison samples of children (~7-14) and adults (~20-30).

Methodological issues in these studies

The results from these two studies however cannot be considered conclusive in the context of normative development from childhood to adulthood. This is because whilst Boersma et al. conducted a longitudinal study, it was primarily of developmental changes in large-scale networks in children aged 5 to 7 years. This may explain the observation of significant differences in global synchronisation and clustering across all frequency bands. EEG oscillations in young children age 5 and 7 are unlikely to correspond to later development. In contrast, the developmental study by Micheloyannis et al. focused on age- related changes in children and young adults, addressing both rest and task-based network organisation.

However, this study has two major limitations. These are first, that the measure used to estimate functional connectivity (i.e. synchronisation likelihood) is sensitive to cross-talk in neighbouring nodes, which many (Brookes, Woolrich, & Barnes, 2012; Hillebrand & Stam, 2014; Stam et al., 2007b) believe is a major problem in sensor-level (i.e. node/electrode level) data (See Chapter 2, section 2.6.4, for a detailed discussion of this problem). Second, the developmental changes in network organisation were reported using non-normalised graph measures, which have been shown to display bias between subject network comparisons (van Wijk, Stam, & Daffertshofer, 2010).

Non-normalised graph-based network measures are highly dependent on the number of nodes in a network and the network density (.e. the number of connections in a network, Stam et al., 2014; van Dellen et al., 2013), which have been shown to differ between participants. For instance, densities have been shown to change considerably with development (Gong et al., 2009). Hence, in childhood, where over-connectivity is prominent, results based on non-normalised clustering are likely to also show higher clustering. Therefore, normalisation of these measures is required (van Wijk et al., 2010). However although they are commonly used and reported on, normalisation metrics as alternative measures do not completely solve the problem (see van Wijk et al., 2010 for a discussion).

A solution to the problem of the above issues is to use the minimum spanning tree (Boersma et al., 2013; Olde Dubberlink et al., 2013; Stam et al., 2014). In a weighted and undirected graph *G*, a spanning tree is defined as a sub-graph *G* (i.e. tree) of loop-less connected vertices of *G*(Figure 6, Chapter 2) (Mares, 2008; Olde Dubbelink et al., 2013). A minimum spanning tree therefore is a spanning tree of a minimum total weight among several spanning trees (van Steen, 2010). A full discussion of minimum spanning tree is outside the scope of this chapter (for a comprehensive discussion of minimum spanning tree, including history, application and formulas, see Jackson & Read, 2010; Mares, 2008). Given that a minimum spanning tree connects all nodes without cycles (Figure 5, Chapter 2), obtained networks have an identical number of connections, facilitating unbiased comparison of functional brain networks, because only important connections are taken into account (van Diessen, Otte, Braun, Stam, & Jansen, 2014). As a computation tool, the application of minimum spanning tree analysis has proved useful in capturing group and state-based functional as well as the structural organisation of brain network activity. (Boersma et al., 2013; Demuru & Fraschini, 2013; Otte et al., 2015; Schoen et al., 2011; van Diessen et al., 2014).

Like graph theoretical analysis, measures obtained using minimum spanning tree analysis are useful in modelling sub-graph organisation (i.e. tree topology). Using different imaging techniques, data have been found to exhibit characteristics that are associated with efficient information processing. In a spanning tree sub-graph representation of weighted brain networks, tree node (leaves) correspond to a brain region, while the edge parallels the functional connectivity between brain regions. As discussed in Chapter 2, while describing spanning minimum spanning tree general characteristics, spanning trees exhibit organisation properties that can be interpreted as either line-like, star-like or as intermediate between these two extreme topologies (Tewarie et al., 2014, See Chapter 2, section 2.7.2., for a graphical representation of these topologies). A line-like minimum spanning tree topology is understood to correspond to a more regular network, whilst a star-like network corresponds to a random network configuration (Stam et al., 2014).

Recently, in the first, and so far unique, high temporal resolution study using EEG, Boersma et al. (2013) characterised the age-related changes of the whole-brain network topological organisation. Using data previously reported by Boersma et al. (2011), the researchers reported a significantly increased minimum spanning tree diameter (i.e. the longest distance connecting any two nodes on a tree), and eccentricity (importance of a node), decreased leaf number (number of nodes with links), and hierarchy (measure of hierarchy organisation of a tree) within the alpha band across development (i.e. longitudinally from 5 to 7 years).

The authors propose that the above results suggest a shift with development towards a more elongated, decentralised, or line-like network topology. This study demonstrates that minimum spanning tree analysis, an unbiased application for characterising complex networks, is able to capture and discriminate between age-related changes in underlying neural networks. Given that minimum spanning tree measures are mainly concerned with global network topological organisation, the normal developmental topological trajectory would be expected to correspond to the results reported on an age-related reorganisation of long-distance functional connections. Furthermore, in the context of neurodevelopment, given that the minimum spanning tree is concerned with core weighted functional connections it is likely that with development, higher frequencies are likely to be crucial in terms of complex organisation of whole-brain connectivity, given their role in higher order cognitive functions.

It is, however, important to highlight that although weighted connections are bias-free, and the fact that weighted connections in the original network that result in loops during tree construction are discarded, the direct quantification of local organisation using minimum

spanning network analysis is challenging (Tewarie et al., 2014). This means that on its own, the application of minimum spanning tree analysis to characterise the functional organisation of large-scale brain networks is limited in giving information about the local organisation of large-scale networks.

In addition, minimum spanning tree analysis is a relatively novel approach; and not much is known about what its measures imply in terms of neurodevelopment. This, and the fact that more is known about the link between conventional graph theoretical measures and cognitive functioning, as well as behavioural functioning, explains why the studies in Chapters 4, 5, and 6 used minimum spanning tree metrics along with conventional or classical graph theoretical measures.

<u>Planned analysis</u>

Note that although 57 participants were recruited for this study, the age distribution was not homogeneous. Results in Figure 4.4 revealed that there were very few participants in the age-ranges 16 to 19 and 37-57. Hence to obtain insights into age-related changes in organisation of functional networks, networks were initially constructed for two age ranges (7-13 and 20-30 years), based on previous studies (Fair et al., 2009; Micheloyannis et al., 2009; Smit et al., 2012; Otte et al., 2015). The two age-ranges were chosen because these clusters had sufficient number of participants compared to other age-ranges. To then assess the longitudinal developmental changes, functional connectivity and network parameters were assessed linearly across a broad age-range.

<u>Hypotheses</u>

1. Children will show a more random topological organisation of functional networks compared to adults.
2. Local clustering (i.e. short-range/local functional connections), will be significantly greater in children, while long-distance functional connections will be greater in adults.
3. More decentralised line-like minimum spanning tree topology will be present in adults, corresponding to a shift towards less random and more efficient network configuration.
4. Efficiency in information processing in short and long-distance functional connections will correlate with broad measures of behavioural problems

5. Children will show a less efficient hierarchical topological organisation compared to adults

4.3. Method

4.3.1 Participants

Typically, developing children and adolescents were recruited through advertisements placed in the Aston University newsletter and Think-Tank Birmingham Science Museum, and they received an Amazon voucher for their participation. This sample was recruited as gender and chronological age (CA) matched controls, for comparison to children with a diagnosis of ADHD (Chapter 5) and those with learning difficulties (Chapter 6).The inclusion criteria for children were their ability to assent to testing, age between 7:0-17 years, and attendance in a mainstream educational school.

The adult sample was recruited from the student and staff population at Aston University through advertisements in the University newsletter, and through the undergraduate 'Research Participation Schema'. Students were awarded course credits for their participation. The inclusion criteria for healthy adults were the ability to consent to testing, no history of neurological impairment, and being aged between 18-59 years. All participants, children and adults, had normal or corrected to normal vision.

31 children and 41 adult subjects participated in the two elements of this study. Nine children and seven adults were excluded from further analysis due to: (1) disclosure of a diagnosis of dyslexia (i.e. three children and one adult), ADHD (one adult), stated on the behaviour functioning questionnaires; and (2) excessive artifacts in MEG signal, resulting in a failure to identify at least four artefact-free MEG epochs (six children and five adults). Subsequently, the final sample consisted of 56 participants, comprising 22 children aged between 7 and 17 years (M = 11.8, SD = 3.2; 2 left, 20 right handed: and 11 boys, 11 girls); and 34 adults aged between 18 and 57 years (M = 34.2, SD = 1.9; 8 left, 26 right handed; and 15 males and 19 females).

4.3.2. MEG recording protocol

Data were acquired continuously using procedures described in Chapter 3. Briefly, MEG recordings were acquired during resting-state (RS) conditions. For the eyes-open (EO) state,

subjects were instructed to keep their eye gaze fixed on a white cross projected centrally on a black screen and to try not to blink excessively. During the eyes-closed (EC) RS, they were asked to rest quietly with their eyes closed and to try not to move for the duration of the scans. Note that all data reported in this study refer to MEG time-series acquired only during EC, as nearly all graph metrics derived from the EO state (See Chapter 3, Figure 3.4) yielded lower ICC scores).

Due to system-based mechanical and electrical issues, including trapped flux, and damage, five sensors were disabled during MEG recordings for ten participants. Given the documented restriction in comparing networks of varying numbers of nodes (Stam et al., 2014), five sensors, roughly corresponding to those that were damaged, were excluded from all further analysis for all participants. Hence, unless otherwise stated, all networks presented in this study were constructed from 97 nodes (i.e. magnetometer sensors), represented by the data from spatially separate magnetometers).

4.3.3. Data pre-processing

Pre-processing steps are described in Chapter 3. Briefly, noise and artefact suppression was achieved with the temporal signal-space separation (tSSS) Taulu & Hari, 2009), with movement compensation implemented in the MaxFilter software (Elekta Neuromag). Following the MaxFilter process and a visual inspection of data, four artefact-free epochs (10 s, 4096 samples) were obtained and segmented for each subject, in line with Douw et al. (2011).They were converted to ASCII files for further computation using the BrainWave software (Stam; http://home.kpn.nl/stam7883/brainwave.html) version 0.9.116. All further computation was estimated per epoch and frequency band. Results from each epoch in different frequency bands were subsequently averaged for each person, and later for each age group (for the first planned analysis) and entire sample (for the second planned analysis).

Spectral power

Raw MEG signals were converted into the frequency domain using a Fast Fourier Transformation (FFT) implemented in the BrainWave software (Resolution: 1/4=0.25 Hz). For each epoch, mean frequency-based power was computed for each scalp magnetometer. PLI measures, graph and tree network metrics were computed per epoch and subsequently averaged per subject within frequency bands of interest.

Estimating functional connectivity (FC) using Phase Lag Index PLI)

Cross-correlations in classic frequency bands were digitally filtered offline in the commonly adopted ranges of theta (4-8 Hz), lower alpha (8-10 Hz), upper alpha (10-13 Hz), beta (13-30 Hz), and gamma (30-45 Hz), in line with both software recommendations and previous studies (Dimitriadis et al., 2013; Micheloyannis et al. 2009). The alpha frequency was evaluated in the two commonly applied sub-bands of lower alpha/alpha1 and upper alpha/alpha2. This differentiation is in line with studies that show different neural activity in the two sub-bands (Klimesch, 1999). The FC between MEG time series was estimated using the phase lag index (PLI), an FC measure that insensitive to the confounding effects of volume conduction (Stam et al., 2007b) compared to traditional FC measures such as coherence and synchronisation likelihood (see Chapter 3 for a description of PLI computation).

Graph theoretical network analysis

All network analysis, including FFT, FC, and network computations were performed using the BrainWave package. In computed networks, magnetometer sensors represent nodes while associations (estimated using PLI) between the sensors form the links/edges. This resulted in a 97 x 97 connectivity matrix for each epoch and frequency range. To enable independent network comparison across studies, graph measures derived from the original networks were compared to the average of 50 random networks, a process known as normalisation (see Chapter 2, section 2.7.1., for a detailed review), as previously described by Stam et al. (2009). For this reason (as set out in Chapter 2) the main graph-based findings reported in this thesis were those relating to normalised clustering coefficient (gamma) and path length (lambda), that correspond to short-range (local) and long-distance (global) functional connections respectively.

Minimum spanning tree network analysis

Using the BrainWave software, for each participant's graph (consisting of 97 nodes), a minimum spanning tree (for each epoch and frequency band of interest) was computed using Kruskal's algorithm (Kruskal, 1956) (See Chapter 2, section 2.7.2., for a discussion of Kruskal's algorithm). Researchers including van Dellen et al. (2013), Boersma et al. (2013), and Tewarie et al. (2014) have previously described this procedure. Minimum spanning tree topology was quantified with four metrics including eccentricity, diameter, hierarchy, and tree number. These are discussed in detail in Chapter 2, section 2.7.2.

All statistical analysis was computed using SPSS version 21. Most measures reported in the study had non-normal distributions (Kolmogorov-Smirnov test, p<.05). Transformations using both log and square root did not sufficiently improve data distribution. Subsequently, non-parametric analyses and specific Mann-Whitney tests statistical analyses were performed to compare network differences between children and adults. For the analysis comparing large-scale functional networks across a broad age range, Spearman's rank correlation coefficients (r_s) were computed in assessing the relationship between network parameters and development. Spearman's rank correlation coefficients were also used to assess relationships between network parameters and behavioural measures. All analyses were computed separately for each frequency band.

4.4. Results

4.4.1. Network analysis 1: the organisation and development of functional brain networks in neurotypical children and adults

All analysis computed in this first part of the study explored age-related changes in the topological organisation of functional brain networks in children (aged 7-13 years) and adults (aged 20-35 years). Results in Table 4.1 illustrates the demographic characteristics for two age-groups, i.e. children (age range 7-13, $M = 10.5$, $SD = 2.1$ years: 7 boys and 10 girls) and adults (age range 20-35 years, $M = 26.7$ $SD = 3.8$ years: 6 males and 13 females). To measure cognitive and behaviour functioning, assessments, age-appropriate group of Wechsler scales and the Achenbach System of Empirically Based Assessment (ASEBA) questionnaires was administered. The former was used to derive verbal and performance ability, while the latter was used derive measures of attention, internalized, and externalised behaviour functioning. (See Chapter 2 for a description of the standard procedure for administering both psychometric and adaptive behaviour assessments). Psychometric test scores were converted to scaled scores to standardise the data across normed age groups ($M = 10$, $SD = 3$), as per the standard recommendations of Wechsler instructions. Similarly, adaptive behaviour scores were converted to standard T scores, based on norms of gender, age, and type of informant. T scores < 65 are considered to be in the normal range (Achenbach, McConaughy, & Rescorla, 2011).

Psychometric assessments were only administered to children and not adults. As stated in Chapter 2, in instances where the child was not able to complete all four subtests (i.e. similarities, vocabulary, matrix reasoning, and block design), the similarities, and matrix reasoning subtest were administered. For the behaviour test assessment, the focus was on the three behaviour problem scores: namely, attention, internalising and externalising. The internalising problems summarise the scores of the anxious/depression, withdrawn/depressed and somatic complaints scores, while the internalised problems summarise scores of the aggressive and rule-breaking behaviour problems.

Psychometric data were available for 14 of the 17 child participants for the core subscales of verbal (i.e. similarities) and performance abilities (i.e. matrix reasoning), while behaviour data were available for 16 of the 17 children, and 17 of the 19 adults. All the children scored in the average range of verbal and performance reasoning scales, with scores in the range of the normative averages for standardised samples. Similarly, on the behaviour problem scales, child and adult subjects had low scores.

However, subjects with T scores ≥ 63 were not excluded from further analysis. This was because community studies often demonstrate the presence of clinical symptoms in healthy individuals especially when behaviour is assessed using a dimensional construct (Mazefsky, Anderson, Conner, & Minshew, 2011; Hutchison et al., 2013; Tan, Dedrick, & Marfo, 2007). T-scores for both children and adults were very low (i.e. closer to 50 and 65), indicating low levels of behaviour problems. In the current study, only the relationship with the behaviour checklists was investigated. This is because these were acquired from both adults and children. See Chapter 5 for the correlations between cognitive ability and network topological organisation.

Table 4. 1 Demographic characteristics

Measure	Children (7-13)	Adults (20-35)	
	Mean (*SD*)	Mean (*SD*)	*p*
N	17	19	
Gender (M:F)	7:10	6:13	
Age in years	10.5 (2.1)	26.7 (3.8)	
Handedness (R:L)	16:1	14:5	
Years of education	9 (2)	23 (2):	
Verbal IQ (SI)	12.7 (2.6)	*NA*	
Non-verbal IQ (MR)	11.2 (2.1)	*NA*	
Int problems	50.1 (7.6)	52.2 (10.1)	*NS*
Ext problems	45.9 (9.1)	46.4 (10.3)	*NS*
Att problems	53.0 (3.8)	53.5 (4.0)	*NS*

Participant demographic characteristics for the children vs. adult subjects. Key; M = male; F = female; SI = similarities; MR = matrix reasoning; NA = not applicable; NS = not significant; Int = internalising; and Ext = externalising, and Att = attention. IQ scores were not available for 3 children, due to difficulties encountered during the experiment, including children displaying physical signs of tiredness, or simply refusing to participate.

4.4.1.1. Developmental changes in whole-brain functional connectivity network

Results of whole-brain functional connectivity Figure 4.3 Illustrates the untransformed mean global functional coupling (phase-lag-index) in children and adults. Mann-Whitney tests were used to investigate age-related changes in network measures (Table 2). Medians (*Mdn*) and Cohen's *d* effect sizes were reported. Adults showed a decrease in mean phase-lag-index (See Figure 4.2) in the beta band (*Mdn* = .09), *z* = -2.33, *p* = .019, *d* = .62. No differences were observed in any other frequency range.

Table 4. 2 Group differences in global whole-brain functional coupling (assessed using the phase lag index) across frequency bands of interest

	Children n = 17	Adults n =19	*p*
Theta	.16[.05, .17]	.16[.15, .17]	*NS*
Alpha1	.25[.23, .26]	.24[.23, .25]	*NS*
Alpha2	.19[.19, .20]	.20[.19, .21]	*NS*
Beta	.10[.10, .09]	.09[.08, .08]	*
Gamma	.09[.09, .09]	.09[.09, .09]	*NS*

The results of Mann-Whitney U- test, *p < .05, used to assess the mean phase-lag-index values for each frequency band. Values are medians with Bca bootstrap 95% confidence intervals (CIs) in square brackets. Significant differences were observed for the beta band and no other frequency range. Key, NS = not significant.

For age-related changes in global topological functional coupling strength, the results in Table 2 reveal statistically stronger whole brain functional connectivity in children compared to adults, for the beta band. A visual articulation of the results in Table 4.2 is presented in Figure 4.1.

Figure 4. 1 Mean group differences in whole-brain functional connectivity
Mean group differences in global functional coupling strength estimated using the phase lag index, across different frequency bands of interest, in children and adults during eyes-close rest. Results reveal significantly decreased global functional coupling in the beta band in adults. Error bars are ± 2 standard error. * p < .05.

4.4.1.2. Age-related changes in the global topological organisation of functional networks: a graph theoretical and minimum spanning tree network analysis

As discussed in Chapter 2, non-normalised graph theoretical analysis-based measures (i.e. clustering and path length) do not provide genuine estimates of network measures. As a result, only computations using normalised graph-based measures and the minimum spanning tree network were reported. All networks, including those of children, revealed small-worldness, suggesting a non-random functional organisation, which does not support the hypothesis made about networks in children in the current study. The results in Table 4. 3 revealed that normalised characteristic path length (lambda) was significantly high in children (*Mdn* = 1.03), *z* = -2.06, *p* = .04, *d* = .54)), compared to adults within the beta band. No other statistically significant graph-based results were observed. For minimum spanning tree analysis, results within the gamma band revealed significantly higher leaf number (*Mdn*

= 0.54), *z* = -2.01, *p* = .04, *d* = .30)), as well as a higher hierarchy in children (*Mdn* = 0.40), *z* = -1.97, *p* = .05, *d* = .78)), compared to adults, (See Table 4.3).

Table 4. 3 Results of weighted graph theory and minimum spanning tree network analysis

		Theta *Mdn* [95% CI]		Alpha1 *Mdn* [95% CI]		Alpha2 *Mdn* [95% CI]		Beta *Mdn* [95% CI]		Gamma *Mdn* [95% CI]	
Gamma	Children	1.05	[1.05, 1.05]	1.08	[1.07, 1.08]	1.06	[1.05, 1.07]	1.05	[1.05, 1.06]	1.04	[1.04, 1.05]
	Adults	1.05	[1.05, 1.05]	1.07	[1.06, 1.08]	1.06	[1.05, 1.07]	1.05	[1.05, 1.05]	1.04	[1.03, 1.05]
	P	NS		NS		NS		NS		NS	
Lambda	Children	1.03	[1.02, 1.03]	1.05	[1.05, 1.06]	1.04	[1.03, 1.05]	1.04	[1.03, 1.04]	1.02	[1.01, 1.03]
	Adults	1.03	[1.03, 1.03]	1.04	[1.04, 1.04]	1.04	[1.03, 1.04]	1.03	[1.02, 1.03]	1.02	[1.02, 1.03]
	P	NS		NS		NS		*		NS	
Sigma	Children	1.02	[1.02, 1.02]	1.03	[1.02, 1.03]	1.02	[1.02, 1.02]	1.02	[1.02, 1.02]	1.02	[1.02, 1.03]
	Adults	1.02	[1.02, 1.02]	1.03	[1.03, 1.03]	1.02	[1.03, 1.03]	1.02	[1.02, 1.03]	1.02	[1.02, 1.02]
	P	NS		NS		NS		NS		NS	
Eccentricity	Children	0.14	[.13, .14]	0.13	[.13, .14]	0.14	[.13, .14]	0.13	[.12, .13]	0.14	[.13, .14]
	Adults	0.14	[.13, .14]	0.13	[.13, .14]	0.13	[.13, .14]	0.13	[.13, .14]	0.14	[.13, .14]
	P	NS		NS		NS		NS		NS	
Diameter	Children	0.19	[.18, .19]	0.18	[.17, .18]	0.18	[.18, .19]	0.17	[.17, .18]	0.19	[.17, .19]
	Adults	0.18	[.17, .19]	0.18	[.17, .18]	0.18	[.17, .19]	0.18	[.17, .18]	0.18	[.18, .19]
	P	NS		NS		NS		NS		NS	
Leaf Number	Children	0.54	[.53, .55]	0.58	[.57, .60]	0.55	[.53, .55]	0.54	[.53, .55]	0.54	[.53, .54]
	Adults	0.55	[.55, .56]	0.57	[.56, .57]	0.55	[.54, .58]	0.54	[.54, .54]	0.52	[.52, .54]
	P	NS		NS		NS		NS		*	
Hierarchy	Children	0.38	[.38, .39]	0.4	[.40, .4]1	0.39	[.38, .40]	0.38	[.37, .39]	0.38	[.38, .39]
	Adults	0.39	[.38, .39]	0.41	.40, .41]	0.4	[.40, .41]	0.38	[.38, .40]	0.37	[.36, .38]
	P	NS		NS		NS		NS		*	

Functional network (assessed using graph measures) and sub-network (assessed using minimum spanning tree measures) medians and Bca bootstrap 95% CIs (represented in square brackets). Group differences were assessed with non-parametric testing (Mann-Whitney U-test, *p< .05). Key: NS: not significant, gamma: normalised clustering coefficient, lambda: normalised characteristic path length, and sigma: small-world index. Networks in both children and adults revealed small-world topology. Normalised path length (lambda) was significantly different between the two groups, as was minimum spanning tree leaf number and hierarchy.

Significant graph theoretical-based measures and minimum spanning tree results in Table 4.3 are visually represented in Figure 4.2. Both types of network analysis were highly influenced by development-related changes in high frequency beta and gamma respectively. Development significantly reduced the long shortest paths between any two nodes (lambda), number of leaves, and hierarchy of trees.

Figure 4. 2 Comparison of network topology measures for children and adults
Age-related changes in the network topological organisation of functional brain networks using A) graph-based normalised path length, B) minimum spanning tree leaf number, and C) hierarchy. Only significantly altered network measures across frequency bands of interest were represented. Error bars are +/- 2 standard error. Age-related network topological organisation significantly differed in the high frequency beta and gamma band. *p < .05.

<u>Relationships between age-related changes in the topological organisation of functional networks and adaptive behaviour checklist</u>

Findings from Table 4.1 showed that there was no statistically significant difference between children and adults for attention, internalising, and behaviour problem scores. Hence frequency-based investigations of correlations between functional networks and dimension-based measures of adaptive and problem behaviours were computed across the entire sample cohort, using a Bonferroni corrected α of .0024 (.05/21 corrections) as a means of minimising Type 1 errors. No statistically significant correlations were observed between network measures and behaviour problem scores for the theta and alpha1 band.

Table 4. 4 Correlations between network measures and adaptive behaviour for children vs. adults

(n = 33)	Theta							Alpha1							Alpha2							Beta							Gamma						
	Gamma	Lambda	Small-world	Eccentricity	Diameter	Leaf number	Hierarchy	Gamma	Lambda	Small-world	Eccentricity	Diameter	Leaf number	Hierarchy	Gamma	Lambda	Small-world	Eccentricity	Diameter	Leaf number	Hierarchy	Gamma	Lambda	Small-world	Eccentricity	Diameter	Leaf number	Hierarchy	Gamma	Lambda	Small-world	Eccentricity	Diameter	Leaf number	Hierarchy
Att	.27 (.13)	.06 (73)	.17 (.35)	.16 (.37)	.12(.52)	.30(.10)	.32(.07)	.14(.45)	.08(.63)	.06(.74)	-.25(.16)	-.16(.39)	.30(.09)	.29(.11)	.07(.68)	.10(.60)	-.15(.40)	-.07(.71)	-.10(.59)	-.27(.12)	-.34(.06)	-.21(.25)	.10(.56)	-.30(.09)	.08(.66)	.10(.58)	-.42(.02)*	-.16(.36)	.02(.92)	.01(.95)	.10(.58)	-.10(.56)	-.07(.69)	.37(.03)*	.30(.09)
Int	.15 (.41)	.04 (.84)	-.00 (.99)	.01(.97)	.02(.92)	.21(.25)	.22(.23)	-.19(.29)	-.24.19)	.05(.77)	-.17(.34)	-.19(.30)	.10(.60)	.11(.54)	.03(.87)	.01(.95)	-.03(.85)	-.02(.90)	-.08(.64)	-.20(.26)	-.20(.27)	-.21(.25)	-.11(.54)	.06(.76)	-.05(.79)	-.08(.63)	-.27(.12)	-.20(.29)	-.18(.33)	-.17(.34)	.02(.91)	.02(.93)	-.01(.97)	-.13(.47)	.03(.88)
Ext	.14 (.43)	-.20 (.28)	.02 (.91)	.13(.47)	.07(.35)	.10(.61)	-.17(.35)	-.04(.85)	.06(.74)	-.07 (.71)	-.07(.71)	-.14(.44)	-.03(.88)	.14(.43)	-.18(.32)	-.29(.10)	.20(.28)	.16(.38)	.16(.38)	-.40(.04)*	-.25(.16)	-.05(.77)	.07(.70)	.02(.91)	-.32(.07)	-.33(.06)	-.10(.62)	-.10(.61)	.01(.96)	-.24(.18)	.35(.05)*	.18(.33)	.22(.22)	.13(.47)	.18(.33)

Spearman's rank correlation coefficients (r_s) for network measures and adaptive behaviour in frequency bands of interest. Key, Att = attention, Int = Internalising, and Ext = Externalising, = normalised clustering, lambda = normalised path length, and small-world = small-world index. *p <.05, **p <.0024 (Bonferroni-corrected α value).

For upper alpha and beta bands, the minimum spanning tree leaf number showed significant moderate negative correlations with externalising problem scores (r_s = -.40 [-.65, .03], p = .04, see Figure 4.3, top left scatter plot) and attention (r_s= -.42 [-.69, -.11], p = .02, see Figure 4.3, top right scatter plot) respectively. For the high frequency gamma band, graph-based small-world index and minimum spanning tree leaf number showed significant weak positive correlations with externalising (r_s= .35 [-.01, .61], p = .05, see Figure 4.3, bottom left scatter plot) and attention (r_s= .37 [.02, .67], p = .03, see Figure 4.3, bottom right scatter plot) respectively. These relationships were not statistically significant at the Bonferroni-corrected α level of .0024.

Figure 4. 3 Correlations between age-related functional network topological organisation and adaptive measure checklist

Spearman's correlations was statistically significant at *p<.05 but not at the Bonferroni-corrected α level of .0024. Top scatter plots correlations represent relations for the upper alpha and beta bands while the bottom scatter plots show relationships for the higher gamma band.

4.4.2. Analysis 2: organisation and development of large-scale functional brain networks across a broad age range

As stated earlier (See section on methodological issues in these studies, Aim 1) the second part of the analysis presented in the current study investigated functional brain networks related to normal ageing in a larger sample of 56 participants, aged 7 to 57 years (See Table 4. 5 for the demographic data).

Table 4. 5 Demographic data for the entire sample with adaptive behaviour scores

Measure	n = 56
	Mean (*SD*)
Gender (M:F)	26:30
Age in years	25.9 (14.2)
Handedness (R:L)	46:10
Attention problems	53.4 (4.3)
Internalised problems	49 (9.2)
Externalised problems	45.1 (9.7)

The demographic characteristics of the entire sample including children, teenagers, and adults. Key M:F = male:female, R:L = right:left.

4.4.2.1. Relationship between global functional network connectivity and healthy ageing

To investigate the effect of healthy ageing on whole brain functional coupling, Spearman's rank correlation coefficients were computed with age as a continuous variable. See Table 4.6 for the correlations between phase-lag-index (i.e. the measure of functional connectivity) across the frequency bands of interest. No significant relationship was observed between global functional connectivity and typical ageing.

Table 4. 6 Correlations between global functional connectivity and typical development

	Theta	Alpha1	Alpha2	Beta	Gamma
Mean Phase-lag-index	.13 (.35)	.13 (.36)	-.11 (.42)	-.24 (.07)	-.13 (.36)

*Results are Spearman's rank correlation coefficients (r_s), and *p<.05, across the frequency bands of interest. Global network functional connectivity showed a trend towards a weak negative correlation with ageing within the beta band.*

Results in Table 4. 6 revealed a trend towards a weak negative correlation between global functional connectivity and typical ageing for the beta band (r_s = -.24 [-.49, .05], *p* = .07, see Figure 4.4). This relationship was not significant at the α level of .05.

Figure 4. 4 Correlations between typical ageing and beta band whole-brain functional connectivity
Spearman's rank correlation coefficient showed a weak trend towards a negative relationship between typical ageing and global functional connectivity.

4.4.2.2. Relationship between changes in functional network topological organisation and healthy ageing

Associations between development and network architecture parameters were assessed with Spearman's bivariate correlation coefficients. Analysis was performed separately for each of the five frequency bands at a Bonferroni-corrected α value of .0071 to minimise Type 1 errors (Table 4.7). Within the beta band, normalised characteristic path length showed a significant weak negative correlation with age (r_s = -.32 [-.54, -.06], p = .02, see Figure 4.5). Also within the beta band, the small-world index showed a significant weak positive correlation with age (r_s = .35 [.12, .56], p = .01, see Figure 4.5). However, these correlations were not statistically significant at the Bonferroni-corrected α level of .0071.

Table 4. 7 Correlations between network/tree topological organisation and development across frequency bands of interest

	Theta							Alpha1							Alpha2							Beta							Gamma						
	Gamma	Lambda	Small-world	Eccentricity	Diameter	Leaf number	Hierarchy	Gamma	Lambda	Small-world	Eccentricity	Diameter	Leaf number	Hierarchy	Gamma	Lambda	Small-world	Eccentricity	Diameter	Leaf number	Hierarchy	Gamma	Lambda	Small-world	Eccentricity	Diameter	Leaf number	Hierarchy	Gamma	Lambda	Small-world	Eccentricity	Diameter	Leaf number	Hierarchy
Age	.09 (.53)	.03 (.81)	.06 (.68)	-.10 (.45)	-.14 (.32)	-.04 (.75)	.11 (.40)	-.17 (.21)	-.05 (.69)	-.17 (.21)	.13 (.34)	-.14 (.32)	-.10 (.45)	.03 (.82)	-.08 (.57)	-.10 (.46)	.06 (.67)	-.01 (.97)	-.03 (.83)	.00 (.98)	-.05 (.71)	.04 (.78)	-.32 (.02)*	.35 (.01)*	.08 (.54)	.06 (.69)	.18 (.19)	.11 (.40)	-.14 (.30)	-.19(.17)	.06 (.64)	-.03 (.82)	-.05 (.74)	-.15 (.26)	-.08 (.54)

Spearman's rank correlation coefficients (r_s) *p<.05, **p<.0071 (Bonferroni-corrected α value). Value are correlation and significance level. Key, gamma = normalised clustering, lambda = normalised path length, and small-world = small-world index.

Figure 4. 5 Correlations between age and beta band-based network architecture parameters

Spearman's rank correlations were statistically significant at p<.05 for both measures. However, both correlations were not statistically significant at the Bonferroni-corrected α level of .0071.

Correlation with adaptive behaviour scores across development in five frequency bands

Behaviour data were available for 45 of the 56 participants, justifying a correlation analysis of relationships between behaviour checklist and functional network topological parameters. For each frequency band, correlation computations were assessed on attention, and internalising and externalising behaviour problem scores, across the entire cohort and using a Bonferroni corrected α of .0024. For the lower alpha band, hierarchical organisation showed a weak positive correlation with attention problems scores (r_s = .34 [.06, .57], p = .02, Figure 4.6, top left). Within the upper alpha, leaf number showed weak negative correlations with internalising (r_s = -.34 [-.06, -.04], p = .02, see Figure 4.5, top middle) and externalising (r_s = -.30 [-.57, .00], p = .05, Figure 4.6, top right). Finally the beta band showed a weak and moderate negative correlation with normalised clustering coefficient (r_s =-.32 [-.60, -.04], p = .03, Figure 4.6, bottom left) and leaf number (r_s = -.40 [-.64, -.08], p = .01, Figure 4.6, bottom right) respectively. However, these correlations were not statistically significant at the Bonferroni-corrected α level of .0024.

Table 4. 8 Correlations between network measures and adaptive behaviour across the entire sample in frequency bands of interest

(n = 45)	Theta							Alpha1							Alpha2							Beta							Gamma						
	Gamma	Lambda	Small-world	Eccentricity	Diameter	Leaf number	Hierarchy	Gamma	Lambda	Small-world	Eccentricity	Diameter	Leaf number	Hierarchy	Gamma	Lambda	Small-world	Eccentricity	Diameter	Leaf number	Hierarchy	Gamma	Lambda	Small-world	Eccentricity	Diameter	Leaf number	Hierarchy	Gamma	Lambda	Small-world	Eccentricity	Diameter	Leaf number	Hierarchy
Att	.28 (.86)	-.10 (.45)	.16 (.29)	.04 (.82)	.03 (.84)	.21 (.18)	.16 (.31)	.19 (.20)	.14 (.37)	.12 (.43)	-.14 (.38)	-.06 (.68)	.27 (.07)	.34 (.02)*	.02 (.90)	.05 (.73)	-.13 (.41)	.11 (.49)	-.07 (.61)	-.27 (.07)	-.19 (.22)	-.32 (.03)*	-.04 (.78)	-.28 (.06)	.14 (.35)	.17 (.26)	-.40 (.01)*	-.20 (.18)	-.11 (.49)	-.12 (.45)	.25 (.16)	-.07 (.63)	-.06 (.72)	.19 (.22)	.07 (.66)
Int	-.06 (.71)	-.08 (.57)	-.05 (.76)	07 (.66)	.05 (.73)	.11 (.46)	.15 (.34)	-.09 (.55)	-.24 .11)	.14 (.35)	-.10 (.53)	-.12 (.42)	.08 (.59)	.22 (.14)	-.04 (.80)	-.05 (.77)	-.14 (.37)	.16 (.28)	.11 (.45)	-.34 (.02)*	-.07 (.66)	-.20 (.18)	-.14 (.37)	-.10 (.53)	-.00(.99)	-.05 (.77)	-.24 (.12)	-.16 (.30)	-.20 (.18)	-.16 (.31)	.06 (.73)	-.08 (.58)	-.09 (.56)	-.13 (.38)	-.13 (.40)
Ext	.01 (.97)	-.19 (.21)	.05 (.73)	.21 (.16)	.24 (.12)	-.07 (.64)	-.17 (.25)	.15 (.32)	.11 (.49)	.13 (.39)	-.18 (.24)	-.21 (.16)	.14 (.35)	.24 (.11)	-.01 (.93)	-.20 (.21)	.24 (.12)	.14 (.36)	.14 (.36)	-.30 (.05)*	-.13 (.40)	-.08 (.61)	.04 (.82)	-.04 (.82)	-.11 (.48)	-.10 (.06)	-.19 (.22)	-.04 (.80)	.05 (.75)	-.22 (.15)	.17 (.35)	.14 (.36)	.17 (.27)	.06 (.70)	.07 (.65)

Spearman's rank correlation coefficients (r_s) for network measures and adaptive behaviour in frequency bands of interest. *p <.05, **p <.0024 (Bonferroni-corrected α value). Behaviour problems are Att = attention, Int = Internalising, and Ext = Externalising, while normalised graph measures included gamma = normalised clustering, lambda = normalised path length, and small-world = small-world index.

Figure 4. 6 Correlations between adaptive behaviour and network architecture measures across development

Spearman's rank correlations were statistically significant at p<.05 but not at the Bonferroni-corrected α level of .0024.

4.5. Discussion

To the knowledge of the author, this is the first study to use graph theoretical measures along with minimum spanning tree analysis to investigate age-related changes in the functional organisation of large-scale brain networks. The effect of development was primarily found in the higher frequency beta and gamma bands. Generally, development significantly reduced the wiring distance between any two nodes in a network, the number of leaves and the hierarchical structure. It is however important to note that the effect of development disappeared when networks were explored across the broad age range. This was most likely to have been as a consequence of cross-sectional sampling and the diversity of changes occurring within different age-ranges.

<u>Developmental changes in complex large-scale whole-brain functional networks</u>

For the children vs. adults, analysis, the non-parametric Mann-Whitney test revealed significantly increased whole brain functional coupling strength in young children in the beta band (See Figure 4.1). Across a broad age-range (7-57 years), the Spearman's rank correlation revealed a weak negative relationship between whole brain functional coupling strength and neurotypical ageing (See Figure 4.4). Both results suggest that whole brain coupling strength decreased across development. This is consistent with results reported in the only other high temporal resolution study covering roughly the same age ranges as the current study, conducted by Micheloyannis et al. (2009).

Across a broad age-range, correlations in the beta band revealed a weak negative relationship between whole-brain functional connectivity and development. A closer visual inspection of these results (See Figure 4.4), revealed a correlation pattern of a U-curve shaped developmental trajectory. From ~ 37 years, the results suggest that whole-brain functional connectivity was no longer decreasing linearly.

Typically, with development, the formation of more localised functional connections parallels a reduction in global connectivity (Supekar et al., 2009). From the present study, it is proposed that increased whole-brain functional connectivity in childhood may operate as a compensatory mechanism, allowing for more wiring in higher frequencies that is necessary for cognitive experiences and challenges. Specifically, children may require higher global brain synchronisation to compensate for the less 'optimal' long-range connections, whose role in the high frequency beta band may be crucial to develop cognitive abilities.

Network organisation characterisation using graph theoretical analysis

With regard to children vs. adults groups, the Mann-Whitney test revealed that development significantly reduced the shortest distances between any two nodes (i.e. normalised characteristic path length). Similarly, across a broad age-range (7-57 years), the Spearman's rank correlation revealed a weak negative correlation between the shortest distances between any two nodes (lambda) and development (See Figure 4.5). In addition, Spearman's rank correlation revealed a weak positive correlation between small-world network topology and development (See Figure 4.5). However, these correlations were not statistically significant when a strict α level was applied. In the context of small-world network topology, information processing in short and long-distance functional connections is regarded as being 'economical' because it is achieved at minimum wiring cost, while still sustaining dynamic and complex processing (Bassett & Bullmore, 2006).

To recap, a network with high average clustering coefficient is perceived to be characterised by dense connected local network clusters, while one with low characteristic path length corresponds to short long-range distances between any two nodes in a network (Supekar et al., 2009; Bullmore, & Sporns, 2009). Together these two mechanisms support high specialisation (i.e. information processing in local network connectivity) and global integration (i.e. information processing in global network connectivity) (Bassett & Bullmore, 2006).

Discussion

Results in the current study therefore suggested that early childhood is associated with high levels of information processing within long-distance functional connections. Information processing in these connections is responsible for network integration of spatially distributed, but functionally linked brain regions. However, in the context of a small-world brain networks, topology is characterised by dense local clustering of neighbouring connections (in regions/nodes) and efficient path lengths between distant connections that results from fewer, but optimal longest paths between nodes (Bassett & Bullmore, 2006). According to Otte et al. (2015), higher shortest path length between any possible pair of nodes, (such as that observed in children in the beta band) suggests a less integrated and less efficient topology. The results reported in this study hence confirm that at sensor/node level, the organisation of brain functional networks in adults were more efficient in providing better support for distributed or integrated information processing. In other words, the results reflect a shift from a random network topology towards a more refined functionally efficient

organisation along with typical brain maturation. The results supported the hypothesis stating that with development, more structured and stronger long-range connections would be formed.

Biologically, it has been suggested that age-related changes in short and long-distance functional connections are highly influenced by synaptic pruning and myelination of axonal fibre tracks through the process of development (Kuhn, 2006; Hagmann et al., 2010; Whitford et al., 2007). Increased network performance is often linked to underlying neuronal migrations of white matter axonal myelination corresponding to axonal conduction (Thatcher et al., 1986) and to grey matter atrophy throughout development (Whitford et al., 2007). With maturation, the brain eliminates connections that are not used, while preserving and strengthening those connections associated with efficient information transfer (Wu et al., 2012). This is believed to result in a reduction of local connections in parallel with the formation of more specialised long-distance links (Boersma et al., 2011; Supekar et al., 2009). In the current study design, it was expected that the topological organisation of functional networks in children would be less efficiently organised. The significantly short average path length in adults suggests that within typical neurodevelopment, whole-brain over-connectivity in childhood is followed by synaptic pruning, resulting in more specialised but stronger and fewer long-distance functional connections.

Network organisation characterisation using Minimum spanning tree analysis

To test the hypothesis of increased network efficiency, while controlling for network comparison bias, minimum spanning tree analysis was applied to resting-state weighted matrices. Significant results were only found in the analysis investigating network topology in children vs. adults. Using Mann-Whitney tests, results were confined in the gamma band, where through development, the minimum spanning tree network topology of children was characterised by significantly lower leaf number and tree hierarchy (See Figure 4.2).

Discussion

As is the case with networks derived from graph theoretical analysis, the tree topological organisation too, must ensure efficient communication between all vertices (Olde Dubbelink et al., 2013). The results in the current study revealed a shift towards a more line-like organisation with development. Development from mid-childhood to adulthood was characterised by a significant reduction in the number of leaves and hierarchy. A shift towards a more line-like or decentralised topology is believed to reflect the weakening of

dense local connectivity in neighbouring nodes (Boersma et al., 2013). The authors discuss the view that such a topology represents increased cost-effective processing in long-distance connections (i.e. short path length between distantly connected nodes), that corresponds to a shift towards a more ordered/structured network configuration. Several studies have previously demonstrated that the standard hallmark of typical functional networks is the formation or development of fewer specialised distant connections and a weakening of local connections (Boersma et al., 2011; Fair et al., 2009; Smit et al., 2012; Wu et al., 2012). Hence, the minimum spanning tree results observed in the current study appear to confirm findings in classical network metrics. The functional role of these observations in a frequency specific context is discussed at a later stage.

Minimum spanning tree results discussed in the current study are partly consistent with Boersma et al. (2013), albeit in a different frequency band. The authors reported significantly increased diameter, eccentricity, and decreased leaf number and hierarchy in the alpha band, in relation to development from 5 to age 7. It is however important to note that the study conducted by Boersma et al. (2013), investigated developmental changes in very young children at 5 and again at 7 years. It should also be emphasised that unlike the current study, Boersma et al. studied networks in unconventional frequency ranges (i.e. theta: 4-6 Hz, alpha: 6-11 Hz, and beta: 11-25). The alpha band reported by these authors was averaged between theta and alpha, which may have increased the power within which minimum spanning trees were later constructed. In addition, this study revealed changes in eccentricity and diameters in relation to development. Such changes were not observed in the current study. It is likely that these two measures are not changing in the age-range studied here.

The role of higher frequency beta and gamma band neural synchrony in the development of functional networks

Several studies have shown that different frequency oscillations in large-scale networks reflect different brain connections (Siegel et al., 2012; Uhlhaas et al., 2006, 2008; von Stein & Sarnthein, 2000). In the current study, all significant age-related network changes, using both graph and minimum spanning tree analysis, were observed in high frequency beta and gamma bands. Researchers have highlighted that beta oscillations are crucial for optimal network performance in development, both during rest (Micheloyannis et al., 2009; Schafer et al., 2014) and during task performance (Siegel et al., 2012). These oscillations are crucial for the coordination of large-scale neural activity (Uhlhaas et al., 2008), which would explain

why long-distance functional connections were significantly more efficient in adults in the beta band.

Furthermore, data from Micheloyannis et al. (2009) revealed that within the beta band, healthy adults whose networks were characterised by lower whole brain synchronisation, and short average path length between distant electrodes/nodes, performed more efficiently on a cognitive task. Beta oscillations are traditionally associated with several cognitive skills, most prominently attention control (Schnitzler & Gross, 2005). Therefore, in the current study, where differences between the two groups were more sensitive in the beta band, they appear to demonstrate cognitively immature systems in children compared to adults.

With respect to minimum spanning tree analysis, the results presented in the current study suggest a shift towards a more line-like topology in the gamma band in adults, which has been interpreted as an indication of fewer long distance interactions across development in the gamma band. Gamma oscillatory activity has been implicated in top-down attention (Siegel et al., 2012) and high cognitive abilities (van den Heuvel et al., 2009). In task-based studies, gamma neural oscillations often shown to be associated with high-level information processing. Hence, it is reasonable to propose that a more decentralised network configuration or line-like topology corresponds to highly distributed information processing within such a topology, which may explain higher cognitive skills in neurotypical adults, as such functional connections reflect global network integration efficiency.

Relationship between topological organisation of functional brain networks and behaviour measures

There is increasing evidence to suggest development plays a crucial role in the re-configuration of functional networks, with more optimal topological organisation linked to cognitive and behavioural functions. Given that in this study cognitive ability was only assessed in children, its relationship with network measures was not addressed in this Chapter. See Chapter 5 for such computations. In the current part of the study, Spearman's bivariate correlation tests were carried out to explore the relationship between network measures and adaptive behaviour scales (i.e. attention, internalised, and externalised problems) in each frequency band.

However, as presented in the discussion, all reported correlations between network parameters and behavioural problems scales were not confirmed when a strict α level of .0024 was applied. In addition, the correlations were largely weak, with very few reaching

moderate strength. In brief, analyses of children and adults (See Figure 4.3) and across a broad age range (See Figure 4.6), in upper alpha and beta bands, showed moderately negative correlations between leaf number and externalising as well as attention. Visual representations in Figure 4.4 show that with the exception of one subject, all participants scored well under the clinical boundary. It is possible that lack of evidence in the current study to strongly demonstrate that network parameters coincide with behaviour measures is attributable to the fact that only healthy participants were studied.

<u>Limitations</u>

Caution must be exercised when interpreting some of the results presented in the current study. First, results reported in this study only represented neurophysiological changes at node/sensor level during development, and not neuroanatomical structural estimations. As a result, no direct conclusion can be drawn regarding the underlying sources of reported neurophysiological age-related changes.

 Second, it is also worth addressing the implication of the frequency band weightings employed in the current study. Selecting appropriate frequency band (especially with respect to the alpha band) remains a challenge in neurophysiological studies, especially in studies involving children. Researchers, such as Boersma et al. (2011; 2013) have previously opted to average low frequencies together among children, arguing that splitting low frequencies (especially alpha) into sub-bands splits low frequencies at crucial peak points, which may bias observed results.

A third limitation concerns the application of minimum spanning tree analysis. By discarding connections that form loops, this analysis provides a robust and more conservative strategy for network analysis (Otte et al., 2015), however, because this type of analysis is primarily concerned with 'core' connection edges (Stam et al., 2014), it is likely that valuable information about network topology is lost. Then again, using simulations Stam et al. (2014) recently demonstrated that minimum spanning tree analysis is as sensitive as conventional graph-based measures to changes in network topology.

Fourth, unlike graph theoretical analysis, minimum spanning tree analysis is a relatively novel approach to neuroscience, and as such, its usefulness in providing applicable interpretations with respect to neurodevelopment remains to be established (Stam et al., 2014). Further empirical studies are needed for better understanding of the relationship between tree topological changes and underlying functional networks derived from classical

graph-theoretical analysis. Fifth, associations between classical graph theory-based network metrics and minimum spanning tree results remain difficult to explain. For instance the study by Boersma et al. (2013) suggested that high clustering and longer path length correspond to line-like minimum spanning tree topology, while low clustering and short path length correspond to a minimum spanning tree topology of short diameters and high leaf number.

In contrast, the study by Olde Dubbelink et al. (2013) found that lower clustering and shorter path length were associated with a more line-like minimum spanning tree topology. The discrepancy in these two studies concerning the relationship between classical graph theoretical analysis-based network measures and minimum spanning tree suggests that caution must be exercised when making neural functional links between the two types of network analysis. Sixth, the current study is cross-sectional in design and could potentially have failed to capture subtle changes in neural networks associated with processes of typical development. Hence, future research would benefit from longitudinal studies as a means of understanding the developmental trajectories of functional networks. Related to study design is also the question of sample size. The small number of participants in certain age-clusters in the current study resulted in a much more focused sample of children (7-13 years) and adults (20-35 years). As a result, the contribution of this study is limited regarding the nature of progressive changes occurring in functional networks during adolescence or beyond 36 years.

4.6. Conclusion

The use of classical graph theory analysis, alongside minimum spanning tree measures to characterise topological organisation of functional networks, provided new insights into the effect that development has on the complex brain. Tree-based measures were equally highly influenced by development as classical metrics in neurotypical children and adults. Results in the current study generally suggest that development is associated with refinement/re-organisation of wiring distance (including integration, hierarchical organisation, and network leaves) in large-scale whole-brain networks in higher frequencies. The results suggest a shift towards more effectively preserved and stronger long-range connections in adulthood, which is associated with stronger information processing in long-range nodes in adults compared to children.

It is proposed that the results reported in the current study suggest that neural activity in the higher frequency oscillatory beta and gamma band may serve as an important biological marker of brain development and efficiency. It appears that synchrony in these frequencies

is important in the reorganisation of cortical connections throughout development, particularly between childhood and adulthood. In line with the results discussed, it is proposed that atypical developmental may potentially hinder the progressive maturity of functional integration, resulting in less efficient information processing in the global integration-based functional connections that are responsible for network integration often implicated in age-appropriate and controlled cognition.

To conclude, this part of the study demonstrated that the application of minimum spanning tree analysis to characterise large-scale whole-brain functional network was able to successfully support other results derived from classical graph-based network analysis, and was crucially able to capture developmental changes in network organisation between children and adults, This provides practical and scholarly backing for minimum spanning tree as a suitable tool for investigating network organisation along with classical graph-based network analysis.

5. Altered whole-brain resting-state functional connectivity and network topology in attention-deficit/hyperactivity disorder (ADHD) children and adolescents

5.1. Chapter summary

Attention-deficit/hyperactivity disorder (ADHD) is one of the most common neurobehavioural disorders in children and has been shown to persist into adulthood. Abnormalities in underlying brain systems have been implicated as the likely cause of behaviour and cognitive impairments. Given the burden, that ADHD imposes on those affected, and on families, and society, a better understanding of its causes, and clarification of underlying neural-biological mechanisms is of significant importance in applied neuroscience. Biological descriptions might be useful as potential predictor variables for ADHD. In the current study, the topological organisation of large-scale whole-brain functional brain networks was investigated in children with ADHD and controls, using graph theory and minimum spanning tree (MST) analysis of eyes-closed sensor-level resting-state MEG data.

In the beta band and in no other frequency, lower whole-brain functional connectivity, normalised clustering coefficient, characteristic path length, higher eccentricity, and diameter were observed in children with ADHD compared to controls. Furthermore, in the ADHD group, the lower path length in the beta band was associated with poorer scores on verbal performance, while higher eccentricity and diameter in the beta and gamma band, was associated with poorer non-verbal performance. Finally, higher scores on the small-worldness index in the beta band was associated with poorer non-verbal performance scores as well as higher attention problem scores.

Large-scale functional networks in children with ADHD revealed a less local efficiency (i.e. normalised clustering coefficient) integrated (lower normalised path length), line-like tree topology (i.e. higher diameter, and eccentricity) in the beta band as compared to normal controls. At a neural level, the results offer support to the idea that the transfer of information in large-scale functional brain networks in children with ADHD is less efficient in the beta band. Reported associations between network parameters and cognition/behaviour

functioning suggest that these measures hold promise as potential markers of cognitive and behaviour impairments as reported in children with ADHD.

5.2. Introduction

Regarded as one of the most complex neurodevelopmental disorders (Cao et al., 2012), attention-deficit/hyperactivity disorder (ADHD) is characterised by developmentally inappropriate inattention, impulsivity, and motor restlessness (Barkley, 1990). Studies of neuroimaging (Sripada, Kessler & Angstadt, 2014; Wilson et al., 2011; Wong, & Stevens, 2012), neuropsychological (Wong & Stevens, 2012; Xia, Foxe, Sroubek, Branch, & Li, 2014) and neurochemical (Berridge et al., 2006; Volkow et al., 2001) have generally implicated differences in cortical and subcortical brain systems as the neural basis of ADHD-related impairments. The transfer of information in cortical and sub-cortical networks occurring between grey matter regions takes place via systems of white matter tracts (Murias, Swanason, & Srinivasan, 2007). Reports of reduced white matter volume in children with ADHD (Silk, Vance, Rinehart, Bradshaw, & Cunninghton, 2009b) offer support to the suggestion that it is the impaired interactions and not specific brain regions that underlie ADHD (Murias et al., 2007).

Despite overwhelming theoretical support for a neural basis of ADHD (See Castellanos et al., 2002; Krain & Castellanos, 2006; Sripada et al., 2014, for a review), the standard assessment and subsequent diagnosis of ADHD is based on structured diagnostic interviews and Conner's questionnaires (Hulme & Snowling, 2009). These are used to help to determine whether the child meets the criteria or may alternatively have a condition other than ADHD that may explain reported symptoms (Goldman et al., 1998). However, this way of assessing children's competencies and behavioural problems can be compromised by assessor bias (Biederman et al., 1993; Geller et al., 2004; Piper, Gary, Raber, & Birkett, 2014) often resulting from serious misconceptions. As a result, misdiagnosis of unaffected children as well as over-medication is common in children with ADHD (Pennington, 2009).

The application of empirically based instruments free from such bias offers a solution to this issue (Biederman et al., 1993). The current study undertook the use of task-independent measures of brain function to investigate whole-brain functional connections along with commonly applied measures of behaviour and cognitive functioning. If identified, biological risk markers could help in the identification of at-risk children at an earlier age. This could mean early assessment, diagnosis, and implementation of interventions children with ADHD.

Data from structural magnetic resonance imaging studies have shown consistent abnormalities in the frontostriatal network (Ashtari et al., 2005; Silk et al., 2009b, also see Bush, Valera, & Seidman, 2005; Cubillo, Halari, Smith, Taylor & Rubla, 2011, for a review). The pathways of the frontostriatal circuit connects regions in the frontal lobe (including dorsolateral prefrontal areas, dorsal anterior cingulate) with the basal ganglia (striatum) through the thalamus. The observations of reductions in total brain volume of frontal cortex (Bush et al., 1999; Mackie et al., 2007; Seidman et al., 2011) and the striatum (Silk et al., 2009a) have prompted some (Mostofsky, Cooper, Kates, Denckla, & Kaufmann, 2002; Silk et al., 2009a; Valera, Faraone, Murrary, & Seidma, 2007) to propose that underlying clinical symptoms, as well as cognitive impairments, may represent abnormalities of the frontal lobe and stratum.

Evidence for this view (i.e. abnormalities of the frontal lobe) comes primarily from task-based neuroimaging studies. For instance data from Franzen et al. (2013), and Murias et al. (2007), have previously revealed lower connectivity between brain regions forming the frontostriatal circuit during cognitive task performance. Note however that task-based findings in neuroimaging provide no clear distinction regarding the functional interactions between brain regions. Therefore, as it stands, a clear and complete picture of the neural underpinnings of ADHD remain largely unavailable (Bush et al., 2005). A solution to this is to study the interactions between spatially discrete brain regions. This strategy provides a powerful tool for examining differences in brain regions, where similar patterns of activations relate to functional roles (van den Heuvel & Hulshoff Pol, 2010).

Evidence for altered functional interactions in ADHD

Data from diffusion MRI tractography studies such as Ashtari et al. (2005) and Silk et al. (2007b) have offered support to the suggestion that reduced functional connectivity is likely to be associated with significant reductions in structural white matter integrity across major tracts in children and adolescents with ADHD. For instance, the study by Silk et al. (2009b) reported white-matter abnormalities in several distinct local clusters within left fronto-temporal regions, as well as in right parietal-occipital regions, which the authors attributed to deceased branching in white-matter pathways.

However the primary focus of many ADHD functional connectivity studies has been to investigate the strength of functional connectivity between specific regions (Cao et al., 2013; Cocchi et al., 2012) and/or connectivity between networks chosen a priori, typically the default mode network (Franzen et al., 2013; Tomasi & Volkow, 2012; Wilson et al., 2011).

Each study reported altered connectivity strength. However, the results reported from such studies have yielded inconsistent conclusions.

For instance, Castellanos et al. (2008), Wilson et al. (2011), and Uddin et al. (2008), reported 'hypoconnectivity' (i.e. lower connectivity strength) between medial prefrontal cortex regions and the posterior cingulate precuneus cortices of the default mode network in those with ADHD compared to controls. In contrast, data reported by Tian et al. (2008) revealed that compared to controls, those with ADHD showed 'hyperconnectivity' (i.e. greater connectivity strength) in several regions, one of which was the bilateral thalamus. Functional connectivity between the thalamus and posterior cingulate cortex in the default mode network were recently reported to be weaker in neurotypical participants who were more conscious of the present state (Wang et al., 2014). Hence, the increased activity reported by Tian et al. (2008) is not consistent with previous reports of decreased coupling strength in regions forming the default mode network. Therefore, based on previous functional connectivity studies, no concrete conclusions regarding underlying neural mechanisms can be drawn.

By not focusing on specific regions, the present study hoped to provide information about the pathophysiological mechanisms of brain networks at a global scale, which is appropriate in ADHD since this disorder is highly heterogeneous. Using graph theory, and MST measures would inform the state and pattern of local and global structures in children with ADHD. A reported loss of efficiency is likely to affect how well brain networks in children with ADHD exchange information, in both local and global network structures, which in turn may underlie help explain behavioural and cognitive impairments.

To summarise, despite an increasing number of ADHD studies choosing to investigate functional connectivity and in some cases using resting-state paradigms (Franzen et al., 2013; Rubia et al., 2009; Tian et al., 2008; Wison et al., 2011; Wong & Stevens, 2012; Xia et al., 2014), the majority often chose to focus on connections between specific regions and/or networks that based on previous literature derived from task-based findings.

Aims of the current study in respect of ADHD

1. To provide a more comprehensive profile of connectivity patterns in children with ADHD and in age-matched controls, this study investigated whole-brain resting-state functional connectivity, using conventional graph theoretical analysis and a novel unbiased approach of characterising networks.

2. Furthermore, to better understand any differences in whole-brain functional networks between controls and children with ADHD, the relationship was examined between local/global network efficiency and functional measures of behaviour problem rating scales and of cognitive ability.

<u>Hypotheses</u>

1. Whole-brain functional connectivity analysis in children with ADHD will reveal increased whole-brain theta functional connectivity, as well as decreased beta coupling strength. This hypothesis is based on consistent reports from resting-state EEG studies in children with ADHD of increased theta power (Chabot & Serfontein, 1996; Lazzaro et al., 1998, 1999; Matsuura et al., 1993) and decreased beta power (Mann, Lubar, Zimmerman, Miller, & Muenchen, 1992; Matsuura et al., 1993).
2. Network parameters will reveal a loss of local and global efficiency in the theta and beta band in children with ADHD.
3. Less global and local network efficiency will be associated with worse intellectual and behavioural functioning

5.3. Method

5.3.1. Participants

The Study Protocol (including this specific element) was reviewed and approved by the ethical review committee of Aston University and by the NHS. Language-appropriate informed assent and consent forms were signed by all participating children and parents/guardians in line with the Aston University Ethics Committee.

Children with a diagnosis of ADHD were recruited with the help of clinicians working within the Child and Adolescent Mental Health Services (CAMHS) at the Worcestershire Health and Care NHS Trust. All 14 children (aged between 7- 17 years) referred, had undergone structured clinical diagnostic interview assessments and received a diagnosis of ADHD, with associated deficits of attention, and/or hyperactivity/impulsivity, prior to their referral to the Aston Brain Centre by a clinical psychologist/ psychologist (See Figure 5.1, Chapter 2 for the referral pathway).

The inclusion criteria for children with ADHD included an ability to assent, the child being aged between 7:0 and 17:11 years and having a primary diagnosis of ADHD confirmed by an ADHD specialist. The exclusion criteria were the child being outside the age-range, having a primary diagnosis of a major psychiatric disorder or of other disorders such as brain damage that would preclude a primary diagnosis of ADHD, or a history of substance abuse. Given that this was a clinical sample, at the time of the study all (but two) ADHD children were on medication aimed at relieving ADHD symptoms.

Prescribed medication in the clinical sample of ADHD children fell into two broad categories: those containing the active ingredient methylphenidate (i.e. Concerta XL, Equasym XL, and Medikinet XL), and atomoxetine (i.e. Strattera) (See Table 5.1, for medication descriptions). The former are central nervous system (CNS) stimulant drugs while the latter is a non-stimulant, selective noradrenalin reuptake inhibitor (Volkow et al., 2001). More than half (i.e. seven children) of children with ADHD indicated sleep-related problems on the Achenbach System of Empirically Based Assessment) (ASEBA) questionnaire. Three children were receiving Circadin (melatonin) medication. Circadin contains melatonin a hormone produced by the pineal gland, and is associated with normal control of circadian rhythms, specifically sleep.

Two children with a diagnosis of ADHD were excluded from further analysis: one due to excessive artefacts in data, and the other due to overlapping learning difficulties (data for the latter participant was added to the comorbid groups. See Chapter 6). The final sample consisted of 12 children with ADHD and 21 age-matched typically developing controls. The sample of typically developing controls has been described in Chapter 4. All participants had normal or corrected to normal vision and no history of epilepsy or other neurological abnormalities. Using the ASEBA form, parents were asked to disclose whether or not the child in question had received a diagnosis of any other neurodevelopmental disorder. All participants volunteering for the study received Amazon vouchers as compensation for their participation.

Participant matching

Children with ADHD were assigned at least one chronological age (CA) matched control. In cases where this was not possible, an older CA child was allocated

5.3.2. MEG recording

MEG recordings were acquired during resting-state conditions. Procedures have been described in Chapters 3 and 4. Briefly, children were instructed to remain still, relax, and await instructions of when to close and open their eyes. Resting-state paradigms lasted approximately two minutes or as long as the child was willing to sit in the magnetically shielded room.

5.3.3. MEG data pre-processing

Continuous MEG recordings were max-filtered and visually inspected, similar to the procedures described in Chapter 4. For younger children, extra care was taken to visually inspect epochs of the eyes-closed rest condition for eye-blinks in sensors corresponding to the occipital regions. For each child, four epochs of eyes-closed were converted to ASCII files of 4096 samples and exported into the BrainWave software package v0.9.76 for all further computations. These were conducted separately for each frequency band including, theta (4-8 Hz), lower alpha/alpha1 (8-10 Hz), upper alpha/alpha2 (10-13 Hz), beta (13-30 Hz), and gamma (30-45 Hz) frequency bands. See Chapter 2 for a discussion of the computation sequence.

The phase lag index measure was used to estimate functional coupling between pairs of magnetometers in five frequency bands. No threshold was applied to matrices, hence network parameters, using both graph theory and minimum spanning trees, were constructed from weighted graphs. As was reported in previous chapters, graph measures were normalised to control for the effect of varying network size with random surrogate networks. In the light of findings in Chapter 3 regarding graph metric reproducibility, data presented in this study are primarily concerned with eyes-closed task-independent brain activity.

Statistical analysis

All statistical analysis was computed with SPSS (version 20). Given that the distributions of most measures did not meet the assumptions of normality prior to and after log transformations, group differences were assessed using non-parametric tests. Mann-Whitney U-tests for independent samples were applied to assess group differences in network measures. Associations between network measures and intellectual/behavioural

performance were assessed using Spearman's rank correlation coefficients (r_s). Unless otherwise stated, significance levels of α < .05, with the Cohen's *d* effect sizes were reported. The trends described correspond to α level of >.05 ≤ .10.

<u>Participant characteristics</u>

Cognitive abilities: Clinically, the relationship between ADHD and IQ has been of interest to researchers, with low IQ being associated with poor treatment (Buitelaar, Van der Gaag, Swaab-Barneveld, & Kuiper, 1995), and negative long-term outcomes (Hechtman, 1999; Wallander, 1988). Data from Kuntsi et al. (2004) revealed that the ADHD symptoms and lower intelligence abilities covaried in children. The aetiology of the association/co-occurrence of ADHD and lower IQ has genetic origins (Kuntsi et al., 2004), that may be reflected in the level of functional network efficiency.

To investigate in the current study whether ADHD is associated with IQ deficits, verbal and non-verbal intellectual abilities were assessed using a battery of either two and/or four age-appropriate Wechsler intelligence subtests: namely, similarities (SI), vocabulary (VC), matrix reasoning (MR) and block design (BD). For children aged between 6 years 0 months and 16 years, the Wechsler Intelligence Scale for Children 4th UK Ed (WISC-IV[UK]) (Wechsler, 2003) was administered, while for those over 16 years, the Wechsler Abbreviated Scale of Intelligence (WASI) (Wechsler, 1999) was administered. As stated earlier, SI and MR were the core verbal and non-verbal subtests, while VC and BD were supplementary measures, obtained if the child was willing and able to participate. As a result, only the SI and MR scores are reported as these were obtained from almost all children.

Behaviour maturity: ADHD manifests itself as a childhood disorder with differences from norms in behaviour. As a result, diagnosis is typically based on age-appropriate maturity. In this study, the Child Behaviour Checklist/6-18 (CBCL/6-18) (Achenbach, 1991) questionnaires was completed by parents to assess various behavioural, emotional, and social problems in children and adolescents. The CBCL/6-18 is part of the Achenbach System of Empirically Based Assessment (ASEBA). Although the questionnaires cover several behaviour problem areas, the current study focused on attention behaviour and the two broad problem subscales of internalised problems (encompassing, withdrawn/depressed, somatic complaints and anxious/depressed scales) and externalised problems (encompassing delinquent and aggressive behaviour). Scores were reported using population standardised T scores (*M* = 50, *SD* = 10). Hence, a score of ≥ 63 is 1.5 *SD* above the mean, suggesting a high risk of maladaptive behaviour

5.4. Results

Demographic data in Table 1 revealed poorer performance on verbal and non-verbal intellectual assessments compared to age-matched controls. In addition, children with ADHD were reported by their guardians to have more attention, internalising and externalising problem behaviours than did controls.

Table 5.1 also displays results of theta, beta, and theta/beta power. These are reported because one of the most consistent findings in ADHD literature is elevated slow wave (predominately theta power) and decreased fast wave (i.e. beta power) (Chabot & Serfontein, 1996; Clarke, Barry, McCarthy, & Selikowitz, 2002; Mann, Lubar, Zimmerman, Miller, & Muenchen, 1992). What is more, the theta/beta ratio (TBR) has been found to be elevated in those with ADHD (Arns et al., 2013; Clarke, Barry, McCarthy, & Selikowitz, 2001) and often regarded as discriminatory feature distinguishing those with ADHD from controls (Arns, Conners, & Kraemer, 2013; Ogrim, Kropotov, & Hested, 2012). Although not significant, the results in Table 5.1, showed trends in children with ADHD towards an elevated power of slow wave theta, an increased theta/beta ratio, and a decreased power of fast wave beta.

Table 5. 1 Demongraphic data including intellectual, behaviour, and oscillatory activity characteristics for children with ADHD and typically developing controls (TDC)

	ADHD	TDC		
	(n=12)	(n = 21)		
	M ± SD	M ± SD	*p*	
Age (years)	12.4 ± 3.5	11.6 ± 3.1	*NS*	
Gender (M:F)	10:1	10:11		
Hand (L:R)	2:10	2:19		
SI	8.3± 2.3	12.7 ± 2.5	**	**ADHD Medication** (n)
MR	9.1 ± 2.4	11.3 ± 1.9	**	Equasym XL (2)
Att	78.5 ± 10.36	52.4 ± 3.6	**	Concerta XL (2)
Int	65.8 ± 11.02	48.5 ± 8.0	**	Strattera (2)
Ext	70.3± 8.6	44.5 ± 8.9	**	Medikinet XL (1)
Theta power (θ)	.20 ± .03	.19 ± .03	*NS*	Concerta XL & Circadin (1)
Beta power (β)	.19 ± .05	.20 ± .04	*NS*	Unmedicated (2)
θ/β ratio	1.12 ± .36	1.00 ± .35	*NS*	Strattera, Concerta XL, Circadin (2)

Demographic data for the two groups. Key: M = mean; SD = standard deviation; MR = matrix reasoning subtest; SI = similarities subtest; Att = Attention, Int = internalised; Ext = externalised, θ/β = theta/beta ratio power and NS = not significant. IQ scores are standardised scaled scores (M = 10, SD = 3), while ASEBA problem measures scores are age, gender, informant, and society normed T scores (M = 50, SD = 10). Group differences were assessed using non-parametric Mann-Whitney U- tests: * p < .05, **p < .01.

5.4.1. Mean whole-brain functional brain networks in controls and in children with ADHD

Whole brain functional connectivity was computed by averaging all pair-wise phase-lag-index values across all magnetometers. Mean whole-brain functional connectivity in the beta band for children diagnosed with ADHD (*Mdn* = .09) was significantly lower than in the control group (*Mdn* = .10), (U = 62.00, z = -2.39, p < .05, d = -.92). Non-significant trends in the opposite direction were observed in the lower alpha band (p = .80). No other clear differences were found in other frequency bands (See Table 5.2 and Figure 5.1, for whole-brain functional connectivity in other frequencies).

***Figure 5. 1 Mean whole-brain functional connectivity
differences across frequency bands***

*Mean group phase lag index averaged over magnetometer sensors in frequency bands of
interest. Mean PLI was significantly lower in children with ADHD, compared to TDC, in the
beta band. No other significant differences were found in other frequency bands. Error bars
are ± 2 SE, standard error. * p < .05. Key: ADHD= Attention-deficit/hyperactivity disorder;
TDC = typically developing controls.*

5.4.2. Group differences in short and long-distance functional connections

Although results in Chapter 3 revealed that non-normalised first-order metrics (i.e. C_W and
L_W) were highly robust, these metrics do not represent 'pure' measures of network topology
(Stam et al., 2009) as discussed in Chapter 3. Briefly, this is because they are more likely to
be affected by changes in the average coupling strength (i.e. average phase lag index),
(Boersma et al., 2012), whereby lower average coupling strength results in decreased C_W,
but longer L_W, regardless of network structure (Stam et al., 2009). Placed in the context of
ADHD, where Figure 5.1 revealed significantly lower average coupling strength in the beta,
this would mean that C_W would be lower and this would be coupled with higher L_W in children
with ADHD compared to TDC. This was in fact confirmed for the beta band (See Appendix
A.1). For this reason, as with Chapter 4, reported main findings concern normalised classical
metrics and minimum spanning tree network measures.

Both groups showed small-worldness in all frequency bands (i.e. an optimal balance between segregation and integration in functional brain network), which is formally demonstrated by values that were > 1 (Sporns et al., 2004; Watts & Strogatz, 1998). However, local and long-distance functional connections were significantly lower in the beta band in children with ADHD compared to controls (See Table 5.1 for results of other network parameters).

Normalised clustering coefficient (gamma) was significantly lower in the beta band in children with ADHD (*Mdn* = .1.0) compared to TDC (*Mdn* = 1.06), *U* = 64.50, *z* = -2.30, *p* < 05, *d* = -.85 (See Figure 5.3A). Also in the beta band, the normalised characteristic path length (lambda) was significantly lower in children with ADHD (*Mdn* = 1.03) compared to TDC (*Mdn* = 1.04), *U* = 41.50, *z* = -3.14, *p* < .01, *d* = -1.10 (see Figure 5.3B).

Table 5. 2 Group differences in global functional connectivity and network topological organisation

		Theta	p	Alpha1	p	Alpha2	p	Beta	p	Gamma	p
Mean PLI	ADHD	.16 [.16, .16]	NS	.24 [.23, .27]	NS	.21 [.20, .23]	NS	.09 [.08, .10]	*	.09 [.09, .10]	NS
	TDC	.16 [.16, .16]		.24 [.23, .25]		.20 [.20, .21]		.09 [.09, .10]		.09 [.09, .10]	
Gamma	ADHD	1.06 [1.05, 1.07]	NS	1.08 [1.07, 1.08]	NS	1.07 [1.05, 1.08]	NS	1.04[1.04, 1.05]	*	1.04 [1.04, 1.05]	NS
	TDC	1.05 [1.05, 1.06]		1.07 [1.06, 1.08]		1.06 [1.05, 1.07]		1.06 [1.05, 1.06]		1.04 [1.04, 1.04]	
Lambda	ADHD	1.04 [1.03, 1.04]	NS	1.04 [1.03, 1.07]	NS	1.04 [1.03, 1.06]	NS	1.03 [1.02, 1.03]	**	1.02 [1.02, 1.03]	NS
	TDC	1.03 [1.03, 1.03]		1.05 [1.04, 1.06]		1.04 [1.04, 1.05]		1.04 [1.03, 1.04]		1.02 [1.01, 1.03]	
Small-world	ADHD	1.02 [1.01, 1.02]	NS	1.03 [1.01, 1.04]	NS	1.02 [1.01,1.03]	NS	1.02 [1.02, 1.03]	NS	1.02 [1.01, 1.03]	NS
	TDC	1.02 [1.02,1.03]		1.02 [1.02, 1.03]		1.02 [1.01, 1.02]		1.02 [1.01, 1.03]		1.02 [1.01, 1.03]	
Eccentricity	ADHD	.14 [.13, .15]	NS	.13 [.13, .14]	NS	.13 [.12, .14]	NS	.13 [.13, .14]	*	.14 [.13, .14]	NS
	TDC	.14 [.13, .14]		.13 [.13, .14]		.13 [.13, .14]		.13 [.12, .13]		.14 [.13, .15]	
Diameter	ADHD	.18 [.17, .20]	NS	.17 [.16, .19]	NS	.17 [.16, .19]	NS	.18[.17, .19]	*	.18 [.17, .20]	NS
	TDC	.18 [.17,.19]		.18 [.17, .18]		.18 [.17, .20]		.17 [.17, .18]		.19 [.17, .20]	
Hierarchy	ADHD	.39 [.37, .42]	NS	.41 [.40, .43]	NS	.41 [.40, .42]	NS	.38 [.37, .40]	NS	.38 [.37, .40]	NS
	TDC	.38 [.38, .39]		.40 [.40, .41]		.39 [.38, .40]		.38 [.38, .39]		.38 [.37, .39]	
Leaf number	ADHD	.56 [.54, .57]	NS	.58 [.56, .60]	NS	.57 [.55, .60]	NS	.55 [.54, .56]	NS	.54 [.51, .55]	NS
	TDC	.54 [.53, .56]		.57 [.56, .60]		.55 [.55, .57]		.54 [.53, .55]		.53 [.53, .54]	

Whole-brain functional connectivity and network parameters derived from both classic network metrics and minimum spanning tree measures. Results are displayed as medians and bootstrap 95% CIs (in square brackets). Group differences were assessed with non-parametric testing (Mann-Whitney U-test, *p< .05, ** p < .01. Differences between the two groups were found in the beta band and no other frequency.

Figure 5. 2 Mean group difference in normalised short-range and long distance connections in children with ADHD and TDC

Mean group differences in A) normalized weighted clustering coefficient and B) path length. Significant results were found for the beta band and no other frequency range. * p < .05, ** p < .01. Error bars are ± 2 SE. Networks in both groups showed small-world organisation, an index for optimal local and global connectivity.

Group differences in minimum spanning tree (MST) measures

Similar to classic network metrics, significant differences in MST measures were observed in the beta band and in no other frequency (See Table 5.2 for the results of other measures). From the four measures assessed, significant group differences were found for MST diameter and eccentricity (Table 5.2, Figure 5.3), which showed that these measures were significantly higher in children with ADHD. Eccentricity in the ADHD (*Mdn* = .13) was significantly higher compared to TDC (*Mdn* = .13), *U* = 61.00, *z* = -2.43, *p* < .01, *d* =.10). In addition, MST diameter in children diagnosed with ADHD (*Mdn* = .18) was significantly higher than in typically developing children (Mdn = .17), *U* = 67.00, *z* = -2.21, *p* < .05, *d* = .67.

Figure 5. 3 Mean group differences in MST eccentricity and diameter, in children with ADHD and TDC

*Graphs of group differences in A) eccentricity, and B) diameter over frequency bands derived from MST. Significant group differences were only observed in the beta band and in no other frequency range. Error bars are ± 2 SE. * p < .05.*

<u>To what extent can the significant classic network metrics and minimum spanning tree measures in the beta band predict group membership?</u>

Planned analysis: discriminatory analysis was used to determine group membership, (i.e. TDC or ADHD), based on normalised classic or graph-based measures and minimum spanning tree metrics that were significantly different between these two groups (i.e. normalised clustering coefficient, normalised path length, eccentricity, and diameter). This analysis is used as follow-up to multivariate analysis of variance (MANOVA) or as a mean test for predicting membership of groups based on several predictive variables. Predictive analysis (e.g. regression or multivariate analysis of variance) often relies on groupings to try and predict differences among single or multiple variables (Morgan, Vaske, Gliner, & Harmon, 2003). In contrast, the aim of the present study was to try to predict membership in a group using two types of network analysis. Therefore, statistical analysis using a discriminant function offered an appropriate strategy to investigate whether the predictor variables of normalised clustering coefficient, normalised path length, eccentricity, and diameter within the beta band would allocate children into the appropriate group (i.e. TDC or ADHD).

Graph-theoretical analysis predictors (i.e. normalised clustering coefficient, normalised path length) in the beta band:

Discriminant analysis revealed one discriminant function that significantly differentiated the groups. This was Wilks' Lambda (λ) = .78, $X^2(2)$ = 7.44, p = .02. Correlations between outcomes and the discriminant function revealed that classic network metrics for short and long-distance functional connections loaded fairy highly. Long-distance functional connections loaded more highly onto the function (r = .98) compared to short-range connections (r = .79). Overall, cross-validated classification revealed that 66.7% of children were appropriately classified. Specifically, the function using the GTA predictors correctly predicted 33.30% of children with ADHD and 85.7% of TDC.

Minimum spanning tree predictors (i.e. eccentricity and diameter) in the beta band:

The second discriminant analysis was computed using the MST predictors to predict whether a child was a control or had a diagnosis of ADHD. Analysis revealed one discriminant function that significantly differentiated the groups, Wilks' Lambda (λ) = .81, $X^2(2)$ = 6.25, p = .04. Correlations between outcomes and the discriminant function revealed that MST measures loaded fairy highly. Eccentricity loaded more highly onto the function (r = .83) compared to diameter (r = .67). Finally, cross-validated classification revealed that 66.7% of children were appropriately classified. Specifically, the function correctly predicted 41.70% of children with ADHD and 81.00% of TDC. In other words, the predictor function was better at identifying TDC group membership.

Relationship between network characteristics and cognitive abilities for each frequency band

The finding that verbal and non-verbal reasoning abilities were significantly different between groups (See Table 5.1) justified investigating the relationship between cognitive abilities and network parameters independently for each group. The correlations between network measures and cognitive abilities that were significant at a level of .05, albeit not statistically significant at the Bonferroni-corrected α level, are visually articulated in Figure 5.4. Although, these were observed in the ADHD group, Figure 5.4 displays relationships across the entire sample cohort of children.

No significant correlations between network measures and cognitive abilities (See Table 5.3) were observed in the theta, lower or upper alpha bands.

In the beta band, eccentricity showed a strong negative correlation with non-verbal reasoning (r_s = -.63 [-.95, .10], p = .027). Similarly, diameter showed a strong negative relationship with non-verbal reasoning (r_s = -.72 [-.97, .02], p = .008). The results in the beta band also revealed trends towards moderate negative correlations between small-worldness and non-verbal reasoning (r_s = -.53 [-.89, .23], p =.076), as well as between diameter and verbal reasoning (r_s = -.57 [-.93, .13], p = .052). Finally path length in the beta band showed a trend towards a moderate positive correlation with verbal reasoning (r_s = .55 [.21, .77], p =.064). None of the correlation coefficients listed above was statistically significant at the more strict Bonferroni-corrected α level of .001.

In the gamma band, eccentricity showed a strong negative correlation with non-verbal reasoning (r_s = -.75 [-.93, -.30], p = .005). A similar trend was observed between diameter and non-verbal reasoning (r_s = -.76 [-.98, -.03], p = .005). In contrast, leaf number showed a moderate positive relationship with non-verbal reasoning (r_s = .53 [.07, .87], p = .075). Similar to correlations for the beta band, the relationships listed above were also not statistically significant at the Bonferroni-corrected α level.

Network correlation with cognitive abilities in the control group

No correlations or trends were observed between network parameters and cognitive ability in the lower alpha, beta, or gamma bands (See 4 Table). The correlations observed in the theta and upper alpha bands were moderate, although not statistically significant at the Bonferroni-corrected α level of .001.

In the theta band hierarchy showed a moderate positive correlation with verbal reasoning (r_s = .44 [-.03, .75], p = .069), while in the upper alpha band a positive relationship between leaf number and non-verbal reasoning was observed (r_s = .52 [-.02, .86], p = .02).

Table 5. 3 Correlations between network measures and cognitive abilities for children with ADHD

(n = 12)	Metric	SI	MR
Theta	Gamma	.43(.17)	.12(.72)
	Lambda	-.02(96)	-.21(.52)
	Small-world	.22(.49)	.18(.58)
	Eccentricity	-.37(.24)	-.03(.92)
	Diameter	-.45(.14)	-.02(.94)
	Leaf number	.31(.32)	.08(.80)
	Hierarchy	.34(.28)	.15(.65)
Alpha1	Gamma	-.08(.80)	-.20(.54)
	Lambda	-.03(.94)	-.36(.25)
	Small-world	-.08(.80)	-.47 (.12)
	Eccentricity	.17(.60)	-.20(.54)
	Diameter	.11(.73)	-.30(.34)
	Leaf number	-.04(.90)	.26(.41)
	Hierarchy	.36(.26)	-.02(.95)
Alpha2	Gamma	.03(.93)	-.30(.35)
	Lambda	.15(.65)	-.17(.59)
	Small-world	.10(.78)	.20(.95)
	Eccentricity	.16(.62)	.43(.17)
	Diameter	.28(.38)	.36(.25)
	Leaf number	-.13(.69)	-.32(.31)
	Hierarchy	-.26(.41)	-.16(.61)
Beta	Gamma	.27(.39)	-.05(.88)
	Lambda	.55(.06)	.07(.83)
	Small-world	-.40(.20)	-.53(.08)
	Eccentricity	-.42(.18)	-.63(.03)*
	Diameter	-.57(.05)	-.72(.01)**
	Leaf number	.02(.94)	-.08(.80)
	Hierarchy	.12(.72)	-.05(.87)
Gamma	Gamma	.40(.20)	.48(.11)
	Lambda	.10(.77)	.07(.83)
	Small-world	.37(.23)	.36(.25)
	Eccentricity	-.12(.70)	-.75(.01)**
	Diameter	-.14(.68)	-.76(.01)**
	Leaf number	-.12(.71)	.53(.08)
	Hierarchy	-.11(.74)	.05(.87)

*Spearman') rank correlation coefficients (r_s) for network measures and cognitive abilities in each frequency bands. Key: SI = similarities (i.e. verbal skills), MR = matrix reasoning (i.e. non-verbal skills), network metric gamma = normalised clustering coefficient, lambda = normalised characteristic path length, and small-world = small-world index. *p <.05, ** p < .01, ***p <.0024 (Bonferroni-corrected α value).*

Table 5. 4 Correlations between network measures and cognitive abilities for typically developing children

(n = 21) Metric	SI	MR
Theta Gamma	.36(.12)	.13(.60)
Lambda	-.04(.87)	.19(.43)
Small-world	.31(.19)	-.22(.34)
Eccentricity	-.31(.18)	-.02(.94)
Diameter	-.26(.26)	.02(.95)
Leaf number	.36(.12)	.18(.45)
Hierarchy	.44(.07)	.16(.49)
Alpha1 Gamma	-.16(.51)	-.04(.88)
Lambda	-.34(.15)	-.12(.63)
Small-world	.20(.39)	.28(.24)
Eccentricity	-.04(.86)	-.18(.46)
Diameter	-.01(.10)	-.18(.45)
Leaf number	-.07(.79)	.13(.58)
Hierarchy	.07(.76)	.15(.52)
Alpha2 Gamma	.00(.99)	.23(.35)
Lambda	.17(.47)	-.22(.35)
Small-world	-.15(.52)	-.23(.33)
Eccentricity	-.15(.54)	-.15(.52)
Diameter	-.21(.38)	-.23(.33)
Leaf number	-.16(.49)	.52(.02)
Hierarchy	-.16(.50)	.37(.10)
Beta Gamma	.11(.62)	-.18(.45)
Lambda	.26(.28)	.12(.61)
Small-world	-.09(.72)	-.28(.23)
Eccentricity	-.37(.11)	-.04(.88)
Diameter	-.33(.16)	-.06(.81)
Leaf number	-.15(.95)	.06(.81)
Hierarchy	-.21(.37)	.22(.35)
Gamma Gamma	-.31(.20)	-.24(.31)
Lambda	-.23(.34)	-.26(.28)
Small-world	.10(.71)	.03(.92)
Eccentricity	.15(.53)	-.03(.90)
Diameter	.15(.53)	-.04(.86)
Leaf number	.31(.18)	-.03(.92)
Hierarchy	-.23(.32)	.06(.80)

Spearman's rank correlation coefficients (r_s) for network measures and cognitive abilities in each frequency bands. *p <.05, p <.01, ***p <.0024 (Bonferroni-corrected α value). Key: SI = similarities (i.e. verbal skills), MR = matrix reasoning (i.e. non-verbal skills), network metric gamma = normalised clustering coefficient, lambda = normalised characteristic path length, and small-world = small-world index

Figure 5. 4 Correlation coefficients between network organisation measures and individual verbal and non-verabl performance variations, in controls, and children with ADHD

Correlations between non-verbal reasoning ability and MST network parameters in beta (top) and gamma band (bottom). These represent only the correlations that showed strong ($r_s >$ .6) relationships. The solid line is the fit-line for the entire sample cohort.

Relationship between network characteristics and behaviour measures for each frequency band

In Table 5.1, ADHD children were shown to have more problem behaviours than controls for the three behaviour measures, allowing for independent investigations of the groups. Consideration of such a relationship in healthy participants, described in Chapter 4, revealed

mostly weak relationships. Hence, the computation focus in this section focused on the ADHD group. No significant correlation was observed for any frequency band between network parameters and behaviour measures in children with ADHD (See Table. 5.5).

In the lower alpha, path length showed a moderate trend towards a positive correlation with attention (r_s = .50 [-.21, .95], $.p$ = .10). A similar trend was observed for the correlation between small-worldness and attention (r_s = .50 [-.08, .86], $.p$ = .096) in the beta band. Finally in the gamma band, the clustering coefficient showed a trend towards a positive correlation with externalised behaviours (r_s = -.53 [-.91, .15], $.p$ = .08).

Table 5. 5 Correlations between network measures and behaviour problems for children with ADHD

(n = 12) Metric	Theta							Alpha1							Alpha2							Beta							Gamma						
	Gamma	Lambda	Small-world	Eccentricity	Diameter	Leaf number	Hierarchy	Gamma	Lambda	Small-world	Eccentricity	Diameter	Leaf number	Hierarchy	Gamma	Lambda	Small-world	Eccentricity	Diameter	Leaf number	Hierarchy	Gamma	Lambda	Small-world	Eccentricity	Diameter	Leaf number	Hierarchy	Gamma	Lambda	Small-world	Eccentricity	Diameter	Leaf number	Hierarchy
Att	.14(.66)	.06(.85)	.25(.43)	-.18(.56)	-.16(.61)	.11(.73)	-.26(.42)	.29(.36)	.50(.10)	-.46(.14)	.10(.75)	.24(.46)	.06(.87)	.00(1.00)	-.11(.74)	-.15(.65)	-.18(.57)	-.30(.35)	-.15(.64)	-.09(.78)	-.24(.46)	.44(.16)	.07(.83)	.50(.10)	.08(.80)	.20(.55)	.42(.18)	.08(.80)	-.25(.43)	.02(.96)	-.13(.70)	.24(.45)	.18(.58)	-.07(.84)	-.26(.41)
Int	-.11(.73)	-.18 (.58)	.34(.28)	.11(.74)	.22(.48)	-.23(.47)	-.40(.20)	-.14(.66)	-.24(.45)	-.32(.31)	.03(.94)	.14 (.66)	.07(.83)	-.12(.71)	-.24(.46)	-.25(.41)	-.29(.37)	-.41(.19)	-.38(.22)	-.11(.73)	-.28(.38)	-.05(.88)	-.34(.29)	.47(.12)	.17(.60)	.33(.30)	.06(.86)	-.26(.42)	-.53(.08)	.00(1.00)	.45(.14)	.13(.68)	.14(.66)	-.05(.88)	-.32(.31)
Ext	.06(.85)	-.18(.57)	.32(.31)	-.13(.70)	-.03(.92)	-.16(.61)	-.47(.12)	-.05(.87)	.10(.76)	-.11(.73)	.15(.64)	.26(.41)	-.00(.99)	-.04(.89)	-.25(.44)	-.31(.33)	-.09(.78)	-.10(.77)	.01(.97)	-.07(.83)	-.23(.48)	.11(.73)	-.33(.30)	.45(.14)	.13(.70)	-.30(.34)	.10(.78)	-.16(.62)	-.40(.20)	-.10(.78)	.22(.50)	.18(.58)	.21 (.51)	-.07(.83)	-.30(.35)

Spearman's rank correlation coefficients (r_s) for network measures and adaptive behaviour for each frequency band. No significant correlations were observed. Key: Att = attention, Int = Internalising, and Ext = Externalising, metric gamma = normalised clustering, metric lambda = normalised path length, and small-world = small-world index. *p <.05, **p < .01, and **p <.0024 (Bonferroni-corrected α value).

5.5. Discussion

To the best of the author's knowledge, this study is the first to describe the functional network organisation in ADHD using minimum spanning tree. Furthermore, this is first study to investigate changes in the large-scale whole-brain functional organisation of resting-state MEG, using normalised conventional graph metrics. Applying both network analysis strategies in the present study revealed differences in network organisation between the two groups that were confined in the beta band. In this frequency, lower normalised clustering coefficient, shorter normalised path length, larger diameter, and higher eccentricity were characteristic of functional networks in the ADHD cohort. Furthermore, higher eccentricity and diameter, and decreased path length in the beta band were associated with poorer non-verbal reasoning performance in children with ADHD.

Whole-brain functional connectivity

In children with ADHD, the results confirmed the prediction of altered whole-brain functional connectivity in the beta band, but not in the theta band. The results revealed significantly lower beta band coupling strength when compared to age-matched controls (See Figure 5.1). Substantially decreased activity in the beta band is in line with previous resting-state EEG studies (Clarke et al., 2002; Mann et al, 1992; Matsuura et al., 1993). In structural networks, long-distance connectivity is related to low frequencies, while short-range connectivity is related to synchronisation of fast beta and gamma frequencies (Ahmadlou et al., 2011; von Stein et al., 2000; von Stein & Sarnthein, 2000). Therefore the observation (in relation to controls) of lower whole-brain connectivity coupling strength in the ADHD group is an indication of a lower level high beta band oscillation functioning, which could in turn suggest that information processing in local neighbourhood clusters in ADHD is disrupted. Placed in the context of previous studies investigating network-specific connections, the results observed allude to a 'hypo connectivity' of whole-brain structures in ADHD.

Functional network organisation using conventional graph theoretical measures

Both children with ADHD and controls showed small-world organisation, a measure of the optimal balance between high clustering and short path length (i.e. normalized clustering > 1 and normalised path length ≈ 1) (See Figure 5.2). The finding of preserved small-world topology is in line with previous studies, in which data have shown the small-world topology to be resilient in relation to both age-related (Fair et al, 2009; Supekar et al., 2009) and pathology-related network alterations (Cao et al., 2012; Xia et al., 2014). The preservation of

this topology may be attributed to the fact that it has a stronger biological basis compared to other measure. Moreover, data reported by Smit et al. (2008) revealed a strong genetic basis for features of small-worldness in pairs of dizygotic and identical or monozygotic twins. Furthermore, the fact that small-world networks have also been reported in non-biological systems (See Latora & Marchiori, 2001, for a review), prompts the suggestion that higher clustering and cost-effective global processing in small-world networks, is fundamental to information exchange in any complex dynamic system.

However, the results in the present study revealed differences between the two groups in the local and global topological organisation of functional networks in the beta band (See Figure 5.2). Functional brain networks in children with ADHD were characterised by lower normalised clustering coefficient and normalised path lengths, suggesting lower levels of local integration along with a loss of global connectivity efficiency in the beta band. Studies in medicated children and adolescents previously found that ADHD was associated with reductions at both the global and regional level (Batty et al., 2010; Carmona et al., 2005). This may explain the reduced whole-brain functional connectivity coupling observed in children with ADHD in the current study. The process of such mechanisms remains unknown. That said, some (El-Sayed et al., 2003; Shaw et al., 2007; Sripada et al, 2014) have attributed such changes to a delay in cortical maturation, predominantly in the prefrontal regions.

The current study results suggest that compared with age-matched controls, the functional brain network topology of children with ADHD indicates a deviation towards a more random topological organisation. Compared to the 'small-world', random networks lack an optimal balance between segregation and efficient global information exchange. The graph-theoretical results reported in this study are not in line with a previous EEG study that investigated among other things, whole-brain connectivity using graph analysis in medicated ADHD children at sensor-level (Ahmadlou et al., 2011).

Focussing on the both hemispheres as well as the organisation of whole-brain coupling strength, Ahmadlou et al. (2011) did not observe any differences in whole-brain beta band functional connectivity. Instead, the authors reported altered clustering and path length in the left hemisphere in the delta band. The differences between these results and those reported in the present study could be explained by methodological differences. First, different measures were used to estimate functional connectivity coupling. The authors applied the fuzzy synchronisation likelihood (See Ahmadlou & Adeli, 2011, for a discussion of this measure), while functional connectivity in the current study was based on phase lag index.

As discussed in Chapter 3 (see Discussion), different estimates of functional connectivity have been reported to generate different conclusions (i.e. measures that sensitive to the effects of field spread and common sources are likely to revealed higher coupling strength, because zero-lags are not excluded from estimated functional connectivity). Furthermore, although fuzzy synchronisation quantifies both linear and non-linear synchronisations, this measure is sensitive to the effects of volume conduction.

Second, unlike the current study, Ahmadlou et al. (2011), reported changes in network structure using non-normalised clustering and path length. Changes in these measures are influenced by average connectivity strength (Stam et al., 2007), and as a result are unlikely to represent genuine differences in network comparisons. As a result, reported findings using such measures are challenging to interpret when bias arising from differences in network structure and density in two different populations is not addressed (see results in Figures 5.1, 5.2 and Appendix A.1 for an illustration).

Comparison of findings in the current study with other previous ADHD neuroimaging studies, is challenging, because not only do these studies examine functional connectivity and network organisation in task-dependent activities (Murias et al., 2007; Rubia et al., 2009; Xia et al., 2014), they also often focus on network differences in specific regions or networks that are determined a priori (Franzen et al., 2013; Sripada et al., 2014; Wilson et al., 2011; Wong & Stevens, 2012). Reported network efficiency is therefore regional or network-specific.

However, despite the differences in methodology, the results in the current study seemed to mirror some of those reported in earlier task-based studies. For instance, decreased local efficiency reported in those with ADHD is consistent with data reported by Xia et al. (2014) in the visual attention networks, while less global efficiency observed in the present study closely aligns with previously reported lower global integrity of the default mode network (Uddin et al., 2008; Wilson et al., 2011).

Functional network organisation using minimum spanning tree (MST)

Results in the present study revealed that beta band MSTs differed in the beta band between the two groups. The global measures of eccentricity and diameter were statistically significantly higher in children with ADHD (See Figure 5.3), indicating a loss or disruption of global efficiency of brain networks in the beta band. As a measure of centrality, eccentricity corresponds to nodal importance or efficiency. Therefore, higher eccentricity is an indication of a lower level of nodal importance (Old Dubberlink et al., 2014). Hence, the findings in the

current study suggest that in children with ADHD nodal efficiency was lower in the beta band.

Furthermore, as previously stated, efficient communication between vertices requires a star-like topology, corresponding to both the maximum number of leaves and the shortest average path length. The observation of larger diameter in the current study, which is a measure of the longest distance connecting any two nodes on a tree, therefore suggests a shift towards a path or line-like topology in children with ADHD. Collectively MST eccentricity and diameter results suggest that in the beta band, the topology of functional networks in children with ADHD corresponds to a more line-like topology, which according to Boersma et al (2012) indicates a shift to a more regular, less global integrated network.

Beta neural oscillations in ADHD

Given that all significant contrasts in network topological organisation between controls and those with ADHD were observed in the beta band, the current study offers support to the suggestion that network parameters in this frequency offer more sensitive measures capable of capturing network structure differences in typical and atypical neurodevelopment. Discriminant analysis also revealed that these measures were able to significantly differentiate the groups. However cross-validated classification revealed that in the beta band controls were much likely to be appropriately classified than were children with ADHD. This observation makes intuitive sense especially when considering the highly complex and heterogeneous nature of ADHD. That said the discriminant analysis results must be interpreted with caution. Given that the sample sizes were not equal, one may argue that cross-validated classification power of the reported predictors may have favoured the group with more participants. That said, ability of discriminatory specificity reported in this study is an interesting observation, that future studies could consider exploring further.

Differences in beta neural oscillations of resting-state in ADHD (compared with controls) have previously been reported in EEG studies by researchers such as Arns et al. (2013), and Clarke et al. (2001), who have proposed that abnormalities in beta oscillatory activity play a key role in the pathophysiology of this condition. In neurotypical development, beta activity is less frequent in children compared to adults. However for some children with ADHD beta has been reported to be elevated, while in others it has been shown to shown to be reduced (Synder & Hall, 2006).

<u>Association between functional brain network organisation and measures of behaviour and cognitive abilities</u>

For a better understanding of any differences between the two groups in the current study, the relationship was assessed between network measures for each frequency band and measures of behaviour and cognitive functioning. Exploratory analysis of the relationship between functional brain connectivity and measures of behaviour, as well as cognition, revealed that overall decreased global efficiency (i.e. normalised path length, eccentricity, and diameter) was associated with poorer cognitive abilities and increased severity of attention and behaviour problems in ADHD.

<u>Relationships between network measures and cognitive abilities</u>

In the ADHD group, associations between network measures and cognitive abilities were observed in the beta and gamma band. Interestingly, although not significant, the trends reported in controls revealed similar patterns to those in children with ADHD, providing further support for the view that network parameters contain important neurobiological information and that the efficiency of network organisation plays a crucial role in cognition.

Lower non-verbal performance scores were associated in the ADHD cohort with higher beta and gamma band eccentricity, and diameter. As stated earlier, in an optimal network topology, eccentricity is low if the node is central in a tree (Boersma et al., 2013b). Higher eccentricity corresponds to a higher shortest path length/distance between pairs of nodes in a tree, which suggests a shift towards a less integrated and efficient tree topology (Otte et al., 2015). Loss of integration is the result of nodes becoming less central in a network (Tewarie et al., 2014), which in the current study was found to be associated with poorer cognitive abilities. The association between loss of centrality and poorer cognition was recently reported by Tewarie et al. (2014), who applied MST to investigate brain networks in early relapsing remitting multiple sclerosis patients.

Another interesting finding, though not statistically significant, in both controls and children with ADHD, was that beta and upper alpha bands showed trends towards a positive correlation between leaf number and non-verbal performance. As stated earlier, the maximum number of leaves is one of the requirements for the most efficient exchange of information in a star-like topology. Therefore, results revealing that increased leaf number was associated with better non-verbal performance offer support for the proposition that

increased global efficiency is a likely marker of reported cognitive impairments in typical and atypical populations.

In addition, in the present study decreased path length, a measure of global connectedness of a network in the beta band, was associated with poorer verbal performance. The results are in line with previous neuroimaging studies (Song et al., 2008; van den Heuvel et al., 2009). Data from these studies previously revealed that in healthy participants more efficiently connected functional brains at a global scale showed higher levels of intellectual performance. In addition, the results observed in the current study, are also in line with a recent study by Olde Dubberlink et al. (2014) investigating brain network topology in those with Parkinson's disease, albeit in a different frequency band. The researchers revealed that decreased path length was associated with poorer performance on a cognition assessment scale. The difference in frequency band of the observed relationships is likely to be attributed to the different roles that slow and fast frequency oscillations play in the two conditions. (I.e. ADHD and Parkinson's)

However as with an earlier resting-state functional MRI study in healthy adults (van den Heuvel et al., 2009), results in the current study revealed no correlation, or trend, between normalised clustering coefficient and intellectual performance. This suggests that intellectual abilities may possibly not be related to the efficiency of information transfer in local network clusters.

None of the associations reported between network measures and behaviour functioning was significant (See Table 5.5). However, some behaviour associations with network efficiency appeared to align with those observed in relation to cognitive performance. For instance, in children with ADHD, in addition to increased beta band small-worldness being associated with poorer non-verbal performance scores, topology in these networks was also associated with higher attention behaviour problem scores. Biological networks, like many other efficient systems, tend to operate in a manner that ensures optimal information processing at a low cost (Stam, 2004). In the light of the small-world association with non-verbal performance and attention problem behaviour, the results observed in the current study suggest a role for high frequency small-worldness in maintaining normal functioning of behaviour and cognitive abilities, which are crucial for age-appropriate development. Small-worldness may therefore provide a potential clinical marker of behaviour and cognitive impairments in children with ADHD.

Evidence from pervious structural studies has implicated atypical developmental maturation such as white matter integrity, processes as the likely cause of behaviour problems in children with ADHD. For instance, a structural neuroimaging study of boys with the ADHD-hyperactivity subtype (Semrud-Clikeman et al., 2000) reported a negative association between white matter volumes and higher CBCL externalising scores, while Castellanos et al. (2001) reported that small white matter volumes in girls with ADHD was associated with higher CBCL anxiety-depression scores. Together the two studies appear to suggest that underlying neuronal processing such as myelination of axons connecting brain regions involved in local and global network communication seems to affect the behaviour functioning in individuals.

Finally, an intriguing finding in the present study was that oscillations play important but distinct roles in the associations between network topology and cognition and behaviour functioning. For example, in the beta band decreased path length was associated with poorer verbal performance. On the other hand, with regard to behaviour, decreased gamma band clustering and upper alpha path length were associated with fewer attention behaviour problems.

Limitations

Whilst this study demonstrates the potential for use of network analysis techniques in sensor-level resting-state MEG and contributes to our understanding of specific neurobiological functions in ADHD, the findings reported may not be generalisable to the wider ADHD population. Given the small sample of ADHD children (n = 12), it was not possible to investigate the effects of symptom sub-types on network topology. Although the decision to investigate children with ADHD as a single cohort in not in line with convergent reports of there being symptom-related subtypes, it is worth mentioning that many in the field (Baeyen et al., 2006; King & Young, 1982; Milich, Balentine, & Lynam, 2001) have questioned the validity of DSM-IV oriented ADHD subtypes. In addition, concerning gender, the current ADHD sample was very homogeneous (11 male and 1 female). Although ADHD is three times more likely to affect boys than girls (McGrath & Peterson, 2009), future studies using equal gender samples could provide better insights as to whether gender is associated with network topology.

Furthermore, due to the small sample in the current study, developmental effects were not considered. Future studies would benefit greatly from investigating network measures in different age groups. This is because an age-dependent decline in ADHD-related symptoms

has been proposed (Faraone, Biederman, & Mick, 2006; Goldman, Genel, Bezman, & Slanetz, 1998), leading some to suggest that ADHD is a childhood condition of developmental delay (Lara et al., 2009). Although the mechanisms of such a decline remain unknown, future studies would benefit from comparing those with ADHD with both age-matched and younger controls, to help determine whether changes in neural functional networks are the result of delay or of deviant neural mechanisms.

Another limitation concerns the issue that children with ADHD were not treatment-naive. Although the standard protocol would be to investigate brain function in treatment-naive participants (Cao et al., 2012; Cocchi et al., 2012; Wilson et al., 2011), in reality, the majority of those diagnosed as having ADHD are typically prescribed psycho-stimulant drugs (Meltzer et al., 2003) that have been shown to have a response rate of approximately 70% (Goldman et al., 1998). It therefore makes practical sense to investigate network topology in medicated children. As a matter of fact some EEG studies (Lubar et al., 1999; Swartwood et al., 1998) investigating the effects of Methylphenidate, a commonly prescribed stimulant, reported no clear changes in global coherence, a measure of frequency, following treatment administration in children. This aligns with a large morphological study conducted by Castellanos et al. (2002) involving 152 children and adolescents. These researchers too found no significant differences between medicated and drug-naive participants. However, others such as Tomasi and Volkow (2012) have speculated that medication may normalise short-range functional connections. Hence, the results reported in the current study might have been partially influenced by increased levels of drug-related dopamine. Future studies in drug-naive children would help elucidate the role of medication, especially because some like Rubia et al. (2009), and Wong and Stevens (2012) have proposed that medication-related improvement in connectivity in children with ADHD is related to improved working memory that is attributed to significantly increased extracellular dopamine levels (Volkow et al., 2001).

5.6. Conclusion

The findings of lower beta band normalised clustering coefficient, decreased normalised path length, larger diameter, and increased eccentricity in children with ADHD confirmed predicted alterations in this range. The results suggest an overall degree of loss (i.e. a reduction) of local and global efficiency of functional brain networks in the beta band. Interestingly, the present study demonstrated that changes in network topology were associated with levels of cognitive and behaviour functioning. These findings need further

research in a larger ADHD cohort to clarify the role of network analysis and its association with clinical phenotypes.

Given that it is now generally agreed that ADHD has primarily a biological basis (Murias, et al., 2007; Wilson et al., 2011; Sripada et al, 2014), future resting-state MEG studies, using network analysis, would provide an opportunity to further the knowledge of potential neurobiological risk factors that would in turn aid in the identification of more specific diagnostic markers. Defining the characteristics of whole brain topology, as was the case in the present study, is the first step in developing novel biological markers possibly for underlying symptoms that lead to impairment in children with ADHD.

6. Graph theoretical analysis of MEG resting-state functional connectivity networks: a differential study of resting-state functional connectivity in dyslexia, ADHD, and typically developing children

6.1. Chapter summary

This study investigated whole-brain connectivity strength and network organisation differences between two groups of children and adolescents with ADHD and dyslexia and typically developing controls as well as those with comorbid conditions. Using eyes-closed resting-state MEG data, connectivity strength, and network parameters were quantified using graph theory analysis and minimum spanning tree.

The dyslexic readers showed elevated whole-brain connectivity in the theta band compared to the three groups, commonly reported in the dyslexia literature. Compared with controls, the clinical groups with a single neurodevelopmental diagnosis demonstrated lower clustering coefficient, path length, and higher eccentricity, and diameter in beta band activity, suggesting similar underlying pathophysiological mechanisms in the two behaviourally different condition. Interestingly, compared to the two 'pure' clinical groups, those with comorbid condition, demonstrated network efficiency disruptions that revealed a trend in the opposite direction. This preliminary suggests different underlying pathophysiological mechanism in those with 'pure' vs. comorbid developmental conditions. Correlation analyses provided further support for the crucial role of network communication efficiency in high frequency beta and gamma network topology in cognitive and behavioural functioning

Results were in agreement with the proposition that impaired local and global efficiency are characteristic features of atypical development. Given association with measures of cognitive and behaviour functioning, reported network changes appear to reflect clinically relevant biological metrics, that hold promise as markers of impaired functions in atypical neurodevelopment.

6.2. Introduction

Attention-deficit/hyperactivity disorder (ADHD) and dyslexia are two of the most prevalent neurodevelopmental disorders (Boarda, Willcutt, & Pennington, 2012). Developmental disorders are typically conceptualised as products of interactions between genetic and environmental risk factors (Hulme & Snowling, 2009).

Although they are typically viewed as separate neurodevelopmental disorders, ADHD and dyslexia co-occur more often than is expected by chance in childhood, with co-morbidity estimates of between 25-40% (Pennington, Willcutt, & Rhee, 2005; Willcutt & Pennington, 2000). Coined by Feinstein (1970), the term comorbidity refers to the co-occurrence of two or more different conditions in the same individual.

Recent years have witnessed an upsurge of interest in the co-occurrence of the two developmental disorders (See Boada, Wilcutt, & Pennington, 2012; Semrud-Clikeman et al., 1992, for a review). As a result, it is now well acknowledged that 'pure' neurodevelopmental disorders are rare and that co-morbidity is frequent (Hulme & Snowling, 2009). With respect to specific documented difficulties, behaviourally, ADHD and dyslexia are slightly different disorders. Whilst ADHD is characterised by developmentally inappropriate symptoms of inattention, and/or impulsivity/hyperactivity (DSM-IV-TR, 2000; Franzen et al., 2013; Konrad & Eickoff, 2010), dyslexia, is viewed as a specific learning disability characterised by problems related to word recognition, decoding, and spelling, despite normal intelligence, schooling and motivation (Knivsberg et al., 1999; Kraus, 2012; Pennington, Van Orden, Smith, Green, & Haith, 1990). For those with ADHD, the difficulties experienced typically, result from a deficit in regulatory control processes (Barkley 1990; Douglas, 1983), while for most (Boada et al., 2012; Goswami, 2011; Stanovich, 1988; Svensson & Jacobsson, 2006) dyslexia is the result of a deficit in underlying phonological processing.

Another factor differentiating children with ADHD from those with dyslexia is performance on achievement tests (e.g. intelligence tests). Individuals with ADHD typically underperform, leading many to suggest that key factors such as intellectual abilities may co-vary with ADHD (Kuntsi et al. (2004). In contrast, children with dyslexia are often reported to have normal intelligence (Pennington et al., 1990) despite having reading-related problems.

The difficulties in regulatory or control processes in ADHD, such as impulsiveness (Faraone & Biederman, 2005; Matza, Paramore, & Prasad, 2005) and the difficulties associated with a lack of phonemic awareness (such as reading accuracy) in dyslexia (Bruck, 1990; Maughan

& Hagell, 1996; Svensson & Jacobsson, 2006), persist into adulthood. Given the lifelong burdens imposed on individuals and society, it is important to understand the causes of these two highly comorbid conditions and develop more effective interventions that effectively address their underlying causes (Konrad & Eickhoff, 2010; Macdonald, 2010) and help in the early identification of at-risk individuals.

The co-occurrence of ADHD and dyslexia can be classified as heterotypic comorbidity (i.e. co-occurrence in two different diagnostic groups: see Angold, Costello, & Erkanli, 1999). It is known that behaviourally the two conditions are different. Furthermore, although it is true that one can identify those affected using phonological (dyslexia) or inattention-hyperactivity/impulsivity scores (ADHD), this does not tell us why the conditions occur. But if one were to find that there is a difference in terms of brain function in the two conditions, then a biological metric could help discriminate or connect the underlying pahophysiolgical mechanisms in those with ADHD and dyslexia. This would be a significant means of indicating something regarding the underlying causes.

Magnetic resonance imaging (MRI) studies have revealed that the brains of children and adolescents with ADHD (Castellanos et al., 1996; Mackie et al., 2007; Seidman et al., 2011) and those with dyslexia (Brown et al., 2011; Eckert et al., 2003; Eliez et al., 2000) show significant differences when compared to typically developing age-matched controls. For some, reported differences are region-specific (See Bush, 2010 ; Richlan, 2012, for reviews of dyslexia and ADHD respectively), while for others (Franzen et al., 2013; Schurz, Wimmer, Richlan, Ludersdorfer, Klack, & Kronbichler, 2014) it is the circuits involving several regions that are believed to underlie reported abnormalities (Beaulieu et al., 2005; Bush 2010; Klingberg et al., 2000).

Further support for a neurobiological basis of ADHD and dyslexia comes from studies that have revealed grey and white matter abnormalities in these children (Ashtari et al. 2005; Richlan, Kronbichler, & Wimmer, 2013; Silk et al., 2009b). In children and adolescents with ADHD, reductions in white and grey matter have been reported in both right and left hemispheres, typically in the right frontal cortex (Ashtari et al., 2005; Silk et al., 2007b). A study by Overmeyer et al. (2001) investigating cortical abnormalities in children with ADHD and controls reported reductions in both grey and white matter. Grey matter differences were observed in regions including the right frontal and cingulate gyrus. Observed white matter reductions were reported predominantly in the left hemisphere.

In dyslexia, like ADHD, different levels in both white and grey matter have also been

reported (Eliez et al., 2000; Vinckenbosch, Robichon, & Eliez, 2005). An MRI study by Brown et al. (2001) investigating neural correlates of dyslexia revealed decreases in grey matter in dyslexic male subjects, predominantly in the left temporal lobe, temporo-parieto-occipital junction (implicated in high-level reading and comprehension functions, including visuo-spatial recognition, symbol processing, writing, language, working memory and reading De Benedictis et al., 2014), as well as in the frontal lobe, cerebellum, and thalamus. Reduction in all but the bilaterally temporo-parieto-occipital (TPO) junction has been reported in ADHD by researchers such as Mostofsky et al. (2002).

The changes in spatially distributed grey and white matter may result from a defective structure and functioning of myelin sheath in the brain, including atrophy (Overmeyer et al., 2001) and/or deceased branching in white-matter pathways (Silk et al., 2009b). It is believed that white matter tracks determine the strength of communication between brain areas involved in several high-level functioning information processing elements. It is likely that their alteration disrupts the efficiency of information processing in widely distributed cortical areas. Observed morphologic alterations in distributed brain regions have been proposed as the underlying mechanisms contributing to the reported deficits associated with the two conditions (See Bush, 2010; Eckert et al., 2005; Richlan, 2010, for reviews on both developmental disorders).

To demonstrate the association between brain structure and functioning, several studies have examined the relationship between brain volumes and measures of functioning and cognitive ability. As discussed in Chapter 5, in ADHD, researchers have previously reported associations between smaller white matter volumes and higher externalising behaviour problems in ADHD boys (Semrud-Clikeman et al., 2000), and anxiety-depression in girls with ADHD (Castellanos et al., 2002).

In dyslexia, individual differences in reading ability have been shown to correlate with white matter structure, measured using DTI in both adults (Klingberg et al., 2000) and children (Deutsh et al., 2005). Using DTI to examine integrity of white matter in adults with poor reading abilities, Klingberg et al. (2000) revealed an association between decreased temporo-parietal white matter structure levels (measured using anisotropy) and reading difficulty. A similar trend was observed in children, i.e. lower anisotropy was associated with poorer performance scores on reading, spelling, and rapid naming (Deutsh et al., 2005). With regard to grey matter, a recent meta-analysis (Richlan et al., 2013) revealed that reduced grey matter volume in dyslexic readers was prominent in the reading-related regions of right superior temporal gyrus and left superior temporal sulcus.

Although the standard protocols for diagnosing and characterising ADHD and dyslexia involves the use of behavioural and cognitive measures, converging evidence from several neuroimaging studies such as those discussed above points to a neurobiological basis of ADHD (Mostofsky et al., 2002; Overmeyer et al., 2001) and dyslexia (Eliez, Rumsey, Giedd, Schmitt, Patwardh, & Reiss, 2000; Klingberg et al., 2000). However, despite several imaging neuroscience studies, the underlying pathophysiological neural mechanisms and pattern of brain-related abnormalities in these conditions remain largely unknown.

An alternative strategy to investigate the neural underpinnings of developmental disorders, coinciding with emerging conceptualizations of neuropsychiatric conditions (such as schizophrenia, autism, and Alzheimer's schizophrenia) has emerged in neuroscience. This has shifted the focus from specific regional brain abnormalities to a conceptualization of neuropsychiatric disorders occurring as a result of dysfunctions/disconnections in distributed network organizations (Konrad & Eickhoff, 2010). Viewed this way, the 'brain systems' approach considers functions (e.g. cognitive and behavioural) as emerging from an interaction of specialised brain regions (Konrad & Eickhoff, 2010; Richlan, Kronbicherler, & Wimmer, 2009; Schurz et al., 2014).

For this reason, quantifying brain connectivity has become a crucial means of gaining further insight into brain function. The present study aimed to investigate functional connectivity during task-independent states in subjects with two of the most common neurodevelopmental conditions. The question being explored is whether the two conditions differ qualitatively from each other or simply diverge within a uni-dimensional pattern.

Recent advances in network science, specifically, graph theory; have permitted the characterisation of topological properties of complex networks. Using these approaches researchers have shown that network configurations in children with ADHD (Ahmadlou et al., 2011; Cao et al., 2012) and dyslexia (Dimitriadis et al., 2013; Gonzalez et al., 2015) are characterised by less local information processing and global integration. However, as discussed in Chapter 5, the majority of functional imaging studies of people with both developmental disorders have tended to use task-based activation paradigms to reveal dysfunctions of brain interactions that are associated with various functions including cognition and attention. These studies are based on the assumption that developmental conditions result from disconnections between regions supporting crucial networks, typically the default mode.

With respect to this strategy, dyslexia studies have suggested that reported difficulties represent a disconnection syndrome, involving altered communication in local and/or global efficiency (i.e. short and long-range functional connections) (See Paulesu et al., 1996; Richlan et al., 2009; Richlan, 2012; Schurz, et al., 2014; Vourkas et al., 2011, for a review).

Using whole-brain sensor-level MEG data and phase lag index to estimate functional connectivity, Vourkas et al. (2011) examined task-based (i.e. pseudo-word reading and letter-sound naming tasks) brain network connections in children with severe reading difficulties and in controls. Using graph theory concepts, the authors found that children with severe reading difficulties showed significantly lower local clustering in the alpha (8-13 Hz) range and lower global efficiency in both the alpha and gamma (20-30 Hz) range, suggesting a less organised network. The study provided evidence that short and long-range functional connections are altered in children with severe reading difficulties. This study also reported significant correlations between graph measures and reading ability.

Recently, and alternatively focussing on specific regions and networks in the left hemisphere, the study by Schurz et al. (2014) reported lower connectivity in dyslexic readers between the left posterior temporal areas and left inferior frontal gyrus. Lower connectivity was observed during both task performance and rest. However, the authors also reported higher connectivity in reading-based areas and those of the default mode network particularly the precuneus, when compared to children without impaired reading.

The brain connectivity-function relationship in ADHD was discussed in Chapter 5 of this thesis (See Introduction). Briefly, the evidence in relation to ADHD has been inconsistent. For instance, as noted in Chapter 5, Castellanos et al. (2008) reported lower functional connectivity between the anterior cingulated cortex and regions of the default mode network such as the precuneus, while Tian et al. (2008) reported higher resting connectivity in sensory cortices, believed to be crucial for attention deficits in ADHD. As explained in Chapter 5, previous neuroimaging studies have two major limitations. Either they focus on task-based activations or rely on pre-defined regions or networks chosen based on a priori literature.

In the current study, these limitations were addressed by examining whole-brain interactions as correlates of dyslexia and ADHD, using a task independent paradigm. The study specifically aimed to investigate whether the application of network analysis derived from resting-state MEG data could differentiate children with dyslexia from controls and from those with ADHD. A secondary task was to determine whether network measures could

predict cognitive/behavioural functioning in dyslexics, those with ADHD, and controls.

<u>Aim</u>

The study focused on a task-independent functional network level of explanation to examine whether there is a difference, in terms of over network connectivity vs. under network connectivity, as a potential metric to discriminate between children with ADHD and those with dyslexia, for both in relation to neurotypical controls. Understanding the role of task-independent brain network measures has the potential to shed light upon the neurodevelopmental mechanisms involved and how these changes present themselves in the two conditions, ultimately affecting the symptom profile of these children.

<u>Hypothesis</u>

1. Whole brain resting-state theta connectivity strength will be increased in dyslexic readers compared to controls and those with ADHD.
2. Resting-state functional network parameters will discriminate controls from those with dyslexia.
3. Disrupted network parameters will mirror those observed in ADHD but not comorbid conditions.
4. There will be correlations between MEG disrupted network measures and cognitive/behavioural functioning. Higher global efficiency will be associated with better cognitive performance.

6.3. Methods

6.3.1. Participants

Four groups of children aged between 7 and 17 years participated in the current study. These included typically developing children (previously described in Chapter 4 & 5), those with a diagnosis of ADHD (described in Chapter 5), those with dyslexia, and those with comorbid conditions.

The 16 children with developmental dyslexia were an opportunistic sample recruited by a research associate from the Dyslexia and Developmental Assessment Unit (DDAU) at the Aston Brain Centre. The children were part of a separate cross-sectional study investigating

genetic links associated with literacy difficulties, predominantly developmental dyslexia. Diagnosis was confirmed by educational psychologists undertaking a range of cognitive and literacy assessments with the purpose of determining eligibility for special education. Following the assessments, families were approached by the research associate to explore the prospect of participating in a neuroimaging study. It was strongly emphasised that declining to participate would have no implication for the family's current or future relationship with the clinic. Those interested were offered a scheduled visit to the centre. Consent, withdrawal, data protection, and confidentiality issues were discussed with the families prior to their visit. All children had received their diagnoses based on their performance on a battery of oral, fluency, and word reading efficiency tests.

Inclusion criteria included the ability to provide assent, the child being aged between 7:0 and 17:11 years, and having a diagnosis of reading difficulties confirmed by an educational psychologist. The exclusion criteria included children being outside the age-range, a primary diagnosis of a major psychiatric disorder or neurologic disease, a history of substance abuse and children whose parents/guardians did not consent to their taking part.

Due to significantly higher scores in whole brain connectivity strength in the beta band, three of the sixteen children with developmental dyslexia were excluded from this group and placed into the comorbid condition group. See Chapter 5 and 6 for sampling information for the controls, and for children with ADHD respectively.

The fourth group classified as the comorbid condition group, comprised children who had initially been recruited in the two clinical groups. The comorbid group comprised six children between the ages of 7 and 16 years. Of these, two had ADHD and dyslexia (un-medicated), one had dyslexia and developmental co-ordination disorder (dyspraxia, affecting motor coordination in children), and finally there were three children, who were initially recruited as dyslexic readers. Compared to the dyslexia group, these three children scored very highly on some of the network analysis measures (> 3 SD). Rather than exclude them completely they were included in the comorbid condition. Developmental disorders are more often present with comorbid conditions. See Table 6.1 for the demographic data. Each child in the clinic group was matched with at least one control.

Procedure

With the exception of children with dyslexia, who had to return to the Aston Brain Centre for this study following earlier confirmation of a diagnosis, all testing for the other groups was done in one session often lasting up to 3 hours.

6.3.2. MEG data acquisition

MEG recordings were carried out in corroboration with another researcher. MEG recordings were acquired during resting-state conditions (in a procedure similar to that described in previous chapters). The data reported are only about eyes-closed resting state, in line with the evidence from Chapter 3 showing that networks in state have higher reproducibility across repeated measurements compared to the eyes-open state.

6.3.3. MEG data pre-processing

Continuous resting-state data were MaxFiltered and visually inspected following the procedures described in Chapter 3.

<u>Intellectual and behavioural assessments</u>

As described in Chapter 5, behavioural functioning in children with ADHD and controls was assessed using the CBCL/6-18 behaviour questionnaire (Achenbach et al., 2011, see Chapter 5, Method section for a discussion of the CBCL questionnaire). Behavioural functioning in children with dyslexia was examined as part of their full psychological assessments during their clinical visits. This was done using a brief version of the CBCL/6-18, known as the Brief Problem Monitor. The Brief Problem Monitor for ages 6-18 (BPM/6-18, Achenbach et al., 2011) that was adapted for children with dyslexia, is part of the Achenbach System of Empirically Based Assessment (ASEBA; www.aseba.org) questionnaires. It offers an abbreviated questionnaire comprising of attention, internalising, externalising, and total problems sub-scales. These are derived from the CBCL/6-18; Teachers' Report Form (TRF), and Youth Self-Report (YSR) (Manual for the ASEBA Brief Problem Monitor, 2011). It consists of 19 items that take approximately two minutes to complete (Manual for the ASEBA Brief Problem Monitor, 2011), and has been shown to have high test-retest consistency (r = 0.73-0.82).

Compared to other brief assessment instruments such as the 12-item Brief Problem Checklist (BPC: Chorpita et al., 2010), the BPM offers additional items to assess attention (Piper, Gray, Raber, & Birkett, 2014). As with the CBCL, the BPM item questionnaire has been standardised separately for gender, age group, informant (i.e. parent, teacher, or child) and society. Standardised T scores > 65 (i.e. 93rd percentile) are noted as being high enough to raise concern (Manual for the ASEBA Brief Problem Monitor, 2011).

To avoid repeated testing, children's scores of attention internalised and externalised behavioural functioning were derived from this scale. As was the case in previous chapters, only the attention, internalised and externalised problem scores were considered. These scores were compared to those derived from the CBCL/6-18 in controls and in children with ADHD. In controls, internalised and externalised behavioural functioning scores were available for 20 of the 21 participants, while for the dyslexia group, behaviour data on the three measures were available for 11 of the 13 participants.

Cognitive abilities

Procedures for IQ assessments were described in chapter 5. All children with dyslexia completed age-appropriate Full Scale IQs, as part of their clinical assessments, administered by an educational psychologist using the WISC-IV^{UK} (Wechsler, 2003). However, only their SI and MR scores are reported in the current study. As previously stated in Chapter 2, section 2.4., children with developmental dyslexia had received full IQ assessments as part of their evaluation during their earlier visit to the centre. These children were therefore not tested again. Their scaled scores on the SI and MR subtests were used, as it was considered unlikely that cognitive ability would have changed by the time of the current study tests.

Statistics

SPSS 20.0 package for Windows was used to compute all statistical analyses. Assessing the normality of network metrics using the Kolmogorov-Smirnov test showed a non-normal distribution. Hence, the non-parametric Kruskal-Wallis H test was used, and where appropriate this was followed by Mann-Whitney U-tests for pairwise comparisons, with the Bonferroni correction.

Unless otherwise noted, all planned post-hoc testing concerned comparisons between: first, the dyslexia group and the controls and, second, the ADHD group and the dyslexia group. No contrasted comparisons were computed with the comorbid conditions group. Because of the varied profiles in the comorbid group, no consistent and informed decision on appropriate network measures could be made.

Correlations between network measures and cognitive ability, as well as behavioural measures were computed using Spearman's rank correlation coefficient. All statistical

analysis was computed separately for each frequency band. Due to multiple correlations, only r_s values with $p <.01$ were reported.

6.4. Results

For the ADHD group, data on cognitive abilities were available for all participants. For the dyslexia group verbal performance scores were available for 11 of the 13 participants, while non-verbal scores were available for 12 of the 13 participants. For children in the comorbid condition group, non-verbal performance scores were available for 3 of the 6 participants, while no verbal scores were available for this group. For this reason, computations were primarily assessed in relation to controls, those with ADHD and children with dyslexia.

Data in Table 6.1 showed group differences in measures of behavioural and cognitive functioning across the three groups. Planned post-hoc testing (Bonferroni-corrected α level of .025) indicated that cognitive abilities between the control group children and those with dyslexia were not significantly different for verbal (SI: scaled scores: $U = 98.0$, $p = .49$), or non-verbal performance (MR: scaled scores: $U = 79.0$, $p = .08$). In addition, comparisons between children with dyslexia and those with ADHD revealed significantly lower verbal ($U = 20.0$, $p = .004$), but not non-verbal performance ($U = 43.5$, $p = .10$) in children with ADHD.

For measures of behavioural functioning, comparisons revealed that the dyslexia children were reported to have more internalised problems than controls ($U = 35.0$, $p = .001$) and a trend towards higher attention ($U = 68.0$, $p = .08$) and externalised problems ($U = 60.5$, $p = .04$), though these were not statistically significant at the corrected α level of .025.

In the comparisons between children with ADHD and those with dyslexia, the results showed that those with dyslexia were reported to have significantly less attention ($U = 8.5$, $p < .001$) and externalised behaviour problems ($U = 10.0$, $p < .001$). However differences in reported internalised behaviour problems were not statistically different between these two groups ($U = 47.5$, $p = .27$).

Table 6. 1 Demographic characteristics

	ADHD	TDC	Dyslexia	P	Comorbid conditions
	(*n* = 12)	(*n* = 21)	(*n* = 13)		(*n* = 6)
	M ± SD	*M ± SD*	*M ± SD*		
Age (years)	12.4 ± 3.5	11.6 ± 3.1	12.5 ± 3.3		12.1 ± 1.5
Gender (M/F)	10:1	10:11	9:4		4:2
Hand (L/R)	2:10	2:19	1:13		0:6
SI	8.3± 2.3	12.7 ± 2.5	11.8 ± 2.9	*	*NA*
MR	9.1 ± 2.4	11.3 ± 1.9	9.9 ± 2.1	*	10.3 ± 4.9
Att	78.5 ± 10.36	52.4 ± 3.6	58.7 ± 8.9	*	67.2 ± 11.7
Int	65.8 ± 11.02	48.5 ± 8.0	60.6 ± 9.2	*	61.0 ± 6.6
Ext	70.3± 8.6	44.5 ± 8.9	51.55 ± 7.3	*	60.7 ± 12.9

*Values are M ± SD. *p<.05 (Kruskal-Wallis H test). Intellectual ability denoted population standardized scaled scores (M = 10, SD = 3), while measures of behaviour functioning were T scores (M = 50, SD = 10). Key: Att = attention; Int = internalised; Ext = externalised; SI = similarities; MR = matrix reasoning, M/F = male/female, L/R = left/right, TDC = typically developing controls; NA = not applicable.*

6.4.1. Whole-brain functional connectivity between the groups

As described in the previous experimental chapters, whole-brain functional connectivity was assessed using the phase-lag-index for each frequency band, for eyes-closed resting-state data. Kruskal-Wallis H tests revealed that whole-brain coupling strength was statistically significant in slow (theta) and fast (beta) oscillatory networks in the four groups (See Table 6.1 for mean rank scores). Results are discussed in detail below.

<u>Theta band whole-brain functional connectivity</u>

The Kruskal-Wallis H test showed that there was a statistically significant difference in mean phase lag index values between the four groups in the theta band, ($H(3)$ = 10.88, $p <.$ 05, see Figure 6.1). Planned post-hoc contrasts, using Mann-Whitney U-tests (Bonferroni-corrected α level of .025), revealed that the children with dyslexia group showed significantly higher mean whole-brain functional connectivity compared with controls (U = 51.00, z = -3.03, $p <$ .025, d = -1.26), and with children with ADHD (U = 28.50, z = -2.69, $p <$ .025, d = -1.17, see Table 6.2 for mean rank scores). The results revealed increased whole brain connectivity in the theta band in children with dyslexia compared to controls and children with ADHD. In addition, although this was not initially considered in planned analysis, the results in Figure 6.1 revealed that global coupling in dyslexia was also higher compared to

the comorbid group.

Beta band functional connectivity

The Kruskal-Wallis H test showed that there was a statistically significant difference in mean phase lag index values between the four groups in the beta band, (H(3) = 8.70, $p <$. 05, see Figure 6.1). The planned analysis of contrasts revealed no significant differences in functional connectivity in the beta band, when children with dyslexia were compared with controls (U = 132.50, z = -.14, ns, d = -.11). However post-hoc comparison between the two clinical groups revealed a trend towards lower whole brain functional connectivity in children with ADHD compared to those with dyslexia, (U = 43.00, z = -1.91, p = .06, d = -.87, although this was not statistically significant. See Table 6.2 for mean rank scores). Across all four groups, whole-brain functional connectivity was lower in the beta band in those with ADHD compared to the remainder of the groups.

Table 6. 2 Mean rank phase lag index values for the four groups for each frequency band

	Groups	Theta		Alpha1		Alpha2		Beta		Gamma	
			p		*p*		*p*		*p*		*p*
Mean PLI	ADHD	22.63		25.58		30.79		16.08		28.17	
	Dyslexia	38.50		24.23		28.42		28.58		23.23	
	CC	22.17	*	31.75	*NS*	21.33	*NS*	36.17	*	33.58	*NS*
	TDC	22.52		26.93		24.33		28.40		25.55	

Values are mean rank scores of different groups for each frequency band. Kruskal-Wallis H tests (*p <.05, **p <.01) showed that there were statistically significant differences in mean phase lag index scores between the four groups in the theta and beta band. Key: PLI = phase lag index, ADHD = attention-deficit/hyperactivity disorder, CC = comorbid controls, TDC = typically developing controls, and NS = not significant.

Figure 6. 1 Mean group differences in whole-brain functional connectivity

Average whole-brain connectivity strength for each frequency band. Kruskal-Wallis H test (*p <.05) revealed there were significant differences in mean phase lag values between the four groups in the theta and beta band. Error bars are ± 2 SE, standard error. Key: TDC = typically developing controls, ADHD = attention-deficit/hyperactivity disorder.

6.4.2. Characterising functional network organisation using graph theoretical analysis

All participants demonstrated small-world network properties (i.e. normalised clustering and path length values were > 1) across all frequency bands. Differences in network topological organisation in the four groups were assessed independently for each frequency band using Kruskal-Wallis H tests. The tests revealed statistically significant differences in the beta and no other frequency band (See Table 6.3). The results are discussed in detail below. These results only concern network measures for which group differences were observed. For a complete representation of the results in other frequencies, see Table 6.3 and Figure 6.2.

Beta band normalised clustering coefficient (i.e. short-range functional connections)

A Kruskal-Wallis H test showed that there was a statistically significant difference in clustering between the four groups in the beta band, (H(3) = 11.03, p <.05, see Figure 6.2).

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Comparisons revealed that the clustering coefficient values in children with dyslexia were not significantly different from those in the controls (U = 94.00, z = -1.51, *ns*, *d* = .28) or children with ADHD (U = 60.50, z = -.95, *ns*, *d* = -.05). See Table 6.3 for mean rank scores.

Beta band normalised characteristic path length (i.e. long-distance functional connections)

A Kruskal-Wallis test showed that there was a statistically significant difference in path length between the four groups in the beta band (H(3) = 15.54, p <.01, see Figure 6.2). The results from pairwise comparisons revealed a trend towards a lower path length in children with dyslexia compared to controls (U = 80.50, z = -1.99, p = .05, *d* = .41), though this was not statistically significant at the corrected α level. No statistically significant difference were observed in normalised path length between the dyslexics group and those with ADHD (U = 64.00, z = -.76, *ns*, *d* = -.59). See Table 6.3 for mean rank scores.

Mean rank scores in Table 6.3 and results in Figure 6.2 suggest a non-significant but constant trend in the beta band towards decreased normalised clustering coefficient and path length in ADHD and dyslexia compared to controls. However, in comorbid conditions, these trends changed in the opposite direction when compared to controls. In other words, the results suggested that disruptions in short and long-distance functional connection followed a similar trend for those with ADHD and dyslexia but not in those with comorbid conditions.

Though this was not statistically significant (α level of 0.5), the Kruskal-Wallis H test also revealed a trend towards differences in small-worldness between the four groups in the gamma band, (H(3) = 6.68, p = .083, see Appendix A.1).

Table 6. 3 Mean rank scores from Kruskal-Wallis H tests for differences in graph theoretical analysis network parameters

	Groups	Theta	p	Alpha1	p	Alpha2	p	Beta	p	Gamma	p
Gamma	ADHD	31.25		28.00		31.50		17.92		28.33	
	Dyslexia	24.27		24.62		25.00		22.42		23.96	
	CC	23.25	NS	25.08	NS	17.08	NS	40.58	*	33.42	NS
	TDC	26.10		27.21		27.26		29.90		25.05	
Lambda	ADHD	33.25		26.71		29.08		15.79		29.71	
	Dyslexia	28.15		26.50		24.27		21.19		24.04	
	CC	22.50	NS	24.33	NS	19.92	NS	40.83	**	23.42	NS
	TDC	22.76		27.00		28.29		31.81		27.07	
SMW	ADHD	25.04		27.71		29.92		30.88		22.46	
	Dyslexia	19.27		25.46		28.77		24.73		23.35	
	CC	33.58	NS	28.75	NS	27.58	NS	27.33	NS	40.58	NS
	TDC	29.79		25.81		24.55		24.86		26.74	

*Values are mean rank scores of different groups for each frequency band. Kruskal-Wallis H tests (*p <.05, **p <.01) showed that there were statistically significant differences in mean clustering and path length between the four groups in the beta band. Key: gamma = normalised clustering coefficient, lambda = normalised characteristic path length, SWN = small worldness, ADHD = attention-deficit/hyperactivity disorder, CC = comorbid controls, TDC = typically developing controls and NS = not significant.*

Figure 6. 2 Group differeneces in topological network organisation across frequency bands, using graph theoretical analysis

Topological functional networks in A) short-range connections and B) long-distance connections. Kruskal-Wallis H tests (*p <.05) revealed there were significant differences in the local and global efficiency between the four groups in the beta band. Error bars are ± 2 SE, standard error. Key: ADHD = attention-deficit/hyperactivity disorder, and TDC = typically developing controls.

6.4.3. Characterising functional network organisation using minimum spanning tree metrics

Differences in network topological organisation in the four groups were assessed independently for each frequency band using Kruskal-Wallis H tests. As with graph theoretical analysis, significant findings were observed in the beta and in no other frequency band (See Table 6.4). However, though it was not statistically significant (α level of .05), a Kruskal-Wallis H test revealed a trend towards differences in eccentricity between the four groups in the upper alpha band, (H(3) = 6.31, p = .097).

The results are discussed in detail below. Only those metrics in which significant group differences were observed are discussed. For a complete representation of results in other frequencies, see Table 6.4 and Figure 6.4.

MST beta band eccentricity

A Kruskal-Wallis H test revealed that there was a statistically significant difference in MST

eccentricity between the four groups in the beta band, (*H*(3) = 11.4, *p* =.01, see Figure 6.3A). Pairwise comparisons showed that eccentricity was significantly higher in children with dyslexia compared to controls (*U* = 65.5, *z* = -2.52, *p* < .025, *d* = -.93). However, when compared with the ADHD group, eccentricity in the dyslexia group was not statistically different (*U* = 66.00, *z* = -.65, *ns*, *d* = -.30). See Table 6.4 for mean rank scores

MST beta band diameter

A Kruskal-Wallis H test showed that there was a statistically significant difference in MST diameter between the four groups in the beta band, (*H*(3) = 8.50, *p* <.05 (Figure 6.3B). Pairwise comparisons with adjusted *p*-value revealed a trend towards higher diameter in the dyslexia group when compared to the controls, (*U* = 79.00, *z* = -2.04, *p* = .04, *d* = -.71), although this was not statistically significant at the Bonferroni corrected α level. No statistically significant differences were observed in a post-hoc comparison of diameter in children with dyslexia and those with ADHD (*U* = 70.00, *z* = -.44, *ns*, *d* = -.19). See Table 6.4 for mean rank scores

MST beta band hierarchy

A Kruskal-Wallis H test showed that there was a statistically significant difference in hierarchical structures between the four groups in the beta band, (*H*(3) = 10.80, *p* <.05, Figure 6.3C). Pairwise comparisons, with adjusted *p*-value, showed that hierarchical organisation in children with dyslexia was significantly higher when compared with controls (*U* = 41.00, *z* = -3.39, *p*<.025, *d* = -1.43) and showed a trend towards higher values when compared with those with ADHD (*U* = 39.00, *z* = -2.12, *p* = .04, *d* = -.80). See Table 6.4 for mean rank scores

Table 6. 4 Mean rank scores from Kruskal-Wallis H tests for differences in minimum spanning tree network parameters

	Groups	Theta		Alpha1		Alpha2		Beta		Gamma	
			p		*p*		*p*		*p*		*p*
Eccentricity	ADHD	26.63		27.71		18.50		33.04		28.04	
	Dyslexia	26.04		26.00		33.15		34.58		23.31	
	CC	28.75	*NS*	23.92	*NS*	30.50	*NS*	18.75	**	27.50	*NS*
	TDC	26.07		26.86		25.81		19.98		27.31	
Diameter	ADHD	26.45		27.67		18.00		32.46		27.08	
	Dyslexia	25.53		24.73		31.35		33.15		23.04	
	CC	29.08	*NS*	23.33	*NS*	31.25	*NS*	18.92	*	27.00	*NS*
	TDC	26.33		27.83		27.00		21.14		28.18	
Hierarchy	ADHD	28.67		28.96		30.33		23.33		25.50	
	Dyslexia	30.73		26.46		30.38		38.31		27.35	
	CC	24.17	*NS*	28.83	*NS*	23.83	*NS*	24.83	*	31.00	*NS*
	TDC	23.31		24.45		22.67		21.48		25.26	
Leaf number	ADHD	29.63		26.17		31.71		23.71		26.25	
	Dyslexia	28.77		28.19		28.54		34.42		28.12	
	CC	24.17	*NS*	28.50	*NS*	20.83	*NS*	23.17	*NS*	27.58	*NS*
	TDC	23.98		25.07		23.88		24.14		25.33	

Values are mean rank scores of different groups for each frequency band. Kruskal-Wallis H tests (*p <.05, **p <.01) showed that there were statistically significant differences in eccentricity, diameter, and hierarchical structures, between the four groups in the beta band. Key: ADHD = attention-deficit/hyperactivity disorder, CC = comorbid conditions, and TDC = typically developing controls. NS = not significant.

Figure 6. 3 Group differences in topological organisation across frequency bands using minimum spaning tree

Topological functional networks in relation to A) eccentricity and B) diameter, and C) hierarchical structure. Kruskal-Wallis H tests (*p <.05, *p <.01) revealed there were significant differences in the beta band in these three topology parameters between the four groups. Error bars are ± 2 SE, standard error. Key: ADHD = attention-deficit/hyperactivity disorder, and TDC = typically developing controls.

<u>Network correlation with cognitive abilities in the ADHD group</u>

The association between network organization and functioning was only considered in the control, ADHD and dyslexia groups. Those with comorbid conditions were not investigated because, as stated earlier (See Results), data on cognitive abilities were not available for most children in this group.

Despite results in Table 6.1 showing that there were statistically significant differences in verbal and non-verbal performance scores between the three groups, the planned comparisons indicated the following results. There was no significant difference in verbal or non-verbal performance scores between controls and those with dyslexia. Children with ADHD revealed a significantly lower verbal but not non-verbal performance when compared to those with dyslexia. Observations of lack of significant differences in relation to some of the planned comparisons warranted the further investigation of correlations between cognitive abilities and network measures across the groups (i.e. controls, ADHD, and children with dyslexia). These were computed for each frequency band.

Significant correlations and trends were observed in the beta and gamma bands. However, none of the correlation coefficients discussed was statistically significant when a stricter Bonferroni-corrected α level of .001 was applied. Correlations that were significant at the standard α level of .05 are displayed in Figure 6.5 (See Table 6.5 for a complete list of results). Reported results denote Spearman's rank correlation coefficient (r_s), 95% bootstrap confidence intervals (in square brackets) and significance level.

<u>Beta band</u>

Non-verbal performance scores were positively associated with path length (r_s = -.30 [.01, .55], p = .05, see Figure 6.4), and negatively with eccentricity (r_s = -.37 [-.63, -.03], p = .014, see Figure 6.5B) and diameter (r_s = -.38 [-.64, -.04], p = .01, see Figure 6.5D). Also, a trend towards a negative correlation between non-verbal reasoning and small-worldness (r_s = -.26 [-.56, .04], p = .09) was observed.

Verbal performance scores were negatively correlated with eccentricity (r_s = -.47 [-.65, -.22], p = .001, see Figure 6.5A) and diameter (r_s = -.43 [-.62, -.16], p = .004, see Figure 6.5C), and revealed trends towards positive correlations with the clustering coefficient (r_s = .30 [.00, .54], p = .06) and path length (r_s = 28 [-.03, .56], p = .06).

Gamma band

In the gamma band, verbal reasoning showed a trend towards a negative correlation with path length (r_s = -.27 [-.56, .07], p = .08) and a trend towards a positive correlation with small worldness (r_s = .29 [-.03, .54], p = .06).

Table 6. 5 Associations between network organisation and cognitive functioning in controls, children with dyslexia, and those with ADHD

(n = 44)

Band	Metric	SI	MR
Theta	Gamma	.08(.63)	.11(.47)
Theta	Lambda	-.05(75)	.01(.96)
Theta	Small-world	.11(.50)	.12(.45)
Theta	Eccentricity	-.18(.24)	-.11(.50)
Theta	Diameter	-.15(.34)	-.08(.62)
Theta	Leaf number	.07(.66)	-.03(.86)
Theta	Hierarchy	.05(.77)	-.07(.65)
Alpha1	Gamma	-.12(.45)	-.15(.32)
Alpha1	Lambda	-.16(.30)	-.12(.45)
Alpha1	Small-world	.05(.75)	-.23 (.15)
Alpha1	Eccentricity	.05(.77)	-.10(.53)
Alpha1	Diameter	.07(.65)	-.08(.60)
Alpha1	Leaf number	-.15(.34)	.06(.70)
Alpha1	Hierarchy	-.08(.59)	.00(1.0)
Alpha2	Gamma	-.10(.53)	.02(.91)
Alpha2	Lambda	-.03(.83)	.03(.84)
Alpha2	Small-world	.10(.51)	-.03(.83)
Alpha2	Eccentricity	.25(.10)	.14(.37)
Alpha2	Diameter	.29(.06)	.15(.32)
Alpha2	Leaf number	-.16(.28)	-.06(.70)
Alpha2	Hierarchy	-.13(.42)	-.02(.90)
Beta	Gamma	.30(.06)	-.10(.51)
Beta	Lambda	.28(.06)	.30(.05)*
Beta	Small-world	-.11(.47)	-.26(.09)
Beta	Eccentricity	-.47(.00)**	-.37(.01)*
Beta	Diameter	-.43(.00)**	-.38(.01)*
Beta	Leaf number	.10(.56)	-.03(.85)
Beta	Hierarchy	.01(.94)	-.03(.83)
Gamma	Gamma	-.24(.12)	-.07(.66)
Gamma	Lambda	-.27(.08)	-.05(.74)
Gamma	Small-world	.29(.06)	.03(.87)
Gamma	Eccentricity	.08(.61)	-.11(.49)
Gamma	Diameter	.08(.62)	-.12(.45)
Gamma	Leaf number	-.06(.70)	.01(.94)
Gamma	Hierarchy	-.01(.93)	-.00(.98)

Spearman's rank correlation coefficients (r_s) for network measures and cognitive performance scores in each frequency band. Key: SI = similarities (i.e. verbal skills), MR = matrix reasoning (i.e. non-verbal skills), network metric gamma = normalised clustering coefficient, lambda = normalised characteristic path length, and small-world = small-world index. *p <.05, **p < .01, and ***p <.0024 (Bonferroni-corrected).

The correlation between normalised characteristic path length and non-verbal scores revealed that poorer non-verbal performance was associated with decreased global efficiency. See Figure 6.4 below.

Figure 6. 4 Association between beta band normalised path length and variations in non-verbal performance scores in controls, children with dyslexia and those with ADHD
A positive association (p<.05) is seen between normalised path length and non-verbal performance, though this is not statistically significant at the Bonferroni corrected α level. The solid line is the fit-line for scores across the sample cohorts of controls, children with ADHD and those with dyslexia. Key: ADHD = attention-deficit/hyperactivity disorder, and TDC = typically developing controls

Spearman's rank correlation coefficient shows negative associations between minimum spanning tree diameter/eccentricity and cognitive performance scores. In other words, poorer verbal and non-verbal performance was associated with increased beta band eccentricity and diameter. See Figure 6.5 below.

Figure 6. 5 Association between beta band network organisation measures and variations in cognitive performance scores in controls, children with dyslexia and those with ADHD

Beta band positive eccentricity associations (p<.05) are seen with A) verbal performance, and B) non verbal-performance as well as positive associations of diameter with A) verbal and B) non-verbal performance. Results in Figure 6.5 were not statistically significant at the Bonferroni corrected α level. The solid line is the fit-line for correlations across the sample cohorts of controls, children with ADHD and those with dyslexia. Key: attention-deficit/hyperactivity disorder, and TDC = typically developing controls.

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Association between network topology and measures of attention, and internalised and externalised behavioural functioning

Although earlier in this Chapter, Table 1, results, revealed significant differences between groups, planned comparisons in the present study found no significant differences on the internalised behaviour scores between children with ADHD and those with dyslexia. Comparisons between controls and children with dyslexia revealed trends for higher scores of those with dyslexia, although these were not significant when a corrected α level was applied. Again, these results justified the further investigation of the relationship between network topology and measures of behaviour functioning, across the ADHD group, and the controls,

Significant results and trends were observed in the beta band and in no other frequency, (See Table 6.6 for correlation results in the other frequency bands). Only the correlations that were significant at the α level of .05 are displayed in Figure 6.5.

Beta band

Computed correlations revealed that characteristic path length was negatively associated with attention (r_s = -.44 [-.64, -.20], p <.003, see Figure 6.6A), internalised, (r_s = -.54 [-.71, -.32], p < .0024, the corrected α level, see Figure 6.6B), and externalised behaviour functioning, (r_s = -.37 [-.61, -.09], p = .013, see Figure 6.6C).

Also in the beta band, externalised behaviour functioning was associated with diameter, (r_s = .32 [.01, .58], p = .04, see Figure 6.7A) and eccentricity, (r_s = .32 [-.01, .15], p = .04, see Figure 6.7B). Though they were not statistically significant at the α level of .05, results revealed trends towards a negative correlation between beta band normalised clustering coefficient and attention (r_s = -.30 [-.59, .06], p = .053), as well as internalised (r_s = -.30 [-.57, .04], p = .053) behaviour scores.

Beta band small worldness also showed trends towards positive correlations with internalised (r_s = .29 [-.04, .55], p = .06) and externalised (r_s = .26 [-.08, 56], p = .09) behaviour problems. Finally, beta band eccentricity showed a positive trend toward a correlation with internalised behaviour problems (r_s = .26 [-.05, .51], p = .10).

Table 6. 6 Correlations between network measures and behaviour problems in controls, children with dyslexiaand those with ADHD, across frequency bands

(n = 43)	Metric	Att	Int	Ext
Theta	Gamma	.11(.48)	-.10(.51)	.04(.78)
	Lambda	.11(45)	.02(.92)	.10(.51)
	Small-world	.10(.95)	-.14 (.37)	-.10(.57)
	Eccentricity	-.02(.92)	.04(.80)	.01(.97)
	Diameter	-.12(.94)	.09(.58)	-.05(.74)
	Leaf number	.18(.24)	-.06(.68)	-.10(.56)
	Hierarchy	.10(.51)	.60(.70)	-.70(.66)
Alpha1	Gamma	-.01(.95)	.02(.89)	.10(.54)
	Lambda	.04(.79)	.08(.62)	.14(.39)
	Small-world	-.11(.48)	-.11(.48)	-.13(.42)
	Eccentricity	-.00(.99)	-.03(.86)	.02(.91)
	Diameter	-.03(.83)	-.03(.84)	-.03 (.87)
	Leaf number	.07(.66)	.01(.93)	-.05(.73)
	Hierarchy	.09(59)	-.06(.71)	-.04(.80)
Alpha2	Gamma	-.01(.96)	-.04(.78)	-.04(.80)
	Lambda	-.25(.87)	-.87(.58)	-.18(.26)
	Small-world	-.12(.46)	-.01(.95)	.10(.52)
	Eccentricity	-.18(.24)	-.12(.44)	-.06(.71)
	Diameter	-.20(.21)	-.15(.34)	-.06(.72)
	Leaf number	.12(.44)	-.10(.51)	.03(.87)
	Hierarchy	.20(20)	.14(.37)	.06(.70)
Beta	Gamma	-.30(.05)	-.30(.05)	-.24(.11)
	Lambda	-.44(.00)**	-.54(.00)***	-.37(.01)*
	Small-world	.21(.17)	.29 (.06)	.26(.09)
	Eccentricity	.28(.07)	.26(.10)	.34(.04)*
	Diameter	.25(.11)	.28(.13)	.32(.04)*
	Leaf number	.01(.95)	.12(.45)	-.07(.64)
	Hierarchy	-.04(.82)	.10(.54)	.04(.79)
Gamma	Gamma	.14(.38)	-.01(.97)	.12(.43)
	Lambda	.24(.13)	.08(1.60)	.07(.67)
	Small-world	-.15(.33)	-.12(.45)	.07(.65)
	Eccentricity	.00(1.0)	-.00(.98)	.10(.53)
	Diameter	-.06(.70)	-.04(.80)	.04(.75)
	Leaf number	.06(.70)	.03(.86)	.10(.52)
	Hierarchy	-.03 (.87)	-.12(.45)	.05(.75)

*Spearman's rank correlation coefficients (r_s) for network measures and measures of behaviour functioning for each frequency band. Key: Att = attention, Int = Internalising, and Ext = Externalising behaviour, metric gamma = normalised clustering, metric lambda = normalised path length, and small-world = small-world index. *p <.05, **p < .01, and ***p <.0024 (Bonferroni-corrected α value).*

The correlation between normalised characteristic path length and non-verbal scores revealed that poorer non-verbal performance was associated with decreased global efficiency. See Figure below.

Figure 6. 6 Correlations coefficients between beta band graph-based network orgnaisation and measures of behavioural functioning variations in controls, children with dyslexia and those with ADHD

All associations (p<.05) were observed in the beta band. Associations between graph-based normalised characteristic path length and behaviour functioning of A) attention, B) internalised, and C) externalised behaviour were revealed as negative relationships. The solid line is the fit-line across the sample cohorts of controls, children with ADHD and those with dyslexia. Key: ADHD =attention-deficit/hyperactivity disorder, and TDC = typically developing control.

Figure 6. 7 Correlations between beta band minimum spanning tree network organisation and measures of behavioural functioning variations in controls, children with dyslexia and those with ADHD

All associations (p<.05) were observed in the beta band for externalised behaviour functioning and no other measures. The solid line is the fit-line across the sample cohorts of controls, children with ADHD and those with dyslexia. Key: ADHD = attention-deficit/hyperactivity disorder, and TDC = typically developing controls.

6.5. Discussion

The utility of a diagnostic measure relies highly on its ability to distinguish between those affected and those who are not. In ADHD and dyslexia, two highly co-occurring conditions, the diagnostic measures have been conceptualised as unifying explanations of reported difficulties. For instance, it has been assumed that higher scores on attention measures (in relation to cut-off points for normal functioning) help to diagnose ADHD as well as providing a unifying account of deficit. However, these measures do not tell us why the problems occur in the first instance.

ADHD and dyslexia are behaviourally conceptualised, highly heritable, and multifactorial with regard to causes, underlying neural substrates and poor academic outcomes. Although it is generally believed that regardless of reported reading problems, children with dyslexia have normal intelligence in relation to age-matched peers (Knivsberg et al., 1999; Kraus, 2012; Pennington et al, 1990). Boada et al. (2012) maintains that those with dyslexia are likely to differ from non-dyslexic readers in other language-associated features such as vocabulary level and working memory. The authors suggest that reported under-activations or over-

activations may therefore to some extent reflect the influence of such characteristics regardless of whether the task is explicitly assesses reading ability. Similarly Koyama et al. (2010) has pointed out that there is a lack agreement regarding the optimal task for sufficiently characterising functional connectivity strength underlying reading. Similar problems have been discussed with regard to ADHD and neuroimaging studies (See Chapter 5, Introduction).

To understand the two conditions, a more appropriate measure would need to avoid such uncertainties. The exploratory analysis conducted in this study aimed to identify features of functional network topological organisation that in analysis of sensor-level resting data would be able to capture the similarities and/or differences in typically developing controls and children with developmental conditions. The secondary aim was to test the hypothesis of possible associations between network variables and cognitive/behavioural functioning.

Generally, the results revealed that the application of graph theory, along with minimum spanning tree analysis, is able to capture the differences in functional brain network organisation in controls and in children with dyslexia as well as in those with heterogeneous atypical neurodevelopmental disorders. Both common and different network variables were observed in the two clinical conditions (i.e. ADHD and dyslexia).

Observed alterations in network organisation in those with ADHD and with dyslexia, when compared to controls, generally followed a similar trend. In contrast, when significant group differences were observed, network changes in children with comorbid conditions often revealed differences in an opposite direction compared to ADHD and dyslexia. This suggests that different underlying mechanisms in those with more complex heterogeneous profiles may be at play. The implications are discussed in detail below.

<u>Whole brain functional connectivity</u>

The Kruskal-Wallis H test revealed significant group differences in the theta and beta bands functional connectivity. Post-hoc comparison results showed that the main finding was of differences in the theta band.

With regard to whole-brain functional connectivity, a key finding in the current study was that children with dyslexia revealed significantly higher connectivity strength in the theta band compared to all the other groups (Figure 6.1). This suggests that as a potential biomarker, whole-brain coupling strength in the theta band could possibly be adopted in distinguishing

those with dyslexia. In typical development, it is well documented that a progressive decrease in low frequency oscillations (i.e. delta, theta, and lower alpha) occurs with age (Gasser et al., 1988; Matousek & Petersen, 1973). The results in the current study therefore offer support for the proposition that higher whole brain connectivity in dyslexia may be an indicator of possible immaturity of brain development in the theta band. In brain networks, it is believed that long-distance connectivity is related to synchronisation of low frequencies (von Stein et al., 2000; von Stein & Sarnthein, 2000). Increased theta band activity therefore suggests that in dyslexia there is less efficient information processing in long-distance connections.

Furthermore, increased theta functional connectivity in dyslexia can be interpreted as representing altered neuronal oscillatory dynamics, resulting from disrupted neuronal maturation, which has been attributed in poor readers to a delay or maturation lag (Stanovich, 1988). Studies of children with dyslexia have proposed that theta functional connectivity may function as a potential marker of altered linguistic processing in dyslexia, an interpretation previously alluded to by EEG studies (Klimesch, 1999; Spironelli, Penolazzi, Vio, & Angrilli, 2006) of frequency activity during phonological reading tasks in dyslexic readers.

As was the case in Chapter 5, the results of increased whole-brain functional connectivity in dyslexia are difficult to reconcile with previous functional imaging studies conducted with dyslexic readers. Most studies (Cao et al., 2008; Richlan et al., 2010; Schurz et al., 2013) have focussed on task-based functional coupling, between discrete brain regions, and hemisphere. Results from such studies have suggested disrupted functional coupling in dyslexic readers. This is typically in the left hemisphere reading networks, including coupling between temporal-parietal (thought to be crucial for grapheme-to-phoneme conversion mechanism in the brain), occipital-temporal (believed to be involved in whole-word recognition), and frontal regions (See Richlan, 2012, for a review). More specifically, reported findings often reveal lower level functioning or under-activation of temporal-parietal and occipital-temporal regions. However, according to Boada et al. (2012), frontal related areas show both under connectivity and over connectivity.

In task-based brain activation studies (Coomes, Janelle, Duley, & Conway, 2005; Rippon & Brunswick, 1998; Spironelli et al., 2006, 2008), alterations in region-specific theta activity are believed to index specific reading-related abilities. Specifically, increased theta power in frontal regions (specifically the anterior cingulate cortex) has been associated with increased information processing, better ability in focussing one's attention required for increased task

demands (Gevins, Simth, McEvoy, & Yu, 1997). In non-dyslexic readers, lack of increased theta has been reported to be associated with superior performance on language-related tasks (Coombers et al., 2005).

Local and global graph-based network efficiency

For local and global network efficiency, despite there being a statistically significant difference between the four groups in the beta band, no significant differences were found when children with dyslexia were compared in post-hoc analysis to controls or to those with ADHD. However, a consistent trend in the beta band was that both ADHD and dyslexic participants showed lower normalised clustering coefficient (a measure of local segregation processing) and normalised path length (a measure of integration, Figure 6.2), indicating that network organisation (viewed in the Watts & Strogatz's small-worldness model), in both groups resembled that of a random organisation in the beta band.

In those with dyslexia, reduced local and global efficiency determined using graph concepts was reported in previous studies (Beaulieu et al., 2005; Steinbrink et al., 2008; Vourkas et al. 2011). Focussing on task-based brain activations in sensor-level MEG data, Vourkas et al. (2011) reported significantly lower global and local network efficiency in alpha and gamma frequencies in children with reading difficulties, compared to controls. However, by averaging over standard frequency ranges, Vourkas et al. (2011) reported the 20-30 Hz range as a gamma band. Therefore, their results on task-based reduced local and global efficiency in the 20-30 Hz range are broadly consistent with lower network variables levels found in the current study in the beta band, which corresponded to the traditional 13-30 Hz range.

As noted in Chapter 5, significant lower local and global efficiency in children with ADHD when compared to controls provides evidence supporting the proposition of a disconnection syndrome in the beta band hypothesised about ADHD. The finding of a similar pattern in the current study in those with dyslexia points towards a similar possibility in those with dyslexia, but to a lesser extent. Overall (although to a smaller extent in the dyslexia group compared to ADHD), the results of topological organisation analysis using clustering and path length measures, suggest an immature or altered organisation of neuronal networks involved in beta oscillatory activity.

In typical adults (as discussed in Chapter 4, see Discussion) shorter path lengths in brain networks corresponded to efficient information transfer (See Figure 4.5). Although the shorter path lengths observed in those with ADHD and dyslexia in the current study would

appear to contradict the results of the discussion in Chapter 4, it is important to bear in mind that in neurotypical adults, shorter average long-range connections result from progressive but efficient pruning (Fair et al., 2008). Hence, it was proposed that the lower path length observed in ADHD and dyslexia groups is likely to be pathological and indicative of immature neuronal activity that subsequently underlies less efficient information transfer in brain networks. Evidence about altered neuro processes comes from several neuroimaging studies.

Given that it is now generally agreed that reading requires the interaction between spatially distinct brain regions (Schurz et al., 2014), reports of reduced grey and/or white matter abnormalities (typically reduction) during reading-related tasks (often although not always in the left hemisphere network, see Richlan et al., 2013, for a review), suggest local as well as global brain network alterations. From the associations between structural and functional relationships this would suggest that results reported in this study in large-scale brain networks align with previous reports of altered brain information processing.

Global network efficiency: minimum spanning tree

Besides clustering coefficient and path length, MST network variables too demonstrated sensitivity to network structure that was restricted to the beta and found in no other frequency range. Tree eccentricity, which corresponds to node centrality, was significantly higher in children with dyslexia compared to controls (Figure 6.3A), indicating increasing shortest paths between nodes in this population. As discussed in previous chapters this suggests a less integrated and efficient network topology (Otte et al. 2015). Higher eccentricity corresponds to a loss of nodal importance in networks. With regard to MST diameter (a measure of the largest distance between any two nodes in a tree), post-hoc analysis revealed a trend towards higher diameter in dyslexics compared to controls, but not when compared to those with ADHD (Figure 6.3B). Finally, for hierarchical structure, post-hoc comparisons revealed higher beta band hierarchy in children with dyslexia compared to controls, and a trend towards higher values when compared with those with ADHD.

As stated before, reconciling the MST findings in the present study with previous reports is challenging. To the best of the author's knowledge, only one attempt has been made to investigate network topology using MST in people with dyslexia (reported in a poster paper recently presented at a conference by Gonzalez et al., 2015). In relation to ADHD, no such attempt has apparently been made. The results reported by Gonzalez et al. (2015), are of interest for interpretation of the current results due the similarities in methodologies. First, as

with the current study, functional connectivity in EEG investigations conducted by the authors were estimated using the phase lag index. Second, MST network parameters were derived from sensor-level data in frequency ranges similar to those reported here. The MST results reported by Gonzalez et al. (2015) are consistent with those in the current study, albeit in a difference frequency range (i.e. the theta band). The researchers reported higher eccentricity and diameter as well as higher leaf number in dyslexic readers compared to controls. Of particular interest, the MST values of reported abnormalities in both groups consistently lay within similar ranges.

The difference in the frequency range may be explained by the age of children studied. Gonzalez et al. (2015) investigated network analysis in very young dyslexic readers (i.e. average age 8.46 years) while the current study considered children aged 7 to 17 years. Research on typical development may help explain the differences. As already noted, during normal development a progressive decline in low frequency oscillations takes place. In younger children, higher frequencies are under-developed. Therefore, it is proposed that the inconsistency in the frequency range of reported change is likely to be explained by a developmental effect. For both ADHD and dyslexia, a maturational lag hypothesis has been proposed (See Lara et al., 2009, and Stanovich, 1988, for a review). It is likely that abnormalities in the theta activity in those with dyslexia undergo some form of catch-up growth with development, meaning that abnormalities in low frequencies will therefore be less readily detected in older children. Although the issue was not directly considered in the current study, results reported in Chapter 4 (despite being in a different frequency range) pointed to a developmental effect in both whole-brain connectivity (See Figure 4.4) and global network efficiency (See Figure 4.5).

Overall, it is likely that altered short-range and long-distance connections, possibly resulting from early brain development, play a role in the development of neurodevelopmental disorders. However, it appears MST measures (often referred to as the super highway measures) were more sensitive to changes in dyslexia than in ADHD. It is therefore tempting to speculate that although both conditions revealed less graph-based network efficiency, MST measures conveyed more predictive information in the dyslexia group. This could indicate that underlying network alterations in dyslexia are more specific in comparison to ADHD. Put differently, it is proposed on a preliminary basis that ADHD is associated with more widely distributed large scale network abnormalities, while dyslexia corresponds to less widely distributed more specific abnormalities, which are more reliably detected by more conservative network measures such as those offered by MST. The reported alteration in network communication may be related with slow myelination of the white matter tracts

underlying crucial networks.

<u>The association between brain network organisation and measures of cognitive abilities</u>

The association between network organisation and functioning was considered across the groups (i.e. controls, ADHD, and dyslexia). Correlations and trends (though not statistically significant when a stricter Bonferroni-corrected α level of .001 was applied) were observed in the beta and gamma bands. As predicted, higher global efficiency was associated with better performance on non-verbal subtests in the beta band (Figure 6.4). In contrast, higher diameter and eccentricity in the beta band were associated with poorer performance on verbal and non-verbal performance scores across the three groups (Figure 6.5). Overall, the results suggest that altered global efficiency (lower path, higher eccentricity, and higher diameter) in the beta band is crucial for cognitive abilities.

In children with reading difficulties, higher global efficiency has previously been shown to be associated with better reading abilities (Dimitriadis et al., 2013). Also in investigating altered temporal correlations in sensor-level resting-state MEG data, the researchers observed that altered long-range temporal efficiency in lower alpha and beta frequency bands was associated with poor reading scores (assessed using word Attack and letter-word identification subtest scores) in children experiencing reading difficulties. The majority of previous studies investigating the brain-behaviour relationship have focused on specific regional abnormalities. For instance, an fMRI study by Koyama et al. (2011) investigated the brain-behaviour relationship in specific regions; therefore, their results cannot be applied in this study. Schurz et al. (2014) took a similar approach.

Given that diameter corresponds to the largest distance between any two nodes, while eccentricity is related to node centrality, the negative association between these metrics with verbal and non-verbal performance in the beta band suggests that the line-like, less integrated network configuration in the beta band plays a crucial role in cognitive abilities. This association in the beta band can be explained by the fact that beta power is associated with higher cognitive abilities.

<u>The Association between brain network organisation and measures of behaviour functioning</u>

Computations focussed on controls, dyslexics, and children with ADHD. Studies investigating the association between brain function and behaviour functioning are scarce in relation to dyslexia. Given that the core difficulties often relate to reading, measures of reading

competence often take priority. However the results in Table 6.1 revealed that among children with dyslexia, scores on measures of attention, internalised and externalised behaviour problems were higher than for controls Significant associations between network variables and measures of behaviour functioning were observed in the beta band and in no other frequency range.

Lower normalised characteristic path length in the beta band was associated with higher scores of attention (Figure 6.6A), internalised (Figure 6.6B), and externalised (Figure 6.6C) behaviour problems. Conversely, higher scores of externalised behaviour problems were associated with higher beta band diameters (Figure 6.7A) and eccentricity (Figure 6.7B).

<u>Comorbid conditions</u>

Initial Kruskal-Wallis H tests assessing group differences across the four groups revealed significant differences in whole-brain functional connectivity (in the theta and beta bands) and network variables (in the beta band). Planned post-hoc comparison did not investigate comorbid conditions due to the high heterogeneity of the group. With the exception of functional connectivity in the theta band and hierarchical structure in the beta band, there was a consistent trend for all other network variables (where significant group effects were reported using the Kruskal-Wallis H tests, including clustering, path length, eccentricity and diameter) of dissimilar (in relation to ADHD ad dyslexia) local and global communication disruptions. In other words, children with comorbid conditions showed differences in the opposite direction compared to the two clinical groups (i.e. ADHD and dyslexics), providing evidence to the proposition that network topological disruptions in the beta band are able to identify and discriminate different underlying pathophysiological mechanism in those with single vs. comorbid developmental conditions. The pattern of local and global disruptions demonstrated in this thesis in local and global topologies suggest a disruption of the optimal small-world and a shift towards a more regular topology. However, given that the sample of children in the comorbid group was very diverse, it is not possible to reach any conclusions as to why networks in those with comorbid conditions would present with disruptions reported in the current study.

As stated earlier, pure childhood developmental conditions are rare (Hulme & Snowling, 2009), meaning that the likelihood is high that a child with one condition will also meet the diagnostic criteria for another. Due to a substantial degree of missing data, the associations between cognitive/behaviour functioning and network variables were not assessed in the comorbid group. However, given that their scores on the attention, internalised and

externalised behaviour functioning measures were similar to those in the ADHD group, it is likely that similar patterns to those observed in Figure 6.6 and 6.7 would have characterised the association between behaviour functioning and network topology in this comorbid group.

Methodological considerations

A major methodological limitation of this study is that no reading competence scores were reported. Understanding the association between brain interactions and reading scores would further the debate regarding the role of frequency-based brain connections in relation to reading. However this study was primarily concerned with the clinical application and relevance of task-independent measures of brain activity, i.e. whether such measures have any biologically relevant information.

Viewed this way, it was acceptable to rely on standard measures of behavioural and cognitive functioning and examine their relationship with brain measures. However, that means that it is not possible to state with certainty whether altered network topology of itself may enable the identification of those with dyslexia in different groups. Despite this, using task-independent measures, the present study demonstrated that it is possible to identify network variables that point to immature brain network parameters in neurodevelopmental conditions in the absence of specific behaviour measures.

Another study limitation concerns the fact that due to small population samples, the effect of development (young vs. older children), and of gender on changes in network topological organisation was not assessed. However, despite the small sample sizes, significant effects were reported, supporting the hypothesis that atypical neurodevelopment-related difficulties may be caused by underlying alterations in network communication.

6.6. Conclusion

This study was thought to be the first to investigate potential similarities and differences in resting-state functional connectivity and the brain-behaviour correlations simultaneously, in two highly co-occurring neurodevelopmental disorders. Results revealed that graph analysis and MST are sensitive to disruptions in network topology in children with neurodevelopmental conditions in relation to controls. With the exception of theta band connectivity strength which was significantly higher in dyslexic readers compared to all the other groups, results in this study appear to support to proposition that high co-occurrence of ADHD with dyslexia, may be the result of similar underlying pathophysiological mechanisms.

Hence, as it stands, it appears that network analysis holds promise as the key to unlocking the ambiguity of why two behaviourally different conditions co-occur more often than is expected by chance. Further research with larger sample sizes are required to confirm the results reported in this study.

7. General discussion

7.1. Aims and findings

Healthy brain functioning depends on the efficient communication and integration of information between brain regions, forming complex networks. Communication in brain networks is based on the synchronisation of neuronal activity. As a result, by quantifying synchronisation between brain regions, a connected brain network can be analysed (discussed in Chapter 1 & 2). Recent developments in neuroscience, especially where graph theory is concerned, have proved useful for investigating the neural correlates of some neuropsychiatric disorders. In these disorders, behavioural and cognitive problems experienced have been attributed to disrupted communication within and between brain networks. In atypical development, where conventional diagnosis relies on measures of behavioural and cognitive functioning, a biological means of quantifying possible neuro-risk factors would hold promise as a clinical tool.

Using magnetoencephalography (MEG) to investigate frequency-specific temporal dynamics in subjects at rest, the research presented in this thesis mainly sought to identify potential biological markers derived from sensor-level recordings of whole-brain functional connectivity. Such markers could go on to provide a basis for an objective and quantifiable metric of neurobiological risk factors of great importance in neurodevelopmental disorders. If identified and validated, such markers would hold promise as a more objective measure for use in diagnosis alongside conventional approaches, to identify those at risk and helping determine responses to treatment. However before this later task could be accomplished, two major issues would have to be addressed.

First, to validate the clinical potential of graph theory as a means of improving understanding of the aetiology of neurodevelopmental disorders and their diagnoses would require an assessment of the test-retest reproducibility of graph theoretical metrics. The first experimental study (Chapter 3) therefore sought to validate the method by examining the reproducibility of graph-based functional connectivity and network parameters in healthy adult volunteers, using a repeated measures design. Results revealed that the reproducibility of functional connectivity and network variables was dependent on resting-state (i.e. eyes-open vs. eyes-closed), frequency band, and metric order (i.e. first vs. second order). Eyes-open conditions, higher frequency beta band, and small-world index revealed comparatively low test-retest reproducibility. Generally, network measures derived from eyes-closed rest

conditions showed good-to-excellent reproducibility. For this reason, subsequent experimental chapters examined network efficiency in eyes-closed rest conditions.

Second, graph measures have been shown to depend on several methodological assumptions that bias network comparisons. Whilst solutions such as normalisation and the application of thresholds to generate a binary graph are typically applied as alternatives, a recently developed approach (i.e. minimum spanning tree) offers a computationally more objective solution. MST avoids the application of thresholds, as well as of normalisation processes involving random surrogate networks, that often lead to biases in network analysis and subsequent comparison across participants (Boersma et al., 2012; van Wijk et al., 2010). Subsequent chapters in this thesis therefore applied graph theoretical analysis, along with minimum spanning tree (a network analysis approach that allows for unbiased comparisons) to model network topologies.

However, prior to investigating possible changes that occur within brain networks in atypical development, research in the second experimental study (Chapter 4) focused on age-related changes in functional networks during typical development. This is because understanding the pattern of distributed brain networks in typical controls (i.e. in relation to what is occurring in a neurotypical brain) would help put into perspective the findings for those with atypical developmental conditions. In this study, adopting a cross-sectional sampling approach, functional brain organisation was characterised using network analysis; first comparing children (7-13 years) to adults (20-35 years), and then across a broad age range (7-57 years). The effects of developmental changes in functional large-scale networks were found primarily in high frequency beta and gamma bands.

First, children's brains showed over-connectivity of whole-brain coupling strength and increased normalised characteristic path length. It was proposed that over-connectivity in the beta band functions as a compensatory mechanism, whilst increased path length indicated a less optimal balance between local and global brain network communication in younger children. Second, in the gamma band, children revealed higher leaf number and hierarchical organisation compared to adults. Across a broad age-range, functional connectivity and normalised path length decreased with development, to reach the lowest level at 27 years. Minimum spanning trees metrics were not sensitive to developmental changes in network topology across the broad range. This is likely to be a result of complex changes occurring in the different age-clusters. In Chapter 5, functional network topology was investigated in those with ADHD, in relation to typically developing controls. In the beta band and in no other frequency, lower whole-brain functional connectivity, normalised clustering coefficients,

characteristic path length, and higher eccentricity and diameter were observed in children with ADHD, compared to controls. Furthermore, in the ADHD group, the lower path length in the beta band was associated with poorer scores on verbal performance, while higher eccentricity and diameter in the beta and gamma bands were associated with poorer non-verbal cognition. The results indicated a reduction in local and global communication in the beta band in the brains of children with ADHD. These reported associations between network measures and measures of cognitive functioning provide support for the clinical relevance of network analysis of functional organisation for those with ADHD.

Finally, supplementing earlier investigation of functional network topology, it is the case that that 'pure' neurodevelopmental disorders are the exception rather than the rule in childhood conditions. For this reason in experimental study 4 (Chapter 6) local and global network communications was investigated in ADHD and dyslexia, two of the most prevalent neurodevelopmental disorders. The analysis in this chapter also considered children with comorbid conditions. With regard to whole-brain connectivity strength, the results revealed that functional coupling in the theta band can detect and discriminate differences in whole-brain connectivity between both controls and dyslexic readers, and those with ADHD and dyslexic readers. A difference was observed between the groups in beta band normalised clustering and path length. However although among those with ADHD and dyslexia, clustering coefficients and path length were lower in the beta band compared to controls, the difference in relation to dyslexic readers, when compared to the controls or those with ADHD, was not statistically significant. Interestingly, the comorbid group showed dissimilar local and global communication disruptions suggesting that graph-based network analysis in the beta band can discriminate different underlying pathophysiological mechanism only in those with single vs. comorbid developmental conditions.

Using MST, results showed less integrated network configurations in the beta band in dyslexic readers compared to controls (i.e. higher eccentricity and hierarchy). Similar trends (but to a lesser extent) were observed in those with ADHD. Post-hoc results revealed that those with dyslexia did not differ from those with ADHD. In children with comorbid conditions, MST eccentricity and hierarchy decreased in an opposite trend from that for those with a single neurodevelopmental disorder. Overall, with regard to ADHD and dyslexia, the results suggest that whilst graph theoretical measures and MST global network measures are able to detect disruptions in underlying network communication, these metrics were not able to discriminate the different disruptions underlying possible neurobiological risk factors, as revealed by the post-hoc comparisons.

Correlations were computed to assess the association between network measures and performance, on verbal and non-verbal sub-tests across the groups (i.e. identified ADHD, dyslexia, and comorbid conditions). Associations (although not significant when a stricter Bonferroni-corrected α level of .001 was applied) were observed in the beta and gamma bands. As predicted, in the beta band higher global efficiency was associated with better performance on non-verbal subtests. In contrast, higher diameter and eccentricity in the beta band were associated with poorer performance on verbal and non-verbal performance scores, across the three groups. Overall, the results suggest that altered global efficiency (lower path, higher eccentricity, and higher diameter) in the beta band is crucial for cognitive abilities. Similarly, associations between network efficiency and behavioural functioning across the three groups (i.e. ADHD, dyslexia, and controls) revealed that lower normalised characteristic path length in the beta band was associated with higher scores of attention, and internalised, and externalised behaviour problems. Conversely, higher scores of externalised behaviour problems were associated with higher beta band diameters and eccentricity, providing further evidence for the clinical consequence of altered functional network organisation.

The results offer support to the proposition that measures of network organisation derived from sensor-space MEG data offer qualified and informative insights in helping to identify underlying pathophysiological mechanisms in both typical and atypical development. These results are summarised in Table 7.1.

Table 7. 1 Results summarised in relation to chapter-based findings

Chapters	Findings
3	• Eyes-open, higher frequency beta band and small-world index revealed comparatively low test-retest reproducibility
4	• Increase of whole-brain functional connectivity in the beta band in children (7-13 years) compared to adults (20-35 years) • Longer normalised path length, increased leaf number and hierarchy, restricted to the beta band in children compared to adults • Across a broad age range (7-57 years), decreased whole-brain connectivity and normalised path length was found, which decreased to reach lowest level at ~ 27 years and increased from 37 years
5	• Decrease of whole-brain functional connectivity in the beta band in children with ADHD compared to age-matched controls • Decrease of normalised clustering coefficient and normalised path length confined to the beta band in children with ADHD • Increased eccentricity and diameter in children with ADHD, confined to the beta band • Discriminatory analysis, using the normalised clustering and path length predictors, correctly predicted 33.30% of children with ADHD and 85.7% of controls • Discriminatory analysis, using the normalised clustering and path length predictors, correctly predicted 41.70% of children with ADHD and 81.00% of controls, suggesting that network analysis using the two approaches is able to detect and discriminate differences in potential underlying pathophysiological mechanisms • A positive association was found between beta band normalised path length and performance scores on verbal subtest • A negative association was observed between beta and gamma band eccentricity and diameter and performance scores on the non-verbal sub-tests
6	• Whilst generally ADHD and dyslexia showed similar functional network disruptions, dyslexic readers revealed significantly elevated theta connectivity strength compared to all other groups • Increased eccentricity and hierarchy, confined to the beta band, was found in children with dyslexia, when compared to controls • Children with comorbid conditions showed dissimilar functional network disruptions in the beta band, suggesting that network analysis was able to discriminate between children with a diagnosis of one neurodevelopmental disorder and those with comorbid conditions • Higher global efficiency was associated with better performance on non-verbal subtests in the beta band • Increased diameter and eccentricity in the beta band were associated with poorer performance on verbal and non-verbal performance tests across the three groups • Lower normalised characteristic path length in the beta band was associated with higher scores of attention, and internalised, and externalised behaviour problems • Higher scores on externalised behaviour problems were associated with higher beta band diameter and eccentricity

7.2. General discussion

The main aim of the research presented in this thesis was to investigate possible neurobiological risk markers that could provide objective and quantifiable metrics for identifying and discriminating characteristics of functional brain networks in those with typical and atypical development. To this end, MEG, a child-friendly and non-invasive imaging technique, was used to record intrinsic brain activity during task-independent conditions. Graph theory analysis was applied to investigate the effects of typical and atypical development on the organisation of complex functional brain networks. The main findings from each experimental chapter are presented schematically in Table 7.1.

7.2.1. Test-retest reproducibility of whole brain connectivity strength and network organisation parameters

In the context of the research reported in this thesis, this study provided a template for selecting satisfactorily robust processing approaches for the further investigation of functional brain networks as potential clinical biomarkers. Studies investigating the clinical utility of graph measures typically report altered network disruption in data derived from eyes-closed rest conditions. However, previous research (Jin et al., 2013; Xu et al., 2014) has provided evidence suggesting the possibility of different topological organisations of brain networks in relation to eyes-closed vs. eyes open conditions. According to Xu et al. (2014), the opening and closing of one's eyes is essential for directing attention to processing the external vs. the internal world. Using fMRI, and comparing both conditions, the authors reported higher global but lower local network efficiency in eyes-closed vs. eyes-open conditions. In contrast, an MEG study conducted by Jin et al. (2013) reported enhanced global efficiency in the theta and alpha bands in the eyes-open state compared to the eyes-closed state. Together, these studies would appear to suggest that the topological organisation of brain networks is highly volatile, i.e. dynamically switching, in response to the information processing corresponding to the opening, and closing one one's eyes. This volatility has not been thoroughly considered in previous non-invasive high temporal resolution studies investigating intrinsic brain activity.

To the best of the author's knowledge, only three non-invasive high temporal resolution studies have investigated the reproducibility of network measures. In addition, of these only

one (Jin et al., 2011) considered the reliability of network measures in both eyes-open and eyes-closed conditions. This study reported fair-to-moderate reliability. However, Jin et al. (2011) focussed on nodal centrality measures. Hence, these findings cannot be directly applied to the results in Chapter 3, as this study focussed on global network measures. Therefore, to the best of the author's knowledge, this is the first MEG study to consider reliability in both states. Graph theoretical measures in eyes-closed rest conditions were significantly more reproducible than those derived from eyes-open resting states. As a result, all subsequent network computations were concerned with changes in large scale networks derived from eyes-close rest state. The results were not consistent with Jin et al. (2011). The differences in reported results are most likely to be a consequence of the metrics investigated.

Given that network measures have been reported as markers of cognitive impairment, personality traits, intelligence, and age, good reproducibility revealed in Chapter 3 is encouraging for future high temporal resolution studies exploring the effects of pathology on the functional organisation of the brain networks. However, a surprise (but consistent with previous studies, i.e. Deuker et al., 2009; Jin et al., 2011) finding in this study was the low reproducibility of beta band network and small-world index. This is surprising because small-world organisation in the clinical studies (Cao et al., 2012; Supekar et al., 2009) is often found to be preserved. With regard to the beta band, this frequency reflects one of the largest induced changes one can expect. Activity in beta changes moment-by-moment. One may therefore expect that during rest (i.e. state free from cognitive demands), network measures in this range would be more reproducible. This was not the case. It appears that within the beta band, network measures are changeable.

It is however conceivable that within an individual any measures will not be reliable. We know this because one person's beta is not always the same. Hence, aspects like cognitive states that change beta will most likely fluctuate throughout the day and in relation to events. It is likely that we have something that is not individually reliable, but that does not mean that if we were to sample from the same individual at another point in time the measures in this band would not be useful for the understanding of something clinically relevant.

7.2.2. Functional network topology in typical development

Neuroimaging studies of the developing brain (Gong et al., 2009; Hagmann et al., 2010; Paus et al., 2008;) have been fundamental in understanding the maturation of the structure and function of the brain during childhood and adolescence. These studies have provided evidence to suggest that during childhood and adolescence changes in brain structure are vital for the normal development of functions such as intellectual abilities (Nagy et al., 2005; Raz et al., 2005). Majority of the studies investigating human brain maturation have however mainly focused on structure-related changes occurring from childhood through to later life.

Structural studies such as Fair et al. (2008) that have investigated age-related changes using graph theory concepts, observed no difference in local or global changes. However according to van Wijk et al. (2010), unlike structural connections, functional connections are likely to develop at a more increased rate, meaning that they are better candidates for providing information on topological organisation in brain networks underlying brain function during development. Efficient information processing within and between specialised but spatially distributed functional brain regions underpins the successful development of higher cognitive functions (Douw et al., 2011).

When compared to adults (aged 20-35), whole-brain connectivity strength, normalised path length, leaf number and hierarchy in children (aged 7-13) were significantly higher in the beta band. With regard to whole-brain connectivity, these results suggested that in childhood, networks are highly connected and as children develop, these connections are reduced. Across a broad age range (7-57 years), a decrease in whole-brain connectivity strength during development was observed (Figure 4.4). The decrease appeared to reach their lowest level at ~ 27 years followed by an increase of connectivity strength during aging.

The second main finding from this study was increased global connectivity, as indicated by higher normalised path length, leaf number, and hierarchy in children (aged 7-13). The results are aligned with convergent data from structural imaging studies that reported increased long-distance connectivity during development (Fair et al., 2008; Giedd et al., 2009; Srinivasan, 1999) as well as with Boersma et al. (2013), and Micheloyannis et al. (2009) who investigated network organization using EEG.

It has been suggested that age-related changes in short and long-distance functional connections are highly influenced by synaptic pruning and myelination of axonal fibre tracks through the process of development (Kuhn, 2006; Paus et al., 2008; Whitford et al., 2007). Increased network performance is often linked to underlying neuronal migrations of white matter myelination, corresponding to axonal conduction (Thatcher et al., 1986) and to grey matter atrophy throughout development (Whitford et al., 2007). With maturation, the brain eliminates connections that are not used, while preserving and strengthening those connections associated with efficient information transfer (Wu et al., 2012). This is believed to result in a reduction of local connections, in parallel with the formation of more specialised long-distance nodes (Boersma et al., 2011; Supekar et al., 2009). In the current study design, it was expected that the topological organisation of functional networks in children would be less efficiently organised. The significantly short average path length in adults suggests that within typical neurodevelopment, whole-brain over-connectivity in childhood is followed by synaptic pruning, resulting in more specialised but stronger and fewer long-distance functional connections.

Overall, the results from graph theory analysis and minimum spanning tree analysis suggest a shift, during typical development, from a random network organisation towards a more structured, hierarchical, and line-like network organisation, and in higher frequency beta and gamma oscillatory activity that is indicative, of more efficient integrated information processing in spatially distributed networks. Given that higher oscillatory frequencies appear to reveal age-related changes in functional network topology, they may hold promise as biological markers of progressive functional refinement and network integration through the process of development. These results thus add to informative insights regarding developmental changes in functional network organisation changes in typical development.

7.2.3. Atypical development

Atypical development of large-scale brain functional networks was investigated in two behaviourally different but highly co-occurring conditions, namely ADHD and dyslexia. A comorbid group was also considered, albeit with less focus.

<u>ADHD</u>

Attention-deficit/hyperactivity disorder (ADHD) is one of the most common neurobehavioral disorders in children and has been shown to persist into adulthood. Abnormalities in underlying brain systems have been implicated as the likely cause of behaviour and cognitive impairments. In this study, the topological organisation of large-scale whole-brain functional brain networks was investigated in children with ADHD and with controls, using graph theory and minimum spanning tree (MST) analysis of eyes-closed sensor-level resting-state MEG data. The results reported in this thesis lend further support to the current consensus because they show that lower levels of local and global efficiency are present in such (medicated) cohorts.

In Chapter 4, the observation in typically developing children of higher beta band path lengths was suggested as a compensatory mechanism, while lower path lengths in children with ADHD (reported in Chapter 5) was suggested as indicating a disruption in global network efficiency. Although this appears somewhat contradictory, the finding of a negative relationship between path length and performance on a cognitive abilities sub-test does provide evidence that what is occurring in children is a pattern of neurotypical immaturity of brain networks in the beta band, while what occurs for those with ADHD, represents a deviation from typical network functioning in the beta band.

Overall, large-scale functional networks in children with ADHD revealed less local efficiency (i.e. normalised clustering coefficient), information integration (lower normalised path length), line-like tree topology (i.e. higher diameter, and eccentricity) in the beta band, as compared to normal controls. At a neural level, the results offer support to the idea that the transfer of information in large-scale functional brain networks in children with ADHD is less efficient in the beta band. The findings are consistent with altered functional connectivity and network topology in other neurodevelopmental and neuropsychiatric conditions that affect behavioural and cognitive functioning, such as autism, schizophrenia, and fragile X. It is therefore tempting to speculate that ADHD is a disconnection syndrome. Disruption of distributed functional brain networks may therefore be a crucial feature in ADHD. As a result, network-based descriptions might be useful as potential predictor variables in identifying those with ADHD. The rate at which information is processed in long distance connections is less efficient in networks of children with such developmental conditions.

Associations between network parameters and cognitive/behaviour functioning in the ADHD cohort suggest that network measures could prove useful as potential markers for understanding the underlying mechanisms of impairments. ADHD is the most common comorbidity among those with intellectual disability (Buckley et al., 2006), with scores on the Wechsler intellectual subtests measures of Digit-Symbol Coding, Arithmetic, Block Design, Digit Span, shown to discriminate those with ADHD from normal controls (Goodwin et al., 2011; Gudjonsson et al., 2009; Quinlan, 2001). The ability of IQ to co-segregate with ADHD in families, their affected and non-affected (Rommelse et al., 2008) siblings suggests a possible altered functional pathophysiological mechanism in those with low intellectual performance. Furthermore, such findings have also been observed in the unaffected siblings (Rommelse et al., 2008) of children with ADHD. Although this was not examined in the current study, Goodwin et al. (2011) have previously reported a relationship between intellectual performance and ADHD symptoms. Therefore the finding in the present study, that higher beta band small-worldness was not only associated with lower non-verbal reasoning levels, but was also associated with higher scores in relation to the attention behaviour problems, suggests that the relationship between non-verbal reasoning and ADHD-related attention problems could possibly be caused by disruption or interference within small world network organisation in the beta band.

Dyslexia

Dyslexia is a specific learning disability, characterised by problems related to word recognition, decoding, and spelling problems, despite subjects having normal intelligence, schooling, and motivation (Knivsberg et al., 1999; Kraus, 2012; Pennington et al, 1990). Reading-related problems are believed to be the result of a deficit in underlying phonological processing (Boada et al., 2012; Goswami, 2011; Stanovich, 1988; Svensson & Jacobsson, 2006). Although the two conditions are behaviourally different, ADHD and dyslexia co-occur more often than is expected by chance in childhood, with co-morbidity estimates of between 25-40% (Pennington et al., 2005; Willcutt & Pennington, 2000). Biologically similar brain regions have been implicated as underlying symptom phenotypes.

Although the standard procedures for diagnosing and characterising ADHD and dyslexia involves the use of behavioural and cognitive measures, converging evidence from several neuroimaging studies discussed above points to a neurobiological basis of ADHD

(Mostofsky et al., 2002; Overmeyer et al., 2001) and dyslexia (Eliez, Rumsey, Giedd, Schmitt, Patwardh, & Reiss, 2000; Klingberg et al., 2000). Magnetic resonance imaging (MRI) studies have revealed that the brains of children and adolescents with ADHD (Castellanos et al., 1996; Mackie et al., 2007; Seidman et al., 2011) and those with dyslexia (Eliez et al., 2000; Brown et al., 2011; Eckert et al., 2003) show significant differences when compared to typically developing age-matched controls. Further support for a neurobiological basis of ADHD and dyslexia comes from studies that have revealed grey and white matter abnormalities in these children (Ashtari et al. 2005; Richlan, Kronbichler, & Wimmer, 2013; Silk et al. 2009b).

The study presented in Chapter 6 aimed to determine whether metrics, such as over-network connectivity vs. under-network connectivity, could identify as well as discriminate between (dyslexic readers and controls, and dyslexic readers and those with ADHD. Whole brain connectivity strength was significantly increased in the theta band in dyslexic readers, compared to controls and those with ADHD. This suggested that, as a potential biomarker, whole-brain coupling strength in the theta band could possibly be adopted in distinguishing those with dyslexia from ADHD and controls. In typical development, it is well documented that progressive decreases in low frequency oscillations (i.e. delta, theta, and lower alpha) occur with age (Gasser et al., 1988; Matousek & Petersen, 1973). The results in the current study therefore offer support for the proposition that higher whole brain connectivity strength in those with dyslexia in the theta band may be an indicator of possible immaturity of brain development. Furthermore, in brain networks it is believed that long-distance connectivity is related to synchronisation of low frequencies (von Stein et al., 2000; von Stein & Sarnthein, 2000). Increased theta band activity therefore suggests less efficient information processing in long-distance connections in those with dyslexia.

With regard to local and global communication, network analysis was able to identify disruptions in communication efficiency. Interestingly, despite being behaviourally dissimilar, dyslexic readers showed similar functional network disruptions to those observed with ADHD, whenever a group difference was observed. These disruptions were confined to the beta band and included lower cluster coefficient, path length, and higher eccentricity, diameter, and hierarchy. However, post-hoc computations were not able to sufficiently discriminate differences in underlying pathophysiological mechanisms between the two conditions.

When compared to controls, dyslexic readers showed higher eccentricity and hierarchy in the beta band. The results are consistent with a task-based study by Vourkas et al. (2011), who also reported significantly lower global and local network efficiency in children with reading difficulties compared to controls. However, the disruptions in this study were observed in the alpha and gamma bands. It is however important to point out that Vourkas et al. (2011) focussed on gamma, but their gamma frequency band (i.e. 20-30 Hz), would traditionally be considered as beta band. Therefore, it would appear that the results reported in Chapter 6 are consistent with Vourkas et al. (2011).

The four groups that were considered in Chapter 6 were children with comorbid conditions. As stated earlier, 'pure' neurodevelopmental disorders are the exception in childhood conditions. Typically, the likelihood of a child with one developmental condition also meeting the criteria for another is relatively high. Therefore, a secondary aim was to examine functional topology in these children. However this group was highly heterogeneous, and as a result post-hoc computations were not justified Moreover in instances where the Kruskal-Wallis H test revealed a group difference, children with comorbid conditions showed functional network disruption levels dissimilar from the two clinical groups. This suggests that graph analysis and MST not only hold promise as potential metrics for detecting comorbidities, but that these measures appear to be able to discriminate different pathophysiological mechanisms underlying comorbities in relation to 'pure' developmental comorbities.

Overall, it is likely that reduced short-range and long-distance connections, possibly resulting from early brain development, play a role in the development of neurodevelopmental disorders. However, it appears that while both graph measures and MST are able to detect different underlying physiological mechanisms in controls, compared to those with dyslexia and those with ADHD, these measures were not able to discriminate between the two clinical populations. With the exception of whole-brain connectivity, the results reported in this study, would suggest that functional network topologies in those with ADHD and in dyslexic readers show similar disruptions, primarily confined to the beta band. With regards to comorbidities, further research with a large sample is necessary.

7.2.4. Associations between network parameters and measures of cognitive and behavioural functioning in controls, dyslexic reader and children with ADHD

Computations of correlations between functional network topology and measures of behavioural and cognitive functioning revealed significant relationships alluding at a possibility for the clinical applicability of altered functional network organisation. Higher global efficiency was associated with better performance on non-verbal subtests in the beta band. In contrast, higher diameter and eccentricity in the beta band were associated with poorer performance on verbal and non-verbal performance scores, across the three groups. Overall, the results suggested that altered global efficiency (lower path, higher eccentricity, and diameter) in the beta band might play a crucial in cognitive and behavioural functioning. Previously studies have demonstrated that disrupted or low global efficiency is associated with poor reading scores in dyslexic readers (Dimitriadis et al., 2013). Interestingly, in typical controls, results revealed that lower scores on verbal and non-verbal performance were associated with less efficient global communication.

7.3. Methodological considerations and future possibilities

<u>Relevance of acquired data</u>

Data reported in this thesis were computed at sensor-level. Recordings of synchronized oscillatory activity acquired at sensor level are likely to be contaminated by volume conduction as well as muscle artifacts, which according to (Uhlhaas et al., 2008) can potentially mimic neural synchrony. Given the nature of electromagnetic fields, the accurate mapping of MEG sensor-level data (recorded from the scalp) into underlying structures remains biased (Henson et al., 2009). In this thesis, care was taken to use a measure of connectivity that was not sensitive to the effects of volume conduction and signal spread. The phase lag index (PLI; Stam et al., 2007b), assesses level of asymmetry of the distribution in spontaneous phase differences of two signals and has been reported to be insensitive to the effects of common sources and volume conduction. However, PLI might failure to capture genuine connectivity at zero phase difference that researchers such as Chawla et al. (2001) believe to exist in the brain.

However, although care was taken to ensure that functional connectivity was estimated using a measure that is insensitive to the effects of volume conduction and signal spread, findings are spatially unspecific with regard to the underlying sources of observed activity. Sensor-level analysis is difficult to interpret because it is not clear where observed oscillations are generated. However, this is not to say that sensor-level work is less meaningful. Sensor-level remains a powerful means of understanding brain activations, which in several studies (Boersma et al., 2013; Hardmeier et al., 2014; Olde Dubberlink et al., 2014) continues to provide biologically meaningful insights into the organisation of brain networks. Furthermore, the research presented in this thesis hoped to demonstrate the potential of network analysis as a promising tool for future clinical utility. This was successfully demonstrated, within stated parameters. Of course, there is no denying that source-level analysis in needed for more concrete conclusions regarding the relationship between electrophysiology and anatomy. For this reason, future studies will benefit from adapting computational strategies reported in this thesis at source-level.

Network size with regard to computed nodes

All computed networks in this research were derived from data from magnetometer sensors. The consequence of only using magnetometers was that this strategy significantly reduced both the time and computational memory requirements imposed by considering all 306 sensors in estimating network synchronisation and topological measures. This strategy has previously been adopted by studies investigating sensor-level functional activity using the 306-channel Vecktorview MEG system. For instance, Deuker et al. (2009) only considered using planar gradiometers to examine functional network parameters while Jin et al. (2011) focused on magnetometers. Despite focusing on different sensor types, the studies revealed similar test-retest reliability patterns in relation to functional networks. However filtering out data from gradiometers is likely to have consequences on estimated synchronisation. Tsiaras et al. (2011) has previously discussed the likelihood that processing strategies that filter out data is likely to exclude connectivity-related information. The 306-channel system is arranged in such a manner that each location consists of two planar gradiometers and one magnetometer. There is a possibility that short-distance connections were filtered out. Future studies would therefore benefit from examining whether network measures derived from gradiometers and/or magnetometers differ from those derived when both magnetometers and gradiometers are considered together.

<u>Network comparison in network analysis</u>

With the exception of Chapter 3, only normalised graph metrics and MST results were reported. Although both quantify network topology, metrics based on graph theory consider all connections in the original network, while MST measures capture the most important connections in the original network, thereby ensuring an unbiased network comparison. The primary rationale for using minimum spanning tree analysis in this thesis was the fact that tree-topology measures are independent of many of the computational aspects such as average degree, and density that are likely to affect computed network parameters and bias network comparison. In addition, whilst MST measures are sensitive to network size (Otte et al., 2015), normalisation of these measures is not needed if computed networks have identical size (i.e. nodes, see Tewarie et al., 2014).

MST prevents the imposition of thresholds as well as a normalisation process involving random surrogate networks that often leads to biases in network analysis and the subsequent comparison between participants (Boersma et al., 2012; van Wijk et al., 2010). As a result, MST network analysis provides a more structured way to construct networks from neurophysiological data. By constructing trees using a set number of nodes, this approach ensures an unbiased comparison of networks. Information contained in MSTs is believed to capture the most important connections retained from the original network. As a result, trees are believed to represent 'information highways' (Tewarie et al., 2014; van Dellen, et al., 2013). The use of conventional graph-theoretical approaches, such as small-world properties, along with bias free minimum spanning tree analysis, to characterise local and global organisation of complex functional networks provided further support for a novel strategy of investigating large-scale functional organisation.

Hence, despite discarding connections while constructing MST, this thesis showed that MST measures are as sensitive to alterations in network change as conventional graph measures. However whilst MST offered a bias free novel approach to investigate network topology, further research is needed before MST can be applied as a substitute for a conventional graph- theoretical approach. In addition, the reliability of MST measures was not assessed in this study. This is because unlike graph-based measures, MSTs are mathematically quantified to reflect the more important network properties, and as a result can withstand

connectivity noise and potential dependence on network size (Tewarie et al., 2014). However, future studies would benefit from examining the reproducibility of these measures.

In addition, whilst the application of MST has several advantages over conventional graph network analysis, with MST analysis it is not possible to determine local network efficiency because segregation measures, most notably clustering, are not easy to assess in MST trees (Tewarie et al., 2014), This indicates that the graph analysis, specifically measures of local efficiency such as clustering and modularity, are crucial as they provide insights on local connectivity. It would therefore make sense that future studies, while acknowledging the limitation of graph theory, do not ignore the fact that with this approach one can assess local network efficiency, which is not as straightforward with MST.

Related to network measures, only a few graph and MST measures were considered in the research reported in this thesis. For graph-based networks, efficiency was examined using clustering, path length, and small worldness, whilst for MST global efficiency was examined, based on leaf-number, diameter, hierarchy, and eccentricity. However, as explained in Chapter 3, many of these measures are highly correlated with each other. A decision was therefore taken to only focus on measures that have been extensively investigated in other psychiatric conditions and shown to contained predictive information regarding network efficiency. In the future, studies will benefit from considering several other measures using explanatory analysis.

Sample size issues

The samples sizes for some of the studies were small. Also for the first experimental study (Chapter 3), a high dropout was experienced. For Experimental Study 1, the study was a repeated design. Although students were awarded course credits, this did not apply to the postgraduate volunteers. In the future, an incentive (e.g. a raffle draw) might encourage volunteers to complete both parts of the study.

In addition, sample sizes, particularly in the clinical groups, were limited. As a result, the effects of variables such as medication, gender, age, handedness, and clinical ADHD sub-types were not taken into consideration. Previous studies (Boersma et al., 2013; Gong et al., 2009) have reported that factors such as gender play a crucial role in network organisation.

These studies reported that women and girls showed higher whole-brain connectivity and often the organization of their networks was more efficient. Future studies examining the clinical potential of network variables will therefore benefit from larger and more diverse samples, where the effect of aspects such as medication, gender, and age can be investigated. It would also be beneficial to design longitudinal or follow-up studies to investigate whether network analysis (both graph theory and minimum spanning tree) is able to successfully predict outcome in cohorts with both single and comorbid developmental disorders. Further, to confirm whether network measures are clinical endophenotypes of atypical development, future studies would also benefit from investigating network efficiency in the unaffected siblings of children with neurodevelopmental conditions.

Study design

The research reported in this thesis was cross-sectional. It is therefore not possible to conclude from the results whether observed network disturbances persist throughout development, or whether reorganization of functional networks has a time scale or limit. From these results, it is therefore not possible to predict the long-term outcome of identified disrupted network communication in the context of cognitive deficits and behaviour functioning. To build upon the research presented in this thesis, future studies have three tasks. First, they should conduct longitudinal studies, to determine the progress of disturbances in network function; second, they should use larger samples, and third, they should examine whether functional network alterations measures are specific enough to reliably differentiate those with neuropsychiatry conditions from controls.

Neural oscillations

As with most neuropsychological and region-specific imaging studies, one is inclined to question the specificity of the altered network parameters and neural oscillations reported in this thesis. Are these specific to ADHD and dyslexia? In addition, if they are not, does that imply that their potential clinical utility as biomarkers for atypical neurodevelopment is reduced? In neurodevelopmental conditions, similar altered underlying neurotransmitter systems and brain regions have been reported. For instance, in neuropsychiatry altered global network efficiency in beta oscillations have been shown to be associated with several neuropsychiatric populations, such as those with schizophrenia. This suggests dysfunctional

underlying neurotransmitter systems of beta oscillations. While a finding such as this no doubt reduces potential specific clinical relevance, it does however help explain symptom overlaps in many neuropsychiatry conditions that are sometimes difficult to determine using behavioural measures.

Although the norm is often to study 'pure' disorder conditions, the research presented in this thesis suggests that similarities in underlying network efficiency may be important to enhance understanding of why conditions that appear behaviourally different, such as ADHD and dyslexia, co-occur more often than is expected by chance. For this reason, future studies will benefit from conducting research among clinical groups that co-occur frequently or present with similar behavioural phenotypes. In neurodevelopmental disorders, co-morbidity is the rule rather than the exception, hence investigations of groups such as the one presented in Chapter 6, are important.

Furthermore, one of the main findings of the research presented in this thesis suggested that network efficiency varied depending on frequency band. This indicates that neural oscillations are well suited for further investigation of network efficiency as potential markers of atypical development. Given that much research has already been conducted to determine the underlying mechanisms that generate oscillations, further research would therefore benefit more from using imaging techniques that enable an investigation of network properties across both slow and fast neural oscillations.

However, it is also important to consider that a possible explanation for differences in network efficiency derived from the resting-state condition could be that the resting-state condition in children with atypical neurodevelopment is fundamentally dissimilar from that of controls. By definition, for instance, children with ADHD are inattentive. Perhaps even under resting conditions, underlying spontaneous brain activity is different. While this is a theoretical possibility, instructing children in this study to remain still during scanning was no more difficult for children with developmental conditions compared to controls. Therefore, it is an inference that the results observed were due to genuine biological differences between those groups.

7.4. Conclusion

Graph theory has proved useful for investigating the neural correlates of some neuropsychiatric disorders, where a key feature of the behavioural and cognitive problems experienced is as an effect of disrupted communication within and between brain networks. In those neurodevelopmental disorders where diagnosis is based on measures related to behavioural and cognitive tasks, a consistent set of measures of the underlying biological mechanisms would hold promise as a clinical tool. The research reported in this thesis was able to reliably validate test-retest reproducibility of graph metrics prior to their application in addressing typical and atypical neurodevelopment. The results revealed that connectivity strength and network variables estimated in eyes-closed rest conditions generated more reliable metrics. As a result, subsequent studies computed network measures in this condition. To understand the nature of the underlying physiological mechanisms in atypical development, the research in this thesis first sought to determine the functional network topology in typical volunteers. The results revealed that graph analysis and MST were able to capture network changes in typical development in high frequency beta and gamma ranges. The utility of these approaches was also demonstrated in the clinical populations studied. More importantly, from the results in each chapter (See discussions) it was possible to establish associations between network efficiency and brain volumes integrity. As a result, speculative suggestions were made that changes in functional networks may correspond to typical and/or atypical development of physiological mechanisms. In addition functional network variables correlated with behavioural and cognitive functioning, indicating the potential clinical relevance of altered functional network organisation in atypical development.

In conclusion, the application of mathematically based computational approaches, to quantify neurophysiological disruptions of whole-brain functional connectivity and network activity, proved useful in increasing insight into many underlying pathophysiological mechanisms that characterise neurodevelopmental conditions. The results offered support to the proposition that measures of network organisation, derived from sensor-space MEG data, offer qualified, but informative insights in helping analyse the biological bases of typical brain maturation and neurodevelopmental conditions, with a further possibility of future clinical utility. It has been suggested that the functional networks in higher frequencies, particularly the beta band, may function as vital markers or even 'fingerprints' for untangling

the underlying neurobiological risk factors of atypical development and corresponding cognitive and behaviour impairments. Moving to a more objective biologically driven measure seems vital for improved assessment of neurodevelopmental disorders. This can be seen as a promising future for applied neuroscience, and has already commenced in ADHD diagnosis in the USA, where in 2013, the Food and Drug Administration (FDA) federal agency approved the marketing of the Neuropsychiatric EEG-based Assessment Aid (NEBA), an instrument that provides a measure of the theta/beta ratio, shown to discriminate between those with ADHD and controls. The results reported in the current study suggest a potential utility of functional network topology alongside conventional diagnostic measures. However, the first scientific step should be to attempt to replicate the results reported in this thesis using larger cohorts.

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Appendices

Although results in Chapter 3, revealed that non-normalized/first-order metrics (C_W and L_W) were highly robust, these metrics do not represent 'pure' measures of network topology (Stam et al., 2009). This is because they are more likely to be affected by changes in the average coupling strength (i.e. average phase lag index), (Boersma et al., 2012), whereby lower average coupling strength results in decreased C_W, and longer L_W, regardless of network structure (Stam et al., 2009). When placed in the context of ADHD (See Figure 5.1) where, lower whole-brain connectivity was revealed in the beta band, this would mean that non-normalised C_W would low and L_W would be high in ADHD compared to typically developing controls (TCD). This was in fact confirmed (See appendix A.1). For this reason, we were justified in focusing on normalized C_W and L_W.

Appendix A.1

Figure A. 1 Non-normalised clustering and path length graph metrics

Bar graphs demonstrate mean group differences for the beta band in A) mean clustering coefficient and B) characteristic path length derived from the original networks. Error bars are ± 2 SE, standard error. Topological parameters using non-normalized metrics revealed that local clustering was lower in children with ADHD while path length was significantly higher * p < .05, ** p < .01. Key: ADHD = attention-deficit/hyperactivity disorder, TDC = typically developing controls.

Appendix A.2

***Figure A. 2 Small-worldness organisation in controls and
children with developmental disorders***

*Results of small-worldness in controls and children with developmental conditions. Though
not statistically significant (α level of 0.5), the Kruskal-Wallis H test revealed a trend towards
differences in small-worldness between the four groups in the gamma band. Error bars are ±
2 SE, standard error. Key: ADHD = attention-deficit/hyperactivity disorder, and TDC =
typically developing controls*

Appendix A.3. Regional differences in theta connectivity strength in dyslexics, ADHD and those with heterogeneous phenotypes

Analysis was restricted to frequency range where significant results were observed in post-hoc comparisons (See Figure 6.1). For Chapter 6, this was in the theta. Post-hoc comparisons revealed that mean whole brain connectivity theta connectivity was significantly higher in the dyslexic group compared to controls, those with ADHD and with comorbid conditions.

<u>Inter and intra hemispheric resting-state brain functional connectivity</u>

The justification for this type of analysis was motivated by the possibility that local problems (i.e. sensor clusters) might reveal problems in areas previously considered unaffected, which

may intern affect global network function. Magnetometers were grouped into the four classical cortical regions of interest (excluding the midline sensors) for the left (L) and right (R) hemisphere, consistent with a previous approach reported by Stam et al. (2009). The remaining 96 magnetometers roughly corresponding to frontal (11L, 11R), temporal (12L, 12R), parietal (13R, 13L) and occipital (12R, 12L) in the right and left hemisphere were computed.

Figure A. 3 Topological illustration of the schematic view of magnetometers sensors grouped in the four classical regions
A schematic illustration of sensors grouped to represent 8 cortical regions. Key: L = left, R = right, F =frontal, T = temporal, C = occipital, P = parietal.

The Kruskal-Wallis H test showed that there was a statistically significant difference in standardised mean phase lag index values between the three groups in the left frontal-temporal regions ($H(2) = 6.83$, $p <. 05$) and a trend towards differences in right frontal-temporal magnetometer sensors, ($H(2) = 6.0$, $p >.05$). Post-hoc comparisons of left frontal-temporal magnetometer sensors revealed significant differences between children with ADHD and those with dyslexia ($U = 878.50$, $z = -2.56$, $p <.01$), but not between those with ADHD and comorbid conditions ($U = 447.5$, $z = -1.54$, $p >.05$), or dyslexic readers and those comorbid conditions ($U = 578.5$, $z = -.51$, $p >.05$).

Figure A. 4 Theta band long-distance functional connections in children with dyslexia, those with ADHD and those with comorbid conditions

The y-axis represents mean population standardised z scores for long-distance functional connectivity strength, whilst the x-axis represents hemispheres. Graphs A and B are regions where connections have been reported to be altered in dyslexia, while C and D are implicated in ADHD. Error bars are 2 ± SEM. Kruskal-Wallis H tests (*p <.05).