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A MAGNETOENCEPHALOGRAPHY STUDY OF FUNCTIONAL BRAIN CONNECTIVITY IN CHILDHOOD, ADOLESCENCE AND ADULTHOOD

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Abstract

Functional brain networks are interconnected brain regions that flexibly coordinate their activity to support cognitive demands (Fair et al., 2009). Functional brain connectivity describes a statistical dependency between the activities recorded at spatially distinct brain regions (Friston, 2009). Changes in the pattern of connections and level of activation in functional brain networks are thought to occur across development (Taylor, Donner, & Pang, 2012) but the nature of these changes and their relationship to cognitive development have yet to be delineated clearly.

This thesis seeks to deepen our understanding of the development of functional brain connectivity across the age range 9-25 years. We used magnetoencephalography in conjunction with canonical correlation analysis to explore functional connectivity via amplitude-amplitude envelope correlations in 110 datasets (39 working memory, 33 relevance modulation (attention processing) and 38 resting state).

At the core of this thesis, we have presented novel findings that show non-linear functional connectivity changes across development, with an increase from childhood (age 9-12) to late adolescence (age 17-20) followed by a reduction into young adulthood (age 21-25), resembling an inverted-U-shaped trajectory at least in the females included in this study. Whilst there are subtle yet statistically significant differences in how the functional connectivity profile from 1-100 Hz is modulated by different factors, the overall pattern of functional connectivity development appears to be remarkably consistent across cognitive demands and networks.

Critically, this work is the first example of such findings and suggests that functional brain networks supporting higher order cognitive function are not alone in undergoing functional development; sensory networks that reach structural maturity early on in life also undergo functional development from age 9 to 25.

Publications based on this thesis

Hall, E. L., Smith, H. J. F., Morris, P. G., Groom, M., Liddle, E. B., Brookes, M. J., Liddle, P. F. (in preparation) Electrophysiological brain network changes in neurodevelopment: an MEG study.

Awards

Best short talk award, MEGUK 2014, January 2014.

Building Experience and Skill Travel Scholarship, December 2011.

Declaration

I declare that this thesis is the result of my own work which has been undertaken during my period of registration for this degree at The University of Nottingham. I have complied with the word limit for my degree (as stated in the Quality Manual).

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Presentations

Oral presentations

1. Translational Neuroimaging in Mental Health seminar, April 2014; A MEG study of functional brain connectivity in children, adolescents and adults.
2. MEGUK 2014, January 2014; Development of task-related functional brain connectivity from age 9-25.
3. Centre for ADHD and Neurodevelopmental Disorders Across the Lifespan seminar, June 2012; Investigating functional networks using magnetoencephalography – preliminary findings.
4. Vrije Universiteit Department of Clinical Neurophysiology, Amsterdam, The Netherlands, April 2012; A magnetoencephalography study of task positive functional brain networks.

Poster presentations

1. MEGUK 2014, January 2014; Development of task-related functional brain connectivity from age 9-25
2. Neuroscience at Nottingham, December 2012; Magnetoencephalography - what is it, and how does it work?
3. MRC Impact conference, November 2012; Understanding brain 'networks'.
4. The Institute of Mental Health Foundation Anniversary Celebration, November 2012; A magnetoencephalography study of functional brain networks through development.
5. British Psychological Society Developmental Section Annual Conference, September 2012; A magnetoencephalography study of functional brain networks through development.
6. Medicine and Health Sciences Faculty Postgraduate Research Forum, June 2012; A magnetoencephalography study of functional networks in children, adolescents and adults.

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List of acronyms

ACC	Anterior Cingulate Cortex
AD/HD	Attention Deficit/Hyperactivity Disorder
ANOVA	Analysis of Variance
ANT	Attention Network Task
AP	Action Potential
ASD	Autism Spectrum Disorders
ASRS	Adult AD/HD Self-Report Scale
BOLD	Blood Oxygen Level Dependent
CCA	Canonical Correlation Analysis
CCC	Canonical Correlation Coefficient
CRB	Criminal Records Bureau
dACC	Dorsal Anterior Cingulate Cortex
DAN	Dorsal Attention Network
DC	Direct Current
DLPFC	Dorsolateral Prefrontal Cortex
DMN	Default Mode Network
DTI	Diffusion Tensor Imaging
ECoG	Electrocorticography
EMG	Electromyogram
EPSP	Excitatory Post-Synaptic Potential
ERD	Event Related Desynchronisation
ERS	Event Related Synchronisation
EEG	Electroencephalography
FA	Fractional Anisotropy
FC	Functional Connectivity
FIC	Frontoinsular Cortex
fcMRI	Functional Connectivity Magnetic Resonance Imaging

fMRI	Functional Magnetic Resonance Imaging
FMRIB	Functional Magnetic Resonance Imaging of the Brain
FSL	FMRIB Software Library
GABA	Gamma-Aminobutyric Acid
HR	Haemodynamic Response
HSD	Honest Significant Difference
ICA	Independent Components Analysis
IPS	Intraparietal Sulcus
IPSP	Inhibitory Post-Synaptic Potential
IQ	Intelligence Quotient
LFP	Local Field Potential
MEG	Magnetoencephalography
MNI	Montreal Neurological Institute
mPFC	Medial Prefrontal Cortex
MRI	Magnetic Resonance Imaging
MSR	Magnetically Shielded Room
NHS	National Health Service
OEF	Oxygen Extraction Fraction
PCC	Posterior Cingulate Cortex
PET	Positron-Emission Tomography
PFC	Prefrontal Cortex
PMBR	Post-Movement Beta Rebound
PMC	Premotor Cortex
PPC	Posterior Parietal Cortex
PSP	Post-Synaptic Potential
RAN	Rapid Automatized Naming
RF	Radio Frequency
ROI	Region of Interest
RT	Reaction Time
SMA	Supplementary motor Area

SNR	Signal to Noise Ratio
SPMMRC	Sir Peter Mansfield Magnetic Resonance Centre
SQUID	Superconducting Quantum Interference Device
SWAN	Strengths and Weaknesses of AD/HD Symptoms and Normal Behaviour
TMS	Transcranial Magnetic Stimulation
vACC	Ventral Anterior Cingulate Cortex
VE	Virtual Electrode
vlPFC	Ventrolateral Prefrontal Cortex
WAIS	Wechsler Adult Intelligence Scale
WASI	Wechsler Abbreviated Scale of Intelligence

Chapter 1. Introduction

Functional brain networks are interconnected brain regions that flexibly coordinate their activity to support cognitive demands (Fair, et al., 2009). Whereas structural brain connectivity describes anatomical connectivity between brain regions, functional brain connectivity describes a statistical dependency between the activities recorded at spatially distinct brain regions (Friston, 2009). The roles of functional brain networks with regards to cognition remain unclear, and although potential roles have been proposed, the literature is rightly speculative about some of these.

In order for the adult human brain to be as functionally and structurally specialised as it is the brain undergoes large scale structural and functional change throughout development. Developmental changes in the pattern of connections and level of activation in functional brain networks are thought to occur as cognitive ability develops (Taylor, et al., 2012) but the nature of these changes and their relationship to cognitive development have yet to be delineated clearly. Since no single neuron is responsible for human behaviour, we rely on dynamic interaction and communication between neural assemblies to support cognitive function (Schnitzler & Gross, 2005). Neural oscillations across multiple frequency bands have been proposed as a means of integration and communication between different cortical areas to support sensory and cognitive processing (Basar, Basar-Eroglu, Karakas, & Schurmann, 2001). However, very little is known or understood about how functional connectivity (FC) changes over development and how changes in FC might underpin changes in cognition. In order to understand the nature and development of typical FC, we have chosen to use magnetoencephalography (MEG) to study FC across the age range 9-25 years.

The opening chapter of this thesis will introduce the background to this piece of research and over the following sections I will examine the literature on FC alongside the current understanding of the relationships between functional, structural and cognitive changes in the typically developing brain. I will also present the rationale for using MEG to study the neural correlates of these changes, and the specific hypotheses tested in the study.

Section 1.1. What is functional connectivity?

Put simply, functional brain networks reflect spatially distinct brain regions that exhibit correlated activity. Functional brain networks were primarily characterised using resting state fMRI (functional Magnetic Resonance Imaging) through temporally correlated spontaneous fluctuations in slow frequency (<0.1 Hz) BOLD (blood oxygen level dependent) signal (Biswal, Yetkin, Haughton, & Hyde, 1995; Fox et al., 2005). Because fMRI is based on the premise that blood flow and subsequently oxygen supply follows neural activity, the BOLD signal is regarded as a proxy for brain activity. Despite the excellent spatial resolution on the order of millimetres achieved with fMRI, the nature of this measure means that its temporal resolution is poor, on the order of seconds. The assumption underlying fMRI-based FC is that brain regions that exhibit correlated activity, as measured by BOLD, are working together as a network.

Section 1.1.1. Functional connectivity using BOLD

In a shift from the traditional neuroscientific approach of investigating the function of individual brain regions, the 1990s saw neuroscientific research enter a new phase of exploring interconnected brain regions and how they support cognitive function in the absence and presence of goal-directed tasks. Biswal *et al.* (1995) were the first to show correlated spontaneous fluctuations in BOLD signal between functionally related brain regions associated with motor function in the absence of a motor task (i.e. during resting state, also referred to as 'task-free'). They reported a high degree of spatial agreement between correlated voxels during task-positive and task-free data, indicating that spontaneous connectivity had a functional basis. The network identified is commonly now referred to as the 'sensorimotor network'; it is functionally active before, during and after movement predominantly over the contra-lateral side and is identifiable at rest. The spatial map of the sensorimotor network is demonstrated in Figure 1.1.

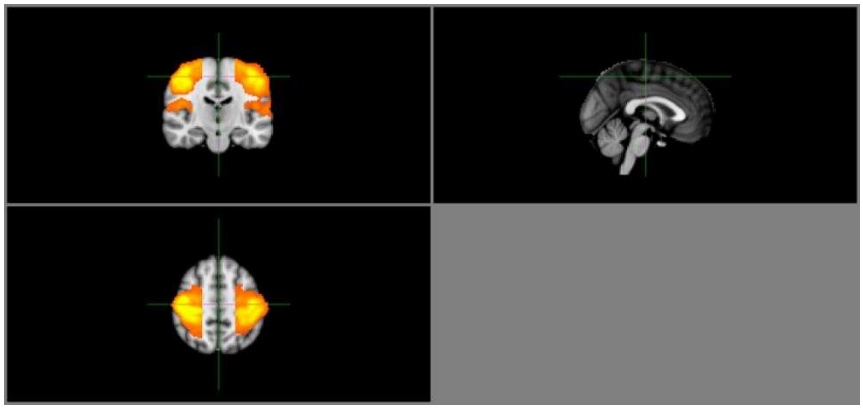


Figure 1.1: The sensorimotor network (based on ICA meta-analysis of resting state fMRI data reported by Smith et al. (2009)).

Cordes *et al.* (2000) built on the work of Biswal *et al.* (1995) demonstrating good spatial agreement between FC analysis of task-positive and task-free fMRI data not only with regards to sensorimotor regions but also in the visual cortex, language regions and the superior temporal lobe (auditory cortex). Brain regions implicated in visual processing are often now referred to as the 'visual network'. Later work showed task-related increases in this network when subjects fixated passively during eyes open task-free positron-emission tomography (PET (Greicius, Krasnow, Reiss, & Menon, 2003; Raichle et al., 2001)), and decreases in activity during eyes closed task-free PET (Raichle, et al., 2001). The spatial map of the visual network is demonstrated in Figure 1.2.

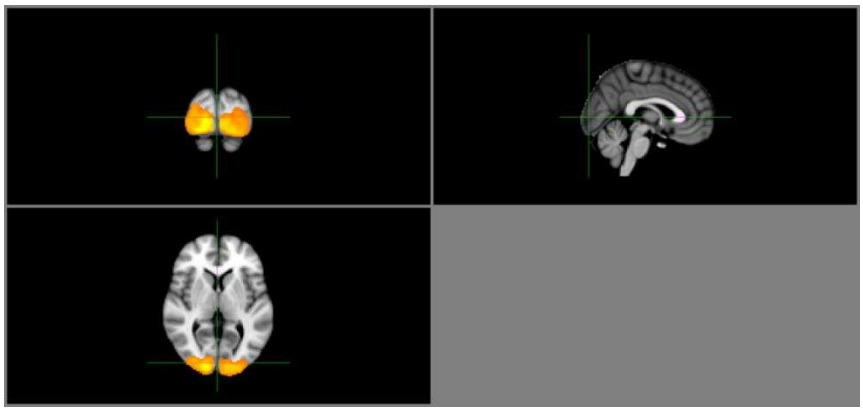


Figure 1.2: The visual network (based on ICA meta-analysis of resting state fMRI data reported by Smith et al. (2009)).

Moving on from the lower level sensorimotor and visual networks, there are several other networks identifiable at rest that have been extensively linked to cognitive processing including the default mode network (DMN), dorsal attention network (DAN) and salience network. The DMN is thought to play a role in supporting higher-order cognitive functions that require internalisation of thought (de Bie et al., 2012) such as emotional and self-referential processing (Gusnard, Akbudak, Shulman, & Raichle, 2001). It comprises several key regions including the mPFC (in particular the ventromedial PFC), PCC (posterior cingulate cortex) and lateral parietal cortex (Fair et al., 2008; Sridharan, Levitin, & Menon, 2008; Uddin, Kelly, Biswal, Castellanos, & Milham, 2009). The DMN is demonstrated in Figure 1.3**.**

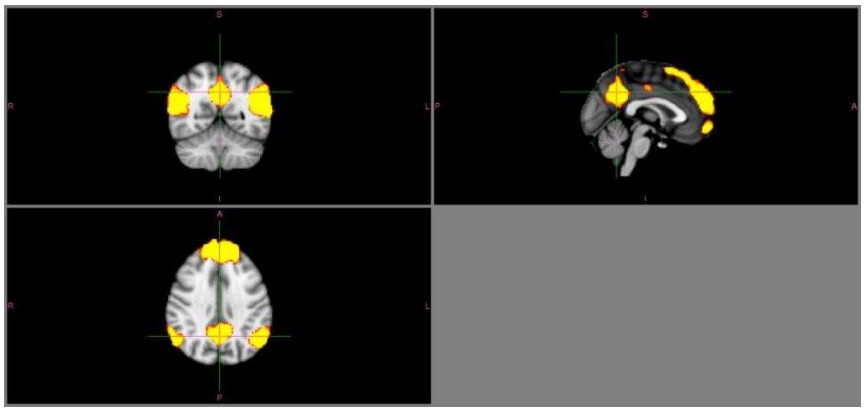


Figure 1.3: The default mode network (based on ICA meta-analysis of resting state fMRI data reported by Smith et al. (2009)).

The DMN is known as a 'task-negative' network because nodes show decreased activity during most goal-directed tasks when compared to baseline (Raichle, et al., 2001; Sridharan, et al., 2008). Activity in the DMN has additionally been found to inversely correlate with activity of 'task-positive' networks such as the DAN and salience network (Sridharan, et al., 2008). Raichle *et al.* (2001) and Fair *et al.* (2009) have hypothesized that this may be because the DMN is responsible for broad information gathering and processing when there is no need for goal-directed cognition i.e. during rest, but when attention needs to be focussed towards a goal, these background cognitive processes are heavily reduced.

Support for this hypothesis comes from many sources. Firstly, researchers have reported that the extent to which DMN network deactivation changes with task difficulty (McKiernan, Kaufman, Kucera-Thompson, & Binder, 2003) suggesting a relationship between deactivation of the DMN and attention. McKiernan *et al.* (2003) suggest that as deactivation of the DMN increases alongside task difficulty, this may reflect a reallocation of processing resources to the task in hand, a hypothesis that has been put forward in other work (Fox, et al., 2005; Greicius, et al., 2003). This is further supported by the report that failure to suppress DMN activity during a goal-directed task is linked to longer RTs (reaction times) considered by the researchers to reflect lapses in attention, although a causal relationship cannot be concluded without further investigation (Weissman, Roberts, Visscher, & Woldorff, 2006).

The DAN (also known as the frontoparietal network and central executive network) is known as a task-positive network because it exhibits task-related increases in activity (Fox, et al., 2005; Greicius, et al., 2003; Seeley et al., 2007). Activity in parietal nodes of the DAN positively correlates with executive task performance (Seeley, et al., 2007) supporting the hypothesis that this network is important for executive function. Nodes in particular include the DLPFC (dorsolateral prefrontal cortex), implicated in working memory (Curtis & D'Esposito, 2003) and the PPC (posterior parietal cortex), implicated in episodic memory and several components of attention (Fan, McCandliss, Sommer, Raz, & Posner, 2002; Seeley, et al., 2007; Sridharan, et al., 2008). The spatial profile of the DAN (also known as the central executive network) is demonstrated in Figure 1.4.

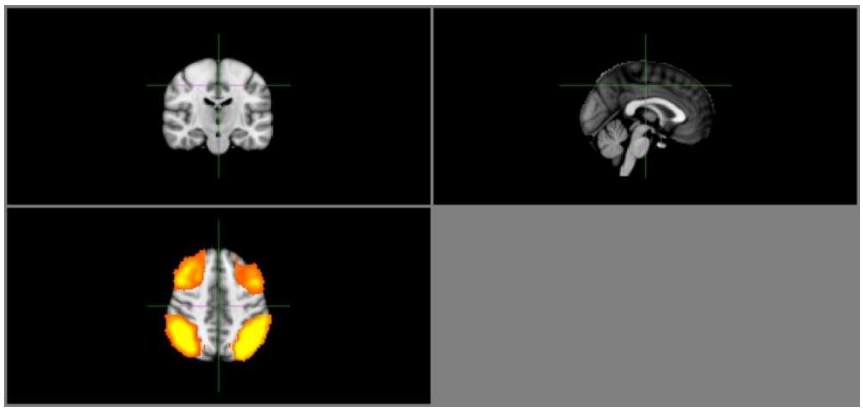


Figure 1.4: The dorsal attention network (based on ICA meta-analysis of resting state fMRI data reported by Smith et al. (2009)).

Seeley *et al.* (2007) more recently presented fMRI data that suggested the presence of two task-positive networks, one termed the 'executive control network' and one termed the 'salience network'. Activity in the salience network (also known as the cingulo-opercular network) has been linked to salience processing such as pain, anxiety and salient auditory and visual stimulation (Seeley, et al., 2007; Sridharan, et al., 2008). Key nodes of the salience network include the dorsal anterior cingulate (dACC) implicated in introspective autonomic processing, right frontoinsular cortex (rFIC) and bilateral insula (Greicius, et al., 2003; Seeley, et al., 2007; Sridharan, et al., 2008), depicted in Figure 1.5 (which does not show the dACC node).

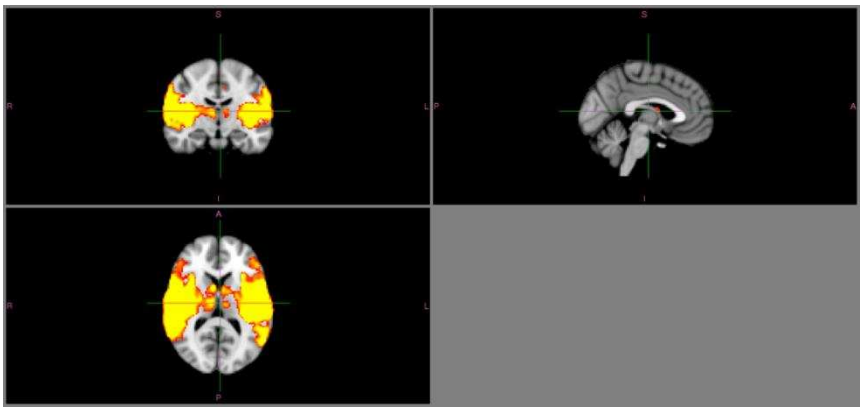


Figure 1.5: The salience network (based on ICA meta-analysis of resting state fMRI data reported by Smith et al. (2009)).

Seeley and colleagues found that pre-scan anxiety correlated with FC in two nodes of the salience network, indicating that networks have a relevance to individual factors which could influence mental health, cognition and personality (anxiety could impact on any of these). They further reported that pre-scan anxiety did not correlate with FC in the executive control network. Additionally, time taken to complete a cognitive task involving working memory and attentional demands correlated inversely with FC in the executive control network (demonstrating that greater FC correlated with better performance on the task). These findings further demonstrate the functional significance of BOLD networks.

Whilst the networks presented here are well established, having been identified across research groups and modalities, it should be noted that functional brain networks are not limited to those presented and studied in this thesis.

Section 1.1.2. Linking BOLD correlations to electrophysiology

Due to the fact that the BOLD signal is regarded as a proxy for brain activity, it is important to link BOLD correlations (an indirect measure of neural activity) to electrophysiological measures of brain activity (direct measures of neural activity). Liu *et al.* (2010) suggested that whilst electrophysiological neural oscillations occur at relatively high frequencies (approximately 1-150 Hz) compared to the BOLD signal (spontaneously fluctuating at <0.1 Hz), the *power modulation* of neural oscillatory activity occurs at a slower rate that generally coincides with the frequency range of the BOLD signal through which resting state networks have been identified in fMRI. They proposed that synchronised power modulation (i.e. synchronised power increases *and/or* decreases) across distinct brain regions reflects the electrophysiological signature of functional brain networks previously characterised using fMRI.

Subsequently, de Pasquale *et al.* (2010) replicated these findings and further found that the most prominently synchronised frequency bands were theta, alpha and beta. They additionally demonstrated that the DMN and DAN are identifiable with MEG using a seed based correlation approach to extract the spatial signatures of the DMN and DAN from MEG data. Seed based correlation is a method whereby a single 'seed' voxel in the brain is chosen either randomly or based on *a priori* hypotheses. The time course of this voxel is compared either with all others in the brain or with selected voxels in the brain (dependent on whether any *a priori* hypotheses exist) to identify those with which it shares a statistical dependency.

More recent work has elucidated several known resting state networks using MEG with temporally down-sampled data to a time resolution similar to fMRI (approximately 2 seconds (Brookes et al., 2011a)). Brookes *et al.* (2011a) illustrated good spatial agreement between fMRI and MEG of several functional brain networks

using ICA, a technique whereby the most prominently and consistently active or co-active regions in the brain can be extracted in the form of spatial maps from acquired data. Such findings indicate that we have a good foundation of spatial information from which we can work to clarify the finer temporal mechanisms of functional brain connectivity and functional brain networks using MEG. They also indicate that the BOLD-delineated networks are not simply an artefact of blood flow, but that they actually represent correlated regions of neural activity.

Furthermore, Brookes *et al.* (2012a) went on to show that task-induced functional brain networks can be identified with MEG. They first used a temporal ICA approach to extract independent components from a range of behavioural data including working memory and selective attention data. These independent components corresponded to functional brain networks such as the visual network, sensorimotor network, salience network and DAN. They then generated time-frequency spectrograms for each of the derived networks, averaged across the voxels included in each network and found task-induced modulations of neural oscillatory activity confirming the functional relevance of these networks (Brookes, et al., 2012a).

In relation to the current study, the research summarised here indicates that functional brain networks originally identified using fMRI can also be observed using MEG. This is of relevance because the current study will identify brain networks using MEG and then determine whether electrophysiological activity in these networks is modulated by the presence and/or absence of goal-directed tasks, and whether these modulations alter with age. To address this research further, we should consider the processes by which the neural oscillations themselves are generated, explored in the following section.

Section 1.1.3. Origins of the neural signal

Arrival of an AP (action potential) at a pre-synaptic cell causes release of neurotransmitters which diffuse across the synapse (a term used for the junction between a dendrite and axon terminal). The movement of ions alters the transmembrane potential of the post-synaptic cell and the neural signal can then be

carried along the dendrite of the post-synaptic cell (Orrison, Lewine, Sanders, & Hartshorne, 1995). This is called a *post-synaptic potential* (PSP).

Post-synaptic potentials occur in two forms: *inhibitory*, which involve inhibitory neurotransmitters such as GABA, and *excitatory*, which involve excitatory neurotransmitters such as glutamate. The current flow caused by synaptic activity that occurs intra-cellularly within a neuron is termed the *primary current* and the current occurring extra-cellularly in the opposite direction is termed the *volume current* (see Figure 1.6B). Combined, these two currents produce a dipole generating a magnetic field that reduces over distance at a rate of $1/r^2$ (see Figure 1.6).

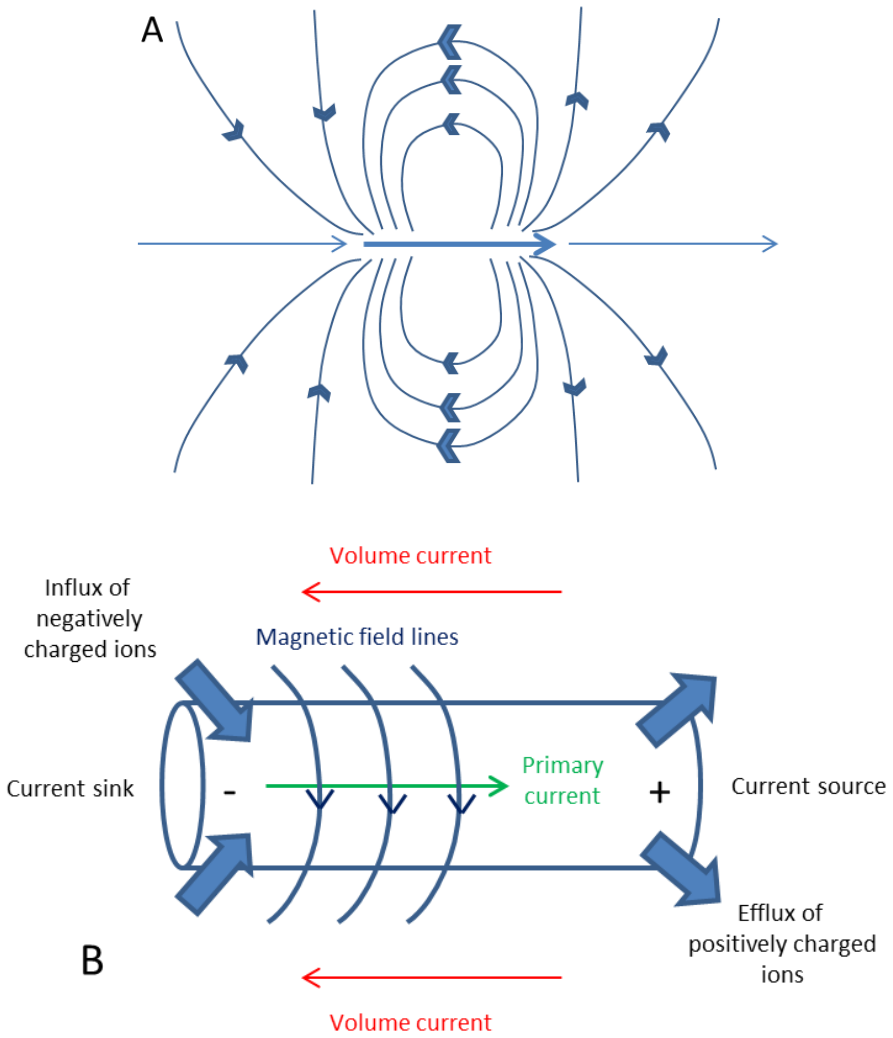


Figure 1.6: (A) The current pattern of a current dipole as generated by a PSP flowing along the dendrite (B) primary and volume currents created by a PSP (adapted from Hale (2012)).

An AP on the other hand, produces a quadrupole (i.e. it travels down the axon as two oppositely directed current dipoles, as demonstrated in Figure 1.7) and therefore the magnetic field produced by an AP reduces over distance at a rate of $1/r^3$.

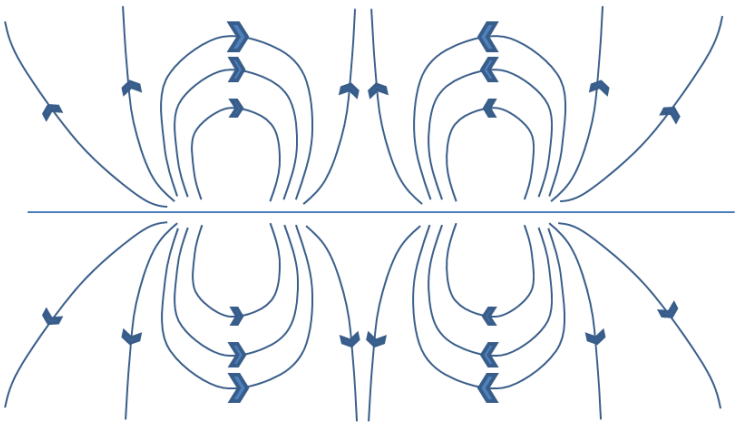


Figure 1.7: The current pattern of a quadrupole as generated by an AP (adapted from Orrison et al. (1995)).

The summation of EPSPs (excitatory post-synaptic potentials) and IPSPs (inhibitory post-synaptic potentials) will produce an AP if they summate to exceed the firing threshold of the AP. If excitation increases, the firing rate of APs will increase but not the amplitude of the APs. It is worth also noting that PSPs occur on a longer time scale than APs (~10 ms and ~1 ms respectively) - in order to produce a magnetic field detectable outside the head, synchronisation of neural activity must occur and the relatively long time scale of PSPs compared with APs means that it is far more likely that PSPs will synchronise. Additionally, given the faster drop-off rate of the magnetic field produced by an AP in comparison with the magnetic field produced by a PSP, the signal detected with MEG is considered to originate from PSPs rather than APs (Hämäläinen, Hari, Ilmoniemi, Knuutila, & Lounasmaa, 1993). It is also important to understand that the signal detected with MEG originates from synchronised PSPs in pyramidal neurons of the cortex which will be explained in more detail below.

Neuronal cell bodies and dendrites are mostly found in the grey matter of the brain (i.e. the cortex and some subcortical regions) whilst axons are mostly found in the white matter of the brain (their myelin gives white matter its colouration). In the

human cortex two types of neuronal cell are found: stellate and pyramidal neurons (Bear, Connors, & Paradiso, 2007). Stellate neurons are identifiable by their characteristic dendrites which fan out from the cell body as illustrated in Figure 1.8A (Bear, et al., 2007). However, because of the arrangement of their dendrites the electrical and magnetic fields produced by current flow in these cells cancel out and therefore do not contribute to the signal detected with EEG or MEG. Pyramidal neurons are identifiable by their characteristic single apical dendrite as illustrated in Figure 1.8B (Bear, et al., 2007) and these neurons align in the cortex of the brain, resulting in a detectable electrical and magnetic field when current flows through these cells.

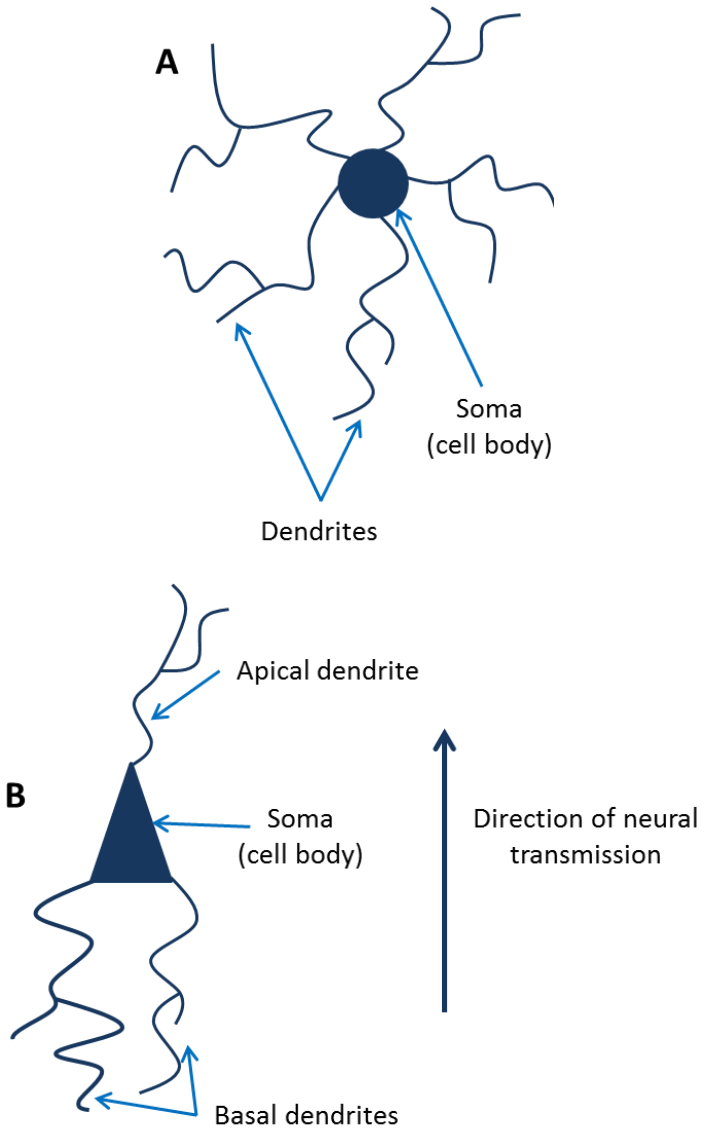


Figure 1.8: (A) A schematic of a cortical stellate neuron and (B) A schematic of a cortical pyramidal neuron (adapted from Bear et al. (2007)).

Whilst EEG offers a direct measure of the electrical fields generated by current flow in pyramidal neurons of the cortex, MEG offers a direct measure of the corresponding orthogonal magnetic fields as demonstrated in Figure 1.9.

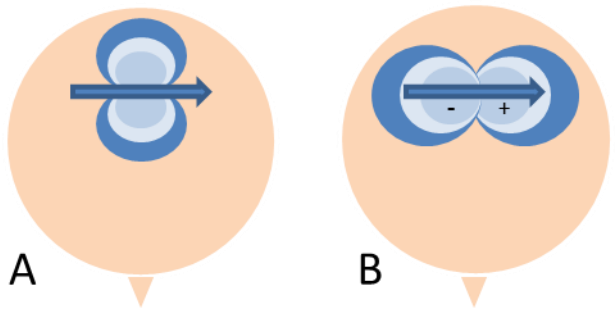


Figure 1.9: A schematic of the relationship between (A) magnetic and (B) electrical fields. Note that the electrical and magnetic fields illustrated are orthogonal to one another but they share the same neural source; the direction of neural current flow is indicated by the arrow (adapted from Hämäläinen et al. (1993)).

Only populations of pyramidal neurons whose dendritic trees are aligned *tangentially* to the surface of the head produce a signal visible to MEG. Sources that are radial to the surface are 'silent' i.e. invisible to MEG (Hämäläinen, et al., 1993). The signal recorded with MEG therefore mainly originates from cortical sulci (folds), rather than the gyri (ridges), as demonstrated in Figure 1.10.

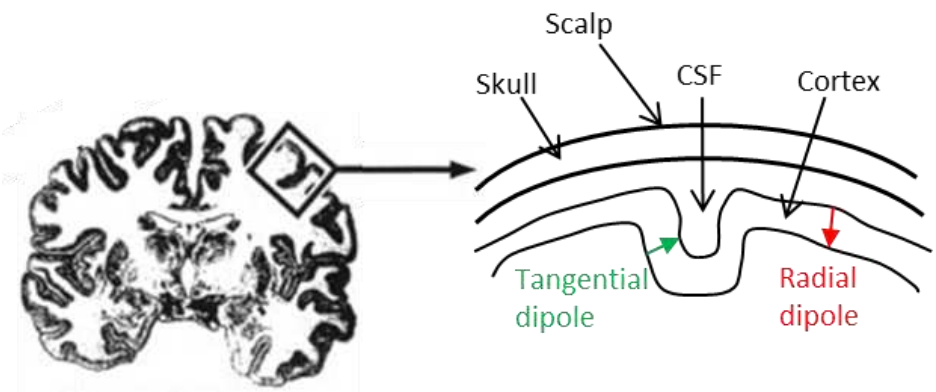


Figure 1.10: The neuromagnetic signal mainly originates from the sulci of the cortex; tangential sources produce external magnetic fields measurable with MEG whereas radial sources do not (adapted from Vrba & Robinson (2001)).

Section 1.1.3.1. Neural oscillations

Neural oscillations reflect the integrated post-synaptic potentials (PSPs) of populations of neurons known as local field potentials (LFPs (Singh, 2012)). Both EPSPs and IPSPs contribute to the LFP, i.e. membrane depolarisation (excitatory) and hyperpolarisation (inhibitory) of post-synaptic neurons (Schnitzler & Gross, 2005). APs firing in the pre-synaptic cells of a given neural population need to be synchronised to produce neural oscillations; if a neuron fires APs at a certain frequency in response to stimulation, the PSPs created will also fluctuate at a certain frequency (Bartos, Vida, & Jonas, 2007). On a larger scale, Schnitzler & Gross (2005) suggest that oscillations thus occur as a result of increased probability or synchronisation of action potential firing rates at the different specific frequencies of neural oscillations, such that LFPs are produced that oscillate at a particular frequency. The branching of dendritic trees is thought to allow this mechanism to travel (Bartos, et al., 2007).

Donner & Siegel (2011) further propose that oscillations arise from local excitatory-inhibitory interactions; input is received at excitatory neurons which stimulate GABAergic inhibitory neurons, but by stimulating GABAergic neurons, further excitation in the given area is inhibited until additional input is received, and the cycle repeats. It has in fact been suggested by Uhlhaas *et al.* (2009a) and Singer *et al.* (2011) that inhibitory interneurons act as the 'pacemakers' for neural oscillations in all frequency bands. The frequencies of neural oscillations can be categorised into distinct frequency bands generally linked to different brain functions, as detailed in Table 1.1.

Name	Symbol	Frequency Range (Hz)	Associated Neural Processing
Delta	δ	1-4	Sleep and cognition
Theta	θ	4-8	Sleep and cognition
Alpha	α	8-13	Consciousness and relaxation
Beta	β	13-30	Motor and sensory processing
Gamma	γ	30-100	Sensory processing and cognition

Table 1.1: Neural oscillatory frequency bands and their associated roles.

Section 1.1.3.2. Neural oscillations and functional connectivity

Neural oscillations across multiple frequency bands have been proposed as a means of integration and communication between different cortical areas to support sensory and cognitive processing (Basar, et al., 2001). Of particular relevance to this thesis, it has recently been shown by separate research groups that FC measured by amplitude-amplitude correlations in specific frequency bands within known functional brain networks appears to peak in the alpha to beta range (8-13 Hz to 13-30 Hz (Brookes, et al., 2011a; Hipp, Hawellek, Corbetta, Siegel, & Engel, 2012)). Several reports suggest that beta band oscillatory activity may mediate long-range integrative brain functions that require the integration of more than one brain region, i.e. involving multiple separate populations of neurons, such as decision making (Donner & Siegel, 2011; Kopell, Ermentrout, Whittington, & Traub, 2000).

Whilst there are many ways to measure FC with MEG, we have chosen to focus on amplitude-amplitude correlations in this thesis given the previous work presented by Brookes *et al.* (2011a) and the subsequent opportunity to build on the body of relevant BOLD research by investigating similar spatial networks in MEG. It should be noted however, that whilst amplitude-amplitude correlations are the focus of this thesis, it may be that amplitude-amplitude correlations simply reflect underlying processes such as phase-phase interactions or phase-power interactions (such as delta phase modulating theta power or theta phase modulating gamma power (Lakatos et al., 2005)) that trigger measurable amplitude-amplitude correlations. Additionally, whilst specific frequency correlations such as beta-beta power correlations are of interest in this thesis, it is brought to the reader's attention that cross-spectral interactions can also be explored and have been highlighted with some significance to cognitive processing (such as nested theta and gamma cycles encoding items into short term memory (Lisman & Buzsaki, 2008)).

'Power', i.e. the amplitude of neural oscillations, reflects the number or scale of neurons acting in synchrony (Klimesch, 1999). In order to explore the power of neural oscillations, we look at the oscillatory envelope or Hilbert envelope because the response is not phase-locked to a stimulus but in doing so we lose phase information. This allows us to examine changes in oscillatory amplitude irrespective of phase. The envelope of an oscillatory signal is demonstrated in Figure 1.11.

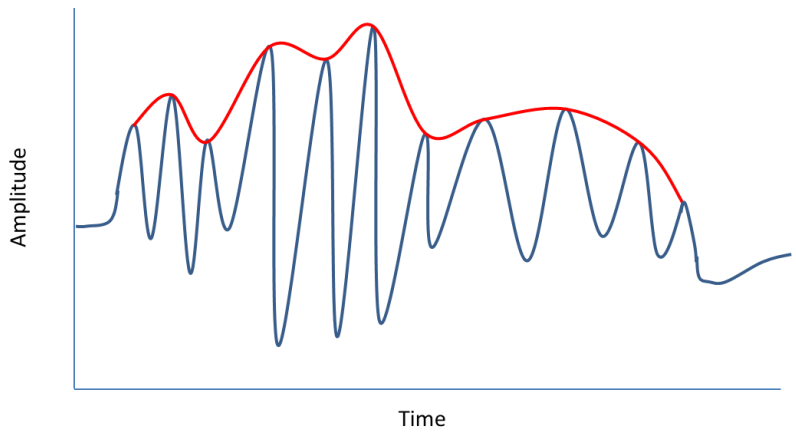


Figure 1.11: The envelope of a Hilbert transformed (in red) band-passed oscillatory signal (in blue; adapted from Hall (2012)).

Section 1.2. How does functional connectivity change over development?

In order for the adult human brain to be as functionally and structurally specialised as it is the brain undergoes large scale structural and functional change throughout development. Changes in cognitive ability that take place in childhood, adolescence and early adulthood are considered the visible extensions of changes taking place in the anatomy and connectivity of brain regions (Taylor, et al., 2012). In order to understand functional changes in brain development concurrently with cognitive development, we must also take into account structural changes that occur parallel to these.

Section 1.2.1. Structural connectivity in the developing brain

Early on in development, over the first few years of life, the cortical surface area doubles and from birth to adulthood our brain mass increases 4 or 5 times over (Johnson, 2001; Taylor, et al., 2012), although by the age of six the human brain is approximately 95% of its adult size (Giedd, 2008). Development of grey and white

matter is known to follow different trajectories. In particular, the development of grey matter is more regionally variable than that of white matter (Giedd et al., 1999a).

Section 1.2.1.1. Grey matter development

The trajectory of grey matter development (cortical thickness and cortical volume) follows an inverted-U-shaped regressive pattern with the greatest decline in cortical thickness reported in adolescence (Giedd, 2004; Shaw et al., 2008; Tamnes et al., 2010). The formation of synapses (synaptogenesis) is a key process in brain development and in most brain regions, synaptogenesis occurs early on in childhood with experience/activity dependent synaptic pruning following shortly afterward through adolescence and in some cases early adulthood (Huttenlocher & Dabholkar, 1997). Gogtay *et al.* (2004) reported grey matter loss first occurring in sensorimotor regions but spreading later to areas linked to higher-order brain function such as the DLPFC. Precise timing of APs and EPSPs reportedly influence synaptic connections (Markram, Lubke, Frotscher, & Sakmann, 1997) and synaptic pruning causes critical reorganisation of neural pathways by reducing the number of synapses by approximately 60% (Casey, Tottenham, Liston, & Durston, 2005; Huttenlocher & Dabholkar, 1997).

Grey matter development is regionally variable (Huttenlocher & Dabholkar, 1997); Gogtay *et al.* (2004) reported that, in general, grey matter develops in a parietal-to-frontal direction, with sensorimotor and additional sensory areas maturing first. Furthermore, Sowell *et al.* (2004) reported regional differences in both cortical *thinning* (found to occur most prominently in the occipital regions and right lateral frontal region of the brain) and cortical *thickening* (found to occur most prominently in the left lateral frontal region of the brain) across development from age 5-9 years.

Huttenlocher (1990) reported that in the visual cortex neuronal density reaches maturation by 5 months of age whereas in the frontal cortex neuronal density does not reach maturation until after the age of 7 years. In the auditory cortex synaptic density reaches peak values by 3 months of age and synaptic pruning comes to completion at the age of 12, whereas in the frontal cortex synaptic density does not

reach peak values until after 15 months of age and synaptic pruning continues into adolescence (Huttenlocher & Dabholkar, 1997). Dendritic length of pyramidal neurons is found to reach adult values by 4 months of age in the visual cortex, however in the frontal cortex the dendritic length of pyramidal neurons is only 50% of the adult values at 2 years of age. Despite this finding, pyramidal neuron dendrites in the frontal cortex are twice as long as pyramidal neuron dendrites in the visual cortex even at 2 years of age. Huttenlocher (1990) believe this to reflect the greater complexity of neurons found in the frontal cortex, known to support higher-order cognitive functioning.

Section 1.2.1.2. White matter development

The trajectory of white matter development follows a more linear, progressive, pattern which peaks in the fourth and fifth decades of life before declining later on in life (Giedd, 2004). White matter development principally involves myelination, a key facilitator of efficient neural processing, which occurs throughout childhood, adolescence and adulthood and is regionally variable. Myelination facilitates neural transmission as it enables self-propagation of neural signals acting as insulation cable surrounding axons, increasing the efficiency of longer distance neural processing. The first regions in the brain to become fully myelinated are the primary sensorimotor areas and the last are the frontal lobes (Taylor, et al., 2012). Paus *et al.* (1999) and Barnea-Goraly *et al.* (2005) suggest that white matter changes in the developing brain play a key role in the development of cognitive abilities, and it is thought that myelination, axonal diameter changes, white matter re-organisation and density changes contribute (Barnea-Goraly, et al., 2005; Schmithorst, Wilke, Dardzinski, & Holland, 2002).

DTI (diffusion tensor imaging) allows assessment of white matter properties such as axonal density, diameter and extent of myelination non-invasively as the technique is based on MRI. FA (fractional anisotropy) provides a measure that reflects diffusion properties where 0 reflects diffusion in all directions, i.e. no restriction and therefore an absence of white matter tracts, and 1 reflects diffusion in one direction only, i.e. total restriction of diffusion by the presence of a completely coherent bundle of fibres within a tract (Barnea-Goraly, et al., 2005). In a comparative DTI study of 8-12

year old children and 20-31 year old adults, children exhibited significantly weaker FA in frontal white matter reflecting the immaturity of white matter tracts in children of this age (Klingberg, Vaidya, Gabrieli, Moseley, & Hedehus, 1999). At the other end of the human life span, in a comparative DTI study of older adults (aged 56-85 years) and younger adults (aged 23-37 years), O'Sullivan *et al.* (2001) demonstrated decline in white matter with old age (measured by decreases in FA) particularly in frontal regions. The decline in white matter with old age furthermore correlated with decline in executive function (measured by a trail making test which is an assessment of attentional set switching).

Following on from the work presented by O'Sullivan *et al.* (2001), Schmithorst *et al.* (2005) also demonstrated correlations between FA (particularly in frontal brain regions) and IQ in younger subjects (aged 5-18). Barnea-Goraly *et al.* (2005) reported significant age-related increases in FA and white matter density in prefrontal brain regions, visual pathways and other regions such as the corpus callosum (consistent with additional reports showing increases in FA between childhood and adolescence (Koerte et al., 2009)) and the arcuate fasciculus in their study of 6-19 year olds.

The corpus callosum (a major white fibre tract consisting of approximately 200 million axons that connects homologous areas of left and right cortex, integrating function of the two cerebral hemispheres (Giedd, 2008)) no doubt plays a vital role in facilitating both structural and FC of the human brain. When this tract is transected, as was the treatment for several intractable epilepsy patients around the 1960s, the ability to integrate sensory information from both hemispheres is lost causing functional deficits such as sensorimotor coordination problems, problems naming objects and problems recognising the tactile properties of objects (Paul, 2011; Sperry, 1968).

The arcuate fasciculus, which connects the temporal and inferior parietal lobes to the frontal lobe, has exhibited age-related increases in FA in several studied such as Paus *et al.* (1999), Schmithorst *et al.* (2002) and Lebel *et al.* (2008). Another recent DTI study highlighted a tendency towards more organised axonal (white matter) fibres in late adolescence compared to early adolescence measured by increased FA which furthermore correlates with measures of cognitive ability, at least in males (Ashtari et al., 2007).

It is apparent from the developmental trajectories of structural brain changes (in respect to both white and grey matter) that sensorimotor areas are the first to mature supporting basic cognitive function, and maturation of areas involved with top-down processing supporting more complex, higher-order cognitive function, follows (Gogtay, et al., 2004; Huttenlocher, 1990; Taylor, et al., 2012). This supports the cognitive progress we see during development, where sensorimotor and visual skills are mastered early on and higher-order cognitive skills such as working memory and attention control improve across adolescence.

It is of potential importance to understand this structural development in order to interpret the functional changes that will be explored in this study. Additionally, the studies aforementioned suggest an important link between structural brain development and cognitive ability and show that in conjunction with visible changes, there are dynamic changes occurring in the structural development of the brain involving both grey and white matter changes. It is also of importance to consider the specific interhemispheric and intrahemispheric changes in the developing brain, as these are also considered to play a significant role in brain development as will be explored in the following section.

Section 1.2.1.3. Interhemispheric and intrahemispheric connectivity in the developing brain

The corpus callosum allows both inhibitory and excitatory communication to occur between the hemispheres, allowing one hemisphere to either suppress the other to maximize functional efficiency of single hemispheric processing or to stimulate the other to maximize functional efficiency dual hemispheric processing (Bloom & Hynd, 2005). Whilst all the fibres of the corpus callosum are present from birth, myelination of these fibres still occurs post-natally and continues into young adulthood, perhaps influencing connectivity via this tract (Franz & Fahey, 2007; Giedd, et al., 1999a; Paul, 2011). Of particular relevance to the functional brain networks introduced in this thesis, the splenium of the corpus callosum (the posterior section of the corpus callosum) connects the left and right visual cortices, whilst the main section of the corpus callosum connects the left and right sensorimotor cortices (for instance, the posterior and frontal nodes of the DAN are

connected via the left and right superior longitudinal fasciculus (van den Heuvel, Mandl, Kahn, & Hulshoff Pol, 2009)). Other functional brain networks rely on white matter tracts other than the corpus callosum (van den Heuvel, et al., 2009).

The corpus callosum is implicated in playing a role in facilitating hemispheric specialization (referring to the designation of cognitive functions to particular cerebral hemispheres (Josse & Tzourio-Mazoyer, 2004)). Knowledge in the field of hemispheric specialization is more prominent with regards to language lateralization than other functions. In particular, language lateralization of function to the left hemisphere increases from age 5-20 then plateaus age 20-25 and declines from age 25 onwards (Szaflarski, Holland, Schmithorst, & Byars, 2006). Fransson *et al.* (2007) suggest that whilst functional brain networks in adults are lateralised (demonstrating stronger intrahemispheric connectivity in adults), they appear to be non-lateralised in new-born infants (demonstrating stronger interhemispheric connectivity in infants), consistent with the theory that development of the corpus callosum could facilitate development of lateralisation.

This is further supported by an absence of transcallosal inhibition in young children (Heinen et al., 1998; Muller, Kass-Iliyya, & Reitz, 1997). It is well reported that in young children mirror movements (also known as associated movements, sensorimotor overflow and synkinetic movements) are often seen when a child attempts to execute a unilateral motor response with one hand, an involuntary contra-lateral movement mirroring the intended movement is observed (Heinen, et al., 1998; Lazarus & Todor, 1987; Muller, et al., 1997). This phenomenon can reportedly be observed up until the age of 10 years when it is thought adult-like inhibition of transcallosal connections are sufficiently matured (Lazarus & Todor, 1987; Muller, et al., 1997). Koerte *et al.* (2009) suggest that unilateral movement may not be mastered until transcallosal inhibitory pathways have matured. They in fact reported that maturation of the corpus callosum (explored using DTI and measured by FA) reflects the degree of transcallosal inhibition. They found correlations between ipsilateral silent period duration and FA in a specific area of the corpus callosum (area III). The ipsilateral silent period is a consequence of TMS (transcranial magnetic stimulation) to the primary sensorimotor cortex which causes a silent period in the EMG (electromyogram) over the ipsilateral target muscle. It is

thought that this ipsilateral silent period therefore reflects a measure of transcallosal inhibition.

Developing this theory, Fransson *et al.* (2007; 2009) found that whilst functional brain networks were identifiable in fMRI of both full-term asleep infants and pre-term asleep and slightly sedated infants at full-term age, networks that included both anterior and posterior nodes were absent suggesting that white matter develops to facilitate anterior-posterior FC rather than transcallosal connectivity. They suggest that in these infants perhaps transcallosal connectivity is stronger than anterior-posterior connectivity, i.e. that interhemispheric connectivity is present and develops earlier than intrahemispheric. Smyser *et al.* (2010) also reported that functional connections present in new-born infants mainly consist of interhemispheric connections between homotopic cortical regions.

The studies summarised here indicate an important link between structural brain development and functional brain development, highlighting the impact that structural and functional development can have on behaviour and cognition. However, it is also important to acknowledge that white and grey matter development alone may well not impact on the development of neural oscillatory activity which is measured with MEG to assess functional connectivity. A simple gain or loss in grey or white matter would not necessarily translate into a change in synchronised pyramidal neuron PSP activity tangential to the surface of the head that would be measured with MEG. In order to understand the development of higher-order cognitive function, it is therefore important to look at the underlying development of functional brain networks, which will be explored in more depth in the following section.

Section 1.2.2. Functional connectivity in the developing brain

Visual and sensorimotor brain regions are known to reach structural maturity early on in life (Gogtay, et al., 2004; Huttenlocher, 1990; Taylor, et al., 2012). Consistent with the notion that sensorimotor areas are the first to mature and that maturation of higher order association areas occurs later, cortical hubs in infants are reportedly

centred around primary sensorimotor and sensory areas (Fransson, Aden, Blennow, & Lagercrantz, 2011) whilst cortical hubs in adults are reportedly centred around higher order association areas (Buckner et al., 2009). Hubs are defined as information convergence areas showing extensive connectivity to multiple areas of the brain and are therefore considered critical information integration units (Fransson, et al., 2011).

In agreement with the cortical hub distribution changes described above, in a study of resting state fMRI conducted in a sample of 5-8 year old children functional networks supporting sensorimotor and visual processing were found to exist in a mature state whereas functional networks supporting higher-order cognitive processing were found to exist in an immature state (de Bie, et al., 2012). Those found in an immature state exhibited weaker within network connectivity and subsequently fragmented architecture. Nevertheless, both functional networks underpinning primary sensorimotor functions and those underpinning higher order cognitive functions have been identified (albeit in immature states) in new-born infants using fMRI (Doria et al., 2010; Fransson et al., 2009; Fransson, et al., 2007; Lin et al., 2008). This gives us confidence that it is a valid approach to investigate such networks in the age range included in this study (age 9-25 years).

Electrophysiological literature indicates that whilst the visual and sensorimotor networks may be restricted in very young children, wider regions may be recruited in older children before refinement in adulthood (Srinivasan, 1999; Wilson et al., 2010). Srinivasan (1999) used 128-electrode EEG to obtain eyes-open and eyes-closed resting state data from children (aged 6-11 years, mean = 8.3) and adults (aged 18-23 years, mean = 19.4). Both children and adults exhibited alpha oscillatory activity over posterior electrodes which peaked during eyes-closed and diminished during eyes-open resting state EEG. However, mean peak alpha frequency was significantly lower in children (8.9 Hz) compared to adults (10.1 Hz) and children appeared to exhibit activity over a wider posterior region than adults (although neither children nor adults exhibited lateralisation), suggesting that peak frequencies vary across development as well as the extent of regional recruitment.

In the sensorimotor network, beta oscillatory changes typically precede movement onset and follow movement termination. Pre-movement event related desynchronisation (ERD) occurs from several hundred milliseconds prior to

movement onset and continues into the movement, and post-movement event related synchronisation (ERS), also known as the post-movement beta rebound (PMBR), occurs for several hundred milliseconds following movement termination (Wilson, et al., 2010). Wilson and colleagues used 248-channel MEG in a simple finger movement task to study these characteristic motor responses in typically developing children and adolescents (range 8-15 years, mean = 11.3). They were able to localise the oscillatory features and found similarities between their sample and previous reports in adults. They first confirmed the presence of spatially and temporally similar pre-movement ERD and PMBR (15-30 Hz) in children and adolescents when compared to adults, but also reported high frequency gamma ERS (74-86 Hz) which was localised to the sensorimotor network far more widely than previously reported in adults (Cheyne, Bells, Ferrari, Gaetz, & Bostan, 2008).

Gaetz *et al.* (2010) also used 151-channel MEG with a simple finger movement task in slightly younger children (aged 4-6), adolescents (aged 11-13) and adults (aged 24-42). With this slightly wider age range, they were able to demonstrate that PMBR was weakest in young children and whilst it was present and more prominent in the adolescents, PMBR was still weaker in adolescents than in adults. Both Wilson *et al.* (2010) and Gaetz *et al.* (2010) reported correlations between age and oscillatory power in the sensorimotor network, suggesting that the mechanisms by which FC may be mediated develop with age.

Similar to the wide recruitment of the visual network in children, Wilson *et al.* (2010) found that children and adolescents appeared to recruit additional regions not typically seen recruited in adults, such as the cerebellar cortices. Within additionally recruited regions however, they found that the youngest children exhibited the strongest beta ERD and that this weakened with age, suggesting that neural activity within these perhaps less functionally relevant regions declines with age. High gamma ERS localised to the contralateral SMA also correlated negatively with age, demonstrating that this activity too, likely dissipates in adolescence. Furthermore, Wilson and colleagues reported that the ipsilateral primary sensorimotor cortex played more of a role in the responses recorded in children, and that again this appeared to weaken with age giving way to lateralisation of function.

Erberich *et al.* (2006) also reported fMRI findings that unilateral motor responses in infants produce similar activity in both the contralateral and ipsilateral primary

sensorimotor cortex, consistent with the notion that infants do not exhibit lateralisation of function but also indicating that the sensorimotor network is well connected in children. Further work with MEG however, was able to exploit the excellent temporal resolution afforded by the technique and Nevalainen *et al.* (2008) were in fact able to show a time lapse between the contralateral and ipsilateral responses of the primary sensorimotor cortex, showing that the ipsilateral neural response had a longer latency than the contra-lateral neural response. The findings indicate that perhaps the ipsilateral neural response exhibited in infants is not a consequence of direct stimulation from thalamo-cortical pathways, but rather that the response is a consequence of corpus callosal activity (Nevalainen, Lauronen, & Pihko, 2014).

The DAN has also previously been identified in new-born infants with some success but is found in a more fragmented state than the visual and sensorimotor networks in children of this age (Doria, et al., 2010; Fransson, et al., 2009; Fransson, et al., 2007). Structurally, regions of the DAN are known to mature later than visual and sensorimotor regions (Gogtay, et al., 2004; Huttenlocher, 1990; Taylor, et al., 2012). Previous fMRI research has indicated that the left and right DAN may develop differently; de Bie *et al.* (2012) found they could identify only the left DAN and not the right in resting state analysis of children aged between 5-8 years. They also found additional regions were recruited into the network in children, consistent with sensorimotor and visual network recruitment in children.

Uhlhaas *et al.* (2009b) demonstrated age related task-induced oscillatory changes across DAN like regions, although given this was a 62-electrode EEG investigation spatial localisation is coarse. They nonetheless recorded EEG from 6-21 year olds whilst completing a Gestalt perception task ('Mooney faces', please see Uhlhaas *et al.* (2009b) for more detailed description) and primarily reported gamma and theta task-induced oscillatory changes over parietal and frontal electrodes in all age groups. Gamma power was maximal over parietal electrodes and greater in the face condition relative to the no face condition whilst theta power was maximal over frontal electrodes and also greater in the face condition relative to the no face condition.

They furthermore reported age-related increases in gamma and beta power with maximal differences between groups found over parietal electrodes, and a

particularly pronounced difference between adolescents and adults given a transitory decrease in late adolescence. They reported age-related increases in theta power averaged across all electrodes (which remained significant when averaged across frontal electrodes only), and no age-related differences in alpha power. They concluded that a general enhancement of long range synchronous activity in theta, beta and gamma frequency ranges occurs across development and suggest that their results indicate that FC development extends well into late adolescence in this network. They propose that late adolescence marks a time of, "heightened vulnerability of the developmental processes".

There are tentative reports of the DMN also being identifiable with fMRI but existing in a fragmented, precursive state in new-born infants (Doria, et al., 2010; Fransson, et al., 2009; Fransson, et al., 2007; Smyser, et al., 2010). With regards to functional development of the DMN, Fair *et al.* (2008; 2009) have reported reduced connectivity between nodes in children when compared with adults, suggesting that FC within the DMN strengthens with age. Thomason *et al.* (2008) reported that in an fMRI study of typically developing children aged 7-12 years old, both working memory task-induced deactivations and resting state activations of DMN regions were found. They therefore propose that whilst the network may not be *fully* formed in children of this age, the network is emergent and showing functionally relevant activity in this age range. In agreement with previous studies (for example, McKiernan *et al.* (2003)), Thomason *et al.* (2008) also reported that task induced deactivations of DMN regions increased with increasing task demands (in this case, working memory load). The DMN has also been found to be fragmented in children aged 5-8 years, split into several sub-systems, compared to adults where it is usually found to be a single coherent network (de Bie, et al., 2012).

Less is known about the development of the salience network compared to other functional networks as it has not been the focus of many studies of this kind. However, de Bie *et al.* (2012) also found the salience network to be fragmented in children whereas we would expect to find the salience network robustly defined in adults indicating immature connectivity within this network in children, similar to what has been found with respect to the DAN and DMN. Fair *et al.* (2007) also used resting state fMRI to look at FC measured by pairwise temporal BOLD correlations across 39 pre-defined ROIs in children (aged 7-9 years, mean = 8.6 years),

adolescents (aged 10-15 years, mean = 11.9 years) and adults (aged 20-31 years, mean = 24.1 years). They demonstrated differences between the strengths of different types of connections (short- and long-range) within two networks (the frontoparietal network also known as the DAN, and the cingulo-opercular network also known as the salience network) analysed in children and adults. In particular they appeared to show that longer range connections increased in strength from childhood to adulthood (consistent with the conclusions drawn by Uhlhaas *et al.* (2009b)) and shorter range connections decreased in strength from childhood to adulthood. They therefore concluded that both segregation (weakening) of short-range functional connections and integration (strengthening) of long-range functional connections contribute to the development of functional brain networks, this hypothesis will be termed the 'local to global hypothesis' for future reference.

Fair and colleagues later used resting state fMRI to look specifically at FC development in the DMN in a sample of children aged 7-9 years and adults aged 21-31 years (Fair, et al., 2008). In this study they used a voxel-wise whole-brain seed based correlation approach to produce FC maps for children and adults for specific pre-defined ROIs in the DMN. They found that, rather than supporting their original local to global hypothesis, there were very few short-range connections present in children and some short-range connections even increased in strength across the age range studied, such as the between the anterior and ventral mPFC. They did however report overall increases in FC within the DMN with increasing age showing that the network develops with age.

Fair *et al.* (2009) followed this work with further graph analysis of the same data presented in 2007 and 2008. They demonstrated that *overall* in children, local anatomically driven connections were prominent whereas by adulthood functional networks had emerged and integrated. They therefore suggested, supporting their local to global hypothesis that functional networks emerge over the course of development via integration of functional network connectivity and segregation of local, anatomically driven connectivity.

However, their work has recently come under criticism after it has emerged that movement artefact in fMRI "increases short-distance correlations and decreases long-distance correlations" (Power, Barnes, Snyder, Schlaggar, & Petersen, 2012), which unfortunately encompasses the main findings presented by Fair *et al.* (2009)

and therefore weakens their conclusions about functional brain development. The findings presented by Power *et al.* (2012) thus raise questions as to whether the findings presented by Fair *et al.* (2007) were truly age-related or whether they are an artefact of confounded movement in younger children. Since various groups have been able to demonstrate that resting state functional brain networks are identifiable in infants (for example, Doria *et al.* (2010)), indicating that long range connectivity exists in children from birth, this provides an additional reason to question the local to global hypothesis proposed by Fair *et al.* (2007). In the current study it will be of significance to consider the findings in relation to the local to global hypothesis, given that it is difficult to now tell whether the findings presented by Fair *et al.* (2007) were truly age-related.

Bringing together ideas of structural and FC development, Whitford *et al.* (2007) conducted an extensive developmental study of 138 subjects aged 10-29 years old. They used 28-electrode resting EEG in concurrence with structural MRI to explore how functional changes present in EEG map onto structural changes present in MRI. They found that both grey matter volume in two regions (frontal and parietal lobes) and EEG power across 3 frequency bands (slow wave 0.5-7.5 Hz, alpha 8-12 Hz and beta 12.5-34.5 Hz) and 4 regions (frontal, temporal, occipital and parietal lobes) reduced with age. They additionally reported a correlation between grey matter volume and EEG power in corresponding regions, supporting their hypothesis that a relationship exists between EEG power and the number of active synapses in a given region. This is of potential relevance to the current study bearing in mind that grey matter thickness follows an inverted-U-shaped developmental trajectory, peaking in adolescence. The indication is that reduced grey matter volume corresponds with reduced EEG power, and increased grey matter volume corresponds with increased EEG power which may have a knock-on effect on FC measured by oscillatory power.

Additionally, Klimesch (1999) and Ward (2003) describe that within frequency bands, peak oscillatory frequencies changes with age. Specifically peak delta (1-4 Hz) and theta (4-8 Hz) decrease as age increases, and peak upper alpha (10-13 Hz, sometimes referred to as 'mu') increases as age increases. Since FC within known functional brain networks appears to peak in the alpha to beta band range (8-13 Hz to 13-30 Hz (Brookes, et al., 2011a; Hipp, et al., 2012)), whether or not this peak correlation

frequency develops with age will be of potential importance to note as it may indicate whether or not the mechanisms that mediate FC also develop across age.

Section 1.3. Cognitive changes over development

As shown by the evidence cited above, structural changes over development are accompanied by changes in FC as observed with fMRI BOLD correlations (both spontaneous and task-induced). Moreover, the limited electrophysiological literature also supports the notion that structural and functional changes occur alongside one another across development, continuing well into adolescence. The question remains how such changes relate to cognitive development and therefore in the sections that follow, a brief introduction to working memory and attention will be covered in addition to exploring current knowledge of their developmental trajectories. We have chosen to focus on these two specific cognitive functions because they are known to develop over the age range studied, and play key roles in everyday cognition. The impact of understanding their development would subsequently be widespread.

Section 1.3.1. Working Memory

Working memory refers to the active capacity to store and manipulate information over short periods of time. This can either be information extracted from long term memory or information just experienced (Curtis & D'Esposito, 2003). Behaviourally it can be thought of as comprising two processes: executive control and active maintenance of information (Cohen et al., 1997). It is an essential component of brain function required for everyday cognitive tasks such as remembering telephone numbers or names, writing a shopping list, travelling from one place to another, and plays a vital role in learning (Taylor, et al., 2012) as well as being linked to mathematical and reading abilities (G. J. Hitch, Towse, & Hutton, 2001). Hence it is a

cognitive domain of key significance to investigate over development – a time when a tremendous amount of learning occurs (Taylor, et al., 2012).

It has been suggested that although working memory components are in place by a young age (Alloway, Gathercole, & Pickering, 2006; Gathercole, Pickering, Ambridge, & Wearing, 2004), processing capabilities develop to utilise this function more efficiently over development (Crone & Ridderinkhof, 2011; Gathercole, et al., 2004; Kwon, Reiss, & Menon, 2002; Taylor, et al., 2012). Such improvements in processing capabilities could include increased rate of rehearsal or increased rate of memory 'scanning' (Gathercole, et al., 2004). In any case, improvements in working memory function have been shown to correlate with age (Rodriguez-Martinez, Barriga-Paulino, Rojas-Benjumea, & Gomez, 2013). Particularly since myelination of the frontal lobes continues into adulthood, it may be that the central executive component of working memory which facilitates orientation of attention and control, does not develop fully until later stages in development.

An early model of memory known as the 'multi-store model' that was proposed by Atkinson & Shiffrin (1968) showed how incoming information, the sensory register (where sensory information first enters memory), short-term memory storage (now a term used interchangeably with working memory) and long-term memory storage interacted. They proposed that incoming information would at first reach the sensory register and information that was attended to would transfer into short-term memory whilst information that was not attended to would be lost. From there, information could be lost through decay (forgetting) or if rehearsed appropriately in short-term memory, the information could be transferred into long-term memory. Whilst very simplistic, this model provided a useful way of conceptualising human memory and offered a starting point for models that followed.

Shortly after the multi-store model, Baddeley & Hitch (1974) focussed in on working memory. They developed the model over 20 years or so and now consider working memory to consist of four interactive components: the central executive, the phonological loop, the visuospatial sketchpad and the episodic buffer. These components form the most influential model of working memory and are thought to interact as modelled in Figure 1.12.

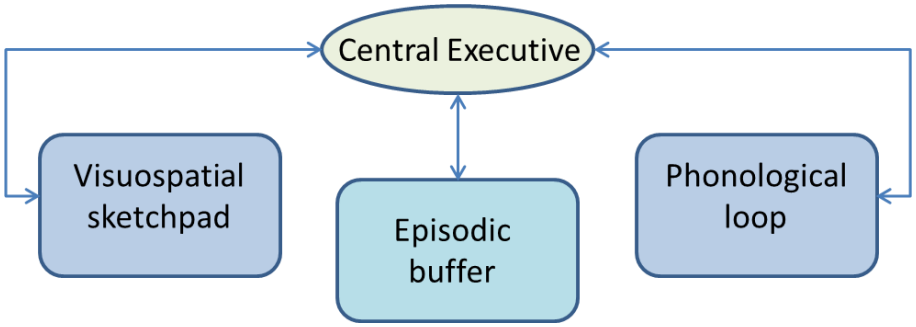


Figure 1.12: The Baddeley and Hitch (1974) model of working memory (adapted from Baddeley (2003)). The 'episodic buffer' is a more recent addition to the model.

The central executive is thought to control resources and direct attention, and is anatomically linked to the frontal lobes, specifically the PFC (Cohen, et al., 1997; D'Esposito et al., 1995). The phonological loop allows us to store phonological information and articulate the rehearsal of such information whether vocally or sub-vocally, and is anatomically linked to left-lateralised inferior parietal areas as well as anterior temporal and frontal areas such as Broca's area and the pre-motor cortex (Henson, Burgess, & Frith, 2000; Paulesu, Frith, & Frackowiak, 1993). Non-phonological information can be re-coded phonologically and rehearsed to counter-act decay of information. Without rehearsal, information decays rapidly. The visuospatial sketchpad allows us to store and manipulate visuospatial information and is anatomically linked to right-lateralised occipital and inferior frontal areas (E. E. Smith & Jonides, 1997).

Whilst influential, the main limitation of this model was that, similar to the multi-store model, it is simplistic. For instance, it does not account for how the serial order of items might be encoded and the functions of the central executive are very broadly described (Baddeley, 2003). In fact, Baddeley (2003) describes the central executive as more of a useful concept rather than a concrete component of the model. He instead describes that the central executive can be considered as defining rather than explaining the processes attributed to it, so there is still plenty to be explored and understood about how working memory information processing occurs in the human brain. By focussing on working memory exclusively, the model also unfortunately neglects the interaction between working memory and long-term memory. The episodic buffer, a more recent addition to the model, was added to

address this criticism. It was proposed that the episodic buffer allows us to bind together 'episodes' of information and integrate them into long-term memory – it is considered to act as a gating mechanism between short- and long-term memory systems (Baddeley, 2003).

More recently, other models of working memory have emerged, such as the slot based and shared resource models (Bays, Catalao & Husain, 2009). The slot based model proposed that 3-4 visual items could be stored into 'slots' (Luck & Vogel, 1997) and that for each visual item, multiple features could successfully be slotted together (Vogel, Woodman & Luck, 2001). This model failed to account for the precision with which items can be recalled from working memory, however, and furthermore failed to account for the role of attention within working memory processing.

The shared resource model thus arose from the concept that the more items there are to hold in working memory, the more variable recall becomes, showing reduced precision when the number of items to be stored is high (Bays, et al., 2009). It was proposed in the shared resource model that a single limited resource is shared among the items being held in working memory; when the number of items is high, there is low precision in recall of the items because the single resource is being shared among many items and vice versa (Bays & Husain, 2008). It was furthermore proposed in this model that the allocation of resource is biased by attention, accounting for how the resource may be distributed across the items to be held in working memory.

Linking functional brain networks to working memory,, it has been suggested that different functional brain networks support different types of working memory (E. E. Smith & Jonides, 1997; Thomason et al., 2009). In particular, a left lateralised verbal working memory circuit is thought to include the PPC (attributed to the phonological store), anterior speech regions including Broca's area, SMA (supplementary sensorimotor area) and pre-motor areas (attributed to phonological rehearsal or maintenance). Other groups have also reported left lateralisation in the context of verbal working memory tasks in both children and adults (Narayanan et al., 2005; Scherf, Sweeney, & Luna, 2006; Thomason, et al., 2009). Together these findings strongly suggest a more significant role of the left DAN compared to the right DAN in supporting verbal working memory function.

The pre-motor area has furthermore been noted for involvement in working memory tasks more recently (Kwon, et al., 2002; Narayanan, et al., 2005; Scherf, et al., 2006) and whilst the functional significance of this is unclear, differences in activation across development have been shown with children exhibiting less activation than adolescents or adults (Scherf, et al., 2006). These findings indicate that task-dependent activity may not be limited to the DAN and could in fact extend into the sensorimotor network but the functional significance of sensorimotor involvement will need to be explored carefully, considering the possibility that involvement is limited to motor response execution.

Kwon *et al.* (2002) found age to be a significant predictor of activity in a frontoparietal network not dissimilar from the DAN during a visuospatial working memory task in fMRI. They believe their findings suggest that left lateralised phonological processing areas and right lateralised visuospatial processing areas mature hand in hand to support improvements in working memory processing. In particular, the brain regions they saw become increasingly active across age included the left and right DLPFC, left vlPFC (ventrolateral prefrontal cortex), left PMC (premotor cortex), and left and right PPC. Whilst the PPC has been implicated itself as playing a role in supporting working memory function, communication between parietal cortex and PFC has also long been recognised to play a role in supporting working memory function (Cohen, et al., 1997; Jolles, Kleibeuker, Rombouts, & Crone, 2011; Narayanan, et al., 2005; Sarnthein, Petsche, Rappelsberger, Shaw, & von Stein, 1998). Scherf *et al.* (2006) additionally showed differences in recruitment of parietal areas between children, adolescents and adults.

The PFC is one of the last brain regions to reach maturity (Huttenlocher, 1990; Huttenlocher & Dabholkar, 1997) and is heavily implicated in supporting higher cognitive function. It has previously been reported that in particular the DLPFC has a key role to play in supporting working memory function (Cohen, et al., 1997), but that children fail to recruit this area to the extent of adolescents and adults despite all groups showing spatially similar recruitment of brain regions (Scherf, et al., 2006). Taken in conjunction with the developmental trajectory of myelin growth and synaptic pruning in the PFC (Crone & Ridderinkhof, 2011) it might be conceived that weaker activity would be exhibited in the younger participants. Since synaptic pruning in the PFC occurs over the course of adolescence we may expect to see

further changes in FC in adolescents that reflect this dynamic process. This may be apparent in decreases in FC between areas, as connections are refined and pruned.

In an fMRI study of visuospatial working memory, Scherf *et al.* (2006) found that prefrontal and posterior parietal areas of the brain formed a core network (quite possibly equivalent to the DAN) which was activated in children, adolescents and adults, albeit to different extents. This core network was differentially activated in younger volunteers with both increases and decreases in regional activity identified. Younger volunteers additionally failed to activate regions that adults and adolescents did, such as the left DLPFC. In another fMRI study of working memory the DLPFC was found to show increasing activation with age (Taylor, et al., 2012). Significant differences were also noted between the recruitment of frontal regions in children with autism spectrum disorders and typically developing children (ages 7-14). This could reflect decreased efficiency of underlying neural processes required to support working memory processing in children with developmental problems.

Curtis & D'Esposito (2003) further emphasize the importance of the DLPFC in working memory processing and suggest that it may play a role in directing attention to internal representations during working memory maintenance. They also suggest that these internal representations may be accessed through posterior areas, suggesting a need for FC between prefrontal and posterior areas perhaps reflecting the importance of the DAN in supporting working memory function.

Section 1.3.2. Attention

"Everyone knows what attention is… the taking possession by the mind… of one out of what seem several simultaneously possible objects or trains of thought… It implies withdrawal from some things in order to deal effectively with others" (James, 1890). This quote, whilst put to paper over a century ago still accurately summarises attention as we consider it today. Parasuraman (2000) put this into more detail, describing attention as a name given to a multitude of processes that flexibly interact with one another as well as with other cognitive processes to support cognitive, sensorimotor and perceptual functions.

Models of attention have evolved since the 1980s when the spotlight and zoom lens models were proposed. The spotlight model first proposed by Posner, Snyder & Davidson (1980) described how attention has a focus which may vary in size and that attention is enhanced for items that fall within the spotlight focus. They suggested orienting as a key component of attention whereby the spatial location of the attention spotlight is selected (although it is somewhat unclear how this model relates to attending to information presented solely in other sensory modalities) and detecting as the coming together of the attention system and the input.

Given the spotlight model was rather simplistic, the zoom lens model was subsequently generated by Eriksen & St. James (1986) which added detail to the spotlight model. They proposed that at a low zoom, a wider field of view would be achieved (relating in reality to increased magnitude but decreased detail attended to) but at a high zoom, a narrower field of view would be achieved (relating in reality to decreased magnitude but increased detail attended to). The low zoom lens represents a similar distribution of attention processing resources to the entire visual field, whereas the high zoom lens represents allocation of attention processing resources to a particular item within the visual field.

Whilst the zoom lens model provided more detail, it still did not give a complete model of attention and both the spotlight and zoom lens models were heavily based on visual attention, lacking detail pertaining to other sensory modalities. More recently, a salience model has been put forward by Corbetta & Shulman (2002) where both prior information and the salience of a stimulus influences the allocation of attention processing resources, accounting for differences in top-down (i.e. cognitive selection) and bottom-up (i.e. salience based selection) processing. It is easier to apply this current model to different sensory modalities. Attention can now therefore be described as involving two basic forms of processing: automatic/passive processing (bottom-up) and controlled/voluntary processing (top-down (Katsuki & Constantinidis, 2013)). Top-down processing for instance allows us to reallocate information processing resources to selected attended items whilst suppressing our attention to irrelevant items – this is a vital process in handling our brain's processing limits as it would be impossible to attend to and process every stimulus in our environment all of the time (Katsuki & Constantinidis, 2013). Bottom-up processing ensures that our attention is drawn automatically to salient stimuli in our

environment for further processing (Corbetta & Shulman, 2002; Katsuki & Constantinidis, 2013).

In the 1990s, Posner & Petersen also proposed a model of attention processing which had more of a basis in functional brain networks. They hypothesised that the attention system was a distinct system which could interact with other systems, that it relied on a network of brain regions rather a single brain region acting in isolation, and that regions involved in the attention network could support different functions (Posner & Petersen, 1990). They considered these different functions to include orienting (foveating a stimulus), detecting (resolving conflict among responses; this function has more recently been referred to as executive attention (Petersen & Posner, 2012)) and alerting (sustaining a state of alertness to process high priority stimuli). They hypothesised that orienting was supported by the visual attention system (the visual cortex) and frontal parietal regions, detecting by an anterior attention system (in particular the mPFC and adjacent ACC), and alerting by an arousal system (based around projections from the locus coeruleus and right frontal/parietal brain regions (Petersen & Posner, 2012)).

They subsequently developed the Attention Network Task (ANT (Fan, et al., 2002)) to tap into the different networks and functions they proposed existed, assuming these functions are distinguishable. The ANT is based on a flanker task (B. A. Eriksen & Eriksen, 1974) however it incorporates cues to guide attention in different ways prior to each trial. These cues consist of: central (the cue is presented centred on the screen, so therefore does not divulge information about the whereabouts of the stimulus but gives notice that a stimulus will be presented shortly), double (two cues are given, one above and one below a central cross hair where the stimulus could appear in either location, so similar to the central cue this only gives information that a stimulus will be presented shortly), spatial (one cue is given either above or below which gives spatial information about where the stimulus will appear and also information that a stimulus will appear shortly) or no cue at all.

In the ANT, alerting is accessed by subtracting the mean RT of the double cue condition trials from the mean RT of the no cue condition trials. Orienting is accessed by subtracting mean RT for spatial cue condition trials from the mean RT for centre cue trials. Executive control is accessed by subtracting the mean RT for all congruent trials (summed across the different cue types) from the mean RT for all incongruent

trials. One issue here is that there is mixing of trials which supposedly tap into the executive system (congruent vs. incongruent trials) and trials which supposedly tap into the orienting or alerting systems (spatial cue vs. centre cue trials and double cue vs. no cue trials, respectively). Additionally, the task is built on the assumption that these functions can be isolated (Redick & Engle, 2006) and the main reason given for this approach is that scores on the three domains do not correlate (Fan, et al., 2002) however, Redick & Engle (2006) point out that this may actually be due to the unreliability of the measures.

Regardless of task however, attentional control develops across childhood and adolescence, and can usually be identified by quicker RTs and improved accuracy with age (Bunge, Dudukovic, Thomason, Vaidya, & Gabrieli, 2002; Konrad et al., 2005; Rueda et al., 2004). Fair *et al.* (2007) proposed that adult attentional control relies on DAN (also implied by (Petersen & Posner, 2012)) and salience network activity, and although they studied these networks in the absence of a goal-directed task they were able to show that both of these networks emerge and strengthen over the course of development. In particular, they reported that in children the dACC (commonly noted as a component of the salience network in adults) was in fact more strongly connected to the DAN, but that this connection gave way over the course of development and the dACC was incorporated into the salience network by adulthood. Greater activation in the left insula in children and right insula in adults (both well-known nodes of the salience network) has also been shown to correlate with improved performance on a flanker task (Bunge, et al., 2002).

In an fMRI study using a modified flanker task to study a group of children (aged 8-12 years) and adults (aged 19-33 years), Bunge *et al.* (2002) reported that the right lateralised vlPFC was significantly more active in adults however the left vlPFC was significantly more active in children (and to an equal extent of the right vlPFC in adults), supporting the theory of development of lateralisation over time. They additionally reported that in children the inferior parietal lobule was more active than in adults. The PPC has also been linked particularly to selective attention processing (Booth et al., 2004; Katsuki & Constantinidis, 2013). In an fMRI study of 9-11 year old children, lower accuracy on a selective attention task measured by false alarms and missed targets was associated with greater activation in the PPC

(specifically the superior parietal lobule) in addition to the right motor cortex (Booth, et al., 2004).

The DLPFC has also been linked to executive control of attention (Konrad, et al., 2005). In an fMRI study of children (aged 8-12 years) and adults (aged 20-34 years), children exhibited significantly reduced activity in the DLPFC in relation to executive control of attention elicited during the ANT and recruited additional regions outside of those recruited in adults (Konrad, et al., 2005). Functional group differences were found in conjunction with structural group differences in grey matter in the frontal, parietal and temporal lobes where adults exhibited decreased volume compared to children (Konrad, et al., 2005). Konrad and colleagues reported reduced activation of key areas (the right inferior frontal gyrus and left superior parietal cortex) in children compared with adults, and therefore suggest that functional yet immature frontoparietal networks exist in children. Furthermore, Konrad *et al.* (2005) reported more lateralisation in adults compared to children, similar to Bunge *et al.* (2002).

Section 1.4. Aims and hypotheses

The foregoing survey of the research literature on the developmental trajectory of FC and of the emergence of brain networks reveals that a great deal is now known about brain networks as delineated by BOLD, the oscillatory patterns as delineated by electrophysiological studies, and the development of structural connectivity. However, there are many open questions regarding the development of FC. We hope that through studying the relationship between different aspects of cognition and neural development we may aid clarification of the roles of functional brain networks. We have chosen to focus our work in particular on working memory and selective attention, as we know these functions develop during mid to late childhood and adolescence so we are likely to see differences in ability and neural activity in our intended sample of volunteers aged 9-25. These functions are also likely to stimulate activity in brain networks of interest because of their reliance on integration of several brain regions.

Given the relatively early maturation of sensorimotor areas of the brain we can expect to find little difference between the activity of the visual and sensorimotor networks between children, adolescents and adults. Contrastingly, given the relatively late maturation of frontal areas of the brain that support higher-order cognition we can expect to find differential activity in the DAN in children, adolescents and adults. We will be using MEG to measure and compare the connectivity between regions of the functional brain networks delineated using amplitude-amplitude correlations in MEG both during rest and whilst participants complete tasks that tap into cognitive functions we know to change in the age range 9-25.

In addition to the currently minimal pool of research addressing this area, inferences drawn from existing literature reporting developmental findings of resting-state BOLD may be confounded by movement artefact. The question remains as to whether the local to global pattern of development reported by Fair *et al*. (2007; 2009) in which the characteristic fMRI networks found in adults emerge from a pattern of BOLD correlation dominated by local correlations in childhood is accurate or whether it simply reflects greater movement-induced local correlations in younger participants. EEG, the most commonly used electrophysiological measure, has poor spatial resolution; MEG, in contrast, has sufficiently great spatial resolution to allow voxel-space correlations to be performed, and indeed, this ability has allowed us to confirm that the BOLD network patterns can also be observed using amplitude-amplitude correlations in MEG data (Brookes, et al., 2011a). MEG therefore offers an ideal opportunity to study network development, as movement artefacts can be readily identified and removed from the data. The precise aims and hypotheses of this thesis are outlined below.

The aims of this exploratory study were to:

1) Delineate age-related changes in FC at rest and during goal-directed cognitive tasks defined as amplitude-amplitude correlations using MEG;
2) Create a rich data source for future developmental analyses.

The hypotheses pertaining to FC in this thesis were:

1) FC would vary by frequency, age group and task;
2) Interhemispheric pairs of network regions would show different patterns of development. In particular, the visual and sensorimotor network region pairs were expected to undergo less development than higher-order network region pairs belonging to the DAN, given that visual and sensorimotor areas of the brain are thought to reach structural maturity prior to higher-order association areas (Gogtay, et al., 2004);
3) Pairs of network regions within the DAN would show different FC developmental trajectories. In particular, previous studies have highlighted the possibility that the left and right DAN may develop differently (de Bie, et al., 2012);
4) Interhemispheric pairs of network regions would show different FC developmental trajectories compared to intrahemispheric pairs of network regions. In particular, we might expect to see that intrahemispheric connectivity is strongest in late adolescents/young adults given the development of hemispheric specialisation (Szaflarski, et al., 2006) and reportedly strong interhemispheric connectivity between homologous cortical areas in infants (Fransson, et al., 2007).

Chapter 2. Magnetoencephalography for developmental studies

This chapter outlines the principles of magnetoencephalography and procedural considerations when conducting an MEG study.

Section 2.1. Why use magnetoencephalography?

The research tools available for measuring functional brain connectivity non-invasively include indirect or direct measures of regional cerebral blood flow (fMRI or O15 PET), and electrophysiological measures such as EEG and MEG. ECoG (electrocorticography) is an ideal but invasive electrophysiological measure that in humans can only be done if there are clinical reasons for applying electrodes directly to the cortex.

The relative spatial and temporal resolutions afforded by each of these techniques are summarised in Figure 2.1 and comparison with the invasive technique ECoG can be seen.

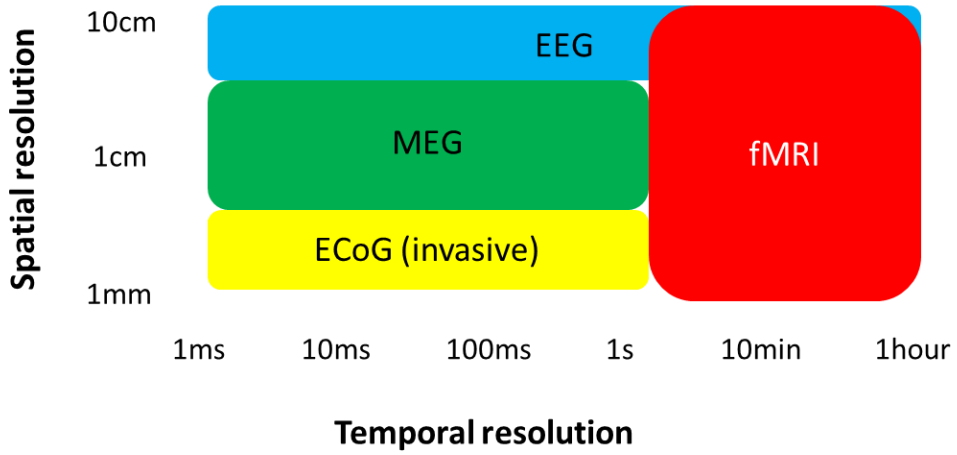


Figure 2.1: The relative spatial and temporal resolutions obtainable non-invasively with EEG, MEG, fMRI and invasively with ECoG (adapted from Huettel et al. (2004)).

FMRI based techniques offer an indirect measure of neural activity based upon BOLD responses, or haemodynamic responses (HRs), which are believed to follow a neural response, albeit in a non-linear fashion. Unfortunately, despite the excellent spatial resolution on the order of millimetres achieved with fMRI, the indirect nature of this measure means that its temporal resolution is poor, on the order of seconds, as the BOLD signal lags the neural response, and is smeared out over several seconds. In contrast, the temporal resolution of neural mechanisms underlying the response is on the order of milliseconds. FMRI is additionally confounded by the issue that the HR appears differently across ages (Richter & Richter, 2003), which presents additional challenges for developmental studies using fMRI.

Although MEG and EEG cannot currently reach the same standards of spatial resolution as fMRI, they provide far superior temporal resolution by allowing direct measures of electrical neural activity (all electric currents generate a magnetic field). Fortunately, work with primates demonstrating that LFPs (which reflect summated PSPs) correlate with the BOLD signal (Logothetis, Pauls, Augath, Trinath, & Oeltermann, 2001), suggests that the BOLD response measured in fMRI is related to underlying electrophysiological activity (as with MEG and EEG). Moreover, recent work demonstrates a close match between spatial ICA maps derived from both fMRI and MEG data (Brookes, et al., 2011a; Brookes et al., 2011b). This demonstrates that although the spatial resolutions afforded by MEG and EEG are inferior to fMRI, similar information about spatial distribution of neural activity can obtained through these techniques.

There are additionally several advantages of the use of MEG over EEG. EEG systems tend to consist of 32, 64 or 128 recording electrodes whereas the CTF Omega 2000 MEG system (VSM MedTech, Coquitlam, British Columbia, Canada) in use at The University of Nottingham, has 275 recording channels. Additionally, the electrical fields recorded using EEG are distorted by the high electrical resistance of the skull. Therefore localisation of neural sources may be inaccurate with EEG, due to the inadequate modelling of these conductivities over the head. MEG avoids this confound partly because of a higher density of sensors as mentioned above, but additionally because the magnetic fields recorded using MEG are less distorted because the relative magnetic permeability of brain, skull and scalp are all

approximately equal. More accurate localisation is therefore possible with the right analysis.

MEG therefore appears to offer the best approach for elucidation of temporal mechanisms, and to a certain extent the spatial organisation, of functional brain networks. However, it is vital that researchers using MEG are aware that measurements are sensitive to magnetic field spread, meaning that a single neural source can affect multiple sensors, which can cause spurious functional brain connectivity. This issue will be covered in more depth in analysis and discussion of results.

Section 2.2. MEG hardware

The 275 channels of the CTF Omega 2000 MEG system (VSM MedTech, Coquitlam, British Columbia, Canada; in which all data contributing to this thesis was acquired) contain gradiometer 'pick-up coils' located in the helmet of the MEG system providing whole-head coverage, and SQUIDs (superconducting quantum interference devices) located in the body of the MEG system, as depicted in Figure 2.2.

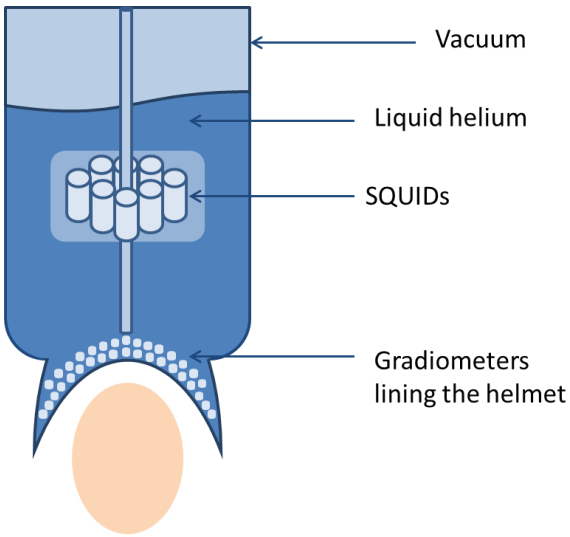


Figure 2.2: A Schematic of the 275 channel CTF Omega 2000 MEG system (VSM MedTech, Coquitlam, British Columbia, Canada; adapted from Brookes & Singh (2013)).

Section 2.2.1. SQUIDS and gradiometers

SQUIDs are superconducting magnetometer devices sensitive enough to measure the extremely small changes in magnetic field on the femtotesla scale (10^{-15} T or 1fT) outside the head (Brookes & Singh, 2013; Hämäläinen, et al., 1993). The DC (direct current) SQUIDs used in MEG systems comprise a loop of superconducting wire interrupted by two 'Josephson junctions' (see Figure 2.3; (Josephson, 1962)).

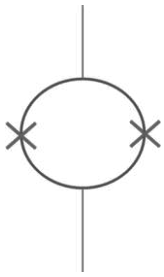


Figure 2.3: A DC SQUID consisting of a loop of superconducting wire interrupted by two Josephson junctions, denoted by X (adapted from Hämäläinen et al. (1993)).

SQUIDs must be kept at extremely low temperatures in order to preserve their superconducting properties (i.e. they need to retain zero resistance) and must therefore be kept in liquid helium at a temperature of ~-270°C (Brookes & Singh, 2013; Hämäläinen, et al., 1993). The SQUIDs and gradiometers housed in the MEG system are coupled so as to produce a voltage output proportional to the current that flows through the pick-up coil (Hämäläinen, et al., 1993). This is achieved through a feedback loop whereby the neuromagnetic field generates a current in the pick-up coil of the gradiometer, which is coupled to the SQUID. The current that is fed to the SQUID from the pick-up coil causes a voltage drop across the Josephson junctions and a feedback current is then applied to counteract this voltage drop. Finally, the magnitude of the feedback current is output and this set-up is demonstrated in Figure 2.4. Gradiometers will be explored in more detail in the following section.

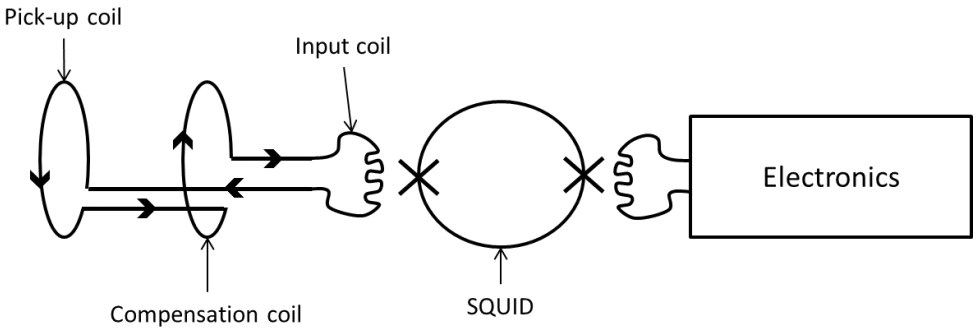


Figure 2.4: A coupled gradiometer → SQUID set-up (adapted from Orrison et al. (1995)).

Since neuronally generated magnetic fields are much smaller than those generated by environmental and biomagnetic noise (Vrba & Robinson, 2001) as detailed in Figure 2.5, ways of minimising the interference of environmental noise must be employed.

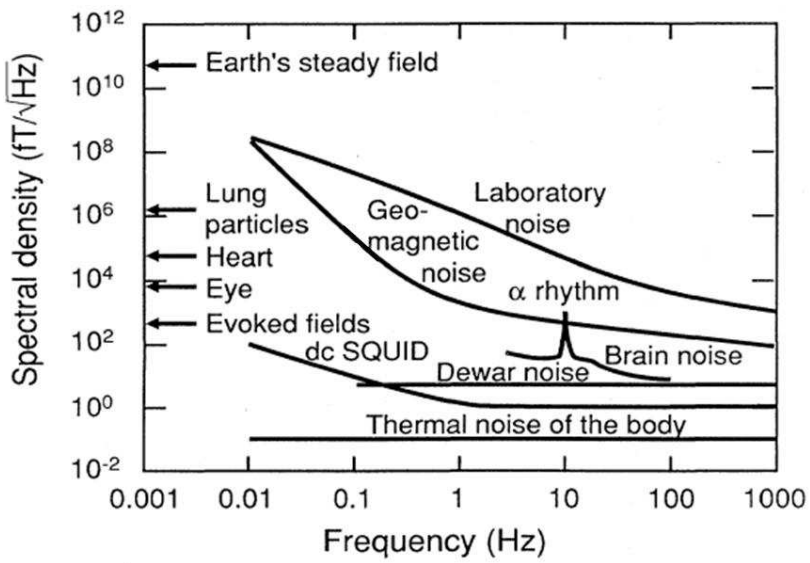


Figure 2.5: The spectral densities and peak amplitudes (indicated by arrows) of environmental noise and biomagnetic sources (adapted from Hämäläinen et al. (1993)).

Gradiometers such as the one depicted in Figure 2.6B are used to reduce interference, whilst the simplest magnetometer form is shown in Figure 2.6A. In the axial first-order gradiometer, the induced current through the compensation coil opposes that induced in the pick-up coil, so the output is reflective of the difference in field at the two loops. The distance between pick-up coils in the system used here is approximately 2.2cm between sensors, with a 1.8cm diameter for each coil and they are placed 1.7cm from the surface of the helmet. The distance between the compensation and pick-up coils is termed the baseline, and is considered a compromise between the capacity to reject external noise and remain sensitive to deep brain sources (Hale, 2012). Bigler (1996) notes that a noise source with uniform magnetic field strength passing through the pick-up and compensation coils at an optimum baseline will result a zero net current due to equal current flows being generated in opposite directions around the coils, however, if the baseline is too small some of the signal of interest may be cancelled out as noise and if the baseline is too large some external noise may not be cancelled out (Bigler, 1996). The baseline used in the CTF MEG system is 5cm.

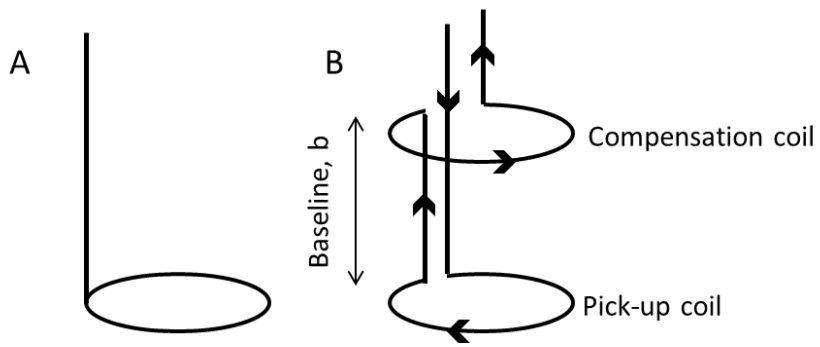


Figure 2.6: (A) A magnetometer (B) An axial first-order gradiometer (adapted from Orrison et al. (1995)).

Gradiometers provide effective noise reduction by the principal that magnetic fields originating from the brain will reduce substantially short distances away from the head and will be measured differentially across the gradiometer pick-up and compensation coils (the magnetic fields originating from synchronous PSP activity in the brain reduce at a rate of $1/r^2$ as previously described). Conversely, magnetic

fields originating further away will be measured almost constant across the gradiometer pick-up and compensation coils and therefore the measured current between the coils will cancel out, effectively cancelling out the interference of the noise on the data recording (Hale, 2012). This principal is illustrated in Figure 2.7 for an axial first-order gradiometer.

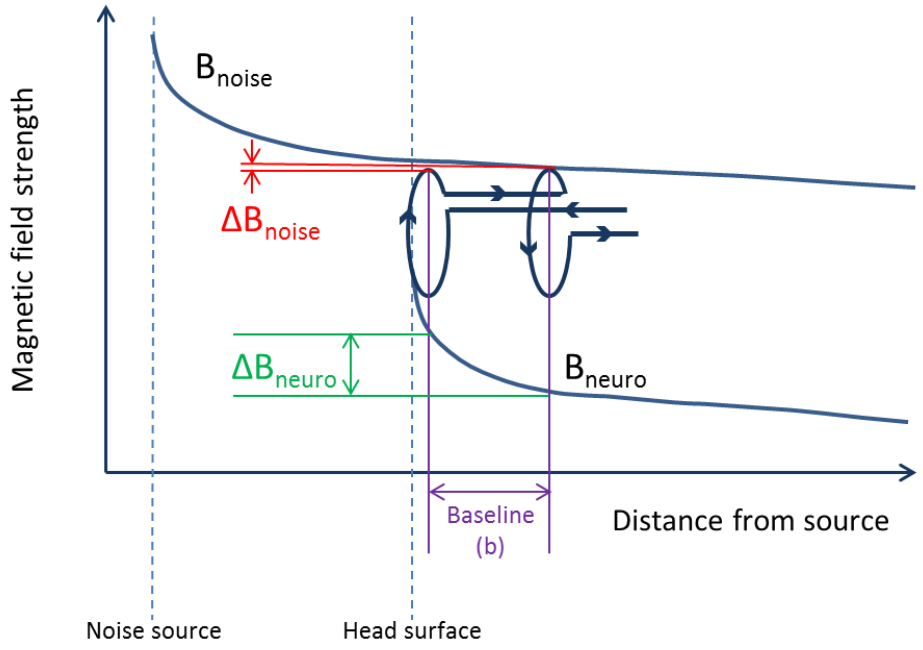


Figure 2.7: Illustrates the differential magnetic fields of neural and noise sources measured across the pick-up and compensation coils of an axial first-order gradiometer (adapted from Hale (2012)).

Higher order gradiometers can be constructed by introducing further compensation coils, however this also increases the physical space each takes up and therefore hardwired third-order gradiometers cannot be incorporated effectively into a whole head MEG system. The CTF MEG system therefore uses hardwired first-order gradiometers and further noise minimisation is facilitated by the use of a synthetic third-order gradiometer set-up, which allows for electronic cancellation of noise in real-time (Hämäläinen, et al., 1993). The synthetic third-order gradiometer system utilises a reference array consisting of 29 sensors placed 35 cm away from the head in the body of the MEG system. The positioning ensures that whilst the signal of interest originating from the head will not be picked up, any external sources of

noise that will also be picked up by the pick-up coils in the sensor array of the helmet will be picked up at this reference array. In this way, the noise interference detected at the reference array can be subtracted from the data and the effects of noise minimised (Hale, 2012).

Section 2.2.2. Practical MEG data acquisition

The MEG system is held in a magnetically shielded room (MSR) to minimise the effects of interference from environmental noise. The MSR is made of mu-metal (nickel and iron) and aluminium, and the model used at the Sir Peter Mansfield Magnetic Resonance Centre (SPMMRC) is AK3b (Vaccumschmelze Hanau, Germany). Volunteers are additionally required to remove all metal from their person before MEG scans so as not to introduce interference inside the MSR. Any metal may be magnetised, and thus produce artefacts in the recording, especially if they move, so items such as bras and jewellery which move with the volunteer could potentially introduce a large amount of noise if not removed prior to the scan.

The electronics rack controlling the SQUIDs, projector, stimulus/acquisition computers, camera viewing screen and speaker/microphone for subject communication are housed outside the MSR to minimise the noise interference during data acquisition. Images can be projected into the MSR from the stimulus computer via mirrors and a ceiling projection screen. Refer to Figure 2.8 for a photograph of the CTF MEG system within the MSR at the SPMMRC at The University of Nottingham and a schematic of the MEG and MSR set up.

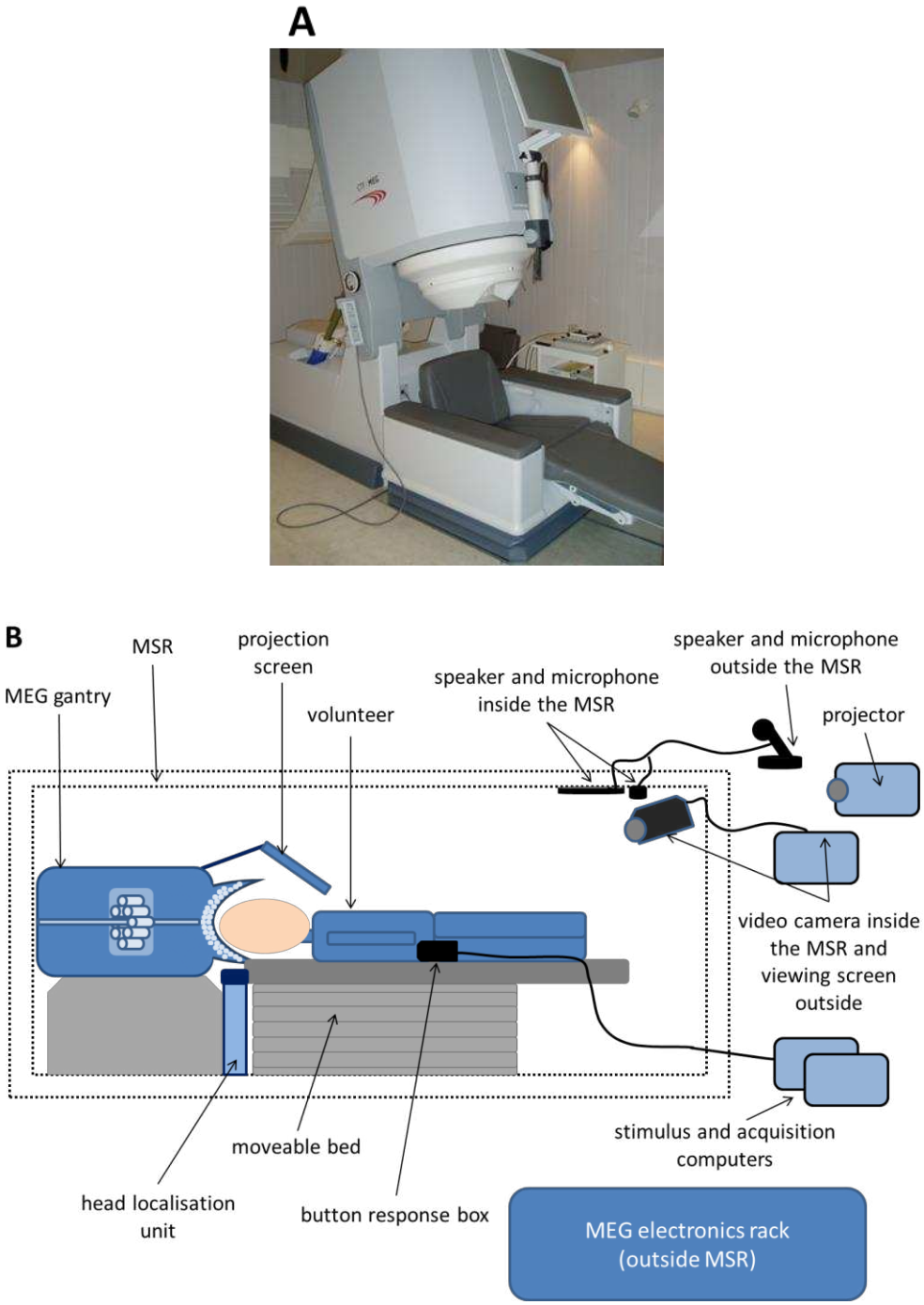


Figure 2.8: (A) The CTF MEG system at the Sir Peter Mansfield Magnetic Resonance Centre (The University of Nottingham) (B) A schematic of the CTF MEG system and MSR set up during data acquisition (not to scale).

Unfortunately, due to the 'one size fits all' helmet of the MEG system, not all head sizes and shapes fit inside the scanner, and for most there is an unavoidable gap

between the head and the helmet which leaves room for movement during data acquisition. This is a particularly important factor to consider when scanning children because we might expect a child to fidget more than an adult and to have a smaller head than an adult. To minimise movement, a combination of verbal encouragement and head padding has been used in this study. Additionally, the option of using a custom made fibre glass insert for the helmet which reduces the size of the helmet was available for this study (see Figure 2.9 for a photograph of the model used in this study).

Figure 2.9: The fibre glass insert created to line the MEG helmet.

Section 2.2.3. Head localisation

So that we can locate the head before/after and during data acquisition and therefore obtain and log information about head movement, a head localisation unit is employed. We are also able to exclude datasets where necessary, rejecting datasets where head movement has exceeded a set threshold. This system, in conjunction with the Polhemus 3SPACE™ FASTRAK® (Polhemus, Colchester, Vermont, USA, http://polhemus.com) with which we create 3D digitised head shapes

for each individual, allows us to coregister MEG sensor geometry with individual MRI scans. A customised Matlab script which performs a least squares fit between the anatomical MRI head shape and the 3D digitised head shape obtained using the Polhemus allows us to coregister MEG data with brain anatomy.

In order to use the head localisation unit, three coils are affixed to the head of each volunteer at the left and right pre-auricular points and the nasion, as illustrated in Figure 2.10. The wires attached to the coils are plugged into the head localisation unit and high frequency electrical currents are sent to the three points during data acquisition to localise their magnetic fields within the sensor array.

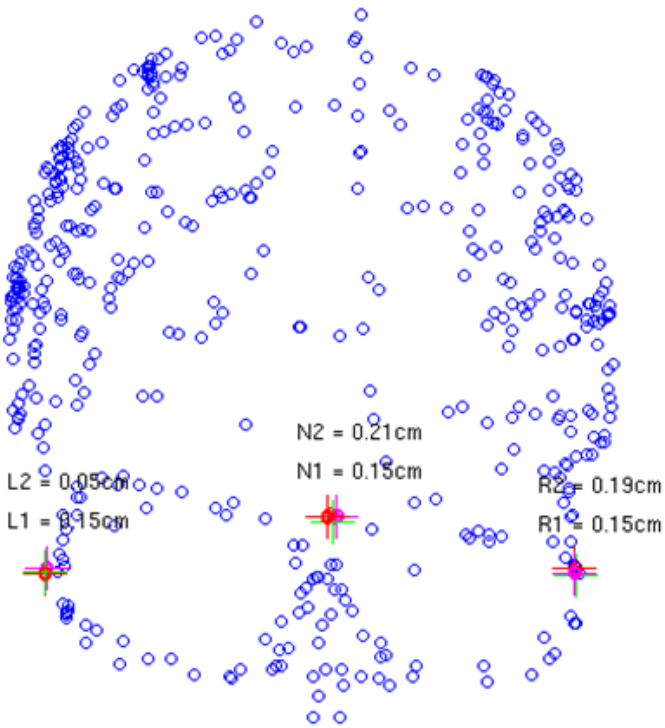


Figure 2.10: A 3D head shape acquired using the Polhemus system. The left and right pre-auricular and nasion points are marked in red/pink/green (L1 and L2/R1 and R2/N1 and N2 show the differences between the measurements taken at the beginning and at the end of the head shape to check accuracy). These points are shown in relation to the head shape consisting of approximately 500 points shown in blue.

The Polhemus system, as pictured in Figure 2.11, consists of an electronic unit, transmitter, receiver and stylus.

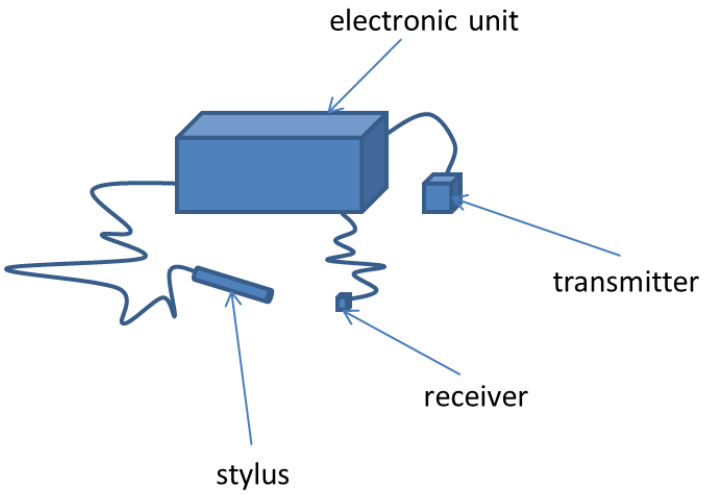


Figure 2.11: The Polhemus 3SPACETM FASTRAK® system (Polhemus, Colchester, Vermont, USA, http://polhemus.com).

The transmitter is placed behind the seated volunteer on top of a plinth providing the coordinate base for the data points to be collected, which is demonstrated in Figure 2.12.

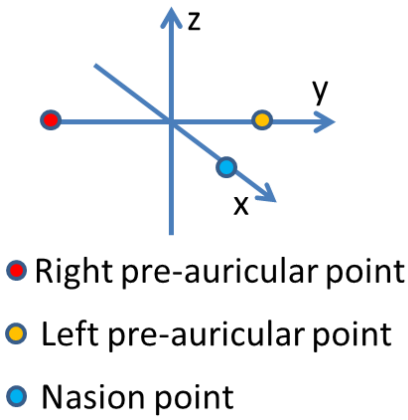


Figure 2.12: The coordinate system used in this thesis and relative locations of the right and left pre-auricular points and the nasion point.

The receiver is held in place on the volunteer's head using a whole-head cap which is fastened around the chin. The stylus is used to locate the three points where the head localisation coils have been attached to the head, and to trace around the volunteer's head to create the 3D head shape. Approximately 500 points are gathered to ensure good coverage, as demonstrated in Figure 2.10. The transmitter produces an electromagnetic field detected by the receiver, and this signal is used to determine the location of the stylus data points and receiver relative to the transmitter. By subtracting the location of the stylus from the location of the receiver, the 3D digital head shape is achieved (Hale, 2012).

Section 2.3. Sensor space to source space in MEG

Section 2.3.1. The forward problem

In order to project the sensor space data into the brain, we need to reconstruct the current density in the brain based upon the measured magnetic field distributions from the data. The forward model refers to the derivation of magnetic field distributions outside the head based upon a known current distribution in the brain, the solution to which can be calculated (Hämäläinen, et al., 1993). Although we do not have prior knowledge of the strength of neural sources that generate the data observed in a MEG study, the first step in the procedure of beamforming (described in Section 2.3.3) is the computation of the signals that would be produced at each sensor by a source of unit strength and specified orientation located in each voxel in the brain. In this thesis the forward solution was based on a multiple local sphere model (Huang, Mosher, & Leahy, 1999) and the forward calculation described by Sarvas (1987).

Section 2.3.2. The inverse problem

The inverse problem refers to the deduction of current distributions in the brain based upon the known measured magnetic field distributions, and there is no unique way to solve this problem so models and assumptions are required (Hämäläinen, et al., 1993). One method of solving the inverse problem is to use 'dipole fitting'. With this method, a dipolar current source is modelled inside the brain and the MEG data then compared to the modelled source. The model is adjusted until it matches the measured signals as accurately as possible. Although this method can give misleading results for cognitive studies due to the fact that one cannot know how many neural sources there are prior to analysis, this method can be used to localise the foci of epileptic neural activity in epilepsy patients (Hale, 2012). In this thesis, we use a method called beamforming (see Section 2.3.3) which is an adaptive data-driven method and is considered an effective approach for exploring induced oscillatory effects in MEG data whilst minimising interference (Hillebrand & Barnes, 2005).

Section 2.3.3. Beamforming

Beamforming is a spatial filtering method applied to MEG data. It was originally created for application to radar, as a means of estimating a signal originating from a specific location, whilst attenuating interfering signals from elsewhere (van Veen & Buckley, 1988). A brief description of beamforming as applied to MEG analysis is given below but please see Appendix 16 Equations relating to beamforming, for a more detailed description and the mathematical equations which describe it.

Each of the 275 sensors in the helmet of the MEG system record magnetic field changes over time, which we need to project into the brain (moving from sensor space into source space) so that we can estimate the time course of neural current at each location (placed at the vertices of a regular 8 mm grid spanning the entire brain). With the *scalar* beamformer used in this thesis, the time course of source strength is estimated using a weighted sum of the MEG sensor measurements for each location in source space and the orientation of each source is determined by a search for the maximum SNR (signal to noise ratio). One of the main assumptions in

beamforming is that no two neural sources are correlated. If a beamformer encounters perfectly correlated sources the power is very small or non-existent.

The lead fields, which are based upon the forward solution, represent the MEG signals that would be recorded for a source of unit strength at a particular location and orientation. The lead fields are represented by a matrix with dimensions M x N, where M is the number of MEG sensors and N is the number of voxels in source space. The lead fields were generated using the analytical derivation of the field from a dipole in a single shell conducting sphere presented by Sarvas (1987). This model takes into account volume current contributions which are important when sensors are not oriented radially (which they are not in the CTF system used here). The head geometry was approximated using a multiple local sphere head model (Huang, et al., 1999).

Beamformer weights depend on both the lead fields and the data covariance matrix. The lead fields represent the sensitivity of each sensor to a neural current source at any given location and orientation in source space (Hillebrand & Barnes, 2005). The data covariance is an M x M matrix with the 'isth' element representing covariance between channels 'i' and 's'. The covariance was calculated using narrow band frequency windows (1-4, 2-6, 4-8, 6-10, 8-13, 10-15, 13-20, 15-25, 20-30, 25-35, 30-40, 35-45, 40-50, 45-55, 50-60, 55-65, 60-70, 65-75, 70-80, 75-85, 80-90, 85-95 and 90-100 Hz) and a time window spanning the entire experiment. Maximising the time window in this way helps to minimise the error in the covariance estimate and therefore ensures beamformer reconstructions are reliable, whilst narrowing the frequency range optimises beamformer suppression of interference since only interference within the band of interest is nulled (Brookes et al., 2008).

In order to ensure that the covariance matrix was well-conditioned we used matrix regularisation with a regularisation parameter of 4 times the minimum eigenvalue of the unregularised matrix (i.e. 4 times the noise estimate). In cases where the condition number of the covariance matrix is high, regularisation is required otherwise the covariance matrix cannot be inverted for beamformer weights calculation. Practically, regularisation adjusts a trade-off between SNR and spatial resolution, with high levels of regularisation giving high SNR estimates but low spatial resolution. This in turn means that high leakage might be expected for high levels of regularisation (hence artifactually increasing connectivity estimates), but

equivalently, low SNR would be expected for no/low regularisation, which would necessarily reduce the reliability of connectivity estimates. Here, regularisation was applied using the Tikhanov method with the regularisation parameter set to 4 times the minimum eigenvalues of the unregularised covariance matrix.

A regularisation parameter of 4 times the minimum eigenvalue of the unregularised matrix was chosen as a compromise between enhancing the SNR of the beamformer projected timecourses (important for subsequent connectivity calculation) and minimising leakage (which itself was reduced further using the regression methodology described below).

Section 2.4. Considerations for using MEG in developmental studies

One of the most important considerations for using MEG in studies that include a developmental cohort is that of head size. Small head size could potentially impair the signal detection of the MEG system, resulting in poorer SNR since the brain is further away from the sensors in the helmet of the scanner. This could potentially bias the results of a developmental study like ours. Head size data will therefore be collected and assessed during analysis so as to address any potential issues arising from differences in head size if any correlation with age is apparent in the sample used in this study. To avoid any bias towards certain regions of sensors it is best practise to have the head as centrally as possible within the sensor array, and this can be achieved using padding around the head and/or use of the fibre glass insert, as pictured in Section 2.2.2 Practical MEG data acquisition, Figure 2.9.

Another important consideration for using MEG in any population is head movement, but this can be particularly problematic when scanning children (Pang, 2011; Wehner, Hamalainen, Mody, & Ahlfors, 2008). Excessive head movement can seriously impair the accuracy of estimating neural sources using MEG (Wehner, et al., 2008). Since we do not have a paediatric MEG system, an adult sized helmet allows even more room for movement of small heads which would lead to inaccurate source localisation. Head movement can be minimised using padding or a dental bite bar. However, tight constriction of movement is uncomfortable and might not only

impair concentration on cognitive tasks but also create a counterproductive urge to move, especially in children. Therefore, in this study we employed light padding adjusted to produce comfort acceptable to the individual. We additionally recorded head localisation continuously during data acquisition in all of our participants so as to enable exclusion of datasets where the head moved excessively during data acquisition. The threshold for head movement used in this thesis was set at 8 mm in line with the dimensions of the grid used to calculate beamformer weights, similar to previous studies (for instance Brookes *et al.* (2011b) used a threshold of 5mm for a 5mm dimensioned grid). It will be important to investigate the relationship between head movement, head size, signal strength and age so as to inform future studies, as well as to support the analyses of this thesis.

Another important consideration is that claustrophobia and anxiety can be an issue particularly in children for neuroimaging studies, however this is far lesser for MEG studies compared to fMRI since MEG systems are silent, more comfortable and less enclosed than MRI scanners (Byars et al., 2002; Pang, 2011). The scanner operator should be aware of this possibility and be prepared to act appropriately in the event of the volunteer having difficulties, to relieve any anxious feelings.

In deciding on the lengths of paradigms to use researchers should take into account several key factors highlighted by Pang (2011). According to Pang (2011) the 'ideal' testing session consists of three things: 1) collection of clean data with high SNR, 2) high performance and good concentration on the tasks, 3) entire session completed in as little time as possible. By keeping the tasks engaging, pitched at an appropriate level of difficulty and kept as short as possible these three key factors of a successful testing session should be achieved. However, there is a trade-off to be made since longer studies allow more averaging and therefore higher SNR.

Section 2.5. Safety considerations

The main safety consideration to take into account when using MEG is the presence of liquid helium within the dewar of the MEG system. When working with cryogens such as liquid helium, the first risk is that of cold burns since helium boils at -270°C so protective gloves and face masks should be worn when filling the scanner. Helium

gas cannot be detected by sight or smell and therefore poses a serious threat if inhaled as helium displaces oxygen within the body which can lead to asphyxiation and death. It is therefore necessary to ensure adequate ventilation. Finally, there is a risk of explosion if boiled off helium gas is not able to exit a dewar, as pressure will build up in the enclosed space the dewar provides. Special care should therefore be taken to ensure pressure does not build up in dewars and this can be achieved by venting dewars when necessary (for instance, before/during/after a liquid helium fill of the MEG system). The air conditioning system within the MSR acts as a precaution to this occurring with the MEG dewar itself, and the scanner operator must check the pressure status of the MEG dewar prior to scanning (Hale, 2012).

MEG is a non-invasive technique used to measure naturally occurring magnetic fields around the head, and is therefore considered safe. Even so, several precautions are taken to ensure the safety of volunteers. An oxygen sensor is placed in the room which is on continually and will alert the scanner operator to any abnormal decreases in oxygen within the MSR. Additionally, the outflow of helium gas is assessed before and between scans to check that the outflow is not blocked (which would be signified by a decrease in gas outflow) and that the dewar is not warming up (which would be signified by an increase in gas outflow).

Chapter 3. Cognitive task development

While there is a large literature on functional brain networks under fMRI task-free, resting state conditions using correlations between brain regions, most of what we know about how the activity of functional brain networks are modulated under task conditions has been carried out by examining the neural correlates of task. While some fMRI studies have examined between-region correlations during task performance, either before or after removing variance attributable to task performance, only one study to date has conducted such a study in MEG (Brookes, et al., 2012a). In the present study we will explore task data as a whole, with the potential for exploring load specific and/or attention specific effects in the future in the same data. The reason for this being to first maximize the use of each data set with a relatively new analytic approach where it is not clear what the impact would be by exploring load effects for instance, where a large amount of data would need to be discarded for analysis. We instead hope to explore broadly how FC might be modulated under each of these task conditions as a whole in this thesis.

We have decided to focus on two cognitive functions known to develop over the age range of the present study (age 9-25), attention and working memory. However, since resting state is a particularly useful way to explore brain activity in children because it does not rely on subject compliance, understanding or the experimental design of a behavioural task, we therefore wished to incorporate resting state into the study alongside 'task positive' scans. Scans recorded during tasks are referred to as task positive, whereas 'task negative' refers to resting state or task free scans.

Over the following sections the development of the behavioural paradigms used in this thesis will be described, and the finalised versions will be detailed. Wehner *et al.* (2008) reported that the effects of head movement and other artefacts such as eye blinks can be reduced by implementing short scanning sessions with regular breaks and easy to follow tasks with good instructions and practise opportunities. We have therefore developed paradigms that last a maximum of 15 minutes each. Each paradigm also includes several minutes of rest during that time. Participants were able to take breaks outside of the scanner in between tasks but not during, and to ensure familiarity with the stimuli we would always have a practice session prior to

the scanning session. The practise versions of each paradigm have been developed alongside the final versions so as to ensure easy to follow paradigms are used.

Section 3.1. Working memory paradigm development

Our working memory paradigm consisted of 6 blocks of 6 trials each, with a short rest at the end of each block (please refer to Figure 3.1). At the beginning of the task participants received the instructions, “Welcome to the alphabet memory game!”, and at the beginning of each block participants received the instructions, “If you see a match, press the left button. If you don’t see a match, press the right button. Keep your eyes on the cross”. All instructions were broken down into small phrases given on separate screens. Depending on the load of the given block they then received the final instruction, “You will see x letters each time. Ready? Good luck”, where x was replaced by the relevant load (2, 4 or 6).

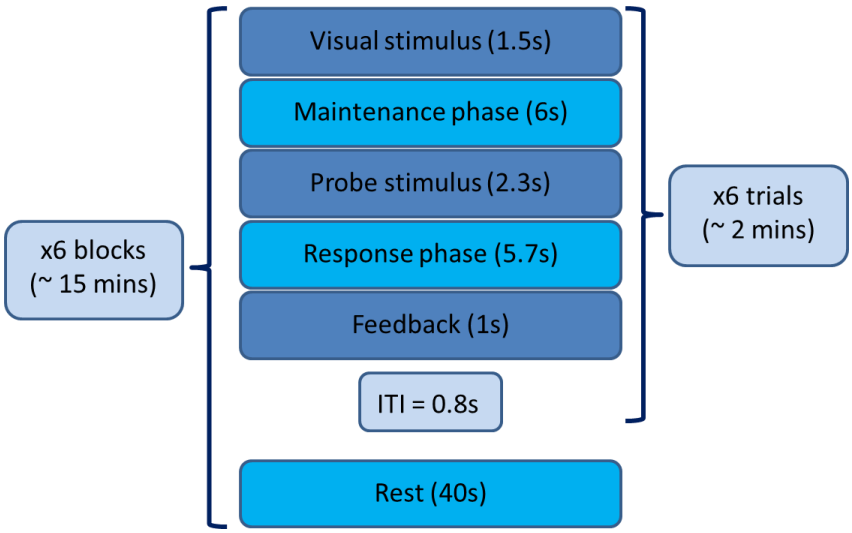


Figure 3.1: Schematic of the working memory paradigm used in this study (ITI refers to inter-trial interval).

Load was varied according to block, but was presented in the following order so as to minimise learning effects: 2-4-6-2-4-6. This was determined after careful consideration and pilot testing; the pilot data (presented in Figure 3.2) showed that mean percentage accuracy increased with age, and all age groups achieved accuracy above chance at load 4 demonstrating that all age groups could understand the task. The behavioural pilot data therefore supports our decision to set the lower age limit for the study at 9 years old. Primarily this decision was based on the logistics of recruiting volunteers and conducting sound, ethical brain imaging research however it is further supported on a behavioural basis that it appears children under age 9 have much more difficulty successfully completing working memory tasks with varying loads.

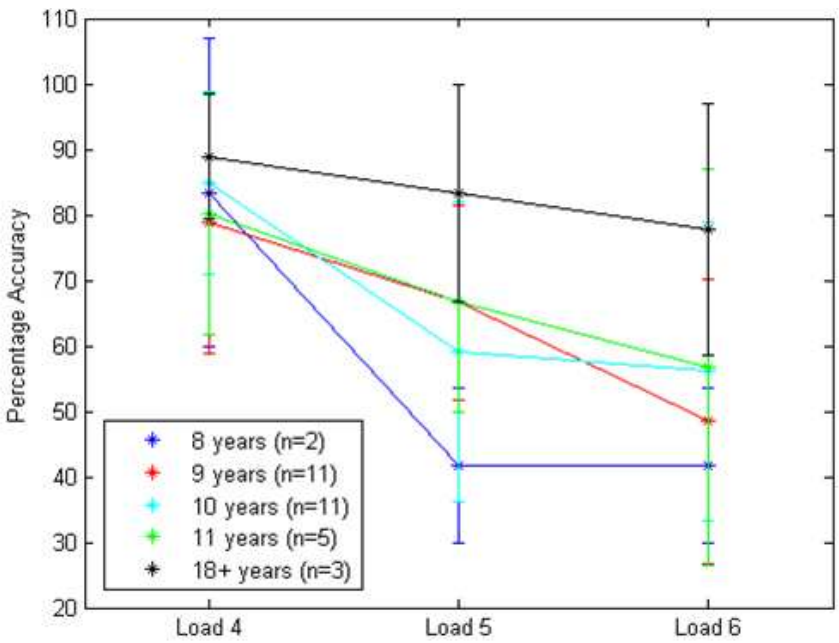


Figure 3.2: Behavioural pilot data collected from individuals aged 8-32 (n=32).

We recognised the importance of developing a paradigm which volunteers could achieve good levels of accuracy on (above 50% and preferably without reaching ceiling effects) so as to ensure volunteers would be engaged in the cognitive processing we were trying to measure. The decision was taken to set the lower load at 2 for the main study because from the pilot data it appeared that even those aged

18+ struggled with load 6. Therefore, in order to achieve a good range we chose to set the lower load limit at 2 and the upper load limit at 6 with the intention of keeping the task within the capabilities of the volunteers. It was additionally encouraging that we found no ceiling effects in any age groups, which gave us confidence in the load levels we decided upon.

The additional benefit of volunteers across the age span achieving high levels of accuracy in the paradigm is that it reduces the ambiguity of results. If low levels of accuracy are achieved in the youngest volunteers but high levels of accuracy are achieved in the oldest volunteers, results could simply reflect performance differences as opposed to genuine age-related differences. We therefore used behavioural pilot testing prior to finalising the paradigm, to ensure levels of accuracy above chance could be achieved even at the youngest age to be included in the study. It has been noted by other researchers that in avoiding the confound of performance differences across different age groups, simplifying cognitive tasks should be avoided since this may not then tap into the cognitive function you wish to investigate (Kwon, et al., 2002). The range of scores achieved (please see Section 5.4.1 Working memory behavioural results) indicate that we achieved a satisfactory balance with this paradigm. Casey *et al.* (2005) note the importance of recording performance data when investigating development of cognition, so as to facilitate controlling for task performance. In this instance, the paradigm has been set up so that performance can be characterised by percentage accuracy on the task (not reaction times, since there will be no instruction given to participants to respond quickly).

Within each of the 6 blocks, each of the 6 trials consisted of presentation of the visual stimuli (2, 4 or 6 white letters on a plain black background; please refer to Figure 3.3) followed by a 6 second maintenance phase where only the fixation cross remained on the screen and participants were required to remember the visual stimuli they had been shown. The letters presented were randomly assigned to each trial in Matlab. The letters ‘O’ and ‘I’ were excluded from the task to reduce ambiguity between themselves and other similar letters such as ‘Q’ or ‘T’. The letter ‘W’ was excluded from the task because it is the only two syllable letter of the alphabet. All letters were presented as capitals, font was clear and each letter used was a graphic (.bmp file created in Paint) so that every presentation was exactly the

same. Letters were arranged closely around the central fixation so as to minimise any need to move the eyes and head to view all the letters presented. Whilst Luna *et al.* (2010) report that most developmental studies use spatial working memory tasks, this is not an appropriate option when using MEG as it is important to keep eye movement to a minimum so as to minimize noise in the data and to keep any head movement to a minimum. We therefore utilised a fixation cross for volunteers to focus on throughout the task (volunteers were additionally instructed to try and fixate on the cross during the practice, prior to data acquisition, and at various point during the scan).

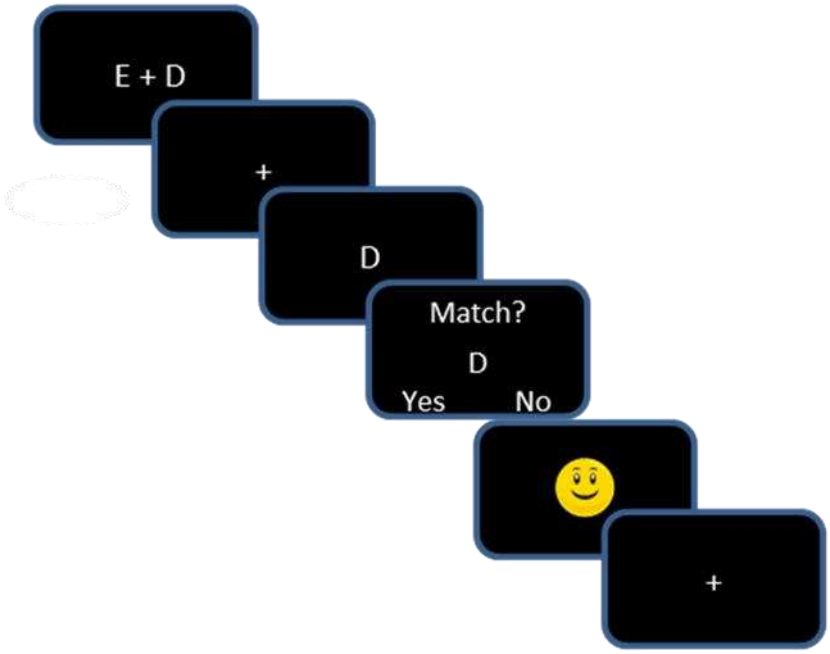


Figure 3.3: On-screen examples of the working memory task (not to scale).

Following the maintenance phase a probe stimulus was presented which was a single letter, and participants were required to assess whether they had seen this letter in the previously presented stimuli or whether they had not seen that letter presented. A response phase took place after initial presentation of the probe. This consisted of a reminder of the task in hand ("Match?" appeared above the probe) and a reminder of the possible responses ("Yes" appeared underneath and to the left of the probe to signify that a 'yes' response should be indicated by pressing the button on the left,

and the word “No” appeared underneath and to the right of the probe to signify that a ‘no’ response should be indicated by pressing the button on the right).

Participants were asked to use their index fingers on each hand to make their responses. We wanted to use this design as opposed to requiring a response for a ‘yes’ and not requiring any response for a ‘no’, so as to reduce the opportunity for participants to lose their concentration on the task. This also enabled us to track their active engagement with the task to ensure reliability of the data. Since some children may experience trouble using a single button response pad with multiple buttons on that single pad because the size of the pad is matched to adult sized hands, we chose to use separate response pads for yes and no and use single fingers on each hand so that hand size would not be an issue. Whilst this response design had the potential to be confusing for younger volunteers, we were able to assess their ability to respond appropriately during the practise session and through behavioural pilot testing and no one showed any signs of being unable to respond in this way.

Following the response phase, participants then received feedback on their response for each trial before the next trial commenced, and this was signified by a yellow happy face cartoon graphic or a red unhappy face cartoon graphic. Additionally, at the end of each block participants received an update on how many points they had scored that block and received a feedback message such as, “You scored 3 points”. This was followed by brief instructions for a short rest period prior to the next block commencing which were, “Have a break but keep still. Keep your eyes on the cross”. They also received a positive message at the end of the rest period such as, “You’re doing so well!”, “Keep up the good work!”, or, “Try your best to get some more points this time”, so as to encourage their continued engagement in the task.

The working memory paradigm for this study was developed as a modified Sternberg delayed response task (Sternberg, 1966). Sternberg’s original task presented a temporal sequence of stimuli to be encoded and actively retained by the participant over a maintenance delay period, ready for retrieval upon presentation of a probe. Due to the time constraints involved in scanning children and the need to maximise the usefulness of the acquired data we decided against following a temporal sequence presentation of stimuli in favour of a single presentation of multiple stimuli at once.

This decision was based on the premise that we would not be exploring working memory encoding in this study, given that encoding is likely to remain stable across the developmental period studied here. We instead decided to concentrate our efforts on exploring working memory maintenance (active retention) given that the ability to maintain information in working memory is more likely to still be developing over the age range studied. Our decision is supported by work published by Hitch *et al.* (1989) which indicates that item identification and rate of rehearsal account for developmental differences in working memory. Since item identification should be easy for all individuals taking part in the study (our stimuli are letters of the alphabet for that very reason), rate or rehearsal is therefore the potentially interesting developmental difference of interest that may be captured through this task.

As stated, we recognised the importance of using stimuli with which subjects would be similarly familiar with to ensure identification and encoding across the volunteers was not confounded by prior experience. In one of the early versions of the task we used Shrek film characters as the visual stimuli, so as to be appealing and engaging for the younger volunteers that would be taking part in the study. This presented significant issues with familiarity as some children had not seen the film, so we decided to use English alphabet letters. We therefore ensured that all volunteers were either first language English speakers or spoke English in the home, to ensure a similar level of familiarity. Furthermore, different strategies may be used at different stages of development whether as a compensatory mechanism in the absence of the most efficient strategy having been learned, or whether by choice of the individual. Either way, a difference in strategy is likely to stimulate different brain regions. Strategic differences must therefore be minimised through careful task design to ensure that if strategic differences occur between subjects, they are not simply due to the preferential strategies of the subject. By using familiar letters of the English alphabet we ensured that stimuli were easily phonologically encoded so as to minimise the possibility of different strategies being used across the subjects.

Some studies of working memory have employed tasks which incorporate basic maintenance and more complex manipulation of stimuli. Despite the scope for investigating the ability to manipulate information maintained in working memory during such tasks, we rejected such a design because of difficulty in establishing if

the participant disengaged from the task or misunderstood the task during complex trials. If a younger volunteer achieved a very low level of accuracy for manipulation trials for instance, would this be because they had disengaged, misunderstood the task or simply found it too difficult and therefore responded incorrectly for many of the trials? At least with a straight forward maintenance task we could be confident that volunteers would understand the task and we would minimise the risk of volunteers disengaging due to task complexity.

We were mindful of the possibility that younger volunteers may have shorter concentration spans and find it more difficult to remain still for the entirety of the scanning session. We therefore kept the paradigm as short as possible, compromising on length whilst still ensuring we would be able to collect enough data. It is imperative that participants are able to keep still for the duration of the scan as otherwise the data may become unusable. Pang (2011) concurs that the most difficult challenge with using MEG with children is their movement, so it was key to avoid lengthening any scans unnecessarily. Where necessary, participants received verbal encouragement to remain still, however any verbal communication was limited to short between-block breaks in data acquisition. Participants received on-screen visual confirmation when they were over half way through the task, and when they were on the last block of the task.

Section 3.2. Relevance modulation paradigm development

Relevance modulation refers to the neural response to task relevance (Brookes, et al., 2012a). To investigate the development of top-down attention, we used a task in which the relevance of the stimulus to the current task was manipulated, in order to allow us to compare neural responses to stimuli that differed in the top-down component of attention, while controlling for bottom-up effects by alternating the relevant and irrelevant stimuli. The task was originally developed to be suitable for use with children and patient populations, so the task is engaging and colourful as well as being straightforward to follow, understand and undertake in a scanning session. The advantage of the task design is that it would enable neural activity

induced by relevant and irrelevant stimuli to be compared without the confound of motor response in an event-related analysis. This is because whilst all relevant stimuli should be attended to and all irrelevant stimuli should be ignored as much as possible, very few relevant stimuli require a motor response. Whilst event-related analysis is not the approach taken in this thesis, it allows subsequent use of the data in several ways after this thesis has been completed.

The relevance modulation task was first described by Brookes *et al. (2012a)* and the precise timings of the task were amended to be suitable for this thesis. Coloured cartoon butterflies and ladybirds were the visual stimuli for the task and the background was photographic green leafy foliage (see Figure 3.4).

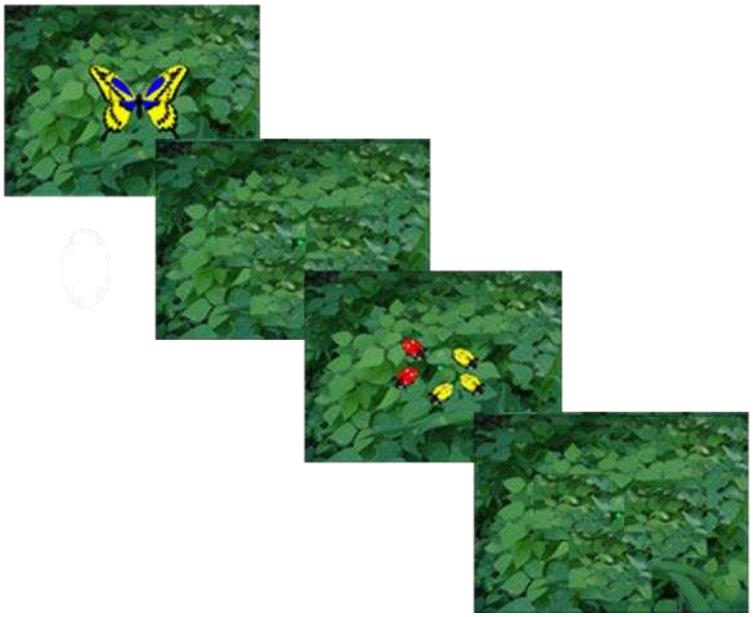


Figure 3.4: On-screen examples of the relevance modulation task (not to scale).

Our relevance modulation task consisted of 5 blocks of 40 trials (please see Figure 3.5). Berger *et al.* (2000) reported that whilst many attention tasks are boring and repetitive, one can successfully make an attention task interesting and enjoyable for children by exploiting their love for games. We have done something similar here. Prior to each block, participants were given instructions for the block which were dependent on the relevant stimulus. Where butterflies were the relevant stimuli the instructions were, “Look carefully at this butterfly and note its shape and colours.

Press the button every time you see a butterfly like this. Ignore the ladybirds and try to keep your eyes on the green star" (the fixation cross was a green star for this task). Where ladybirds were the relevant stimuli the instructions were, "Ignore the butterflies. Check the ladybirds instead. If there are the same number of red and yellow ladybirds press the button and try to keep your eyes on the green star". All instructions were broken down into small phrases given on separate screens.

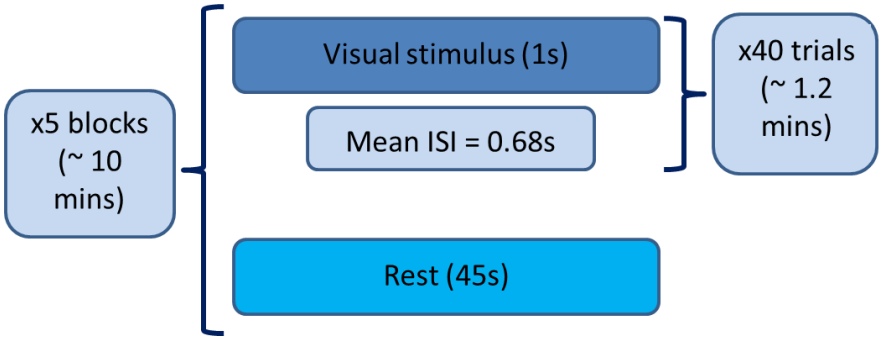


Figure 3.5: A schematic of the relevance modulation behavioural paradigm used in this study (ISI refers to inter-stimulus interval).

Blocks were interleaved using an irregular pattern so that blocks were presented in the following order: butterflies attended (ladybirds unattended), ladybirds attended (butterflies unattended), ladybirds attended (butterflies unattended), butterflies attended (ladybirds unattended), ladybirds attended (butterflies unattended). During each block of 40 stimulus presentations, the stimuli were presented alternately before a short rest at the end of the block. Each rest period was preceded by the instruction, "Now rest for a short time". Inter-stimulus intervals (ISIs) were jittered so as to reduce expectancy of stimuli at regular intervals. For each butterfly presentation the wing shape and two colours were randomly assigned, for each presentation of ladybirds the number of ladybirds presented, their location around the fixation point and numbers of red and yellow were randomly assigned. The random assignments for each stimulus were generated using Matlab.

In deciding to make this paradigm the length it was (and likewise with the working memory paradigm detailed in Section 3.1 Working memory paradigm development) we took into account several key factors highlighted by Pang (2011). As previously

described, the 'ideal' testing session consists of three things: 1) collection of clean data with high SNR, 2) high performance and good concentration on the tasks, 3) entire session completed in as little time as possible. By keeping the tasks engaging, pitched at an appropriate level of difficulty and kept as short as possible we tried to ensure these three key factors of a successful testing session were achieved. Additionally, Byars *et al.* (2002) point out that fatigue and restlessness can be issues in scanning children so we have done our best to avoid the possibility of these affecting the testing session.

During the scan, participants received verbal encouragement to remain still where necessary and were told when they had reached the penultimate block. However, any verbal communication was limited to short between-block breaks in data acquisition. Prior to scanning all participants took part in a short practise of the task outside the scanner room. During the practise run, participants were asked to press the spacebar on the stimulus computer in response to seeing a relevant stimulus and in the scanner they were asked to press a single button on a button response pad with their index finger of the left hand. The practise session helped to minimise learning effects during data acquisition and this also allowed us to assess each participant's understanding of the task prior to data acquisition. Performance will be analysed using RTs for this task.

Section 3.3. Resting state

It was important to include a resting state or 'task-free' scan in the study since this has been the most frequently used way of investigating functional brain networks. There are some significant advantages of using resting state as opposed to task-positive designs because resting state does not require an understanding of a cognitive task and it does not require volunteer compliance. It is therefore particularly accessible and easy to implement in a study of children.

Eyes-open resting state was chosen so as not to encourage sleep, particularly in younger volunteers who may have experienced a long experimental session. The scan was also kept to 5 minutes in the interest of our volunteers given the lower age limit we were planning to scan and the length of the full study session. Participants

were asked to, “Please keep your eyes on the cross and relax for 5 minutes”, prior to a plain background with a small white fixation cross being presented on screen.

Chapter 4. Study methods

Section 4.1. Ethical approval and recruitment

Ethical approval for this study was obtained from the University of Nottingham Medical School Research Ethics Committee (see Appendix 1 Ethics approval, for letter of approval). The study was approved as a student project and the details of the submission are discussed over the following sections. We obtained ethical approval to cover scanning of 90 individuals.

Section 4.2. Recruitment

We obtained approval to recruit 9-18 year olds via approaching local schools and colleges by email to primarily promote the research. Where a school expressed a willingness to be involved, we were able to further disseminate information about the research via text message and the school website so that interested parents/students could contact us directly. For recruitment of 18-25 year olds we obtained approval to post adverts promoting the research in buildings at the University of Nottingham and on the University of Nottingham online portal (the approved poster can be found in Appendix 2 Approved poster of advertisement).

Section 4.3. Informed consent

Ethical approval was obtained for the study to request parent/guardian consent for participants under the age of 16 (for a copy of the consent form, please see Appendix 8 Parent/guardian consent form). Those aged 16 and over were able to give their own consent (please see Appendix 9 Age 16+ consent form for a copy of the consent form).

In order to ensure consent was as fully informed as possible, appropriate information sheets were created for different age groups (age 9-10, 11-15, 16-17, 18-25 and one for parents/guardians; please see Appendix 3 Age 9-10 information sheet, Appendix 4 Age 11-15 information sheet, Appendix 5 Age 16-17 information sheet, Appendix 6

Age 18-25 information sheet and Appendix 7 Parent/guardian information sheet). At the testing session participants were asked if they had read the sheet, if they had any questions, whether they would like another copy of the sheet or to read it again before reading and signing the consent form. All participants received full verbal descriptions of everything that they would be asked to do in the testing session and were encouraged to ask as many questions as they wished at any point. They were then offered a copy of the consent form to take away with them after signing.

Hinton (2002) points out that in obtaining consent from children it can be difficult to truly assess whether the child has been fully informed to an acceptable level. It is the researcher's responsibility to appropriately communicate the procedures involved in their research and ensure a sound understanding of what volunteers are consenting to is reached, prior to signing of the consent form. This process should involve using appropriate language for age and level of prior knowledge of the participant in question, and utilising additional material/tools to help participants understand where necessary. For instance, the ethics committee stipulated that I needed to describe the sound of the MRI scanner as a 'pneumatic drill' in my information sheets, however I did not feel this described the noise accurately. I therefore took to using a sound clip of an MRI scanner so that I could most accurately describe the sort of noise the scanner makes before children went in the scanner. I also ensured I used appropriate language for all my volunteers and checked their understanding verbally whilst making myself as approachable as possible so that volunteers would feel able to ask as many questions as they wished. In particular, Hinton (2002) notes that anyone conducting this kind of research is, "obligated to provide information in a manner that extends beyond that of a simple written form", and I believe I have done this.

In some cases additional amendments were required to the primary ethical approval we received. For instance, one school requested we obtain parent/guardian consent for adolescents recruited from their school up to and including the age of 18 (rather than allowing children aged 16 and over to give their own consent as previously approved). They also wished to obtain a copy of any consent forms received from their students with the view to keeping copies securely on site at the school. In this case, whilst not a usual request or procedure in research, ethical approval was granted and students from the school were made explicitly aware that this would

happen before consenting to taking part in the study. The reason behind this request was that the school also had an ethical and moral obligation to their students and the school would normally require parent/guardian consent rather than student consent for any activity requiring consent. Since recruitment was through the school itself, they felt a need to fulfil their usual requirements for such an activity and we were pleased to oblige once the ethics committee had approved the amendment.

Section 4.4. Procedures

The University of Nottingham Medical School Research Ethics Committee approved our proposal to conduct a study session that consisted of scanning whilst participants rested in the scanner and whilst they completed behavioural tasks in the scanner, with additional pencil and paper assessments (please refer to Section 4.8 Behavioural testing for more information on these).

Following specific ethical guidelines for scanning children (detailed in Appendix 11 Standard Operating Procedure for Scanning Children), we ensured that a copy of all relevant ethics approval paperwork was given to the receptionist at the SPMMRC. Safety forms were completed in accordance with the guidelines and copies of both the consent and safety forms were submitted with the record for each scan in both the MEG and MRI. Please see Appendix 10 Safety questionnaire, for a copy of the standard safety form used prior to every scan for every individual. In the case of children, it was the accompanying parent or guardian that filled in the safety form as they were in a more informed position than the child themselves.

For the purposes of scanning, children were classed as participants aged 18 and under. Adhering to the guidelines, we had at least 2 enhanced Criminal Records Bureau (CRB) checked persons present for all MEG scans (1 of whom was a scanner operator), and 2 enhanced CRB checked persons present for all MRI scans (1 of whom was a scanner operator, 1 of whom was a scanner assistant), for any child scans. No scanning was completed outside of work hours and no scanning of children aged 9-15 was undertaken during school hours on school days. Instead, scans were completed after school or during school holidays. Adolescents aged 16-18 had the option of visiting during free periods or days not usually spent at school (applicable

to those studying at college). Adults aged 18-25 were scanned within working hours but with no other constraints.

Section 4.5. Confidentiality and data security

MEG, MRI and behavioural data were stored using subject numbers rather than names. Data will be stored securely for a minimum of 7 years as detailed on the consent form.

The only times participant names and corresponding subject numbers were stored together was:

- In emails where necessary to obtain the subject number from the SPMMRC volunteer database. Each subject number needed to be generated by the receptionist at the SPMMRC due to the strictly restricted access to the database,
- On the secure SPMMRC volunteer database accessible only to the receptionist at the SPMMRC or her secondary,
- On a password protected document used for scheduling testing sessions and keeping track of booked equipment. This document was for the private use of the researcher only and was a necessary step in keeping track of all the scans and equipment needed for each session as this varied from session to session and person to person,
- On the consent and safety forms.

Any documents which contain participant details are now securely kept at the SPMMRC for their records or at the Institute of Mental Health in a locked filing cabinet in the office of the Ph.D. researcher who presents this thesis. In the case of the one school who required copies of the consent forms for their students, these are stored securely at the school within a locked filing cabinet under the care of Madeleine Varley, Student Support Services Student Administrator, Bilborough College.

Section 4.6. Data acquisition

Fifty-four participants were scanned in total for this study. All participants aged 16-25 gave their informed consent after reading the information sheet and asking any questions. For some exceptions parental consent was additionally obtained (as required by one of the schools through which students had been recruited for the study). For all participants aged 9-15, parent/guardian consent was given after both the participant and parent/guardian had read their respective information sheets and had asked any questions. After consent had been given, a safety form was filled in either by the participant themselves or by the parent where necessary (and in some cases the form was filled in jointly).

Participants were required to fill in their consent and safety forms prior to scanning and these were checked by the scanner operator before any scanning took place. Both MEG and structural MRI scans were required and in all cases the MEG scan was completed prior to the structural MRI scan.

Section 4.7. MEG data acquisition

MEG data were recorded using the third order gradiometer configuration of a 275 channel CTF Omega 2000 MEG system (VSM MedTech, Coquitlam, British Columbia, Canada). Please see Section 2.2 MEG hardware, Figure 2.2 for a detailed schematic of the MEG system and Section 2.2.2 Practical MEG data acquisition, Figure 2.8 for a photograph and detailed schematic of the MEG and acquisition equipment inside the MSR. A sampling rate of 600 Hz was used and a low pass filter of 150 Hz was applied. All scans were completed with the participants positioned supine.

Section 4.7.1. MEG scan preparation

We adhered to good practice guidelines for conducting and reporting MEG research as proposed by Gross *et al.* (2013), to ensure good quality data was acquired. The guidelines propose several steps to ensure good quality data can be acquired with MEG, these steps include:

1) Checking the operating status of the MEG system is satisfactory (completed prior to each scan);
2) Adequate preparation of the participant (as discussed in Section 2.2.2 Practical MEG data acquisition);
3) Experimental design (as discussed in Chapter 3 Cognitive task development).

Section 4.7.2. MRI data acquisition

A structural MRI was acquired for each participant using a Philips Achieva 3T MRI system and 32 channel radio frequency (RF) head coil. The image acquired was a 1mm isotropic MPRAGE (Magnetized Preparation RApid Gradient Echo) image with parameters as follows: echo time (TE) = 8ms, inversion time (TI) = 960ms, repetition time (TR) = 3000ms. The MPRAGE structural scan, which lasted approximately 5 minutes, was acquired after a reference and survey scan were completed, both lasting approximately 1 minute each. Therefore, total scan time was approximately 8 minutes and participants were simply asked to remain as still as possible during this time.

Section 4.7.3. MRI scan participant preparation

On arrival, all participants were asked to fill in a second safety form for the scanner operator (a different scanner operator to the Ph.D. researcher who was the scanner operator only for MEG scans). An additional copy of each consent form was also made and passed on to the MRI scanner operator for their records. Once the safety form had been completed, participants were asked to change into medical scrubs if not already wearing them following the MEG session. They were again asked to remove make-up and any metal from their person.

Once the participant was ready for their scan they were briefly reminded of what the MRI scan would involve and what to expect. More participants were anxious about the MRI scan than were for the MEG scan, so they were also reassured at this point that if they weren't comfortable being on their own for the scan then the Ph.D. researcher (also the MRI assistant) would be able to accompany them in the scanner room for the duration of the scan. When ready, the participant was led into the

scanner room, accompanied by the scanner operator and Ph.D. candidate (MRI assistant). Where participants were overly anxious they were encouraged to try the scanner bed first without having to commit to the scan itself. At this point participants who were happy to continue were given ear protection and asked to lie supine on the scanner bed. The 32 channel RF head coil was then placed over their head and they were moved into position for the scan. Light padding was used at the sides of the head, in between the head and RF coil to minimise any movement during data acquisition. Communication between the participant and the scanner operator was enabled during the scan via a two way intercom. Finally, participants were handed an alarm button that they were told they should press at any time if they wanted the scan to stop.

Section 4.8. Behavioural testing

The pencil and paper tests completed outside the scanners included the Wechsler Abbreviated Scale of Intelligence (WASI (Wechsler, 1999)), an adapted Rapid Automatized Naming test (RAN (Denckla & Cutting, 1999)) and digit span assessments. We additionally had approval to administer the 'Adult AD/HD Self-Report Scale' (ASRS (Kessler et al., 2005)) and 'Strengths and Weaknesses of AD/HD Symptoms and Normal Behaviour' (SWAN (Swanson et al., 2006)). Each of these measures will be described below, with the rationale for including them in the study.

The WASI is an assessment of intelligence quotient (IQ). Here, the two-subtest WASI was used to obtain an estimate of IQ for each participant without the need for devoting a significant amount of time to the assessment (which the full WASI would require). The two-subtest WASI includes tests on vocabulary and matrix reasoning and the assessment takes approximately 15-20 minutes to complete. We chose to measure IQ because it should be an age-independent measure that enables us to assess whether volunteers in each age group are of a similar IQ and exclude any extremely low scores (and therefore minimize the risk of any potential IQ confound).

The RAN is an assessment of processing speed. The RAN test used here was a serial letters test in order to be consistent with the stimuli used in the working memory task. This allowed us to obtain a measure of how quickly each participant could name

letters of the alphabet (i.e. measure their processing speed with regards to letters of the alphabet). This measure should give us an idea about how processing speed might develop through the age groups included in the study. The RAN assessment took approximately 30 seconds to complete.

The digit span assessment is a measure of working memory capacity. The version used here was the digit span subtest of the Wechsler Adult Scale of Intelligence (WAIS (Wechsler, 1997)) so it was not our intention to normalise scores, but obtain a raw score for each individual. This measure should give us an idea about how working memory capacity might develop through the age groups included in the study. Forward and backward digit span tests were used and the assessment took approximately 5 minutes in total.

We finally administered the ASRS for participants aged 16 and over, or the SWAN for participants under the age of 16 (in this case it was the parent or guardian who filled in the form). These questionnaires allowed us to assess any volunteers particularly at risk of displaying AD/HD symptoms, so as to ensure as far as possible that our sample only included typically developing children, adolescents and adults by exclusion of extreme scores indicative of AD/HD. This is particularly important since disrupted FC has previously been identified in AD/HD (for a review see Konrad & Eickhoff (2010)). The ASRS and SWAN both took approximately 5-7 minutes to complete.

In most cases these tests were either completed during breaks outside of the scanner or at the end of the scanning session. All tests were administered by the same researcher under as similar conditions as possible. Although participants were offered the choice of completing the tests at home instead of at the end of the testing session, all participants chose to complete the tests at the testing session. Copies of these tests and details of their respective scoring systems can be found in Appendix 12 Adult AD/HD Self-Report Scale (ASRS), Appendix 13 Strengths and Weaknesses of AD/HD Symptoms and Normal Behaviour (SWAN), Appendix 14 Rapid Automatized Naming test (RAN).

Section 4.9. Data Analysis

We set out to analyse amplitude-amplitude connectivity within networks by computing the canonical correlation between pairs of network regions. This approach was chosen as it is a data-driven method of finding an optimally weighted combination of voxels within a network region for each subject, thus minimising the chance of biasing the analysis by a choice of voxels derived solely from adult models. However, canonical correlation is a highly computationally intensive process, limiting the number of pairs of network regions that would be feasible in exploratory analyses. We therefore selected regions from three networks that are robustly defined in resting state BOLD and MEG and show activation in response to three well-defined aspects of cognitive function: the visual network, representing primary sensory processing; the sensorimotor network, representing motor action; the DAN, representing executive attention. Results would be subjected to various ANOVAs to explore effects of age, task and network region pair. The following sections will detail the MEG data pre-processing and processing steps used in this thesis; Figure 4.1 summarises the main analysis approach used in this thesis; canonical correlation analysis (CCA).

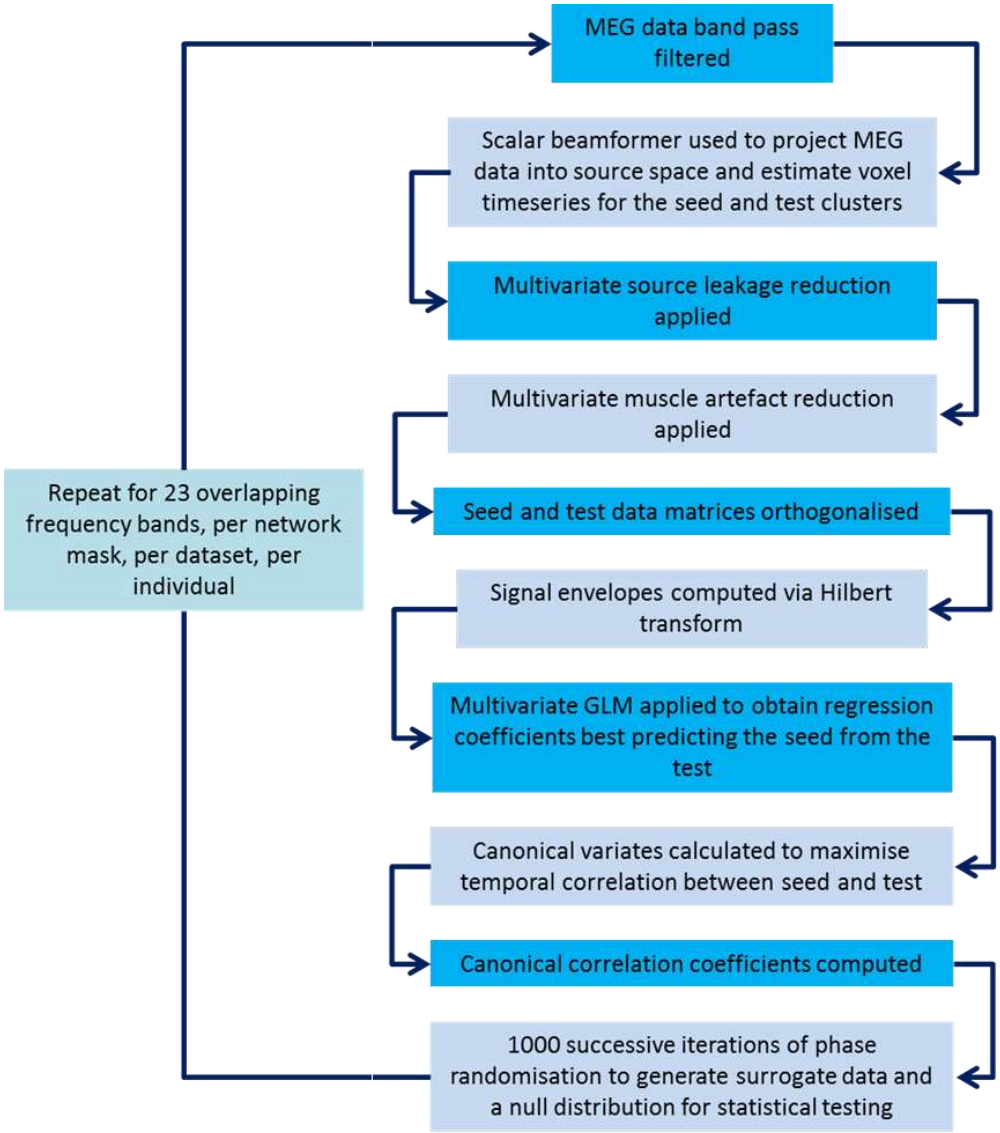


Figure 4.1: Summary of canonical correlation MEG analysis steps (adapted from (Brookes et al., 2014)).

Section 4.9.1. MEG data pre-processing

The MEG system gives us an M x T recording to analyse, where M refers to the number of recording sensors (275) and T refers to the time length of data acquisition, which will vary upon the scan. The sensors record data points at a sample rate of 600 Hz.

All data were inspected visually and trials and/or datasets which contained visually obvious artefacts were removed (such as the data demonstrated in Figure 4.2B). A 'clean' dataset is demonstrated in Figure 4.2A.

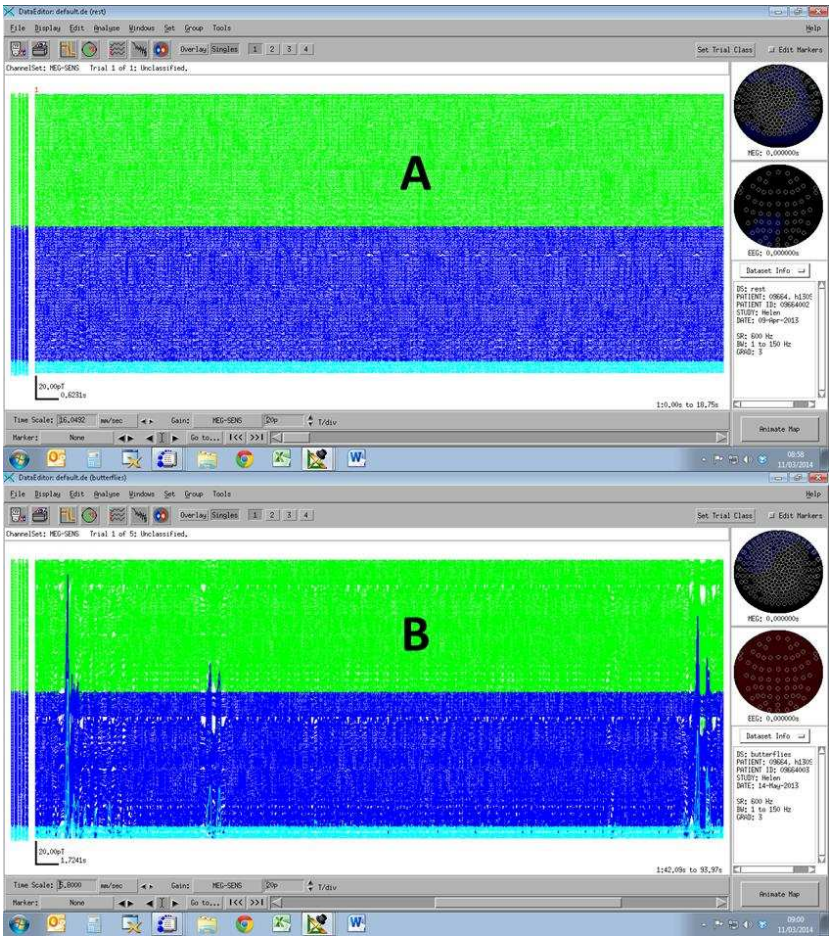


Figure 4.2: (A) Clean MEG data and (B) Excessively noisy MEG data.

Data were broadband frequency filtered (1-150 Hz) and DC offset was removed on a trial by trial basis. Removing DC offset removes the arbitrary baseline value for each sensor from the recorded values. Datasets were excluded for excessive head movement over a threshold value of 8 mm (as previously described in Section 2.3.3 Beamforming), and for excessive and prolonged interference in the data. Only one dataset from each task and the resting state was excluded on this basis and all datasets were for the same participant, aged 16, who exhibited significant unidentified noise.

Each individual's structural MRI scan was then coregistered to their MEG data. As previously described in Section 2.2.3 Head localisation, this allows us to coregister MEG data with brain anatomy using a customised Matlab script which performs a least squares fit between the anatomical MRI head shape and the 3D digitised head shape obtained using the Polhemus (Hale, 2012). Multi-sphere head models were subsequently created for each dataset separately.

Section 4.9.2. MEG data processing

As previously described in Section 1.1.3 Origins of the neural signal, neural activity arising from synchronous activity of neuronal populations generates neuromagnetic fields that are recorded outside the head by sensors in the helmet of the MEG system. This gives a data set in 'sensor space' – i.e. the neural currents are represented by the signals at each MEG sensor, demonstrated in Section 4.9.1 MEG data pre-processing, Figure 4.2). However, in order to extract information about the neural currents at the network regions of interest in this investigation, we need to project the data back into the brain. We therefore translate the data from sensor space into source space using the process of beamforming (Robinson & Vrba, 1998), described in Section 2.3.3 Beamforming.

Section 4.9.3. Head size, movement and lead field calculations

Variations in head size and movement are potential important confounds in any MEG study, but particularly in a MEG study of subjects spanning a large age range and including children (Pang, 2011; Wehner, et al., 2008). Excessive head movement increases the inaccuracy of neural source localisation using the analysis approaches presented and used in this thesis, a key reason why datasets collected with excessive head movement (greater than 8 mm as previously described in Section 2.3.3 Beamforming) were completely excluded from the analysis.

We calculated head size, maximum head movement and the absolute value of the mean lead fields for each individual to ensure we could investigate and quantify head movement in the sample included in the analysis, investigate any significant head size confounds and explore the lead fields for each of the individuals included in the study. Since variation in head size might be expected to affect the SNR and computation of beamformer weights, we computed the lead fields for each subject in order to assess and explore the relationship between head size and the lead fields for data included in this study.

In order to assess head size, several measures were calculated based on the structural MRIs available for each participant. Head size was estimated in three ways: 1) the distance between the farthest anterior and posterior points visible on the MRI scan, 2) the distance between the farthest right and left points visible on the scan, 3) an estimate of the head circumference based on measures 1 and 2.

Maximum head movement for each scan was recorded during data acquisition, identifiable by movement of the fiducial markers which were tracked continuously during data acquisition. We calculated the average maximum head movement for each participant across the scans where they did not exceed the threshold for excessive movement.

The absolute value of the mean lead field was calculated for each individual and used as a measure of signal strength for each participant, since the lead fields reflect the sensitivity of each sensor to a current source at any given location and orientation in the brain. It was important to consider that head size may affect signal strength so we wanted to exclude this as a possible confound if possible by assessing these results in the context of the age groups used in the analysis.

Section 4.9.4. Network mask generation

The next step in analysing our data was to generate 3D spatial functional brain masks of these networks in MNI space, based on a meta-analysis of fMRI data acquired during rest conducted by Smith *et al.* (2009). In the same report it was shown that resting state functional brain networks show good correspondence with task-positive functional brain networks. MEG has additionally been used to demonstrate such

functional brain networks (Brookes, et al., 2012a; Brookes, et al., 2011a; de Pasquale, et al., 2010; Hipp, et al., 2012; Liu, et al., 2010; Luckhoo et al., 2012). Hence we have chosen to use these as the basis for our network masks because they represent robustly defined core functional brain networks present at rest and during active cognitive engagement identifiable through the use of both fMRI and MEG. Please see Appendix 15 ICA of MEG data, for validation of the use of these network masks in the present study - we found spatially similar independent components present across the age range included in our study.

Unfortunately whilst the DMN has been robustly identified using fMRI, questions remain as to its MEG signature (de Pasquale, et al., 2010) and therefore, despite the clear importance of the DMN as a functional network, it will not be a priority of this thesis to explore the development of the DMN. Additionally, the salience network is not a reliable network to explore with MEG, particularly in a developmental cohort, since it consists of deeper cortical structures and the sensitivity of MEG falls off with the square of distance (Barnes, Furlong, Singh, & Hillebrand, 2006). It is also highly susceptible to influence by muscle artefact and for these reasons it is not a network we will be focussing this research on, to avoid any spurious findings. The set of networks used in this thesis are demonstrated in Figure 4.3.

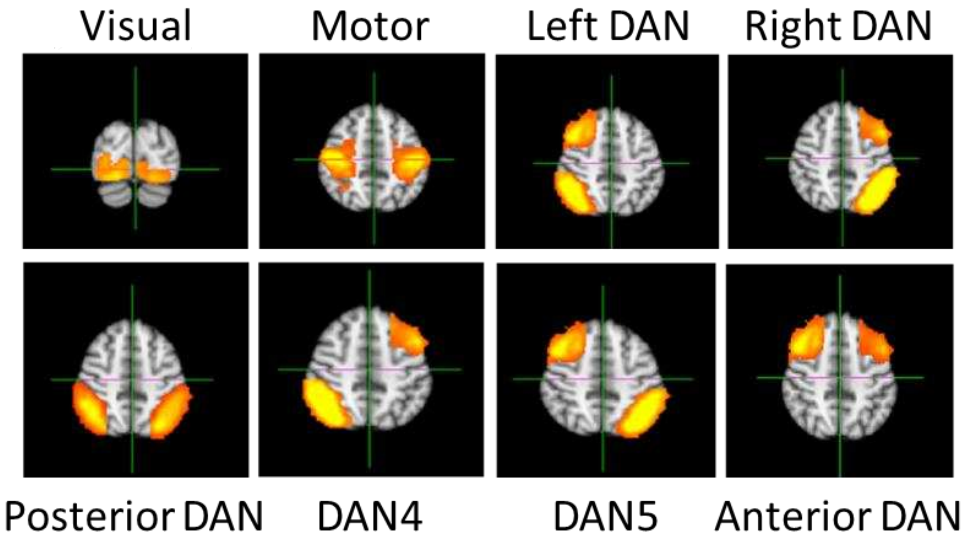


Figure 4.3: Network region pairs based on the findings of Smith et al. (2009).

Masks were first generated in 2 mm MNI space using a customised Matlab script; the masks originally generated by Smith *et al.* (2009) were thresholded in Matlab, based on visual inspection, in order to create a binary mask for each network region. Each binary network mask was then transformed into individual space using FSL (FMRIB Software Library, where FMRIB is an abbreviation of Functional Magnetic Resonance Imaging of the Brain (Jenkinson, Beckmann, Behrens, Woolrich, & Smith, 2012; S. M. Smith et al., 2004; Woolrich et al., 2009)). We used the FLIRT function to do this transform (FMRIB's Linear Image Registration Tool (Jenkinson, Bannister, Brady, & Smith, 2002; Jenkinson & Smith, 2001)).

For each network, we then identified pairs of network regions between which to estimate the canonical correlation (please see Section 4.9.5 Canonical correlation analysis for more information). For the visual network, one pair of network regions was selected, consisting of all masked voxels in left hemisphere (one network region) and all masked voxels in the right hemisphere (the other network region). For the sensorimotor network, the same process resulted in an equivalent left-right pair of network regions. For the DAN, four network regions were selected, consisting of masked voxels in the left and right DLPFC and in the left and right PPC . For this network, the within-network pairs of network regions analysed were: the left PPC and DLPFC, the right PPC and DLPFC, the two posterior DAN regions, 'DAN4' (the left PPC and right DLPFC), 'DAN5' (the right PPC and left DLPFC), and the two anterior DAN regions. The numbering system used here is simply an arbitrary way of naming and identifying the different pairs of network regions.

Section 4.9.5. Canonical correlation analysis

A CCA approach was used, based on Brookes *et al.* (2014) to estimate the connectivity between the two network regions of each pair. CCA allows us to calculate the linear combination of measures that best accounts for the covariance between two sets of variables. In this case, for each pair of network regions, our two sets of variables were the two sets of voxel time-series from the pair of network regions (defined by the network masks as described in Section 4.9.4 Network mask generation). We essentially calculate the linearly weighted combinations of voxels from each network region in the pair that maximises the temporal correlation

between the two sets of time-series, and report the resulting correlation coefficient. In this way, we are able to summarise the FC between two network regions in each narrow frequency band (1-4, 2-6, 4-8, 6-10, 8-13, 10-15, 13-20, 15-25, 20-30, 25-35, 30-40, 35-45, 40-50, 45-55, 50-60, 55-65, 60-70, 65-75, 70-80, 75-85, 80-90, 85-95 and 90-100 Hz).

Following standard data pre-processing (as detailed in Section 4.9.1 MEG data pre-processing), the first step of this analysis was to project data from sensor space into source space and estimate each voxel time series (known as the virtual electrode or VE) within the seed and test regions. This was achieved using a scalar beamformer (please see Section 2.3.3 Beamforming for more information).

Section 4.9.5.1. Signal leakage reduction

Signal leakage refers to the leakage of signal between voxels in source space MEG analysis; signals originating from one cortical location can leak into the estimated MEG signal for several different voxels leading to the possibility of spurious estimates of FC (Brookes, Woolrich, & Barnes, 2012b; Hipp, et al., 2012). It is for this reason that it is important to reduce signal leakage as effectively as possible. Signal from two different voxels that represent leakage will necessarily exhibit zero-phase-lag interactions and this property can be exploited to regress out the leakage signal. Therefore, once the VEs have been obtained for each network region, one of the pair is arbitrarily designated the “seed” region and the other the “test” region, and represented as matrices **X** (referring to the VE time courses for the seed cluster) and **Y** (referring to the VE time courses for the test cluster). Our approach to signal leakage reduction here uses a general linear model to regress the seed signals from the test signals, and thereforewe first calculated the covariance matrices of **X** and **Y**. We subsequently used eigenvalue decomposition to decompose the covariance matrices into constituent components. Please see Appendix 17 Equations relating to signal leakage reduction for the mathematical equation describing eigenvalue decomposition.

A general linear model was finally used to regress the seed signals (defined as any linear combinations of the VE time courses represented by **X**) from the test signals

(**Y**). It should be noted, that whilst this approach ensures that zero-phase-lag interactions (i.e. perfectly temporally correlated signals) are suppressed in the columns of **X** (the seed cluster) and $\mathbf{Y_c}$ (the signal leakage corrected test cluster), we also therefore sacrifice any true zero-phase-lag interactions between the seed and test clusters. Please see Appendix 17 Equations relating to signal leakage reduction, for detailed equations relating to this process.

Section 4.9.5.2. Muscle artefact rejection

The time series for each voxel was band passed into 23 overlapping bands (1-4, 2-6, 4-8, 6-10, 8-13, 10-15, 13-20, 15-25, 20-30, 25-35, 30-40, 35-45, 40-50, 45-55, 50-60, 55-65, 60-70, 65-75, 70-80, 75-85, 80-90, 85-95 and 90-100 Hz). Each of the columns in the matrices X and Y_c were Hilbert transformed to obtain the analytic signal (consisting of real and imaginary parts; a vector of complex numbers). The absolute values of the analytic signal were taken to give band limited VE envelope amplitudes in the 23 overlapping frequency bands. These new envelope VE matrices were labelled $\mathbf{E_x}$ and $\mathbf{E_Y}$. $\mathbf{E_x}$ and $\mathbf{E_Y}$ and were temporally downsampled to 1 second.

We went on to define muscle artefact, which is mainly in the high frequency range of 100 Hz and over, as the envelope of the VE between 100-150 Hz. We used the same general linear model approach as before with signal leakage reduction, to regress muscle artefact from the signal leakage corrected VE envelopes.

Section 4.9.5.3. Canonical correlation coefficient calculations

Eigenvalue decomposition was used to decompose the temporally downsampled envelope data matrices into 5 principal components for each of the seed and test clusters. A general linear model was subsequently used to calculate the canonical correlation coefficients (CCCs) that best predicted the principal components from the seed and test clusters. The explained and unexplained covariance could then be calculated and finally, the CCCs were calculated using Equation 1. Please see Appendix 18 Equations relating to canonical correlation coefficient calculation for more detailed equations relating to this process.

$$r_{can} = \frac{V_1^T V_2}{\sqrt{(V_1^T V_1)(V_2^T V_2)}}$$

Equation 1: The canonical correlation coefficients calculation

r_{can} = canonical correlation coefficients

V_1 = canonical variates of E_{Xo} (where E_{Xo} = orthogonalised version of the envelope VE time course matrix for X (the seed region))

V_2 = canonical variates of E_{Yo} (where E_{Yo} = orthogonalised version of the envelope VE time course matrix for Y (the test region))

(Adapted from Brookes et al., 2014)

The matrix r_{can} is a 5x5 correlation matrix and the CCCs it contains along the diagonal (off-diagonal elements are equal to zero) describe the correlation coefficients between the first 5 eigenmodes of correlation, where the first eigenmode explains most of the variance.

The similarities between Equation 1 and a standard equation of correlation coefficient calculation are demonstrated in Equation 2.

$$r_{xy} = \frac{\sum(x - \bar{x})(y - \bar{y})}{\sqrt{\sum(x - \bar{x})^2 \sum(y - \bar{y})^2}}$$

Equation 2: A standard correlation coefficient calculation (adapted from Kirkwood & Sterne (2003)).

The CCCs describe the correlation coefficients between the eigenmodes of correlation, where the first eigenmode explains most of the variance. We therefore focus on results for the first eigenmode, however for the readers' information Figure 4.4 demonstrates all five eigenmodes of correlation for the sensorimotor network during the working memory task for one individual.

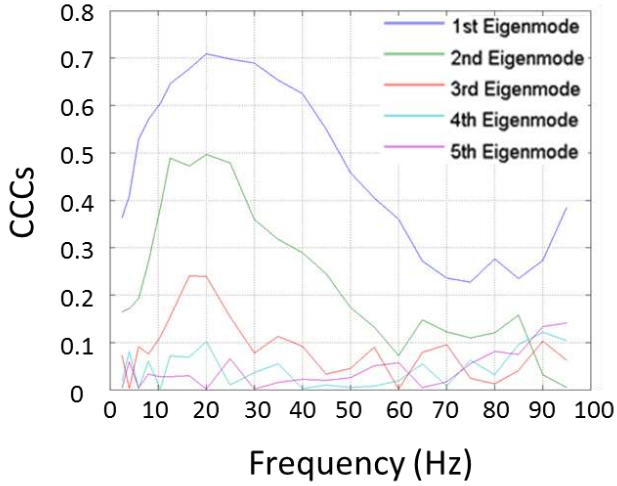


Figure 4.4: All five eigenmodes of correlation for the sensorimotor network during the working memory task for one individual.

Section 4.9.5.4. Statistical testing using phase randomisation

Statistical testing is necessary with CCA so as to assess the extent to which spurious changes in the temporal correlation structure between the seed and test regions are affecting the resultant CCCs. Phase randomisation allows us to generate a 'surrogate' data set based on the original with the same amplitude and cross-correlation structure, but with the phase of each component shifted to a random angle ((Brookes, et al., 2014; Prichard & Theiler, 1994); please see these references for a full mathematical description of this process or Appendix 19 Equation relating to phase randomisation, for the main equation relating to this process).

Since the surrogate datasets should not be correlated, we can use an iterative process (in this thesis we use 1000 iterations) to construct successive surrogate datasets which allow us to create a null distribution. Subsequently, we obtain a statistical threshold on which to base our conclusions of statistical significance of canonical correlation between the seed and test real data.

In Figure 4.5 the first eigenmode is demonstrated against a 95% confidence level (p = 0.05) and a Bonferroni corrected multiple comparisons 99.6% confidence level (p = 0.004). As Figure 4.5 clearly shows, the peak of canonical correlation is well above both thresholds and whilst this is true for most of the results presented, all results

have been corrected using the standard deviation of the value represented by the 99.6% confidence level for each individual, network region pair, and task.

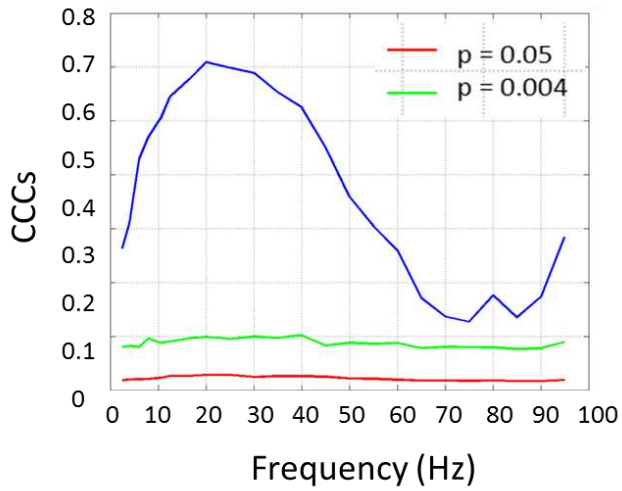


Figure 4.5: The first eigenmode of correlation for the same individual and network with statistical thresholds highlighted.

Section 4.9.6. Statistical analyses of canonical correlations

In order to delineate patterns of FC revealed by the canonical correlations we conducted a series of ANOVAs in which canonical correlations between pairs of network regions were the dependent variables.

We first tested the omnibus hypothesis that connectivity collapsed across pairs of network regions would vary by frequency, age group and task. For this analysis we a priori excluded effects of pair from consideration. We conducted a 4-way repeated measures ANOVA with 3 within-subject factors (task, network region pair and frequency) and 1 between-subject factor (age group) as follows:

- Task: 3 levels (relevance modulation, working memory and resting state);
- Network region pair: 8 levels (visual, sensorimotor, left DAN, right DAN, posterior DAN, anterior DAN, DAN4 and DAN5 region pairs);
- Frequency: 23 levels (1-4, 2-6, 4-8, 6-10, 8-13, 10-15, 13-20, 15-25, 20-30, 25-35, 30-40, 35-45, 40-50, 45-55, 50-60, 55-65, 60-70, 65-75, 70-80, 75-85, 80-90, 85-95 and 90-100 Hz);
- Age group: 4 levels (9-12 years, 13-16 years, 17-20 years, 21-25 years).

Age groupings were defined based on existing literature indicating grey matter peaks in the frontal and parietal lobes around age 11-12 and in the temporal lobe around age 16-17 (Giedd, et al., 1999a) which suggested a basis for splitting the age range equally into the 4 groups chosen. Additionally, the importance of separating late childhood from adolescence has been highlighted as some developmental studies have not done so in the past (Luna, Velanova & Geier, 2010).

To investigate the specific hypothesis that interhemispheric pairs of network regions would show different patterns of development, we conducted a 4-way repeated measures ANOVA with 3 within-subject factors (task, network region pair and frequency) and 1 between-subject factor (age group) as follows:

- Task: 3 levels (as before);
- Network region pair: 4 levels (visual, posterior DAN, sensorimotor and anterior DAN pairs);
- Frequency: 23 levels (as before);
- Age group: 4 levels (as before).

Thirdly, to investigate patterns of development within the DAN itself we conducted two omnibus repeated measures ANOVAs. The first tested the hypothesis that FC between the 6 pairs of DAN regions would differ in their developmental trajectories. We conducted a 4-way repeated measures ANOVA with 3 within-subject factors (task, network region pair and frequency) and 1 between-subject factor (age group) as follows:

- Task: 3 levels (as before);
- Network region pair: 6 levels (left, right, posterior, anterior, DAN4 and DAN5 DAN pairs);
- Frequency: 23 levels (as before);
- Age group: 4 levels (as before).

The second tested the hypothesis that interhemispheric pairs would differ in their developmental trajectory from intrahemispheric pairs. We conducted a 5-way repeated measures ANOVA with 4 within-subject factors (task, pair type, a dummy factor and frequency) and 1 between-subject factor (age group) as follows:

- Task (3 levels, as before);
- Network pair type: 2 levels (interhemispheric vs. intrahemispheric);
- A dummy factor not of interest: 2 levels (left and anterior vs. right and posterior) in order to allow means to be collapsed across pair type;
- Frequency: 23 levels (as before);
- Age group: 4 levels (as before).

To test the hypothesis that performance would vary by load and age group for the working memory task, a two-way repeated measures ANOVA was conducted with load as the within-subject factor, and age group as the between-subject factor. To test the hypothesis that performance would vary by age group for the relevance modulation task, one-way ANOVAs were conducted with mean RT, percentage of misses and percentage of false alarms as the dependent variables, and age group as the between-subject factor.

Where significant interactions between factors were observed, appropriate follow up ANOVAs were conducted to interpret these interactions and, where appropriate, Pearson's r correlation coefficients were computed to assess the relationship between variables. All ANOVA results were Greenhouse-Geisser corrected for non-sphericity.

Chapter 5. Results

Section 5.1. Excluded data

A total of 54 participants were scanned for this study but 11 were removed from analysis, as detailed here. Three adults have been excluded on the basis of scoring higher than 24 on the ASRS (Part A or Part B), which is indicative of AD/HD. One adult did not complete any of the MEG scans for the study. One adult did not complete the MRI scan for the study. One adolescent has been excluded for excessive unidentified noise throughout data acquisition. Four children have been excluded for excessive head movement during data acquisition of either MEG or MRI. Two children withdrew from the study.

Of the remaining 43 participants, two participants did not complete the resting state scan. Additionally 3 resting state datasets, 4 working memory datasets and 7 relevance modulation datasets were excluded for excessive head movement during data acquisition; 2 more datasets were excluded from the relevance modulation analysis for problems with the data files. One dataset was excluded from the relevance modulation analysis for very poor behavioural performance which casts doubt on whether the participant engaged in and/or understood the task. The final sample sizes are summarised in Table 5.1.

	Age 9-12	Age 13-16	Age 17-20	Age 21-25
Males/females (n)	3/7	3/3	4/8	0/11
Working memory sample size (n)	10	6	12	11
Relevance modulation sample size (n)	9	4	12	8
Resting state sample size (n)	7	5	14	12

Table 5.1: Sample sizes included in the data analysis.

Section 5.2. Canonical correlation results

CCCs were calculated between pairs of pre-defined brain regions belonging to known functional brain networks (the visual network, the sensorimotor network and the DAN). For convenience, the network region pairs are shown again in Figure 5.1.

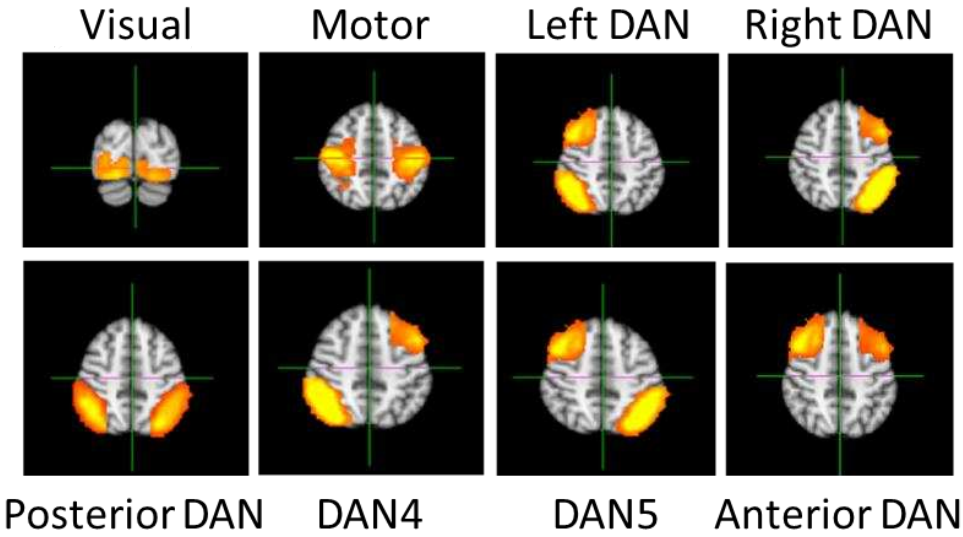


Figure 5.1: Network region pairs used in this thesis.

We first tested the omnibus hypothesis that connectivity collapsed across pairs of network regions would vary by frequency, age group and task. This ANOVA revealed a significant main effect of frequency on mean CCC collapsed over all pairs, tasks and age groups (F (3.787, 87.104) = 105.866, p = 0.000); the mean is shown in Figure 5.2.

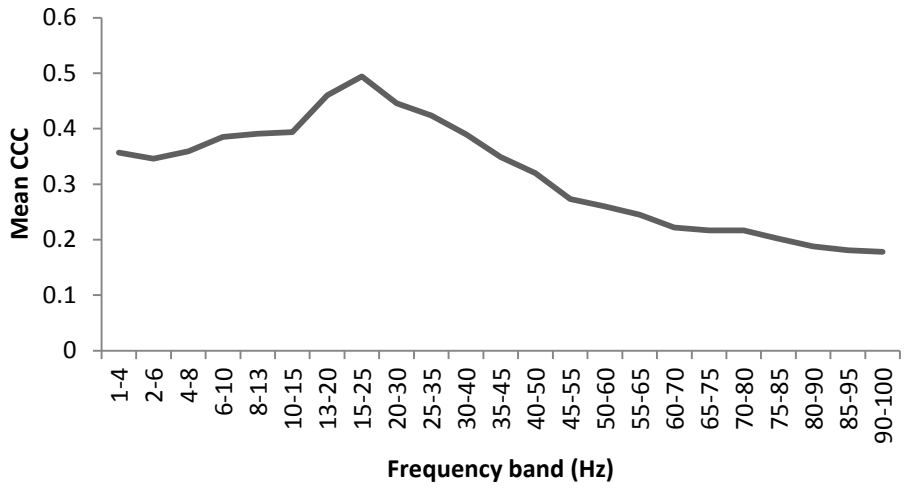


Figure 5.2: Mean CCCs plotted against frequency, collapsed over all network region pairs, tasks and age groups.

There were significant polynomial contrasts for linear (F (1, 23) = 233.384, p = 0.000), quadratic (F (1, 23) = 47.971, p = 0.000), and higher order terms (for example, cubic: F (1, 23) = 115.205, p = 0.000), indicating a systematic tendency for connectivity to vary by frequency. The significant linear contrast reflects the overall decrease in CCCs from low to high frequency, and the significant quadratic term reflects the main peak in the beta band (specifically 15-25 Hz), both of which are visible in Figure 5.2.

There was also a significant interaction between task and frequency (F (6.080, 139.845) = 3.450, p = 0.003) which can be seen in Figure 5.3.

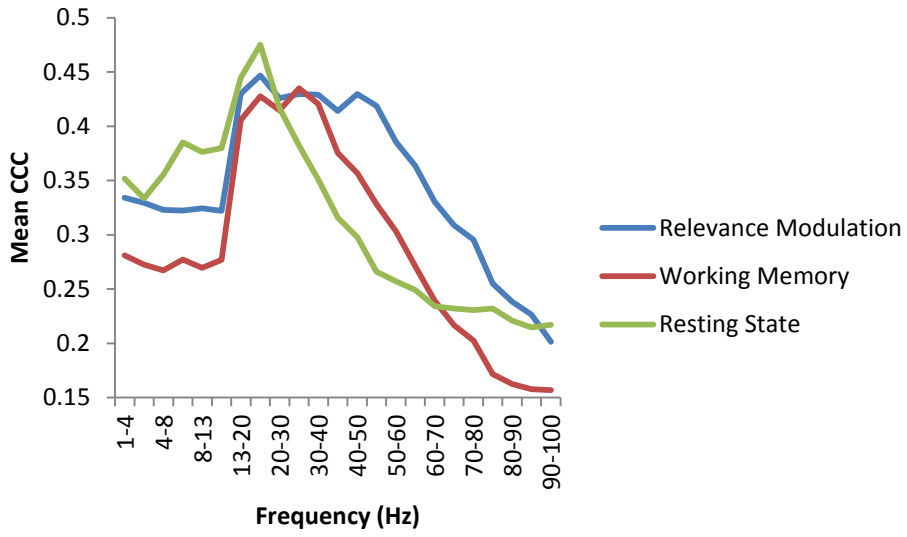


Figure 5.3: Mean CCCs for each task condition plotted against frequency, collapsed over all network region pairs and age groups.

It appears there is a tendency for FC to be strongest over delta to beta for resting state and weakest for working memory over this frequency range. Resting state exhibits the most rapid decline into gamma, followed by working memory and then relevance modulation. There also appears to be a tendency for FC to peak more strongly and exclusively in the beta band for resting state, whereas FC peaks from beta to low gamma for the working memory task and from beta to high gamma in the relevance modulation task. Given the higher level interactions between task and frequency and other factors (age group and pair), these interactions were explored in subsequent analyses.

Section 5.2.1. Developmental effects

Referring to our test of the omnibus hypothesis that connectivity collapsed across pairs of network regions would vary by frequency, age group and task; there was a significant main effect of age group on FC (F (3, 23) = 4.797, p = 0.010). This effect is shown in Figure 5.4.

Figure 5.4: Mean CCCs for each age group plotted against frequency, collapsed over all network region pairs and task conditions.

Post-hoc Tukey HSD corrected tests revealed a significant difference between ages 9-12 and ages 17-20 (p = 0.009), but not between the other age groups. This result revealed that the highest CCC values were in the older adolescent group (aged 17-20 years) and the lowest CCC values were in the child group (aged 9-12). Furthermore, a planned polynomial contrast revealed a significant quadratic term (p = 0.044) indicating a significant tendency for connectivity to peak between the youngest (age 9-12) and the oldest (age 21-25).

Section 5.2.1.1. Interhemispheric connectivity

Referring to our test of the hypothesis that interhemispheric pairs of network regions would show different patterns of development, we conducted a 4-way repeated measures ANOVA, but this time only the 4 interhemispheric pairs were included and we set out to test effects of age group on interhemispheric pair, frequency and task. The 4 pairs were: the visual network pair, posterior DAN pair, sensorimotor network pair, and the anterior DAN pair. This analysis revealed that whilst there was a significant effect of network region pair (F (1.957, 45.014) = 3.994, p = 0.026), this did not significantly interact with age group (F (5.871, 45.014) = 1.345, p = 0.258). This suggests that the visual, sensorimotor, anterior DAN and posterior DAN region pairs undergo similar FC developmental trajectories. There were, however, significant interactions between task and frequency (F (6.393, 147.043) = 3.056, p = 0.006) and task, frequency and age group (F (19.180, 147.043) = 1.678, p = 0.045).

In order to understand the significant interaction between task, frequency and age group, we conducted subsequent 3-way ANOVAs for each level of task. There was a significant interaction between frequency and age group for all task conditions (relevance modulation: F (12.062, 116.600) = 3.838, p = 0.000; working memory: F (12.322, 143.762) = 5.484, p = 0.000; resting state: F (12.591, 142.697) = 4.083, p = 0.000). The interaction between frequency and age group under each task condition can be seen in Figure 5.5.

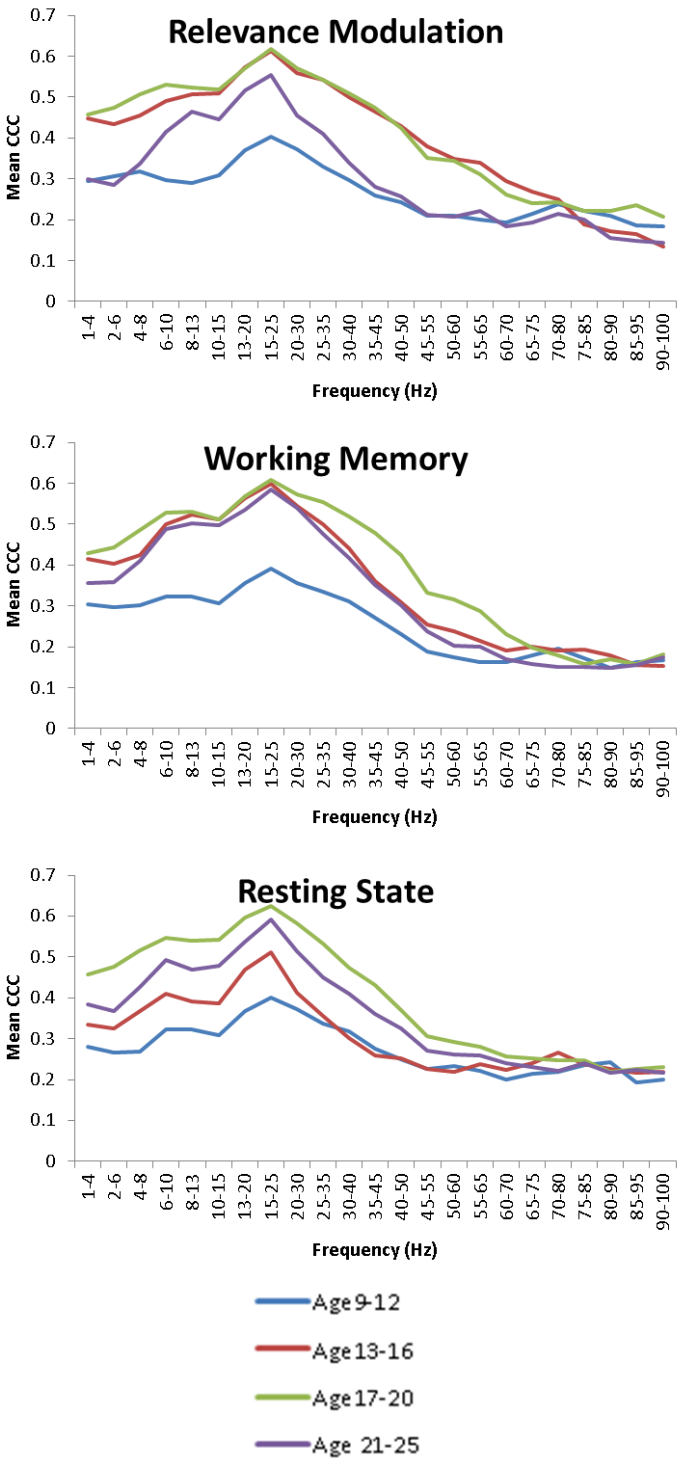


Figure 5.5: Mean CCCs for each task condition and age group plotted against frequency, collapsed over all network region pairs.

It is difficult to test these differences statistically, however visual inspection indicates that across tasks age 9-12 exhibit the weakest FC across the frequency range. For the relevance modulation task there is also a tendency for the adolescents (age groups

13-16 and 17-20) to exhibit stronger FC from delta to high gamma. For the working memory task there is a tendency for the oldest 3 age groups (13-16, 17-20 and 21-25 years) to show a similar FC profile across the frequency range, with adolescents aged 13-16 and 17-20 showing increased FC over low frequencies, and older adolescents aged 17-20 showing increased FC over higher frequencies. For the resting state there is a tendency for FC to be weaker in the age groups 9-12 and 13-16 across the frequency range, and for FC to be strongest in age group 17-20 across the frequency range.

Section 5.2.1.2. Dorsal attention network connectivity

To test the hypothesis that the trajectories of FC development differ between different pairs of regions within the DAN we ran a repeated measures ANOVA on the data for the various pairs of network regions tested within the DAN. This analysis revealed a significant main effect of network region pair (F (3.433, 78.954) = 22.136, p = 0.000) which interacted with both age group (F (10.298, 78.954) = 3.098, p = 0.002) and frequency (F (11.600, 266.796) = 8.117, p = 0.000) and there was a significant interaction between network region pair, frequency and age group (F (34.800, 266.796) = 1.936, p = 0.002).

In order to understand the significant interaction between pair, frequency and age group, we conducted subsequent 3-way ANOVAs for each level of network region pair. There was a significant interaction between frequency and age group for all network region pairs (left DAN: F (11.336, 86.908) = 3.055, p = 0.002; right DAN: F (11.142, 85.419) = 2.869, p = 0.003; posterior DAN: F (12.964, 99.389) = 4.322, p = 0.000; anterior DAN: F (13.449, 103.113) = 3.811, p = 0.000; DAN4: F (12.261, 94.003) = 3.624, p = 0.000; DAN5: F (13.587, 104.170) = 2.920, p = 0.001). The interaction between frequency and age group for each network region pair can be seen in Figure 5.6.

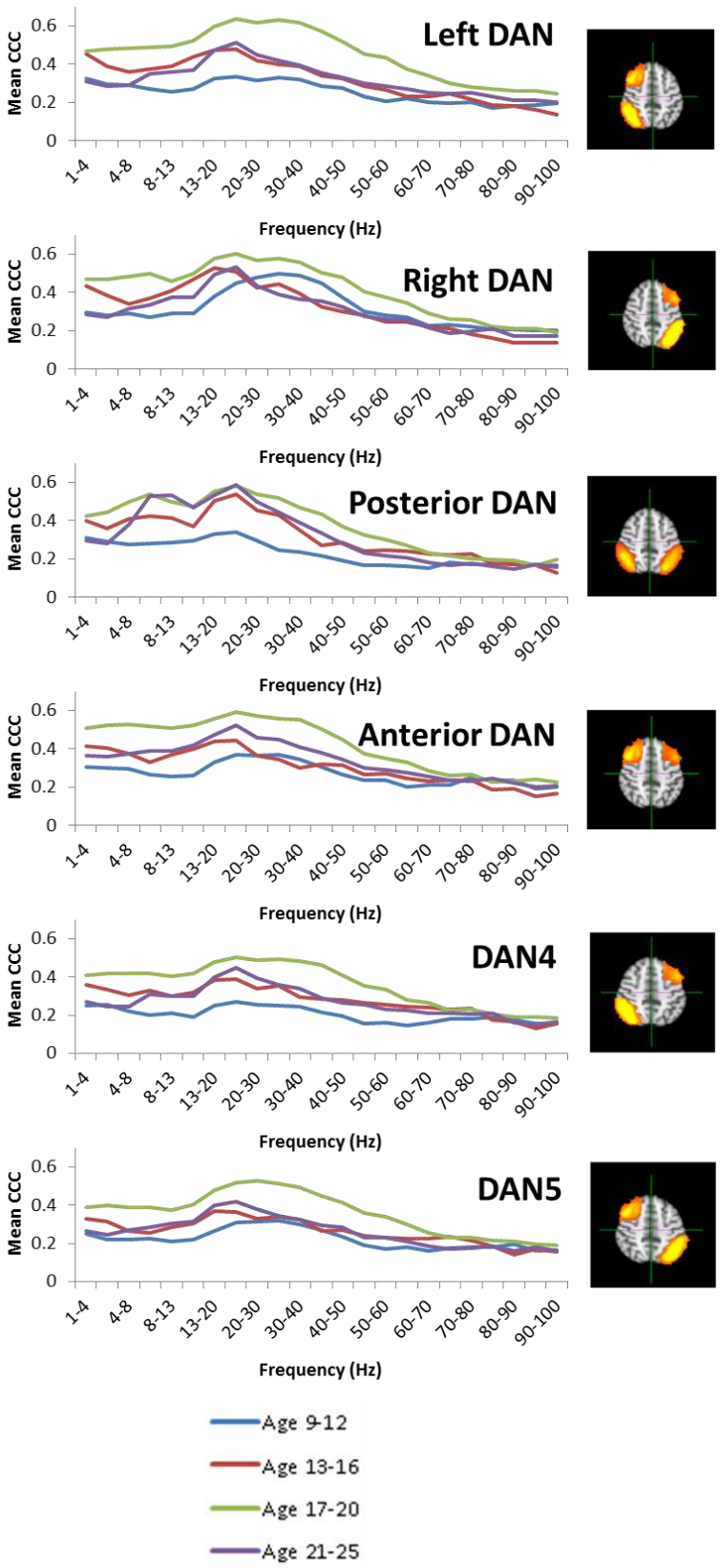


Figure 5.6: Mean CCCs for each age group and each network region pair in the DAN plotted against frequency, collapsed over all task conditions.

It is difficult to test these differences statistically, however on visual inspection there are several common developmental effects across the network region pairs of the DAN, and several unique developmental effects across network region pairs of the DAN. These have been summarised in Table 5.2.

Differences compared to age group 21-25	**Network region pairs**
Age 9-12 exhibits lower FC from δ to θ	Anterior
Age 9-12 exhibits lower FC from θ to low γ	Right, anterior and DAN5
Age 9-12 exhibits lower FC from low γ to high γ	Left, posterior and DAN4
Age 9-12 exhibits higher FC from low γ to high γ	Right
Age 13-16 exhibits higher FC from δ to θ	All
Age 13-16 exhibits lower FC from α to mid-β	Anterior
Age 17-20 exhibits higher FC from δ to high γ	Left, right, anterior, DAN4 and DAN5
Age 17-20 exhibits higher FC from mid-β to high γ	Posterior

Table 5.2: Summary of DAN network region pair developmental effects.

Additionally, there were significant interactions between task and frequency (F (6.207, 142.760) = 3.646, p = 0.002) and task, frequency and age group (F (18.621, 142.760) = 1.709, p = 0.042). In order to understand the significant interaction between task, frequency and age group, we conducted subsequent 3-way ANOVAs for each level of task. There was a significant interaction between frequency and age group for all task conditions (relevance modulation: F (10.669, 103.134) = 2.706, p = 0.005; working memory: F (9.682, 112.953) = 4.249, p = 0.000; resting state: F (11.179, 126.695) = 3.727, p = 0.000). The interaction between frequency and age group under each task condition can be seen in Figure 5.7.

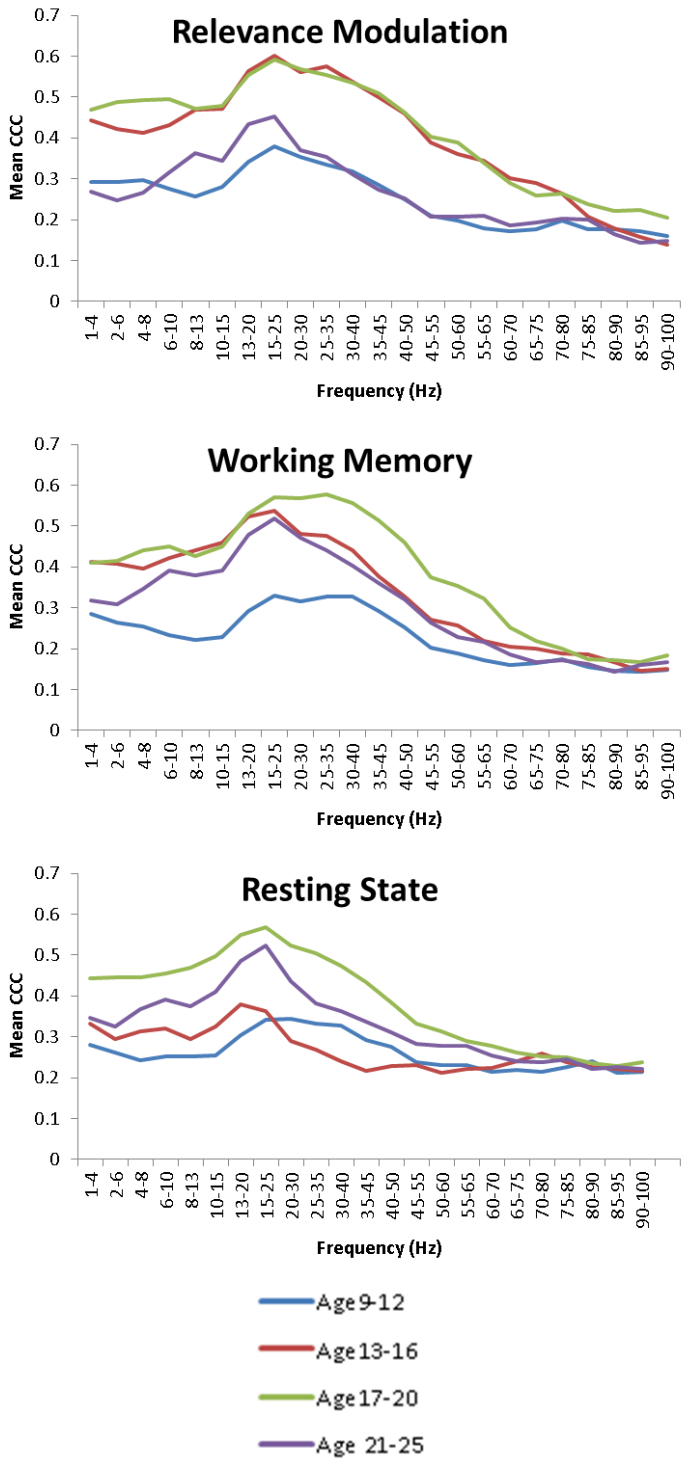


Figure 5.7: Mean CCCs for each task condition and age group plotted against frequency, collapsed over all network region pairs in the DAN.

From visual inspection it can be seen that in the relevance modulation task there is a tendency for age groups 13-16 and 17-20 to exhibit stronger FC across the frequency range, whilst age group 9-12 most notably exhibits reduced FC from alpha to mid-

beta. In the working memory task there is a tendency for age group 9-12 to exhibit weaker FC across the frequency range, whereas age group 13-16 exhibits stronger FC from delta to mid-beta and age group 17-20 exhibits stronger FC across the frequency range. During resting state, it can be seen that yet again the strongest FC is found in the age group 17-20, and the youngest two age groups (9-12 and 13-16) exhibit the weakest FC across the frequency range.

Section 5.2.1.3. Interhemispheric vs. intrahemispheric connectivity within the dorsal attention network

To test the hypothesis that interhemispheric pairs would differ in their developmental trajectory from intrahemispheric pairs, we conducted a 5-way repeated measures ANOVA on the data for the left and right DAN region pairs (intrahemispheric pairs), and posterior and anterior DAN region pairs (interhemispheric pairs) grouped by type (interhemispheric or intrahemispheric). This analysis revealed an effect of type of connectivity which approached significance (F (1, 23) = 4.169, p = 0.053) and significant interactions between type and age group (F (3, 23) = 4.284, p = 0.015), type and frequency (F (4.324, 99.449) = 7.605, p = 0.000), and type, frequency and age group (F (12.972, 99.449) = 2.108, p = 0.020). Figure 5.8 shows the interaction between type and age group.

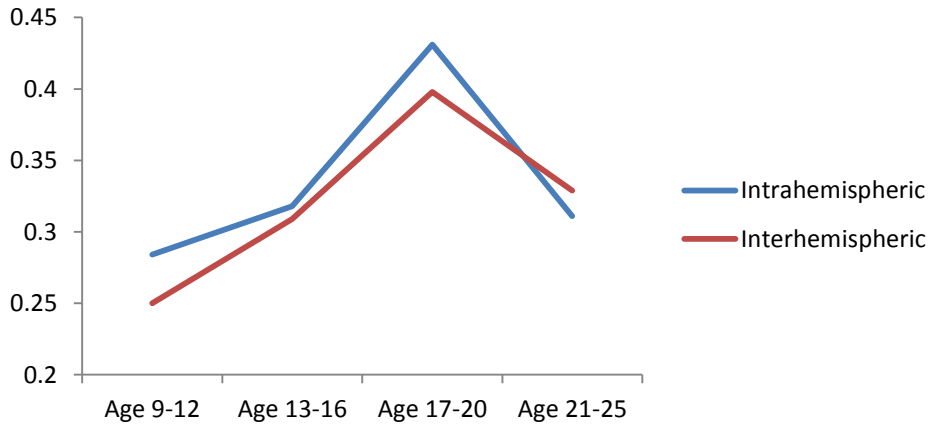


Figure 5.8: The development of interhemispheric and intrahemispheric connectivity.

As Figure 5.8 shows, from age 9-20 intrahemispheric connectivity appears to be weaker than interhemispheric connectivity, however in the oldest age group this relationship is reversed. Figure 5.9 shows the interaction between pair type and frequency.

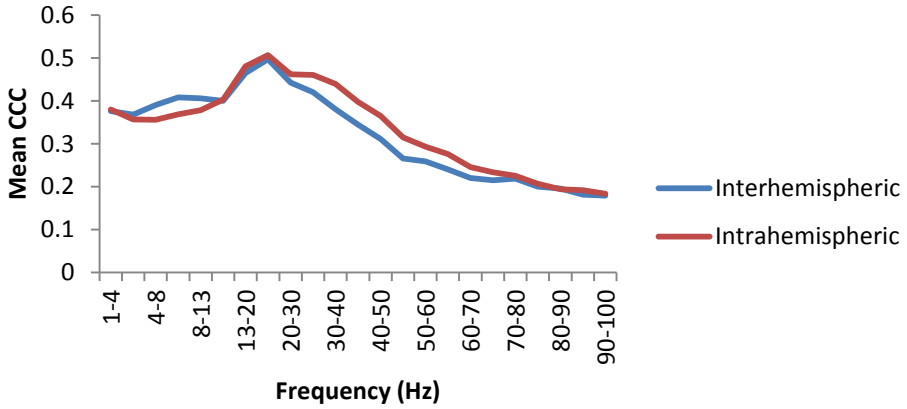


Figure 5.9: Mean CCCs across frequency, collapsed across interhemispheric and intrahemispheric network region pairs in the DAN.

Figure 5.9 indicates a tendency for interhemispheric FC to be stronger over lower frequencies (delta to low beta) but for intrahemispheric FC to be stronger over higher frequencies (high beta to high gamma). In order to understand the significant interaction between type, frequency and age group, we conducted subsequent 4-way ANOVAs for each level of type of network region pair. There was a significant interaction between frequency and age group for both intrahemispheric and interhemispheric pairs (intrahemispheric: F (10.683, 81.904) = 3.035, p = 0.002; interhemispheric: F (12.374, 94.871) = 4.987, p = 0.000). These interactions are shown in Figure 5.10.

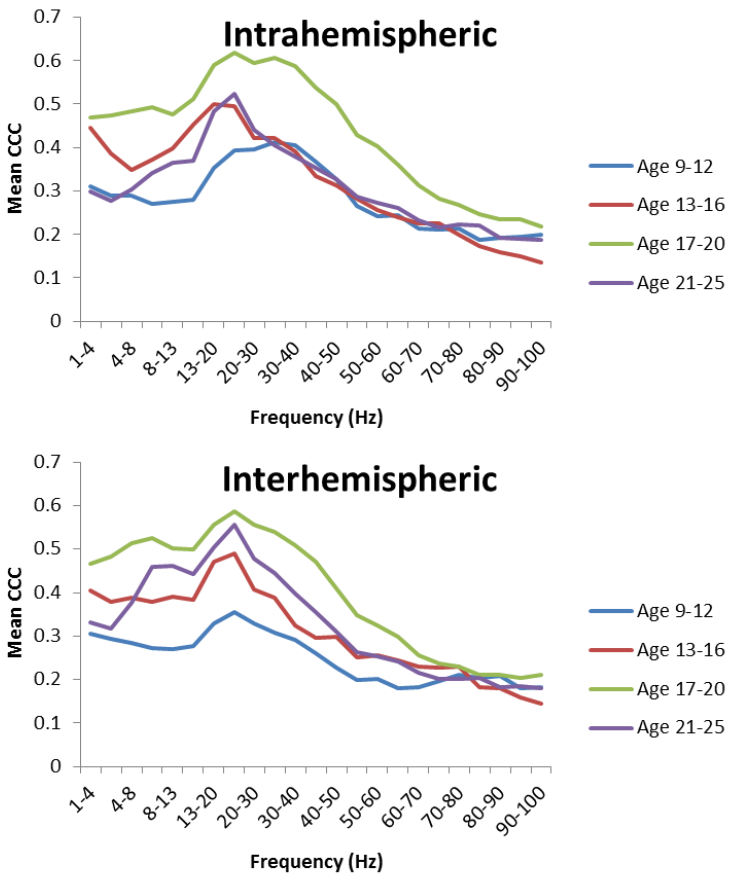


Figure 5.10: Mean CCCs for each age group and each type of network region pair (interhemispheric or intrahemispheric) in the DAN plotted against frequency, collapsed over all task conditions.

Figure 5.10 demonstrates again the tendency for age group 17-20 to exhibit the strongest FC across the frequency range (with regards to both interhemispheric and intrahemispheric FC). In comparison to the oldest age group (21-25), age group 13-16 exhibits stronger delta to theta FC in both interhemispheric and intrahemispheric connections, extending further into the beta band for intrahemispheric connectivity. Additionally, age 13-16 exhibit lower FC from alpha to gamma for interhemispheric connectivity, and age group 9-12 demonstrated lower FC from theta to gamma for intrahemispheric connectivity and from delta to gamma for interhemispheric connectivity.

Section 5.3. Canonical correlation results summary

Overall what these results seem to suggest is that that the profile of FC across frequency varies by task, region and type (intrahemispheric vs. interhemispheric) but nonetheless follows a broadly similar pattern, peaking in the beta band. There are developmental differences in the way the FC profile from 1-100 Hz varies by task, region and type. FC reaches a peak in late adolescence that is particularly marked in the beta band and shows a reduction in the early 20s. Interhemispheric connectivity in the DAN is weaker than intrahemispheric up until late adolescence, when there is a reversal in this pattern.

As a sanity check, we computed mean CCCs for each frequency for groups of 5 subjects at a time in ascending age order (known as a sliding box car; similar to the approach taken by Fair *et al. (2009)*). This summary of results is shown in Figure 5.11.

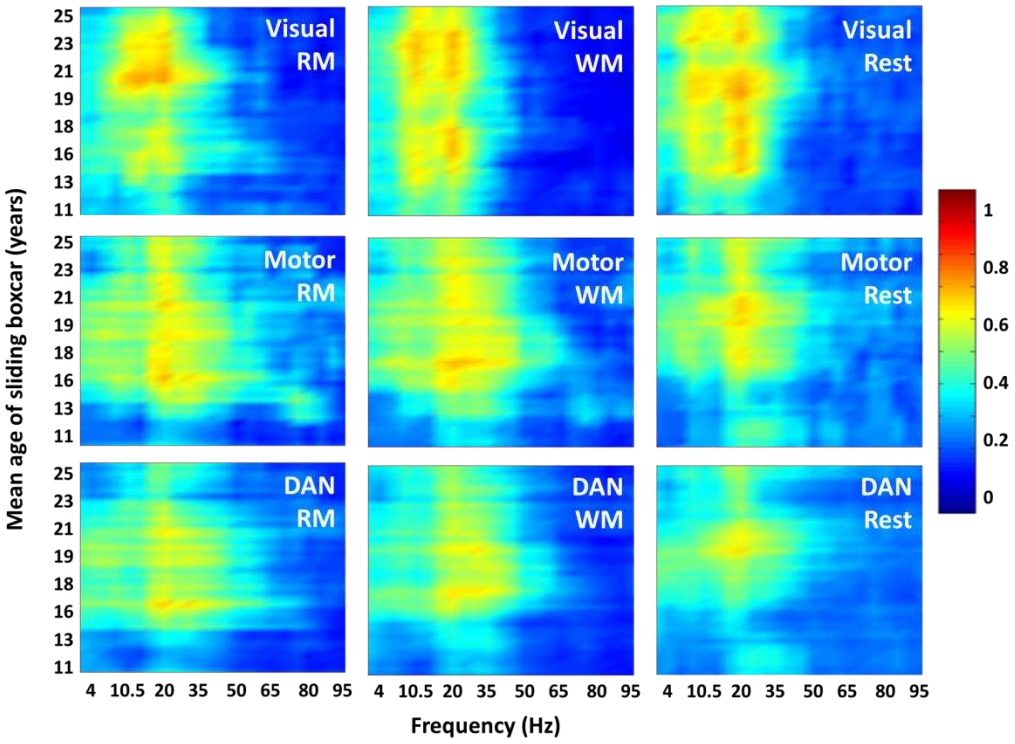


Figure 5.11: Mean CCCs collapsed across sliding boxcars of ascending age for the visual and sensorimotor network region pairs, and the DAN (collapsed across all 6 network region pairs) for the relevance modulation task (RM), working memory task (WM), and resting state (rest).

This exercise supported the interpretation that there is an increase in FC centred around 15-25 Hz which extends into gamma and low frequency bands. There is additionally a most striking alpha increase in the visual network region pair. The results also indicate that the effects seen are not being driven by certain individuals included in the analysis, and finally therefore support the interpretation that there is a notable increase in FC across frequencies in late adolescence, which appears to be refined in the young adults. In addition, it can be seen from Figure 5.11 that the beta peak in FC is very weak in the youngest subjects particularly in the DAN and for the resting state condition where the beta peak appears to be weak for a substantial age range (approximately age 9-16 years). A similar pattern of slightly later development of a strong beta peak in FC during resting state compared to task is also indicated in the sensorimotor network, whilst for the visual network this effect is almost the opposite, with perhaps the earliest development of strongest FC apparent during resting state and the latest development of strong FC seen for the relevance modulation task.

Section 5.4. Behavioural results

Section 5.4.1. Working memory behavioural results

Table 5.3 summarises behavioural performance from the working memory task, according to age group. Two datasets are excluded from performance analysis because the participants were pressing the wrong response button for some of the scan. However, after achieving 100% accuracy in their practise sessions we can be confident they did engage in the task and understand it. We have therefore not excluded their MEG data from analysis.

	Age 9-12	Age 13-16	Age 17-20	Age 21-25
Sample size (n)	8	6	12	11
% Overall accuracy, mean ± std (range)	92.71 ± 5.34 (86.11 - 100)	93.52 ± 4.54 (88.89 - 100)	96.30 ± 3.81 (88.89 - 100)	96.46 ± 3.53 (88.89 - 100)
% Load 2 accuracy, mean ± std (range)	98.96 ± 2.95 (91.67 - 100)	95.83 ± 6.97 (83.33 - 100)	97.92 ± 5.18 (83.33 - 100)	98.48 ± 3.37 (91.67 - 100)
% Load 4 accuracy, mean ± std (range)	97.92 ± 3.86 (91.67 - 100)	97.22 ± 4.30 (91.67 - 100)	95.83 ± 5.62 (83.33 - 100)	98.48 ± 3.37 (91.67 - 100)
% Load 6 accuracy, mean ± std (range)	81.25 ± 13.91 (58.33 - 100)	87.5 ± 6.97 (83.33 - 100)	95.14 ± 5.57 (83.33 - 100)	92.42 ± 10.18 (75 - 100)

Table 5.3: Performance across age groups during the working memory task.

Two-way repeated measures ANOVA revealed a significant effect of load on performance (F (1.437, 47.428) = 21.184, p = 0.000) but no significant effect of age group on performance (F (3, 33) = 1.845, p = 0.158. There was however a significant interaction between load and age group (F (4.312, 47.428) = 3.485, p = 0.012) and given the ceiling effects noted in several individuals at loads 2 and 4, the distribution shows a truncated range and these results should therefore be interpreted with caution. Figure 5.12 shows the interaction between load and age group.

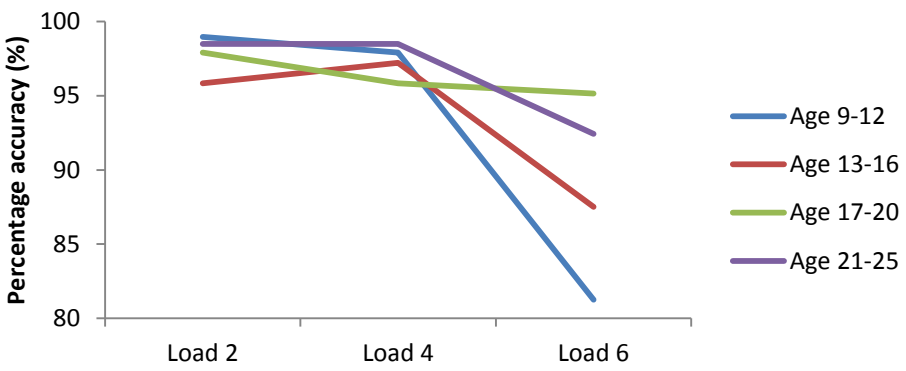


Figure 5.12: Performance on the working memory task, collapsed across load and age group.

One-way ANOVAs revealed no significant difference between age groups at load 2 (F (3, 36) = 0.589, *p* = 0.627) or load 4 (F (3, 36) = 0.742, *p* = 0.535), but a significant difference between age groups at load 6 (*F* (3, 36) = 3.814, *p* = 0.019), as Figure 5.12 would suggest.

Section 5.4.2. Relevance modulation behavioural results

Table 5.4 summarises behavioural performance data from the relevance modulation task, according to age group.

	Age 9-12	Age 13-16	Age 17-20	Age 21-25
Sample size (n)	9	4	12	8
Reaction time on correct trials, mean ± std (range)	799.83 ± 71.93 (696.33 – 900.36)	778.90 ± 114.50 (691.56 – 939.18)	762.71 ± 139.05 (655.76 – 1051.5)	785.21 ± 116.58 (591.93 – 911.24)
% False alarms, mean ± std (range)	26.91 ± 18.43 (0 – 58.82)	23.99 ± 10.73 (9.09 – 33.33)	23.67 ± 23.00 (0 – 78.57)	13.92 ± 19.31 (0 – 58.82)
% Missed targets, mean ± std (range)	10.99 ± 10.65 (0 – 35.29)	8.14 ± 8.45 (0 - 20)	5.85 ± 9.97 (0 – 33.33)	2.30 ± 4.48 (0 – 11.76)

Table 5.4: Performance across age groups during the relevance modulation task.

One-way ANOVA revealed no significant effect of age group on reaction times (*F* (3, 32) = 0.183, *p* = 0.907), percentage of missed targets (*F* (3, 32) = 1.384, *p* = 0.267), or percentage of incorrect responses (*F* (3, 32) = 0.662, *p* = 0.582).

Section 5.5. Possible confounds

One-way ANOVAs revealed no significant difference in IQ across age groups (F (3, 38) = 1.062, p = 0.378) demonstrating that all age groups had comparable IQ scores. As expected, there was a significant effect of age group on forward digit span (F (3, 35) = 3.32, $p < 0.04$), backward digit span (F (3, 35) = 3.48, $p < 0.03$) and RAN (F (3, 35) = 7.32, $p < 0.02$), demonstrating the anticipated development of working memory and processing speed.

Age group statistics relating to head size (estimated head circumference), maximal head movement during data acquisition, and signal strength (represented by the absolute lead field measurement) are summarised in Table 5.5.

	Age 9-12	Age 13-16	Age 17-20	Age 21-25
Sample size (n)	11	6	14	12
Estimated head circumference (cm), mean ± std (range)	53.70 ± 2.26 (48.91 – 57.20)	54.43 ± 1.44 (52.96 – 57.02)	55.89 ± 1.50 (52.38 – 57.74)	54.09 ± 1.68 (50.89 – 56.05)
Mean maximal head movement of included datasets (cm), mean ± std (range)	0.33 ± 0.20 (0.09 – 0.66)	0.36 ± 0.16 (0.16 – 0.53)	0.37 ± 0.16 (0.11 – 0.60)	0.31 ± 0.09 (0.18 – 0.45)
Mean absolute lead field (nAm), mean ± std (range)	0.33 ± 0.27 (0.04 – 0.78)	0.60 ± 0.18 (0.37 – 0.79)	0.42 ± 0.27 (0.06 – 0.91)	0.31 ± 0.24 (0.06 – 0.76)

Table 5.5: Head size, movement and signal strength measurements.

One-way ANOVA revealed a significant effect of age group on head circumference (F (3, 42) = 3.798, p = 0.018), but not mean absolute lead field (F (3, 42) = 2.111, p = 0.114) or mean maximal head movement (F (3, 42) = 0.301, p = 0.824), suggesting the effect of head circumference did not impact on signal strength or head

movement. Furthermore, a non-significant correlation (R = 0.095, p = 0.546) was found between estimated head circumference and mean absolute lead fields. Therefore, despite the significant effect of age group on estimated head circumference there is both a lack of significant effect of age group on absolute mean lead fields and an absence of a significant correlation between head circumference and absolute mean lead field. Critically, this indicates that signal strength remains minimally affected by the significant difference in head circumference between age groups. Furthermore, there are no significant correlations between head circumference, head movement or signal strength and age (R = 0.213, p = 0.171; R = -0.21, p = 0.891; R = -0.77, p = 0.625, respectively).

Finally, whilst most groups exhibit unequal male and female sample sizes, binomial distribution testing revealed that only one age group showed a significant gender imbalance (age 21-25, $p < 0.01$). Four-way repeated measures ANOVA revealed there was no significant main effect of gender (F (3, 42) = 0.167, p = 0.687), nor did gender interact significantly with task (F (1.872, 46.799) = 1.625, p = 0.209), network region pair (F (3.355, 83.871) = 0.576, p = 0.651) or frequency (F (2.777, 69.437) = 1.441, p = 0.240) and there were no higher level interactions of significance involving gender.

Chapter 6. Discussion

In this section, the aims and hypotheses of this thesis will be discussed with respect to the findings of this thesis alongside future directions, implications and limitations of this work. For convenience, the aims and hypotheses are re-stated here.

The aims of this exploratory study were to:

1) Delineate age-related changes in FC at rest and during goal-directed cognitive tasks defined as amplitude-amplitude correlations using MEG;
2) Create a rich data source for future developmental analyses.

The hypotheses pertaining to FC in this thesis were:

1) FC would vary by frequency, age group and task;
2) Interhemispheric pairs of network regions would show different patterns of development. In particular, the visual and sensorimotor network region pairs were expected to undergo less development than higher-order network region pairs belonging to the DAN, given that visual and sensorimotor areas of the brain are thought to reach structural maturity prior to higher-order association areas (Gogtay, et al., 2004; Huttenlocher, 1990; Taylor, et al., 2012);
3) Pairs of network regions within the DAN would show different FC developmental trajectories. In particular, previous studies have highlighted the possibility that the left and right DAN may develop differently (de Bie, et al., 2012);
4) Interhemispheric pairs of network regions would show different FC developmental trajectories compared to intrahemispheric pairs of network regions. In particular, we might expect to see that intrahemispheric connectivity is strongest in late adolescents/young adults given the development of hemispheric specialisation (Szaflarski, et al., 2006) and reportedly strong interhemispheric connectivity between homologous cortical areas in infants (Fransson, et al., 2007).

To briefly recap the findings of this thesis, we have firstly shown that FC varies systematically by frequency with a peak in the beta band (specifically 15-25 Hz) and a tendency to decrease from low to high frequencies. We have also shown that FC varies across different pairs of network regions; in particular there is a striking alpha peak in the visual network pair and FC is furthermore modulated by different cognitive demands (here we used three cognitive demands: working memory, attention and resting state).

Most importantly for the aims of this thesis, we have shown that FC changes across development non-linearly, with an increase from childhood (age 9-12) to late adolescence (age 17-20) followed by a reduction into young adulthood (age 21-25), at least in the females included in this study. This increase in FC is shown as a broadening of the spectrum and an increase in the magnitude of the beta peak (for instance, see Section 5.3 Canonical correlation results summary, Figure 5.11 where this developmental effect is particularly well visualised). In the young adults, the beta peak remains but is more refined than in the older adolescents, with regards to both amplitude and width. Whilst there are subtle yet statistically significant differences in how the FC profile varies by network region pair and cognitive demand, the overall pattern of FC development appears to be remarkably consistent across cognitive demands and networks.

We have additionally shown that interhemispheric and intrahemispheric FC within the DAN appear to follow different developmental trajectories. Specifically, the three youngest age groups (9-12, 13-16 and 17-20 years) showed consistently stronger FC between intrahemispheric pairs compared to interhemispheric pairs but this pattern was reversed in the oldest age group. Finally, we have shown that the reported results are unlikely to be confounded by IQ, head size, head movement or gender.

Section 6.1. Developmental findings

Section 6.1.1. Functional brain connectivity varies by frequency, age group and task

The main aim of this thesis was to investigate age-related changes in functional brain connectivity at rest and during goal-directed cognitive tasks between ages 9-25. We hypothesised that FC would vary by frequency, age group and task.

In line with previous reports such as Fair *et al.* (2007; 2008; 2009), we found FC to undergo significant change across the age range studied in this thesis. In particular we found that children (aged 9-12) exhibit weaker FC between pairs of brain regions belonging to previously identified functional brain networks (the visual network, the sensorimotor network and the DAN) compared to older subjects. However, a key novel component to our findings is that we found that FC follows a non-linear developmental trajectory peaking consistently in late adolescence and reducing into young adulthood. The developmental trajectory of FC therefore appears to follow a somewhat inverted-U-shape (for instance, see Section 5.2.1 Developmental effects, Figure 5.4).

Considering the further implications of this finding, we propose that FC appears to develop intermediately to grey and white matter. For instance, whilst the development of grey matter follows an inverted-U-shape trajectory which peaks earlier than the peak in FC reported in this thesis, the development of white matter (myelination – a key facilitator of efficient neural processing) follows a more linear trajectory continuing to increase past the upper age limit of this study (Giedd, 2004; Giedd, et al., 1999a). So whilst grey matter development peaks slightly earlier in life than the peak in FC we see here, white matter development continuing past the peak in grey matter development (Giedd, et al., 1999a) may account for the later peak in FC that we find. The next step in investigating this relationship further should involve a multi-modal study exploring measures of grey and white matter volume in conjunction with measures of FC. This finding may also have important implications for the knock-on effect of delayed or disrupted development of grey and white matter in the brain impacting on FC development.

Referring to the decrease in FC in the older participants included in this study compared to the adolescents, there is some evidence in the literature to suggest that

reduced activity in certain brain regions is linked to improved performance. In an fMRI study of mirror-reading in adults (reading mirror-reversed text), Poldrack *et al.* (1998) reported that skill-learning was not only accompanied by increased activity of certain brain regions but was also accompanied by reduced activity in some brain regions. In particular, there were skill-learning related reductions in activity exhibited in the occipital lobe (particularly in the primary visual cortex), and the right superior parietal cortex (part of the DAN). Similarly, in an fMRI study of selective attention in children between the ages of 9-12 years old, reductions in brain activity were also shown to accompany improved performance (Booth, et al., 2004). Booth and colleagues reported that increased activity in the bilateral superior parietal lobule and right lateral premotor cortex were indicators of poor performance on a visual search task. Together, these papers support the interpretation that reduced activity in certain functionally relevant brain regions may accompany improved performance or cognitive ability, a process which may underlie the late developmental reductions in FC reported here.

In the current study, it was important to minimize performance differences where possible so as to reduce the possibility of performance confounding age-related effects and at the very least this was certainly achieved for resting state (where there were no performance measures) and relevance modulation conditions (where there were no significant effects of age group on any of the performance measures). Our data also suggest that age group did not have a significant effect on overall performance in the working memory task, however given the ceiling effects in some individuals the results should be interpreted with some caution.

Importantly, we have shown here that FC development extends past adolescence and continues into young adulthood. Given the gender imbalance in our oldest age group (21-25) however, we suggest that whilst we can be confident the results are true for the females included in our study, future work will need to determine whether the same developmental trajectories are found in males by addressing the imbalance in this dataset and determining whether there is a significant gender effect in the final sample.

In concurrence with Uhlhaas *et al.* (2009b) our findings support the interpretation that FC development extends well into late adolescence and quite possibly concurs with their proposal that late adolescence marks a time of, “heightened vulnerability

of the developmental processes". Furthermore, our findings indicate that maturational processes occur in later adolescence and early adulthood that allow FC to stabilise or fine-tune in early adulthood. This presents a sound basis to explore the trajectory of FC past the age range studied in this thesis, so as to determine whether development extends past young adulthood or whether the age group 21-25 represents the final stages of FC development by way of fine-tuning maturational processes, and FC plateaus from young to mid-adulthood.

We believe the work presented in this thesis goes some way to addressing the present issue with the findings originally presented by Fair *et al.* (2007; 2009) which suggested that over the course of development short-range anatomically driven functional connections segregate and long-range functional connections integrate. Whilst the findings here agree with the principal put forward in the aforementioned reports that functional brain connectivity undergoes significant change across development, they contradict the specific developmental patterns elucidated in the study. However, our findings are more in line with the work presented by Fair and colleagues in 2008, where their 'local to global' hypothesis was not necessarily supported, but where they found instead that generally within-network connectivity developed and increased from childhood to adulthood.

As previously mentioned, the work by Fair *et al.* (2007; 2009) has come under criticism after a paper published recently by the same research group highlighted that movement artefact causes precisely the kinds of connections they found so prominent in the children included in their fMRI study (Power, et al., 2012). However, we have demonstrated that head movement is an unlikely confound in the current study (see Section 5.5 Possible confounds) so there are minimal grounds for concern that the results presented here are an artefact of greater movement in younger subjects.

Section 6.1.2. Visual, sensorimotor and higher-order interhemispheric network region pairs undergo similar developmental trajectories

With regards to our second aim, to investigate age-related changes in FC between interhemispheric pairs of brain regions including visual network region pairs, sensorimotor network region pairs and DAN region pairs, we hypothesized that interhemispheric pairs of network regions would follow different developmental trajectories. In particular, we hypothesized that the visual and sensorimotor network region pairs would undergo less development than higher-order network region pairs belonging to the DAN, given that visual and sensorimotor areas of the brain are thought to reach structural maturity prior to higher-order association areas (Gogtay, et al., 2004; Huttenlocher, 1990; Taylor, et al., 2012).

We in fact found no evidence of different developmental trajectories between the visual, sensorimotor, posterior and anterior DAN pairs. Despite extensive prior research indicating that visual and sensorimotor areas reach structural maturity prior to higher-order brain regions, the findings here indicate that they all critically undergo functional development into young adulthood and that there are no significant differences between their developmental trajectories.

The findings therefore point to a somewhat global mechanism of FC development that is broadly similar across brain regions. Critically, this work is the first example of such findings and suggests that functional brain networks supporting higher-order cognitive function are not alone in undergoing functional development over the age range 9-25; remarkably the visual and sensorimotor networks that reach structural maturity early on in life also appear to undergo significant functional development across this age range. Speculatively, even if it is not obvious that these skills are being developed over the age range studied (particularly over the higher end of the age range studied), the findings perhaps indicate that visual and sensorimotor processing skills are being developed, or are at least susceptible to development, until well into late adolescence, similar to higher-order cognitive functions. Uhlhaas *et al.* (2009b) previously defined adolescence as a 'critical period of brain maturation' and our results would support this interpretation in a much broader sense than we would have anticipated prior to this study.

As an aside, the posterior DAN exhibits signs of an alpha peak (for instance, see Section 5.2.1.2 Dorsal attention network connectivity, Figure 5.6) which may alert the reader to considering that signal leakage has occurred from visual areas, given the striking peak in alpha FC that we see for the visual network region pair (most obvious in Section 5.3 Canonical correlation results summary, Figure 5.11). Given the CCA approach of signal leakage reduction, whereby signal leakage reduction is only computed between pairs of network regions and is not computed between different pairs of network regions, it would not be impossible for this to be the case.

Whilst we cannot categorically rule out the possibility of signal leakage from visual areas into posterior areas, we believe there are reasons to suggest this has not occurred. Firstly, if the reader refers to Appendix 20 The visual network region pair, Figure 7.2, and Appendix 24 The posterior dorsal attention network region pair, Figure 7.18, it can been seen that it is in fact more of a theta peak that is exhibited in the posterior network region pair rather than an alpha peak which is the case in the visual network region pair. Secondly, it seems unlikely that signal leakage would present differently in different age groups - referring to the same figures, it can be seen that the peaks are noticeably more defined in the posterior DAN in older compared to younger age groups, and all age groups in the visual network region pair display more well defined peaks in comparison to the posterior DAN. However, as described, the possibility of signal leakage between different network region pairs cannot be ruled out categorically either way.

Section 6.1.3. Network region pairs within the dorsal attention network undergo different developmental trajectories

With regards to our third aim, to investigate age-related changes in FC between pairs of brain regions within the DAN, we hypothesized that in particular the left and right DAN may develop differently in line with previous studies which indicated this might be the case (de Bie, et al., 2012).

We did indeed find a significant interaction between pair, frequency and age group. Although it was difficult to define where this interaction was coming from, the most unique developmental difference apparent from visual inspection was that age 9-12

showed *stronger* FC from low to high gamma in the right DAN, compared to age 21-25. This finding may well be underpinned by significant cortical volume reduction which occurs over development particularly in the right DLPFC (Gogtay, et al., 2004) but again, the next step in investigating this potential relationship further should involve a multi-modal study exploring measures of grey and white matter volume in conjunction with measures of FC.

In all other network region pairs, age 9-12 exhibited weaker connectivity from approximately alpha to low gamma compared to the young adults aged 21-25. At the lower and higher ends of the frequency range there appears to be little difference between these 2 groups, however, in the anterior DAN pair age 9-12 showed weaker FC from delta to theta. Whilst it is difficult to say whether this particular effect is of significance, substantial evidence indicates that theta plays a role in maintaining information in working memory (Jensen & Tesche, 2002; Klimesch, 1999) and consistent with this, the DAN has a strong overlap with brain regions that support working memory, particularly the DLPFC regions that form the anterior DAN pair (Curtis & D'Esposito, 2003). Considering the implications of this finding, it supports the interpretation that the anterior DAN in particular may develop to support working memory function. This cannot categorically be determined from the present study since it was not within the scope or aims of this study to investigate performance-related effects of FC, however future work could look to clarify this finding.

In order to explore this theory further it may be necessary to explore specific performance effects and how these interact with FC between different network region pairs in the DAN. This would require some alterations to the working memory paradigm, given that in the present study we attempted to minimize performance differences where possible so as to reduce the possibility of performance confounding age-related effects. My suggestions for improvement in this case would be that load 2 is unnecessary – all participants found this load very easy. Load 4 is still easy and serves as a good level to start at in order to ease even the youngest children included in this study into the task. I would therefore suggest that the paradigm consist either of blocks of load 4 and 6 or 4, 6 and 7 to explore performance effects.

Section 6.1.4. Interhemispheric and intrahemispheric connections follow different developmental trajectories

Finally, with regards to our fourth aim, to investigate age-related changes in FC between interhemispheric and intrahemispheric pairs of brain regions in the DAN, we hypothesized that intrahemispheric connectivity would be strongest in late adolescents/young adults given the development of hemispheric specialisation (Szaflarski, et al., 2006) and reported strong interhemispheric connectivity between homologous cortical areas in infants (Fransson, et al., 2007). However, whilst we found evidence that interhemispheric and intrahemispheric connectivity within the DAN do undergo different developmental trajectories, we in fact found something quite unexpected. Whilst both intrahemispheric and interhemispheric connectivity followed a non-linear developmental trajectory peaking in late adolescence, from age 9-20 intrahemispheric connectivity was stronger than interhemispheric connectivity but in the oldest age group (age 21-25) this pattern was reversed (please see Section 5.2.1.3 Interhemispheric vs. intrahemispheric connectivity within the dorsal attention network, Figure 5.8).

Whilst the findings appear to be unexpected, it has previously been reported that lateralization follows a non-linear developmental trajectory increasing from age 5-20, plateauing from age 20-25 and then declining from age 25 onwards (Szaflarski, et al., 2006). The trajectory is similar to the trajectories revealed for both interhemispheric and intrahemispheric connectivity in this study, although in the aforementioned report the decline was not seen until age 25. It is not impossible that the results here reflect a similar process seen to occur slightly earlier than was reported by Szaflarski and colleagues (2006), and that it is simply the case that interhemispheric and intrahemispheric connectivity has not been investigated in this way before, comparing amplitude-amplitude correlations. In addition, the literature cited in Section 1.2.1.3 Interhemispheric and intrahemispheric connectivity in the developing brain mainly refers to young children or infants, outside of the age range of this study (Fransson, et al., 2009; Fransson, et al., 2007; Heinen, et al., 1998; Smyser, et al., 2010). However, it therefore remains unclear as to what could be the basis of this finding.

Given that the findings are unexpected, the reader may question whether it is possible that the gender imbalance in the oldest age group may have driven this finding, as the effect of gender on the corpus callosum has been controversial for many years (for instance, see Allen *et al.* (1991)). However, Giedd *et al.* (1999b) reported that in their study of corpus callosum development from childhood to adolescence, when they adjusted results for total cerebral volume, there was no significant effect of gender on mean area of the corpus callosum. Allen *et al.* (1991) additionally reported that whilst they found a gender difference in the shape of the corpus callosum, they did not find evidence to suggest gender differences in the area of the corpus callosum. Furthermore, Allen and colleagues reported that in children aged 2-15 years old the corpus callosum increases significantly in size, but in adults aged 16-79 it decreases significantly in size, therefore following an inverted-U-shaped trajectory. It is subsequently unclear if the gender imbalance in the oldest age group could underpin this finding and it will consequently be of particular importance to investigate this finding further in a balanced sample of males and females across the age range 9-25.

Section 6.2. Non-developmental findings

Given the developmental findings of interest with relevance to the main aims of this thesis, it is also reassuring that we have shown that FC peaks in the beta band, replicating findings in adults that implicate beta as a neural oscillatory frequency mediating integrative brain functions and within-network FC (Brookes, et al., 2011a; Donner & Siegel, 2011; Hipp, et al., 2012). Furthermore, this had previously only been indicated in adults and we have now extended this work to show this is also the case in children and adolescents (for example, see Section 5.2.1.1 Interhemispheric connectivity, Figure 5.5). It should be noted however, that whilst the findings implicate beta as mediating within-network connectivity and that this is a plausible interpretation, it may be that beta power correlations are simply the measure that is best correlated between regions.

We also presented novel findings showing that FC in the visual network peaks not only in the beta frequency but also strikingly in the alpha frequency (see Section 5.3 Canonical correlation results summary, Figure 5.11). Primarily, these findings suggest

that the visual network operates in a unique way. With the accumulating evidence for the role of alpha in inhibition (Klimesch, Sauseng, & Hanslmayr, 2007), the implications of this finding for the role of visual alpha in reducing attention to the external world during internally directed mental processing should be a subject of future investigation.

Section 6.3. Limitations

Section 6.3.1. Recruitment

Since we had trouble recruiting males in the oldest age group (age 21-25) for this study, work is currently underway to recruit additional males to add to the current dataset to even out the gender ratio. This will allow future work to both clarify the findings of this thesis and allow extended use of this valuable dataset in the future. Whilst we reported no significant effect of gender in the current sample, and no significant interactions between gender and other factors, given the imbalance of gender in the oldest age group it is still possible the gender imbalance has impacted on the results, and we therefore suggest the reported results be interpreted with a certain amount of caution.

It was also difficult to obtain an ideal sample size in the 13-16 year old age group, partly due to an unfortunate lack of interest from schools and extra-curricular clubs approached about advertising the research. For those who did hear about the research and were interested in taking part, there was an additional difficulty as a result of the need to exclude individuals on the basis of dental work and braces, which meant they were unsuitable for neuroimaging studies. In this age group, braces in particular are very common and there were several individuals who were interested but who could not take part in the study for this reason. Future work would also therefore look to recruit a larger sample of 13-16 year olds to add to this original dataset.

Section 6.3.2. Head size and movement

It is worth at this point considering the information collected referring to head movement in this study. Most would expect children to move considerably more than adults. In this study we found that the children whose datasets could be included in the analysis (i.e. those datasets where movement did not exceed our threshold of 8 mm) did not move any more than the adults included in the study. In fact, the correlation between head movement and age was found to be insignificant at R = -0.021 (p = 0.891).

It would be true to say however, that collecting useable datasets from children was more difficult for several reasons. It was firstly far more difficult to access and recruit suitable children. We also found that during the MEG scan it would become apparent very quickly whether a child would be able to keep still enough to yield useable data. We were fortunate that ultimately we were able to recruit sufficient numbers of children who were able to remain still enough and complete the scans, despite not completing the scans with some children due to their natural propensity for movement (as detailed in Section 5.1 Excluded data, 4 children were not included in any analysis on this basis). Children who had a natural tendency to move however, chose not to come back to complete the study which suggests that there was something about the set-up of the study (likely being asked to try to keep still) that they did not feel as comfortable with as those who took part.

This raises the possibility that the children included in our study are not fully representative of their age group. In particular, we cannot exclude the possibility that the children we studied had more mature networks than those excluded. However, it should be noted that any bias due to inclusion of children who were able to tolerate the procedure well does not account for finding that those children included nonetheless exhibited less developed connectivity than the adolescents.

It is additionally noteworthy that our finding of an insignificant correlation between head size and age (R = 0.213, p = 0.171) is in fact consistent with previously published data for children in the age range of our study (Nellhaus, 1968). Nellhaus (1968) showed overlaps in the reported head circumference ranges across the age range studied in this thesis. The most important thing is that the correlation between age and the absolute values of the mean lead fields (i.e. the level of signal strength

achieved for each individual) was not significant (R = -0.77, p = 0.625), indicating that signal strength did not correlate with age.

Section 6.3.3. Participant anxiety

Understandably, and as expected, some of the younger participants were anxious about the equipment being used in this research study. Whilst the MEG system is silent, it still requires participants to be left alone in unfamiliar surroundings with communication over an intercom. Fortunately, we only had one 10 year old who was anxious for the MEG scans. She visited several times to familiarise herself with the equipment before undergoing her first scan, and her father joined her in the scanner room for all of her MEG scans (he therefore underwent a safety screening, removed all metal from his person, and was placed in a corner of the room away from the scanner gantry). As previously mentioned in Section 4.3 Informed consent, it was of utmost importance particularly when dealing with the children in this study to offer appropriate explanations of the study to them and to form good relationships with both the participants and parents. In the case of the 10 year old described above, this proved to be an essential factor in successfully completing the scans.

Admittedly, more of our participants were anxious about the anatomical MRI scan, as this was a noisy scanner and more confined than the MEG system which they had previously experienced. It was more appropriate for the researcher (also an approved MRI assistant) to accompany nervous participants for their scan rather than a parent or guardian due to the additional safety concerns involved with MRI.

Given the difference in participant anxiety for the MEG and MRI sessions, we suggest that future studies using MEG offer a particular advantage over fMRI particularly given that anxiety may affect a functional scan (for example Seeley *et* al. (2007) reported that pre-scan anxiety correlated with FC in the salience network). Due to the fact that MEG is less daunting to children and participants of all ages in general, MEG should be exploited in these kinds of studies where an MRI scan need only last a few minutes to match with MEG data.

Section 6.3.4. Analysis limitations

Finally, the analysis pipeline presented in this thesis was extremely time consuming. In particular, the CCA itself took approximately 2 days to complete for each individual dataset included in the analysis (for a total of 110 datasets). As the analysis approach continues to be developed, in future it may be possible to look at different Brodmann Areas to explore how the connectivity between each and every area develops. This is not possible under current computational capacities, but should be a target for future work with this data source. Additionally, Pang (2011) has previously highlighted that the biggest challenge with MEG data collection in adolescents is unidentified noise. We were therefore conscious of first visually inspecting the MEG data, and secondly ensuring all results were corrected for each individual. Any underlying levels of noise in the data would have affected the values with which the results were corrected for each individual via the use of phase randomisation (please see Section 4.9.5.4 Statistical testing using phase randomisation, Figure 4.5). We are therefore confident that noise has been suppressed as effectively as possible in this thesis. Additionally, since beamforming suppresses perfectly correlated sources, artefacts in the data such as eye blinks should be effectively suppressed prior to the CCA pipeline.

Section 6.4. Summary and final conclusions

This study was the first of its kind exploring functional brain development from age 9-25 under different cognitive demands (resting state, working memory and attention) using MEG with CCA to explore FC via amplitude-amplitude envelope correlations. At the core of this thesis, we have presented novel findings that indicate FC increases over the developmental period until mid-adolescence. Thereafter, FC appears to level off and, at least in females, reduces resembling an inverted-U-shaped trajectory. This trajectory is furthermore consistently and robustly found across the different network region pairs and cognitive demands studied in this thesis. The findings therefore point to a somewhat global mechanism of FC development. Critically, this work is the first example of such findings and suggests that functional brain networks supporting higher order cognitive function are not alone in undergoing functional development; sensory networks that reach structural

maturity early on in life also undergo functional development from age 9 to 25. Uhlhaas *et al.* (2009b) previously defined adolescence as a 'critical period of brain maturation' and our results would support this notion. In any case, the paper highlighted the importance of late developmental processes supporting the maturation of cortical networks, which we have mirrored here in all networks of interest.

We suggest that our findings indicate that FC develops intermediately to grey and white matter; whilst grey matter development peaks slightly earlier than the peak in FC, white matter development continuing past the peak in grey matter development (Giedd, et al., 1999a) may account for this. We propose that future research should look to characterise the relationship between structural and functional development in more depth; the next step in investigating this relationship further should involve a multi-modal study exploring measures of grey and white matter volume in conjunction with measures of FC. We have additionally demonstrated that head movement is unlikely to confound the results presented here.

Fundamentally, the findings of this study make a significant contribution to work in this field by not only showing MEG to be a useful and informative technique to use in studies of this kind, but also by furthering our understanding of functional brain development and presenting novel findings pertaining to the development of FC. This thesis additionally provides substantial informed direction for future work efforts to further this research and our overall understanding of human functional brain development. Work in the near future will look to address the gender imbalance in this dataset and to replicate the novel findings of this thesis. Longer term, our data also offers an opportunity to investigate atypical development in comparison to the control data used in this study which would add greatly to the contributions of this original work.

One way to probe the findings of this thesis further to glean more understanding from the results presented would be to look at entropy in the same data within the same network region pairs, to explore complexity of neural processing within the networks studied. Increased complexity of neural processing may reflect increased efficiency of processing. Entropy gives us a measure of 'predictability' or 'complexity' of recorded neural activity; a straight line is very predictable because it does not encode any complex information, whereas a more complex oscillating signal is not as

predictable because it encodes more complex information. At the very least the level of complexity of neural processing could be explored in conjunction with functional brain connectivity development which could help us understand more about what the changes in FC achieve in the developing brain. This measure could furthermore potentially be exploited to draw conclusions about the efficiency of neural processing, perhaps in the future when the approach is more developed and well understood. This is an avenue of analysis that is currently being pursued by this research group.

The other extremely informative route for future work would be that of a multi-modal study. It has become increasingly clear through this thesis that structural brain development may play a fundamental role in underpinning functional development. The findings appear to suggest that a multi-model study incorporating measures of grey and white matter development alongside functional development may elucidate a great deal of the questions that this study has raised with regards to the relationship between structural and functional development.

Finally, this studentship has resulted in collection of a rich data source with which numerous further developmental analyses can be completed. Such analyses could include extended FC analyses exploring different measures such as phase-phase interactions, phase-power interactions, or even between network connectivity (such as between the visual network and DAN). Furthermore, to take advantage of the exquisite time resolution afforded by MEG, event-related analysis of task-positive data (relevance modulation and working memory) could be pursued.

Chapter 7. Appendices

Appendix 1. Ethics approval

Dear Miss Smith

Ethics Reference No: H13092012 CHS Psychiat

Study Title: A Magnetoencephalography study of functional brain networks in children, adolescents and adults

Chief Investigator: Professor Peter Liddle, professor of Psychiatry

Lead Investigators: Dr Maddie Groom, Lecturer, Dr Elizabeth Liddle, Lecturer, Miss Helen Smith, PhD Student, Division of Psychiatry, Dr Matt Brookes, Leverhulme Trust Early Career Fellow, School of Physics and Astronomy.

Duration of Study: 10/12-03/2014 18mths **No of Subjects:** 90

Thank you for your letter dated 24/09/2012 addressing the issues raised by the Committee and enclosing the following revised documents.

- Ethics Amendments 24/09/2012
- Ethics Application form: 9/27/2012
- Invitation letter to pupils ages 16-18 version 2 24.09.2012
- Invitation letter to Parent/Guardian ages 9-15 v2:24.09.2012
- Consent Form – Parental 27/09/12
- Information Sheet (ages 11-15) version 2: 27/09/12
- Information Sheet (Age 18-25) version 2: 27/09/12
- Recruitment Poster 24/09/12
- Detailed Project Proposal 24/09/2012
- Information Sheet (Ages 9-10) version 2: 27/09/12
- Information Sheet (Ages 16-17) version 2: 27/09/12
- Information Sheet Parent/Guardian version 2: 27/09/12

The following documents were reviewed 13/9/2012:

- Ethics Application form: 8/29/2012
- Detailed Project Proposal 29/8/2012
- Information sheet (Age 18-25) version 1: 29/08/12
- Information sheet Parent/Guardian version 1: 29/08/12
- Information sheet (Ages 16-17) version 1: 29/08/2012
- Information sheet (Ages 11-15) version 1: 29/08/12
- Information sheet (Ages 9-10) version 1: 29/08/12
- Consent form dated 29/08/12

- Consent form – Parental 29/08/12
- Recruitment Poster 29/08/2012
- Invitation letter to Head Teacher of School version 2 Date:24.08.2011
- Proposed Information letter from Head Teacher version 2, Date:24.08.2011

On review these are satisfactory and the study is approved.

Approval is given on the understanding that the Conditions of Approval set out below are followed.

Conditions of Approval

You must follow the protocol agreed and any changes to the protocol will require prior Ethics' Committee approval.

This study is approved for the period of active recruitment requested. The Committee also provides a further 5 year approval for any necessary work to be performed on the study which may arise in the process of publication and peer review.

You promptly inform the Chairman of the Research Ethics Committee of

Deviations from or changes to the protocol which are made to eliminate immediate hazards to the research subjects.

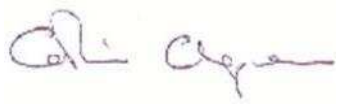
Any changes that increase the risk to subjects and/or affect significantly the conduct of the research.

All adverse drug reactions that are both serious and unexpected.

New information that may affect adversely the safety of the subjects or the conduct of the study.

The attached End of Project Progress Report is completed and returned when the study has finished.

Yours sincerely



Dr Caroline Chapman

Acting Chair, Nottingham University Medical School Research Ethics Committee

Appendix 2. Approved poster of advertisement

The University of Nottingham
UNITED KINGDOM · CHINA · MALAYSIA

Interested in the brain?

Want to help with research?

Then read below!

What are we researching and why?

Brain networks are groups of brain regions that work together in order to support everyday brain function. The exact roles of these networks remain unclear, but problems with the development of brain networks have been linked to several neurodevelopmental disorders. We are therefore investigating both the development and the functions of these networks further.

How do we do this?

We will be using magnetoencephalography (MEG) to look at brain networks in healthy volunteers aged 9-25 whilst they complete computer based tasks and additionally whilst they relax with eyes closed for several minutes.

How can you take part?

If you are interested in taking part and would like some more information, please contact Helen by email (mcxhjsm@nottingham.ac.uk) or by telephone (0115 74 84335).

Researchers: Helen Smith, Maddie Groom, Matt Brookes, Elizabeth Liddle and Peter Liddle

Appendix 3. Age 9-10 information sheet

You have been invited to take part in a research study. This sheet tells you about it. Before you decide if you want to take part or not, it is important that you understand what the study is about and what you will be asked to do if you take part.

What is research?

Research is a careful experiment that can help us find something out.

What are we researching and why?

As we grow up, the way our brains work changes. At the same time, we also become better at remembering things and controlling our actions and thoughts. We are doing this research because we want to find out more about how the brain changes with age and how this can affect us. Our brains are the control centre for our whole body!

Why have I been asked to take part?

You have been asked to take part because you are healthy and you are the right age for our study.

Do I have to take part?

No. It is up to you and your parent or guardian to decide. If you would like to take part and your parent agrees, we will ask your parent to sign a form. You can sign the form as well if you would like to. If you do not want to take part, no one will be unhappy or upset with you. Even if you want to take part but then change your mind, you are free to stop taking part at any time without giving a reason.

What will I need to do if I take part?

We will ask you to come to the University Of Nottingham for a morning or an afternoon during a school holiday, or a visit after school with your parent or guardian. You will be asked to come to a special building called the 'Sir Peter Mansfield Magnetic Resonance Centre'. In this building there are lots of different machines that show us how the brain works. These are called brain scanners. You can see the Sir Peter Mansfield Magnetic Resonance Centre in the picture below.

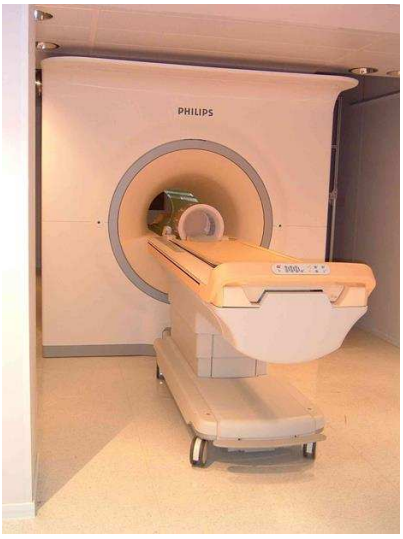
The Sir Peter Mansfield Magnetic Resonance Centre

When you arrive we will take you to a scanner we call a 'magnetoencephalography' scanner. It's a very long word, so we call it 'MEG' for short! You can see the MEG system in the picture below. Only your head goes into this scanner but we will ask you to change into some comfy clothes we have here before you go in. This scanner is silent. While you are in the scanner you will complete some tasks for us that are a bit like computer games. Each game will last about 20 minutes and you will be able to take lots of breaks. During the scan you can speak to us at any time if you are not feeling well.

A MEG system

After this, we will take you to a different scanner called a 'magnetic resonance imaging' scanner. This is also quite a long name, so we call it 'MRI' for short! You can see the MRI scanner in the picture below. A bit more than just your head will need to go into this scanner but this one will be quicker than the MEG scan. This scanner is

noisy, a bit like a drill, so you will get headphones to wear inside this one. Finally, before you go home we will do some short tests with you that just involve paper, pencil and speaking and after your visit we can send you a picture of your scan if you want!



An MRI scanner

Does it hurt?

No. Nothing you will be doing is dangerous or hurts. An adult will be with you the whole time and if you want to stop or take a break you can do so at any time.

Will it be fun?

Yes – we really hope you enjoy the computer games!

Who will know that I'm taking part?

The only people that will know that you are taking part are the adults running this project, your parent or guardian and anyone else you choose to tell.

Who is organising the project?

Dr Maddie Groom at the University of Nottingham is organising this project.

What if something goes wrong?

It is very unlikely that something will go wrong but if you are not happy about any of the things that you are asked to do, you can tell the researchers or you can ask your parent or guardian to tell the researchers for you.

What happens when the research stops?

The researchers will write about the study so that they can share the results with lots of other people but your name will never be told to anyone else.

Has anyone checked the study is OK to do?

Before any research study is done, it has to be checked by a 'Research Ethics Committee'. They make sure the research is fair and that you will be safe. This project has been checked by the University of Nottingham Ethics Committee.

What now?

If you and your parent decide to take part, we will see you soon. If you have any questions, you can ask us yourself or ask your parent or guardian to ask us for you.

Contact details

Helen Smith, Email: mcxhjsm@nottingham.ac.uk, Telephone: 0115 74 84335

Room A10 (PhD office)

The Division of Psychiatry

Institute of Mental Health

The University of Nottingham

Innovation Park

Triumph Road

Nottingham

NG7 2TU

Thank you very much for reading this sheet!

Appendix 4. Age 11-15 information sheet

You are being invited to take part in a research study. Before you decide whether to take part or not it is important that you understand why the research is being done and what you will be asked to do. This information sheet answers some common questions about the study. Please think about this information carefully and talk about it with your family and friends if you want to and please ask us if there is anything you are not clear about.

What are we researching and why?

As we grow up, the way our brains work changes. At the same time, we also become better at remembering things and controlling our actions and thoughts. We are doing this research because we want to find out more about how the brain changes with age and how this can affect us. Our brains are the control centre for our whole body!

Why have I been asked to take part?

You have been asked to take part because you are healthy and you are the right age for our study.

Do I have to take part?

No. It is up to you and your parent or guardian to decide. If you would like to take part and your parent agrees, we will ask your parent to sign a form. You can sign the form as well if you would like to. If you do not want to take part, no one will be unhappy or upset with you. Even if you want to take part but then change your mind, you are free to stop taking part at any time without giving a reason.

What will I need to do if I take part?

We will ask you to come to the University Of Nottingham for a morning or an afternoon during a school holiday, or a visit after school with your parent or guardian. You will be asked to come to a building called the 'Sir Peter Mansfield Magnetic Resonance Centre'. You can see the Sir Peter Mansfield Magnetic Resonance Centre in the picture below.

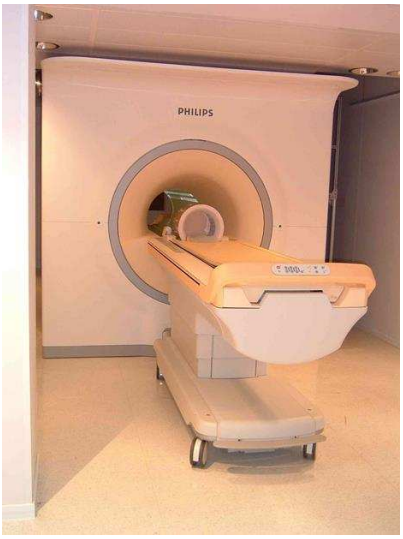
The Sir Peter Mansfield Magnetic Resonance Centre

When you arrive we will take you to a scanner we call a 'magnetoencephalography' scanner. It's a very long word, so we call it 'MEG' for short! You can see the MEG system in the picture below. Only your head goes into this scanner but we will ask you to change into some comfortable clothes we have here before you go in. This scanner is silent. While you are in the scanner you will complete some tasks for us that are a bit like computer games. Each game will last about 20 minutes and you will be able to take lots of breaks. During the scan you can speak to us at any time if you are not feeling well.

A MEG system

After this, we will take you to a different scanner called a 'magnetic resonance imaging' scanner. This is also quite a long name, so we call it 'MRI' for short! You can see the MRI scanner in the picture below. A bit more than just your head will need to go into this scanner but this one will be quicker than the MEG scan. This scanner is

noisy, a bit like a drill, so you will get headphones to wear inside this one. Finally, before you go home we will do some short tests with you that just involve paper, pencil and speaking.



An MRI scanner

What should I do before I arrive?

Please do not wear any make-up on the day of your visit and if you wear a bra, please make sure it does not have any wire in it, or you will need to take if off when you change clothes.

Does it hurt?

No. Nothing you will be doing is dangerous or hurts. An adult will be with you the whole time and if you want to stop or take a break you can do so at any time.

What are the possible disadvantages and risks of taking part?

MRI uses radio waves similar to those used in radio and TV transmission. These have a much lower energy than X-rays and are considered safe. While there is no evidence to suggest that MRI is harmful during pregnancy, the Medicines and Healthcare products Regulatory Agency advises against scanning pregnant women. We have decided not to test for pregnancy as routine but if you think you may be pregnant you should not be scanned. Pregnancy tests are available in the women's toilets. There are no known risks of MEG.

Who will know that I'm taking part?

The only people that will know that you are taking part are the adults running this project, your parent or guardian and anyone else you choose to tell. All information which is collected about you during the study will be kept **strictly confidential**. Any information about you stored on a computer will not have your name stored with it – instead, a unique code will be used in place of your name so that you cannot be identified.

Who is organising the project?

Dr Maddie Groom at the University of Nottingham is organising this project.

What will I get for taking part?

We will pay for your travel expenses and we will pay your parent £15 for the time you have given up to take part. We can send you a picture of your scan after the visit if you want too!

What if something goes wrong?

It is very unlikely that something will go wrong but if you are not happy about any of the things that you are asked to do, you can tell the researchers or you can ask your parent or guardian to tell the researchers for you.

What happens when the research stops?

The researchers will write about the study so that they can share the results with lots of other people but your name will never be told to anyone else.

Has anyone checked this study is OK to do?

Before any research study is done, it has to be checked by a 'Research Ethics Committee'. They make sure the research is fair and that you will be safe. This project has been checked by the University of Nottingham Ethics Committee.

What now?

If you and your parent decide to take part, we will see you soon. If you have any questions, you can ask us yourself or ask your parent or guardian to ask us for you.

Contact details

Helen Smith, Email: mcxhjsm@nottingham.ac.uk, Telephone: 0115 74 84335

Room A10 (PhD office)

The Division of Psychiatry

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The University of Nottingham

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Thank you very much for reading this sheet!

Appendix 5. Age 16-17 information sheet

You are being invited to take part in a research study. Before you decide whether to take part or not it is important that you understand why the research is being done and what you will be asked to do. This information sheet answers some common questions about the study. Please think about this information carefully and talk about it with your family and friends if you want to and please ask us if there is anything you are not clear about.

What are we researching and why?

As we grow up, the way our brains work changes. At the same time, we also become better at remembering things and controlling our actions and thoughts. We are doing this research because we want to find out more about how the brain changes with age and how this can affect us.

Why have I been asked to take part?

You have been asked to take part because you are healthy and you are the right age for our study.

Do I have to take part?

No. It is up to you to decide. If you would like to take part, we will ask you to sign a form. You can sign the form as well if you would like to. If you do not want to take part, no one will mind. Even if you want to take part but then change your mind, you are free to stop taking part at any time without giving a reason.

What will I need to do if I take part?

We will ask you to come to the University Of Nottingham for a morning or an afternoon during a school holiday, or a visit after school with your parent or guardian. You will be asked to come to a building called the 'Sir Peter Mansfield Magnetic Resonance Centre'. You can see the Sir Peter Mansfield Magnetic Resonance Centre in the picture below.

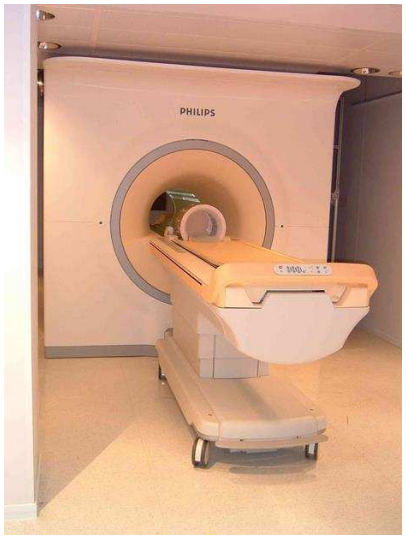
The Sir Peter Mansfield Magnetic Resonance Centre

When you arrive we will take you to a scanner we call a 'magnetoencephalography' scanner. It's a very long word, so we call it 'MEG' for short! You can see the MEG system in the picture below. Only your head goes into this scanner but we will ask you to change into some comfortable clothes we have here before you go in. This scanner is completely silent. While you are in the scanner you will complete some tasks for us that are a bit like computer games. Each game will last about 20 minutes and you will be able to take lots of breaks. During the scan you can speak to us at any time if you are not feeling well.

A MEG system

After this, we will take you to a different scanner called a 'magnetic resonance imaging' scanner. This is also quite a long name, so we call it 'MRI' for short! You can see the MRI scanner in the picture below. A bit more than just your head will need to go into this scanner but this one will be quicker than the MEG scan. This scanner is

noisy, a bit like a drill, so you will get headphones to wear inside this one. Finally, before you go home we will do some short tests with you that just involve paper, pencil and speaking.



An MRI scanner

What should I do before I arrive?

Please do not wear any make-up on the day of your visit and if you wear a bra, please make sure it does not have any wire in it, or you will need to take if off when you change clothes.

Does it hurt?

No. Nothing you will be doing is dangerous or hurts. A researcher will be with you throughout, and if you want to stop or take a break you can do so at any time.

What are the possible disadvantages and risks of taking part?

Magnetic resonance imaging (MRI) uses radio waves similar to those used in radio and TV transmission. These have a much lower energy than X-rays and as such are considered safe. While there is no evidence to suggest that MRI is harmful during pregnancy, the Medicines and Healthcare products Regulatory Agency advises against scanning pregnant women. We have decided not to test for pregnancy as routine but if you may be pregnant you should not be scanned. Pregnancy tests are available in the women's toilets. There are no known risks of MEG.

What happens if you find something abnormal on my scan?

Since you are healthy, it is extremely unlikely that your scan will show any abnormality. Even if there were an abnormality it is unlikely that we would notice it since we are taking these scans for scientific research. They are not the same as scans collected by doctors for medical purposes and the pictures will not be looked at by a radiologist (a doctor qualified to find abnormalities in scans).

If we did suspect that there was something apparently abnormal on your scan then the scan will be sent to a radiologist who will contact you if they decide that the scan is abnormal and requires further investigation.

Who will know that I'm taking part?

The only people that will know that you are taking part are the adults running this project, your parent or guardian and anyone else you choose to tell. All information which is collected about you during the study will be kept **strictly confidential**. Any information about you stored on a computer will not have your name stored with it – instead, a unique code will be used in place of your name so that you cannot be identified.

Who is organising the project?

Dr Maddie Groom at the University of Nottingham is organising this project.

What will I get for taking part?

We will pay for your travel expenses and pay you £15 for the time you have given up to take part. We can also send you a picture of your scan after your visit if you wish.

What if something goes wrong?

It is very unlikely that something will go wrong but if you are not happy about any of the things that you are asked to do, you can tell the researchers or you can ask your parent or guardian to tell the researchers for you.

What happens when the research stops?

The researchers will write about the study so that they can share the results with lots of other people but your name will never be told to anyone else.

Has anyone checked the study is OK to do?

Before any research study is done, it has to be checked by a 'Research Ethics Committee'. They make sure the research is fair and that you will be safe. This project has been checked by the University of Nottingham Ethics Committee.

What now?

If you and your parent decide to take part, we will see you soon. If you have any questions, you can ask us yourself or ask your parent or guardian to ask us for you.

Contact details

Helen Smith, Email: mcxhjsm@nottingham.ac.uk, Telephone: 0115 74 84335

Room A10 (PhD office)

The Division of Psychiatry

Institute of Mental Health

The University of Nottingham

Innovation Park

Triumph Road

Nottingham

NG7 2TU

Thank you very much for reading this sheet!

Appendix 6. Age 18-25 information sheet

You have been invited to take part in a research study. Before you decide whether to take part or not, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with friends and relatives if you wish to. Do ask us if there is anything that is not clear or if you would like more information and take your time to decide whether you wish to take part or not. If you decide to take part, please keep this leaflet for future reference. Thank you for reading this information sheet.

Background

The way the brain operates changes with age and is therefore different between children adolescents and adults. At the same time, cognitive abilities for example memory, action control and flexible thinking also change with age. However, the links between the changes that take place in the brain and the changes in cognitive ability have not yet been fully understood. We want to understand more about the relationship between changes in brain function and cognitive ability with age. It is important to understand this because problems with the development of functional brain networks has been linked to several developmental disorders like attention deficit/hyperactivity disorder (AD/HD), autism spectrum disorders (ASD), and Tourette syndrome (TS). To investigate this, we will measure brain activity using a device called a 'magnetoencephalography' ('MEG') scanner.

Why have you been chosen?

You have been chosen because you are a healthy young adult aged 18-25.

Do you have to take part?

It is up to you to decide whether or not to take part. If you do decide to take part you will be given this information sheet to keep and be asked to sign a consent form. However, it is important that you understand that you will be free to withdraw at any time and without giving a reason.

What does the study involve?

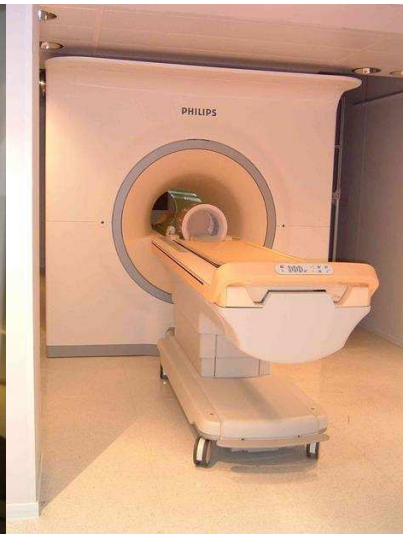
We will ask you to come to the University Of Nottingham for a morning or an afternoon visit lasting approximately 3 hours. If you are still at school this visit will be scheduled during a school holiday, or you will be asked to visit after school one day. You will be shown the MEG system (pictured below) and given an opportunity to ask questions. You will then practice the tasks that we will ask you to complete once in the MEG system.

To prepare for the MEG scan, we will make a map of the shape of your head on our computer and you can just sit comfortably on a chair whilst we do this. You will then

be taken through to the scanner and made comfortable. Only your head goes into this scanner and you will be lying down during the scan. The scanner is completely silent. You will be asked to complete 2 cognitive tasks each lasting up to 20 minutes, with regular breaks. We will ask you to stay as still as possible during each task. Throughout the scan you will be able to communicate with the scanner operator.

After the MEG scan we will take you to a different scanner called a 'magnetic resonance imaging' scanner ('MRI' for short).You can see the MRI scanner in the picture below. A bit more than just your head will need to go into this scanner but this one will be quicker than the MEG scan, although it is noisy. It sounds a bit like a pneumatic drill so you will be given headphones to wear for this one.

A MEG system



An MRI scanner

Finally, before you go home we will do some short tests with you that just involve paper, pencil and speaking. Your travel expenses will be reimbursed (please keep any receipts if possible) and you will be paid an inconvenience allowance of £15 for your time. We will also be able to send you a picture of your scan after your visit if you wish.

You will be asked to change into medical scrubs when you arrive because we will need to make sure there is no metal on you during your scans. We also advise women to refrain from wearing an underwired bra on the day of their visit, or they will need to take it off before the scans. Please do not wear make-up on the day of your visit as some make-up is known to contain metal particles and this can have a very negative impact on our data collection. It is very difficult to remove make-up effectively if it has already been applied on the day, but we do have make-up remover available for you to use should you need it when you arrive.

What are the possible disadvantages and risks of taking part?

Magnetic resonance imaging (MRI) uses radio waves similar to those used in radio and TV transmission. These have a much lower energy than X-rays and as such are considered biologically safe. We scan at up to twice the field used in standard MRI scanners and use more rapidly changing 'field gradients', but these differences are considered safe. We will be following strict national safety guidelines which are designed to prevent the theoretical hazards of MRI which are burns and electric shocks. Such accidents have never occurred in the MR centre and have only very rarely occurred elsewhere.

While there is no evidence to suggest that MRI is harmful during pregnancy, the Medicine and Healthcare products Regulatory Agency advises against scanning pregnant women above 2.5 Tesla (we scan at 3 Tesla). We have decided not to test for pregnancy as routine but if you think you may be pregnant you should not be scanned. Pregnancy tests are available in the women's toilets. There are no known risks of MEG.

What happens if you find something abnormal on my scan?

Since you are healthy, it is extremely unlikely that your scan will show any abnormality. Even if there were an abnormality it is unlikely that we would notice it since we are taking these scans for scientific research. They are not the same as scans collected by doctors for medical purposes and the pictures will not be looked at by a radiologist (a doctor qualified to find abnormalities in scans).

However, in the unlikely event that we do notice something abnormal on your scan, giving you this information might have the benefit of allowing you to start treatment earlier than you would have otherwise.

If we did suspect that there was something apparently abnormal on your scan then the scan will be sent to a radiologist who will contact you if they decide that the scan is abnormal and requires further investigation.

What if something goes wrong/who can I complain to?

In case you have a complaint on your treatment by a member of staff or anything to do with the study, you can initially approach the lead investigator:

Dr Maddie Groom, Room B22, The Division of Psychiatry, School of Community Health Sciences, The Institute of Mental Health, The University of Nottingham, Innovation Park, Triumph Road, Nottingham, NG7 2TU. Telephone: 0115 823 0267. E-mail: maddie.groom@nottingham.ac.uk.

If this achieves no satisfactory outcome, you should then contact the Ethics Committee Secretary:

Mrs Louise Sabir, Division of Therapeutics and Molecular Medicine, D Floor, South Block, Queen's Medical Centre, Nottingham, NG7 2UH. Telephone: 0115 8231063. E-mail: louise.sabir@nottingham.ac.uk.

In the unlikely event that you suffer injury to yourself or damage to your property as a result in taking part in this research, the University does have an insurance policy to cover harm arising as a result of the defect in the design of the study.

Will my taking part in this study be kept confidential?

All information which is collected about you during the course of the research will be kept on a password protected database and is strictly confidential. Any information about you which leaves the research unit will have your name and address removed so that you cannot be recognised from it.

What will happen to the results of the research study?

The results of this research will be published in a PhD thesis towards the end of 2014, and in peer-reviewed scientific journals. You will not be identified in any report or publication.

Who is organising and funding the research?

This research is funded by the Medical Research Council (www.mrc.ac.uk).

Who has reviewed the study?

This study has been reviewed and approved by the University of Nottingham Medical School Ethics Committee.

Contact for Further Information

Helen Smith, Room A10 (PhD office), The Division of Psychiatry, School of Community Health Sciences, The Institute of Mental Health, The University of Nottingham, Innovation Park, Triumph Road, Nottingham, NG7 2TU. Telephone: 0115 74 84335.

E-mail:mcxhjsm@nottingham.ac.uk.

Thank you for taking part in this study!

Appendix 7. Parent/guardian information sheet

Your child has been invited to take part in a research study. Before you decide whether to take part or not, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with friends and relatives if you wish to. Do ask us if there is anything that is not clear or if you would like more information and take your time to decide whether you wish to take part or not. If you decide to take part, please keep this leaflet for future reference. Thank you for reading this information sheet.

Background

The way the brain operates changes with age and is therefore different between children adolescents and adults. At the same time, cognitive abilities for example memory, action control and flexible thinking also change with age. However, the links between the changes that take place in the brain and the changes in cognitive ability have not yet been fully understood. We want to understand more about the relationship between changes in brain function and cognitive ability with age. It is important to understand this because problems with the development of functional brain networks has been linked to several developmental disorders like attention deficit/hyperactivity disorder (AD/HD), autism spectrum disorders (ASD), and Tourette syndrome (TS). To investigate this, we will measure brain activity using a device called a 'magnetoencephalography' ('MEG') scanner.

Why has your child been chosen?

Your child has been chosen because they are healthy and they are aged 9-18.

Do they have to take part?

It is up to you and your child to decide whether or not to take part. If you do decide to take part you will be given this information sheet to keep and be asked to sign a consent form. However, it is important that you understand that your child will be free to withdraw at any time and without giving a reason, even if you sign the consent form and come to see us.

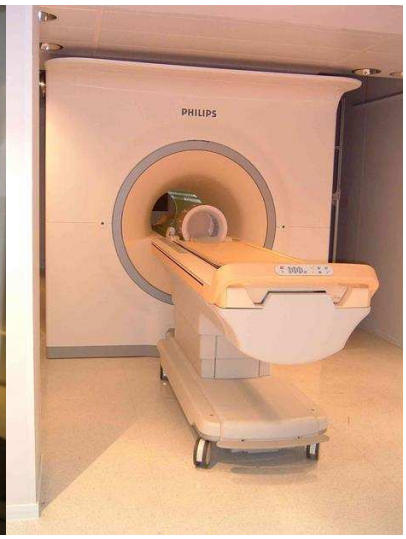
What does the study involve?

We will ask you to come to the University Of Nottingham for a morning or an afternoon visit lasting approximately 3 hours (this visit will scheduled be during a school holiday or after school). You and your child will be shown the MEG system (pictured below) and given an opportunity to ask questions. Your child will then practice the tasks that we will ask them to complete once in the MEG system.

To prepare for the MEG scan, we will make a map of the shape of your child's head on our computer and they can just sit comfortably on a chair whilst we do this. They will then be taken through to the scanner and made comfortable. Only their head goes into this scanner and they will be lying down during the scan. The scanner is completely silent. They will be asked to complete 2 cognitive tasks each lasting up to 20 minutes, with regular breaks. We will ask them to stay as still as possible during each task. Throughout the scan they will be able to communicate with the scanner operator.

After the MEG scan we will take them to a different scanner called a 'magnetic resonance imaging' scanner ('MRI' for short).You can see the MRI scanner in the picture below. A bit more than just your head will need to go into this scanner but this one will be quicker than the MEG scan, although it is noisy. It sounds a bit like a pneumatic drill so your child will be given headphones to wear for this one.

A MEG system



An MRI scanner

Finally, before you go home we will do some short tests with you and your child that just involve paper, pencil and speaking. Your travel expenses will be reimbursed (please keep any receipts if possible) and you (or your child if they are over the age of 15) will be paid an inconvenience allowance of £15 for your time. We will also be able to send a picture of your child's scan to you and your child after the visit, if you wish.

Your child will be asked to change into medical scrubs when you arrive because we will need to make sure there is no metal on them during the scans. We also advise women to refrain from wearing an underwired bra on the day of their visit, or they will need to take it off before the scans. Please ensure that your child does not wear make-up on the day of your visit as some make-up is known to contain metal particles and this can have a very negative impact on our data collection. It is very difficult to remove make-up effectively if it has already been applied on the day, but

we do have make-up remover available for you to use should you need it when you arrive.

What are the possible disadvantages and risks of taking part?

Magnetic resonance imaging (MRI) uses radio waves similar to those used in radio and TV transmission. These have a much lower energy than X-rays and as such are considered biologically safe. We scan at up to twice the field used in standard MRI scanners and use more rapidly changing 'field gradients', but these differences are considered safe. We will be following strict national safety guidelines which are designed to prevent the theoretical hazards of MRI which are burns and electric shocks. Such accidents have never occurred in the MR centre and have only very rarely occurred elsewhere.

While there is no evidence to suggest that MRI is harmful during pregnancy, the Medicines and Healthcare products Regulatory Agency advises against scanning pregnant women above 2.5 Tesla (we scan at 3 Tesla). We have decided not to test for pregnancy as routine but pregnancy tests are available in the women's toilets. There are no known risks of MEG.

What happens if you find something abnormal on my child's scan?

Since your child is healthy, it is extremely unlikely that their scan will show any abnormality. Even if there were an abnormality it is unlikely that we would notice it since we are taking these scans for scientific research. They are not the same as scans collected by doctors for medical purposes and the pictures will not be looked at by a radiologist (a doctor qualified to find abnormalities in scans).

However, in the unlikely event that we do notice something abnormal on your child's scan, giving you this information might have the benefit of allowing you to start treatment earlier than you would have otherwise.

If we did suspect that there was something apparently abnormal on your child's scan then the scan will be sent to a radiologist who will contact you if they decide that the scan is abnormal and requires further investigation.

What if something goes wrong/who can I complain to?

In case you have a complaint on your treatment by a member of staff or anything to do with the study, you can initially approach the lead investigator: Dr Maddie Groom, Room B22, The Division of Psychiatry, School of Community Health Sciences, The Institute of Mental Health, The University of Nottingham, Innovation Park, Triumph Road, Nottingham, NG7 2TU. Telephone: 0115 823 0267. E-mail: maddie.groom@nottingham.ac.uk.

If this achieves no satisfactory outcome, you should then contact the Ethics Committee Secretary: Mrs Louise Sabir, Division of Therapeutics and Molecular Medicine, D Floor, South Block, Queen's Medical Centre, Nottingham, NG7 2UH. Telephone: 0115 8231063. E-mail: louise.sabir@nottingham.ac.uk.

In the unlikely event that you suffer injury to yourself or damage to your property as a result in taking part in this research, the University does have an insurance policy to cover harm arising as a result of the defect in the design of the study.

Will my taking part in this study be kept confidential?

All information which is collected about you during the course of the research will be kept on a password protected database and is strictly confidential. Any information about you which leaves the research unit will have your name and address removed so that you cannot be recognised from it.

What will happen to the results of the research study?

The results of this research will be published in a PhD thesis towards the end of 2014, and possibly in a scientific journal. Neither you nor your child will be identified in any report or publication.

Who is organising and funding the research?

This research is funded by the Medical Research Council (www.mrc.ac.uk).

Who has reviewed the study?

This study has been reviewed and approved by the University of Nottingham Medical School Ethics Committee.

Contact for Further Information

Helen Smith, Telephone: 0115 74 84335, E-mail: mcxhjsm@nottingham.ac.uk

Room A10 (PhD office)

The Division of Psychiatry

School of Community Health Sciences

The Institute of Mental Health

The University of Nottingham

Innovation Park

Triumph Road

Nottingham

NG7 2TU

Thank you for reading this!

Appendix 8. Parent/guardian consent form

Please read this form, **initial each box** once you have read each statement and **sign at the end** once you have read the information sheet and have asked any questions you may have:

- I voluntarily agree for my child to take part in this study.
- I confirm that I have been given a full explanation by the above named and that I have read and understand the information sheet given to me.
- I have been given the opportunity to ask questions and discuss the study with one of the above investigators or their deputies on all aspects of the study and have understood the advice and information given as a result.
- I agree to comply with the reasonable instructions of the supervising investigator.
- I authorise the investigators to disclose the results of my child's participation in the study but not their name.
- I understand that information about my child recorded during the study will be kept in a secure database. If data is transferred to others it will be made anonymous. Data will be kept for 7 years after the results of this study have been published.
- I authorise the investigators to disclose to me any abnormal test results.
- I understand that my child or I can ask for further instructions or explanations at any time.
- I understand that my child is free to withdraw from the study at any time, without having to give a reason for withdrawing.
- I confirm that I have disclosed relevant medical information before the study.
- I shall receive an inconvenience allowance of £ 15. If my child withdraws from the study for medical reasons not associated with the study a payment will be made to me proportional to the length of the period of participation, but if they withdraw for any other reason, the payment to be made, if any, shall be at the discretion of the supervising investigator.
- I understand that my child's MRI scans will not routinely be reviewed by a radiologist, and it is unlikely that any abnormalities that may be present will be detected.
- I understand that if the investigators notice any abnormality on my child's MRI scan they will show my scans to a radiologist based in Academic Radiology at the QMC, who will contact me if further action is required.

Name: **Address:**

Telephone number:

Signature: **Date:**

I confirm that I have fully explained the purpose of the study and what is involved to:

…… I have given the above named a copy of this form together with the information sheet. **Investigators Signature:**
Date: **Investigators Name:**

Study Volunteer Number:

Appendix 9. Age 16+ consent form

Please read this form, **initial each box** once you have read each statement and **sign at the end** once you have read the information sheet and have asked any questions you may have.

- I voluntarily agree to take part in this study.
- I confirm that I have been given a full explanation by the above named and that I have read and understand the information sheet given to me.
- I have been given the opportunity to ask questions and discuss the study with one of the above investigators or their deputies on all aspects of the study and have understood the advice and information given as a result.
- I agree to comply with the reasonable instructions of the supervising investigator.
- I authorise the investigators to disclose the results of my participation in the study but not my name.
- I understand that information about me recorded during the study will be kept in a secure database. If data is transferred to others it will be made anonymous. Data will be kept for 7 years after the results of this study have been published.
- I authorise the investigators to disclose to me any abnormal test results.
- I understand that I can ask for further instructions or explanations at any time.
- I understand that I am free to withdraw from the study at any time, without having to give a reason for withdrawing.
- I confirm that I have disclosed relevant medical information before the study.
- I shall receive an inconvenience allowance of £ 15. If I withdraw from the study for medical reasons not associated with the study a payment will be made to me proportional to the length of the period of participation, but if I withdraw for any other reason, the payment to be made, if any, shall be at the discretion of the supervising investigator.
- I understand that my MRI scans will not routinely be reviewed by a radiologist, and it is unlikely that any abnormalities that may be present will be detected.
- I understand that if the investigators notice any abnormality on my MRI scan they will show my scans to a radiologist based in Academic Radiology at the QMC, who will contact me if further action is required.

Name: **Address:**

Telephone number:

Signature: **Date:**

I confirm that I have fully explained the purpose of the study and what is involved to:

…… I have given the above named a copy of this form together with the information sheet. **Investigators Signature:**
Date: **Investigators Name:**

Study Volunteer Number:

Appendix 10. Safety questionnaire

NAME	Date of Scan	Date of Birth
ADDRESS	Volunteer Number	
	Ethics Code	
Phone number	Weight	Height if applicable

MR scanning uses strong magnetic fields. For your own safety and the safety of others it is **very important** that you do not go into the magnet halls with any metal in or on your body or clothing. Please answer the following questions carefully and ask if anything is not clear. All information is held in the strictest confidence.

1. Do you have any implants in your body? e.g. replacement joints, drug pumps **Y/N**
2. Do you have aneurysm clips (clips put around blood vessels during surgery)? **Y/N**
3. Do you have a pacemaker or artificial heart valve? *(These stop working near MR Scanners)* **Y/N**
4. Have you ever had any surgery? Please give brief details over *(we do not need to know about uncomplicated caesarean delivery, vasectomy or termination of pregnancy)* **Y/N**
5. Do you have any foreign bodies in your body (e.g. shrapnel)? **Y/N**
6. Have you ever worked in a machine tool shop without eye protection? **Y/N**
7. Do you wear a hearing aid or cochlear implant? **Y/N**
8. Could you be pregnant? (Pregnancy tests are available in the female toilets) **Y/N**
9. Have you ever suffered from tinnitus? **Y/N**
10. Do you wear dentures, a dental plate or a brace? **Y/N**
11. Are you susceptible to claustrophobia? **Y/N**
12. Do you suffer from blackouts, epilepsy or fits? **Y/N**
13. Do you have any tattoos? (If yes, you may be asked to read and sign another form) **Y/N**
14. Do you have any body piercing jewellery that cannot be removed? **Y/N**
15. Do you have any skin patches (trans-dermal patches)? **Y/N**
16. Do you have a coil in place (IUD) for contraception? Do you know what type? **Y/N**
17. Do you have any condition that may affect your ability to control your temperature ? (E.g. Do you have a fever, cardiovascular disease, hypertension, diabetes or cerebrovascular disease?) **Y/N**
18. Will you remove all metal including coins, body-piercing jewellery, false-teeth, hearing aids etc. before entering the magnet hall? *(lockers available by the changing rooms)* **Y/N**
19. Is there anything else you think we should know? **Y/N**

I have read and understood all the questions **Signature:**	**Date:**
Verified by: **Scanner Operator Only:**	**Date:**

Appendix 11. Standard Operating Procedure for Scanning Children

SOP for scanning children.

This document defines the additional measures in place when scanning involves subjects who are under 18. It should be read in conjunction with the SPMMRC documentation covering the use of the scanners and scanning of volunteers.

1. Necessary paperwork

1.1 All human scanning must be covered by a valid approval from an appropriate ethics committee. This must explicitly cover the scanning of children and a copy of the approval must be lodged with the SPMMRC receptionist before the start of the study.

1.2 The volunteer information sheet as approved by the ethics committee must be given to the parent/guardian of the volunteer.

1.3 The volunteer must complete the scanning safety questionnaire. This should be done with the parent/guardian who may have more knowledge of relevant history. Anyone else, for example a parent, who needs to enter the magnet hall must also complete this form.

1.4 The parent / guardian may need to sign the consent form as dictated by the ethics committee.

1.5 A copy of the safety questionnaire and consent form must be retained by the SPMMRC for future reference.

2. Scanning

2.1 Where the volunteer is to be under the sole supervision of University staff then at least one of those staff must have successfully completed a CRB check within the last three years. They must remain with the volunteer until they are returned to their parent/ guardian.

2.2 A minimum of one scanner operator and one MR assistant must be present during the scan session. Neither may leave to perform other duties until the scan is complete and the volunteer has left the scanner.

2.3 Scanning of children out of hours must be approved by the SPMMRC operational group. This approval will be specific to the personnel involved. This is to make sure that the staff involved have sufficient knowledge and experience to scan in the absence of the usual support.

Appendix 12. Adult AD/HD Self-Report Scale (ASRS)

Please answer the questions below, rating yourself on each of the criteria shown using the scale on the right hand side of the page. As you answer each question, place an X in the box that best describes how you have felt and conducted yourself over the past 6 months. Please give this completed checklist to your healthcare professional to discuss during today's appointment. [Rating criteria scale on the right hand side of the page gave the reader these options: never, rarely, sometimes, often or very often.]

PART A:

1) How often do you have trouble wrapping up the final details of a project, once the challenging parts have been done?
2) How often do you have difficulty getting things in order when you have to do a task that requires organisation?
3) How often do you have problems remembering appointments or obligations?
4) When you have a task that requires a lot of thought, how often to you avoid or delay getting started?
5) How often do you fidget or squirm with your hands or feet when you have to sit down for a long time?
6) How often do you feel overly active and compelled to do things, like you were driven by a motor?

PART B:

1) How often do you make careless mistakes when you have to work on a boring or difficult project?
2) How often do you have difficulty keeping your attention when you are doing boring or repetitive work?
3) How often do you have difficulty concentrating on what people say to you, even when they are speaking to you directly?
4) How often do you misplace or have difficulty finding things at home or at work?
5) How often are you distracted by activity or noise around you?
6) How often do you leave your seat in meetings or other situations in which you are expected to remain seated?
7) How often do you feel restless or fidgety?
8) How often do you have difficulty unwinding and relaxing when you have time to yourself?
9) How often do you find yourself talking too much when you are in social situations?
10) When you're in a conversation, how often do you find yourself finishing the sentences of the people you are talking to, before they can finish themselves?
11) How often do you have difficulty waiting your turn in situations when turn taking is required?
12) How often do you interrupt others when they are busy?

[The rating criteria carry scores of: never = 0, rarely = 1, sometimes = 2, often = 3, very often = 4. If an adult scores 24 or higher on either Part A or Part B this is indicative of AD/HD.]

Appendix 13. Strengths and Weaknesses of AD/HD Symptoms and Normal Behaviour (SWAN)

Children differ in their abilities to focus attention, control activity, and inhibit impulses. For each item listed below, how does this child compare to other children of the same age? Please select the best rating based on your observations over the past month. Compared to other children, how does this child do the following:

[Rating criteria scale on the right hand side of the page gave the reader these options: far below, below, slightly below, average, slightly above, above, far above.]

1) Give close attention to detail and avoid careless mistakes
2) Sustain attention on tasks or play activities
3) Listen when spoken to directly
4) Follow through on instructions and finish school work/chores
5) Organize tasks and activities
6) Engage in tasks that require sustained mental effort?
7) Keep track of things necessary for activities
8) Ignore extraneous stimuli
9) Remember daily activities
10) Sit still (control movement of hands/feet or control squirming)
11) Stay seated (when required by school rules/social conventions)
12) Modulate motor activity (inhibit inappropriate running/climbing)
13) Play quietly (keep noise level reasonable)
14) Settle down and rest (control constant activity)
15) Modulate verbal activity (control excess talking)
16) Reflect on questions (control blurting out answers)
17) Await turn (stand in line and take turns)
18) Enter into conversations and games (control interrupting/intruding)

The rating criteria carry scores of: far below = 3, below = 2, slightly below = 1, average = 0, slightly above = -1, above = -2, far above = -3. If a child scores an average rating of 2.11 or higher across the 18 questions, this is indicative of AD/HD.

Appendix 14. Rapid Automatized Naming test (RAN)

D	S	A	P	O
S	P	D	A	O
S	A	O	D	P
A	D	O	P	S
D	A	P	O	A
S	P	S	O	D
A	P	S	D	O
D	S	A	P	O

Appendix 15. ICA of MEG data

Figure 7.1 demonstrates good spatial agreement between ICA of the child and adult MEG data included in this study (the total number of data points for each group were matched). Furthermore, the ICA networks are consistent with the network masks used in this thesis and therefore indicate this is a valid approach to the analysis.

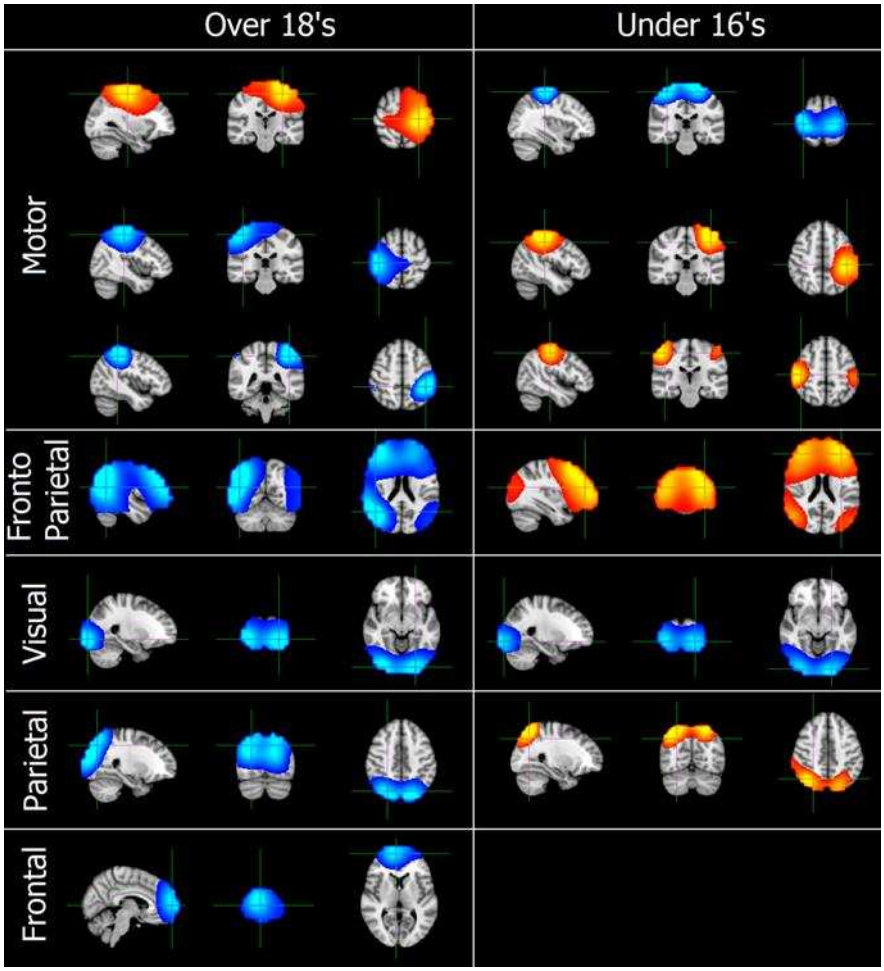


Figure 7.1: ICA of MEG data in children and adults (colours are arbitrary).

Whilst the frontal network regions of the DAN are not found in the ICA of child data, the DAN is found as a coherent network with the frontal network regions present.

Appendix 16. Equations relating to beamforming

The recorded MEG data is related to underlying neural activity as described by Equation 3 where the recorded MEG data is equal to the 'lead fields' multiplied by the strength of neural activity (Hillebrand & Barnes, 2005). The lead fields represent what the recorded MEG signals would be for a source of unit strength at a particular location and orientation.

$$\boldsymbol{B = LQ}$$

Equation 3

B = MEG sensor recordings

L = lead field matrix

Q = estimated strength of neural source

(Hillebrand & Barnes, 2005)

The lead fields are represented by a matrix with dimensions M x N, where M is the number of MEG sensors and N is the number of voxels in source space. They are determined by the configuration of MEG sensors within the helmet, the head model and the source model. They therefore reflect the sensitivity of each sensor to a neural current source at any given location and orientation in source space (Hillebrand & Barnes, 2005). For the purposes of this thesis a 'multi-sphere head model' is used whereby the head is modelled by multiple overlapping spheres rather than the more basic and less accurate single sphere model. Using a beamformer, neural activity can be estimated for any location, orientation and time point using Equation 4, where neural source strength is estimated using the lead fields matrix, the data covariance matrix, the MEG sensor recordings and the source current covariance matrix.

$$\boldsymbol{Q = C_j L^T C_b^{-1} B}$$

Equation 4

Q = estimated strength of neural source

C_j = source current covariance matrix

L = lead field matrix

C_b = data covariance matrix

B = MEG sensor recordings

(Mosher, Baillet, & Leahy, 2003)

The source current covariance matrix is calculated using the lead fields and data covariance matrix, as demonstrated in Equation 5.

$$\boldsymbol{C_j} = \left(\boldsymbol{L_\theta^T C_b^{-1} L_\theta}\right)^{-1}$$

Equation 5

C_j = source current covariance matrix

L = lead field matrix

C_b = data covariance matrix

(Mosher, et al., 2003)

Since the beamformer approach assumes that no two neural sources are correlated so C_j is a matrix with each diagonal element corresponding to location and orientation θ and all non-diagonal elements are 0s. Using Equation 5, which shows how C_j is calculated using the data itself and the lead fields, we can substitute C_j in Equation 4 to give us the beamformer equation as it appears in Equation 6.

$$\boldsymbol{Q_\theta} = \left(\boldsymbol{L_\theta^T C_b^{-1} L_\theta}\right)^{-1} \boldsymbol{L_\theta^T C_b^{-1} B} = \boldsymbol{W_\theta^T B}$$

Equation 6

$Q\vartheta$ = estimate of source power for location and orientation ϑ

L_ϑ = lead field vector for location and orientation ϑ

C_b = data covariance matrix

B = MEG sensor recordings

$W\vartheta$ = weighting parameters for location and orientation ϑ

(Hillebrand & Barnes, 2005)

Appendix 17. Equations relating to signal leakage reduction

Eigenvalue decomposition can be summarised by Equation 7. For each value of λ that the equation can be solved, we obtain an eigenvalue and eigenvector (together they give us an eigenmode) for the matrix, A.

$$\boldsymbol{Av = \lambda v}$$

Equation 7: Eigenvalue decomposition

A = a square matrix

v = a vector (eigenvector)

λ = a scalar (eigenvalue)

We used eigenvalue decomposition to decompose the covariance matrices into their respective eigenvectors and eigenvalues (see Equation 8 and Equation 9).

$$\boldsymbol{C_{XX} = U_X S_X U_X^T}$$

Equation 8

C_{XX} = covariance matrix of X (the seed cluster VE time course matrix)

U_x = columns represent the eigenvalues of C_{xx}

S_x = diagonal elements represent the eigenvectors of C_{xx}

(Brookes, et al., 2014)

$$\boldsymbol{C_{YY} = U_Y S_Y U_Y^T}$$

Equation 9

C_{YY} = covariance matrix of Y (the test cluster VE time course matrix)

U_Y = columns represent the eigenvalues of C_{YY}

S_Y = diagonal elements represent the eigenvectors of C_{YY}

(Brookes, et al., 2014)

We then needed to 'orthogonalise' the matrices X and Y using the derived eigenvectors (see Equation 10 and Equation 11). 'Orthogonal' describes things which, in 2 dimensions, would be perpendicular and in 3 dimensions, would be statistically

independent. A matrix is orthogonal if, when multiplied by its transpose it gives us an identity matrix (a matrix with ones along the diagonal and zeroes for all the off-diagonal elements). This also means that the matrix transpose is equal to the matrix inverse.

$$\boldsymbol{X_o = XU_X}$$

Equation 10

X_o = orthogonalised version of the original seed VE matrix X

X = the seed cluster VE time course matrix

U_X = columns represent the eigenvalues of C_{XX}

(Brookes, et al., 2014)

$$\boldsymbol{Y_o = YU_Y}$$

Equation 11

Y_o = orthogonalised version of the original test VE matrix Y

Y = the test cluster VE time course matrix

U_Y = columns represent the eigenvalues of C_{YY}

(Brookes, et al., 2014)

A general linear model was then used to regress (remove) the seed signals (defined as any linear combinations of the VE time courses represented by X) from the test signals (Y). In this general linear model the estimated term represents the combination of orthogonalised features that best describes the signal leakage between X and Y, and the error term represents the corrected test matrix (see Equation 12 and Equation 13).

$$\boldsymbol{Y_o = X_o\beta_L + Y_{oc}}$$

Equation 12

Y_o = orthogonalised version of the original test VE matrix Y

X_o = orthogonalised version of the original seed VE matrix X

β_L = the combination of features that best describes the signal leakage of X into Y

Y_{oc} = corrected orthogonalised matrix based on the original test VE time course matrix

(Brookes, et al., 2014)

$$\boldsymbol{\beta_L} = \boldsymbol{X_o^+ Y_o}$$

Equation 13

β_L = the combination of features that best describes the signal leakage of X into Y

X_o^+ = Moore-Penrose pseudo inverse of X_o where X_o = orthogonalised version of the original seed VE matrix X

Y_o = orthogonalised version of the original test VE matrix Y

(Brookes, et al., 2014)

Equation 12 refers to an operation called the 'Moore-Penrose pseudo inverse'. This operation is simply a type of matrix inverse. Following on from Equation 12, Equation 13 can be used to give Y_{oc} (the error term in the general linear model given in Equation 14).

$$\boldsymbol{Y_{oc}} = \boldsymbol{Y_o} - \boldsymbol{X_o \beta_L}$$

Equation 14

Y_{oc} = corrected orthogonalised matrix based on the original test VE time course matrix

Y_o = orthogonalised version of the original test VE matrix Y

X_o = orthogonalised version of the original seed VE matrix X

β_L = the combination of features that best describes the signal leakage of X into Y

(Brookes, et al., 2014)

Following Equation 14, the corrected test matrix can be determined using Equation 15.

$$\boldsymbol{Y_c} = \boldsymbol{Y_{oc} U_Y^T}$$

Equation 15

Y_c = signal leakage corrected test VE matrix

Y_{oc} = corrected orthogonalised matrix based on the original test VE time course matrix

U_Y = columns represent the eigenvalues of C_{YY} where C_{YY} = covariance matrix of Y (the test cluster VE time course matrix)

(Brookes, et al., 2014)

Appendix 18. Equations relating to canonical correlation coefficient calculation

To recap the steps of eigenvalue decomposition, we start with Equation 16 and Equation 17 with which the covariance matrices of E_x and E_Y can be computed, after which the respective covariance matrices (C_{EX} and C_{EY}) undergo eigenvalue decomposition into their constituent eigenvalues and eigenvectors.

$$\boldsymbol{C_{EX} = E_X^T E_X = U_X S_X U_X^T}$$

Equation 16

E_X = envelope VE time course matrix for X (the seed region)

C_{EX} = covariance matrix of E_X

U_x = columns represent the eigenvalues of C_{Ex}

S_x = diagonal elements represent the eigenvectors of C_{EX}

(Adapted from Brookes, et al., 2014)

$$\boldsymbol{C_{EY} = E_Y^T E_Y = U_Y S_Y U_Y^T}$$

Equation 17

E_Y = envelope VE time course matrix for Y (the test region)

C_{EY} = covariance matrix of E_Y

U_Y = columns represent the eigenvalues of C_{EY}

S_Y = diagonal elements represent the eigenvectors of C_{EY}

(Adapted from Brookes, et al., 2014)

The columns of U_X and U_Y can then be reduced to 5 principal features (eigenmodes) using Equation 18 and Equation 19 using their respective eigenvectors and eigenvalues.

$$\boldsymbol{E_{Xo} = E_X U_{Xs}}$$

Equation 18

E_{Xo} = orthogonalised version of the envelope VE time course matrix for X (the seed region)

E_X = envelope VE time course matrix for X (the seed region)

U_{Xs} = represents the eigenmodes of C_{EX} (covariance matrix of E_x), for the purposes of this thesis we use the first 5 eigenmodes

(Adapted from Brookes, et al., 2014)

$$\boldsymbol{E_{Yo} = E_Y U_{Ys}}$$

Equation 19

E_{Yo} = orthogonalised version of the envelope VE time course matrix for Y (the test region)

E_Y = envelope VE time course matrix for Y (the test region)

U_{Ys} = represents the eigenmodes of C_{EY} (covariance matrix of E_Y), for the purposes of this thesis we use the first 5 eigenmodes

(Adapted from Brookes, et al., 2014)

A general linear model in Equation 20 was subsequently used to calculate the CCCs that best predict the principal features from the seed and test clusters.

$$\boldsymbol{E_{Yo} = E_{Xo}\beta + \varepsilon}$$

Equation 20

E_{Yo} = orthogonalised version of the envelope VE time course matrix for Y (the test region)

E_{Xo} = orthogonalised version of the envelope VE time course matrix for X (the seed region)

β = matrix of regression coefficients best predicting E_{Xo} from E_{Yo}

ε = the error term

(Adapted from Brookes, et al., 2014)

We then calculated the covariance explained by the term $E_{Xo}\beta$ using Equation 21, the covariance unexplained by the term $E_{Xo}\beta$ using Equation 22, and the ratio of explained covariance to unexplained covariance using Equation 23.

$$\boldsymbol{H} = (\boldsymbol{E_{Xo}\beta})^{\boldsymbol{T}}(\boldsymbol{E_{Xo}\beta})$$

Equation 21

H = explained covariance

E_{Xo} = orthogonalised version of the envelope VE time course matrix for X (the seed region)

β = matrix of regression coefficients best predicting E_{Xo} from E_{Yo}

(Adapted from Brookes, et al., 2014)

$$\boldsymbol{R} = (\boldsymbol{E_{Yo}} - \boldsymbol{E_{Xo}\beta})^{\boldsymbol{T}}(\boldsymbol{E_{Yo}} - \boldsymbol{E_{Xo}\beta})$$

Equation 22

R = unexplained covariance

E_{Yo} = orthogonalised version of the envelope VE time course matrix for Y (the test region)

E_{Xo} = orthogonalised version of the envelope VE time course matrix for X (the seed region)

β = matrix of regression coefficients best predicting E_{Xo} from E_{Yo}

(Adapted from Brookes, et al., 2014)

$$\boldsymbol{D} = \boldsymbol{R}^{-1}\boldsymbol{H}$$

Equation 23

D = ratio of explained to unexplained covariance

R = unexplained covariance

H = explained covariance

(Brookes, et al., 2014)

We further decompose D into eigenvectors and eigenvalues using Equation 24.

$$\boldsymbol{D = AS_D A^{-1}}$$

Equation 24

D = ratio of explained to unexplained covariance

A = eigenvectors of D (canonical vectors of E_{Xo})

S_D = eigenvalues of D

(Brookes, et al., 2014)

The columns of A represent the canonical vectors of E_{Xo} and describe how to combine the columns of E_{Xo} to best explain the variance in E_{Yo}. The canonical vectors of E_{Yo} can be calculated using Equation 25.

$$\boldsymbol{B = \beta A}$$

Equation 25

B = canonical vectors of E_{Yo}

β = matrix of regression coefficients best predicting E_{Xo} from E_{Yo}

A = eigenvectors of D (the ratio of explained to unexplained covariance), also the canonical vectors of E_{Xo}

(Brookes, et al., 2014)

The canonical vectors of E_{Xo} and E_{Yo} (A and B) are then used to calculate the canonical variates. The canonical variates comprise a weighted sum of the columns of E_{Xo} and E_{Yo} that maximise temporal correlation between the seed and test regions. These canonical variates are calculated using Equation 26 and Equation 27.

$$\boldsymbol{V_1 = E_{Xo} B}$$

Equation 26

V_1 = canonical variates of E_{Xo}

E_{Xo} = orthogonalised version of the envelope VE time course matrix for X (the seed region)

B = canonical vectors of E_{Yo}

(Adapted from Brookes, et al., 2014)

$$\boldsymbol{V_2 = E_{Yo}A}$$

Equation 27

V_2 = canonical variates of E_{Yo}

E_{Yo} = orthogonalised version of the envelope VE time course matrix for Y (the test region)

A = canonical vectors of E_{Xo}

(Adapted from Brookes, et al., 2014)

Appendix 19. Equation relating to phase randomisation

$$\widetilde{\boldsymbol{w}}_{\boldsymbol{j}}(\boldsymbol{t}) = \boldsymbol{F}^{-1}\left[\boldsymbol{F}\left(\boldsymbol{w}_{\boldsymbol{j}}(\boldsymbol{t})\right)\boldsymbol{e}^{\boldsymbol{i}(\xi(f))}\right]$$

Equation 28

$\widetilde{w}_j(t)$ *= phase randomised surrogate data corresponding to column j of* E_X *or* E_Y *yielding surrogate datasets* $\tilde{E}_X$ *and* $\tilde{E}_Y$

F denotes Fourier transform

$w_j(t)$ *= column j of the real data matrices* E_X *or* E_Y

ξ(f) = a random sequence of phase rotation angles

(Brookes, et al., 2014)

Appendix 20. The visual network region pair

Figure 7.2 depicts the FC profiles of the visual network region pair for different age groups under the different task conditions studied in this thesis.

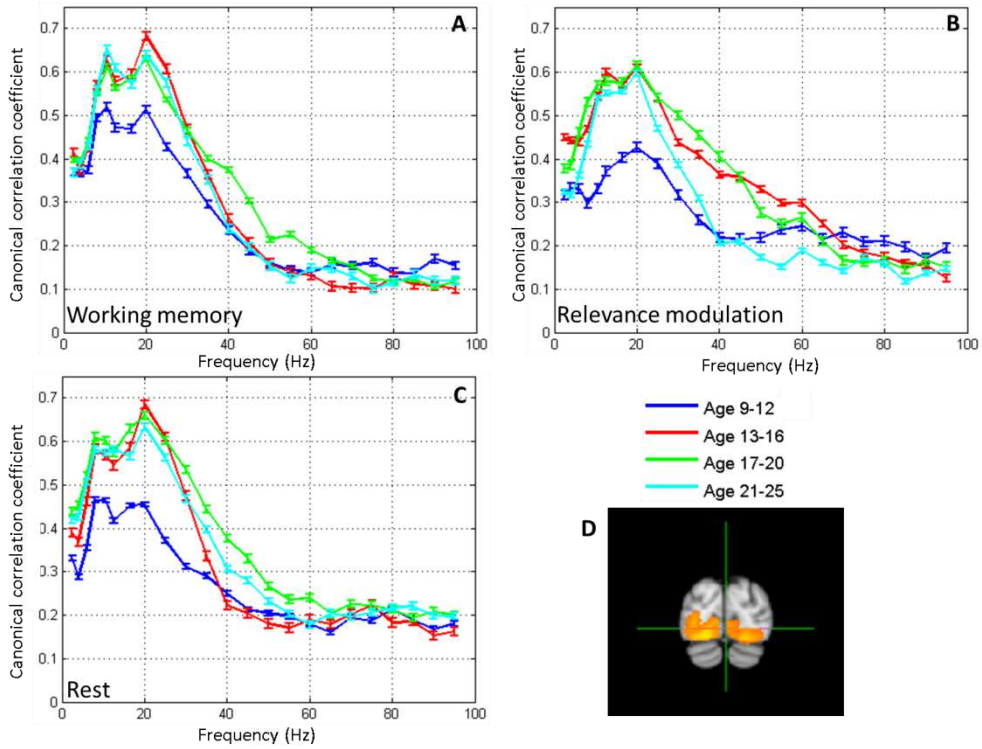


Figure 7.2: (A) The profiles of FC under different conditions from age 9-25 within the visual network (B) The visual network region pair used for the canonical correlation analysis.

FC appears to peak in strength over the alpha and beta frequency bands, and the youngest age group (age 9-12) consistently demonstrates the weakest FC profile in this network across all conditions (working memory, relevance modulation and resting state).

Figure 7.3 shows the developmental profiles of FC over specific frequency bands of interest for the visual network region pair under working memory task conditions. All are second order polynomial correlations. The trajectories of alpha and beta FC appear to correlate most strongly with age in the visual network under working memory task conditions. FC over the alpha frequency appears to peak later than FC over other bands (approximately aged 23 as opposed to age 17-20).

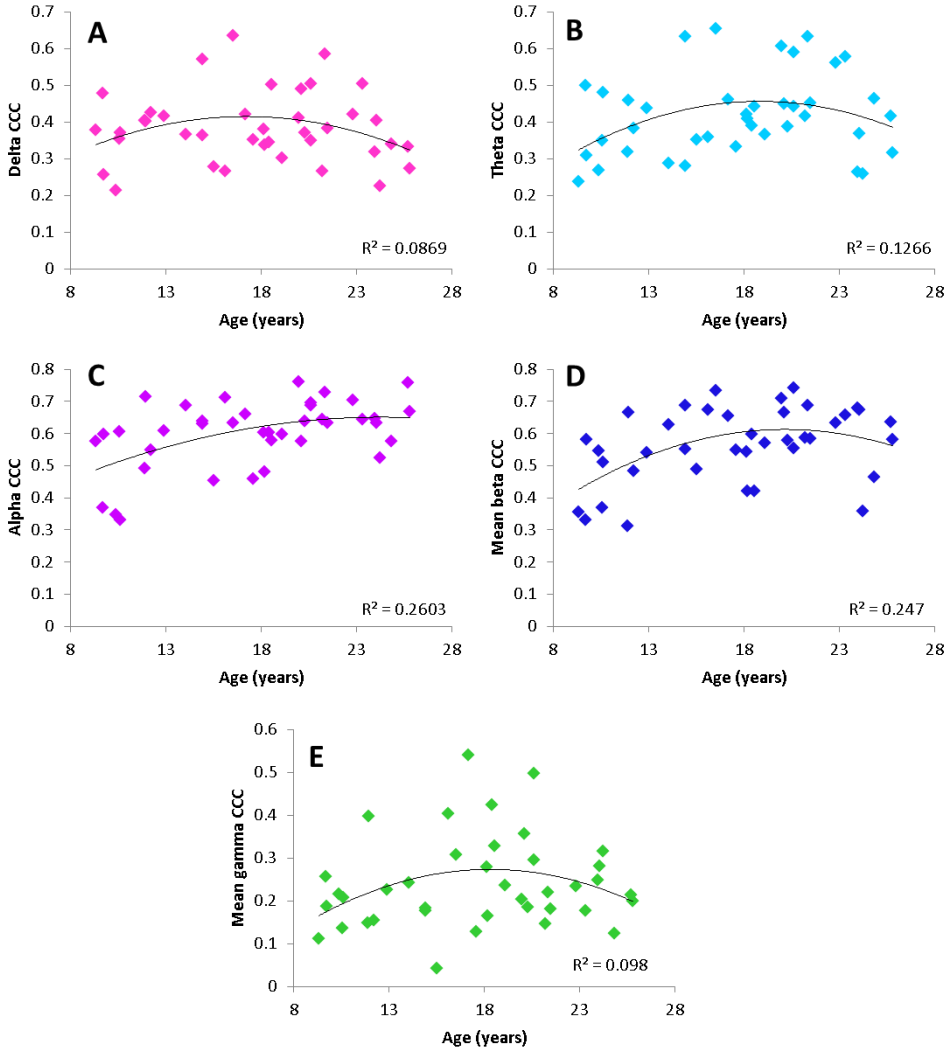


Figure 7.3: Developmental profiles of FC between the visual network region pair under working memory task conditions; (A) Delta; 1-4 Hz (B) Theta; 4-8 Hz (C) Alpha; 8-13 Hz (D) Beta; 13-30 Hz (E) Gamma; 30-60 Hz.

Figure 7.4 shows the developmental profiles of FC over specific frequency bands for the visual network region pair under relevance modulation task conditions. All are second order polynomial correlations. The trajectory of alpha FC development appears to correlate most strongly with age in the visual network under relevance modulation task conditions. FC over alpha and beta frequencies appears to peak later than FC over other bands (approximately aged 20-21 as opposed to age 18).

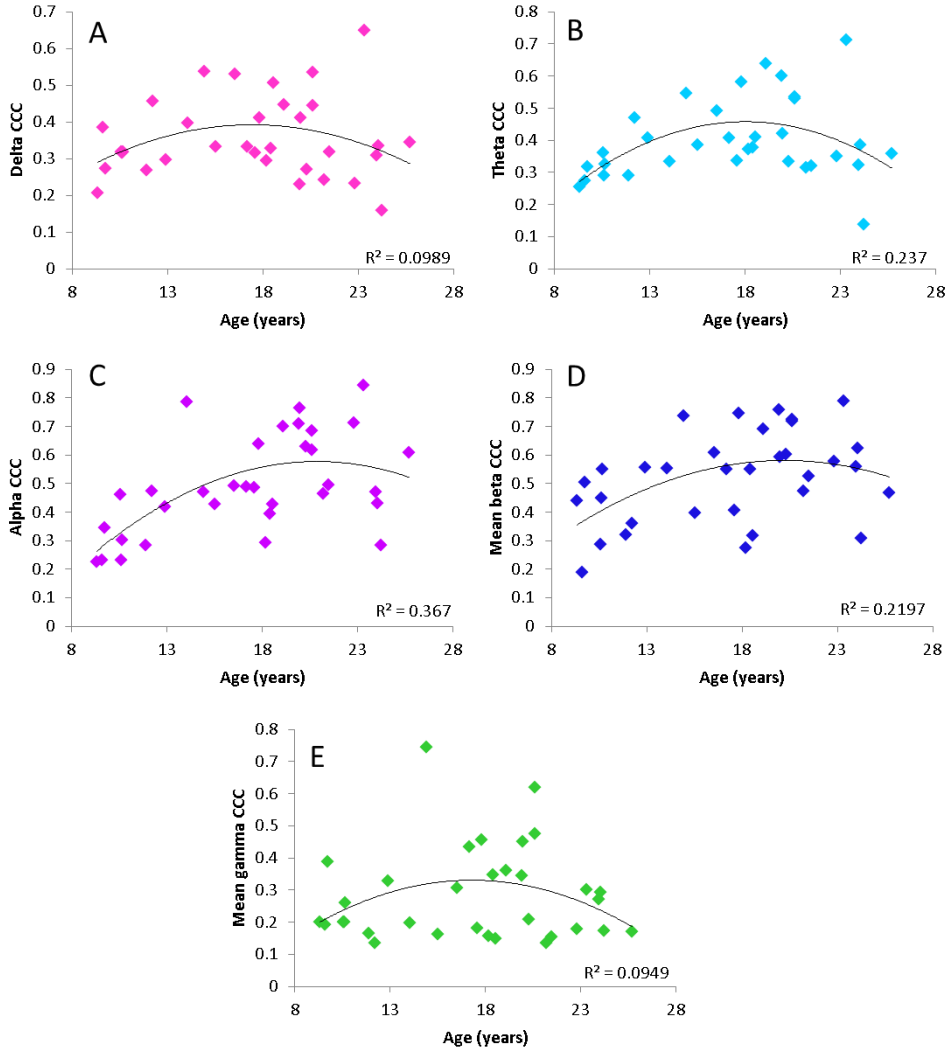


Figure 7.4: Developmental profiles of FC between the visual network region pair under relevance modulation task conditions; (A) Delta; 1-4 Hz (B) Theta; 4-8 Hz (C) Alpha; 8-13 Hz (D) Beta; 13-30 Hz (E) Gamma; 30-60 Hz.

Figure 7.5 shows the developmental profiles of FC over specific frequency bands for the visual network region pair under resting state conditions. All are second order polynomial correlations. The trajectory of beta FC changes appears to correlate most strongly with age in the visual network under resting state conditions. All developmental trajectories appear to peak between the ages of 19-21.

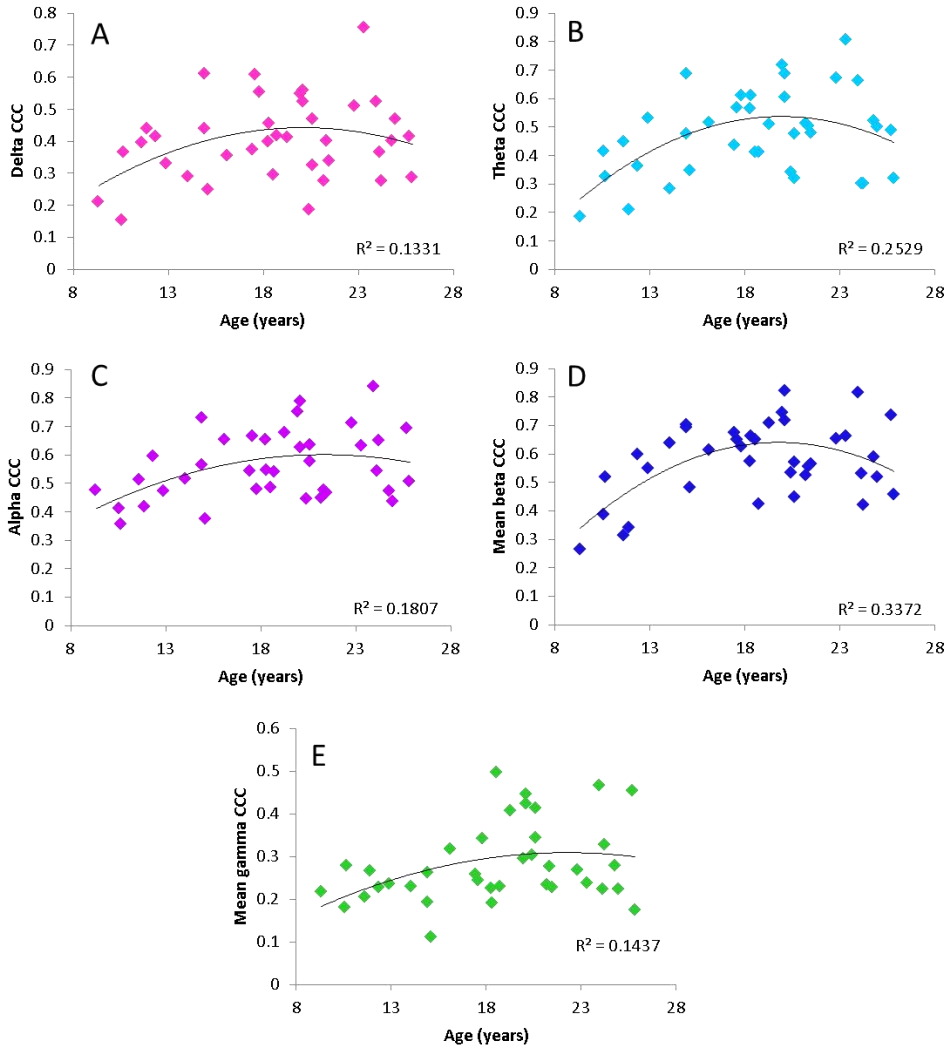


Figure 7.5: Developmental profiles of FC between the visual network region pair under resting state conditions; (A) Delta; 1-4 Hz (B) Theta; 4-8 Hz (C) Alpha; 8-13 Hz (D) Beta; 13-30 Hz (E) Gamma; 30-60 Hz.

Appendix 21. The sensorimotor network region pair

Figure 7.6 depicts the FC profiles of the sensorimotor network region pair for different age groups under the different task conditions studied in this thesis.

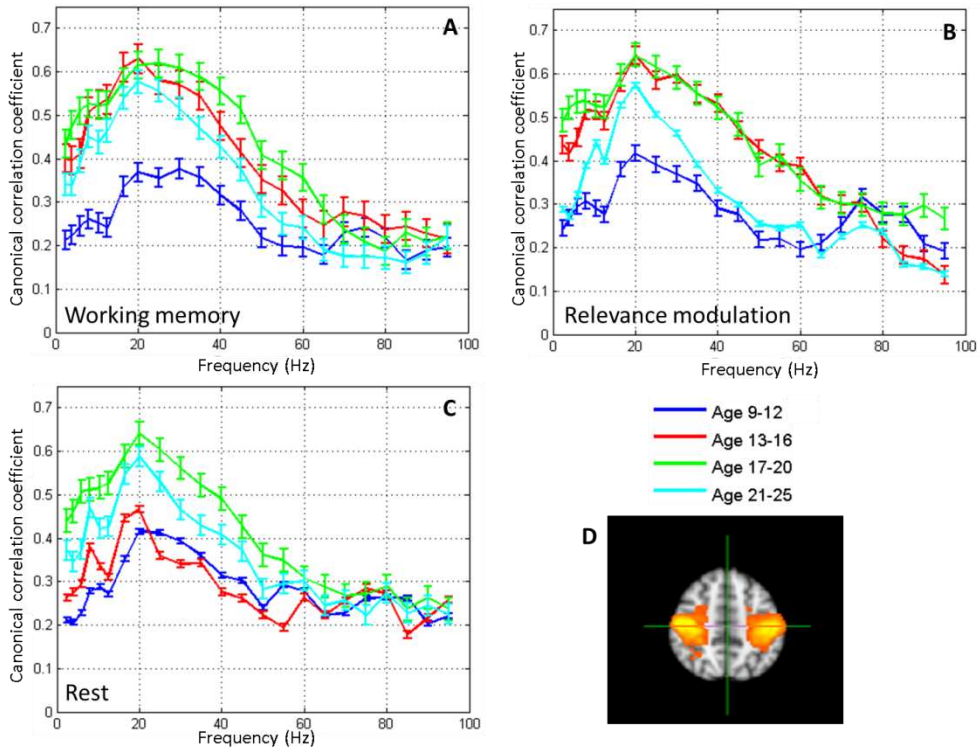


Figure 7.6: (A) The profiles of FC under different conditions from age 9-25 within the sensorimotor network (B) The sensorimotor network region pair used for the canonical correlation analysis.

FC appears to peak in strength broadly over the beta band. The youngest age group (age 9-12) consistently demonstrates the weakest FC profile in this network across the task conditions (working memory and relevance modulation), however, age 13-16 show similarly weak FC to age 9-12 under the resting state condition. Interestingly, the oldest age group (age 21-25) does not demonstrate the strongest FC profile, as it appears the younger and older adolescents (aged 13-16 and 17-20) show the strongest FC under task conditions, and the older adolescents (age 17-20) show the strongest FC under the resting state condition.

Figure 7.7 shows the developmental profiles of FC over specific frequency bands of interest for the sensorimotor network region pair under working memory task conditions. All are second order polynomial correlations. All are second order polynomial correlations. The trajectories of alpha and beta FC development appear to correlate most strongly with age in the sensorimotor network under working memory task conditions. All developmental trajectories appear to peak aged 18-20.

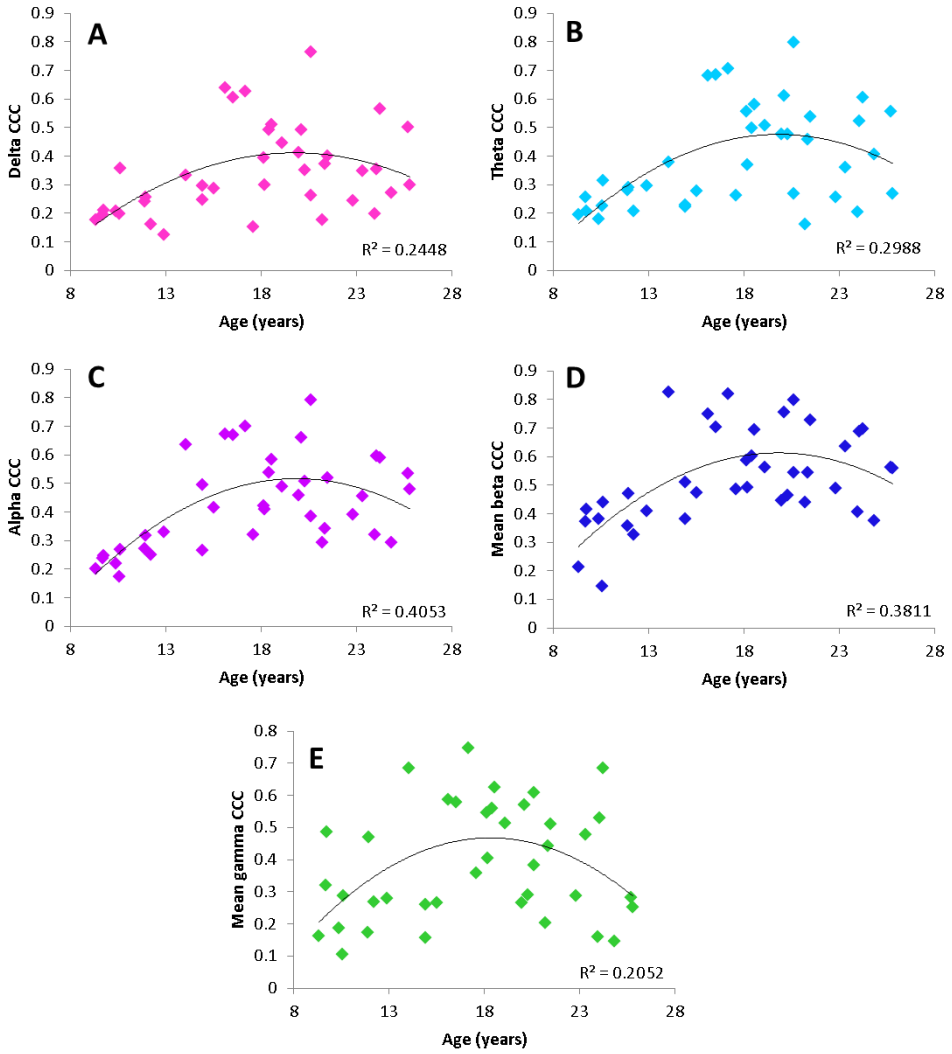


Figure 7.7: Developmental profiles of FC between the sensorimotor network region pair under working memory conditions; (A) Delta; 1-4 Hz (B) Theta; 4-8 Hz (C) Alpha; 8-13 Hz (D) Beta; 13-30 Hz (E) Gamma; 30-60 Hz.

Figure 7.8 shows the developmental profiles of FC over specific frequency bands for the sensorimotor network region pair under relevance modulation task conditions. All are second order polynomial correlations. The trajectories of delta and beta FC development appear to correlate most strongly with age in the sensorimotor network under relevance modulation task conditions. FC over alpha and beta frequencies appears to peak later than FC over other bands (approximately aged 20-21 as opposed to age 18).

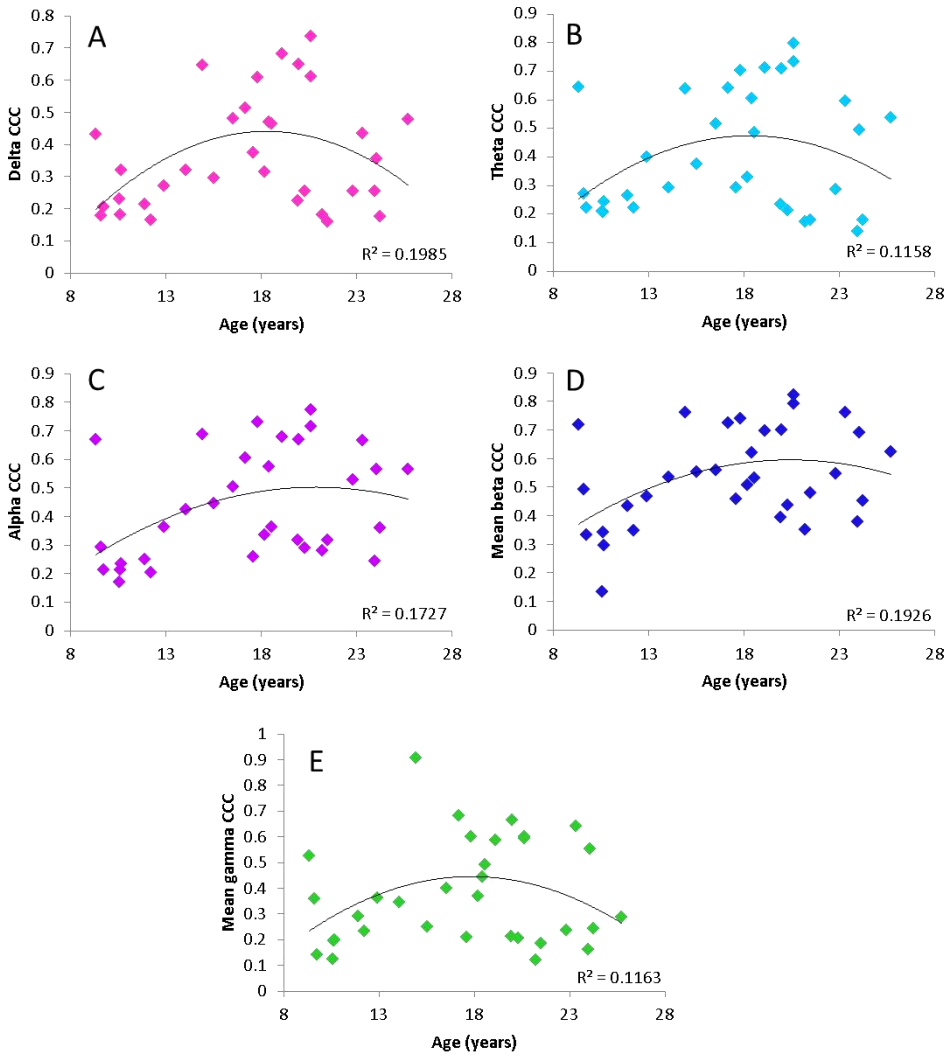


Figure 7.8: Developmental profiles of FC between the sensorimotor network region pair under relevance modulation conditions; (A) Delta; 1-4 Hz (B) Theta; 4-8 Hz (C) Alpha; 8-13 Hz (D) Beta; 13-30 Hz (E) Gamma; 30-60 Hz.

Figure 7.9 shows the developmental profiles of FC over specific frequency bands for the sensorimotor network region pair under resting state conditions. All are second order polynomial correlations. The trajectories of delta, alpha and beta FC changes appear to correlate most strongly with age in the sensorimotor network under resting state conditions. All developmental trajectories appear to peak aged 20-22, aside from gamma which appears to exhibit a smaller change over a longer period possibly peaking at our upper age limit of 25 years.

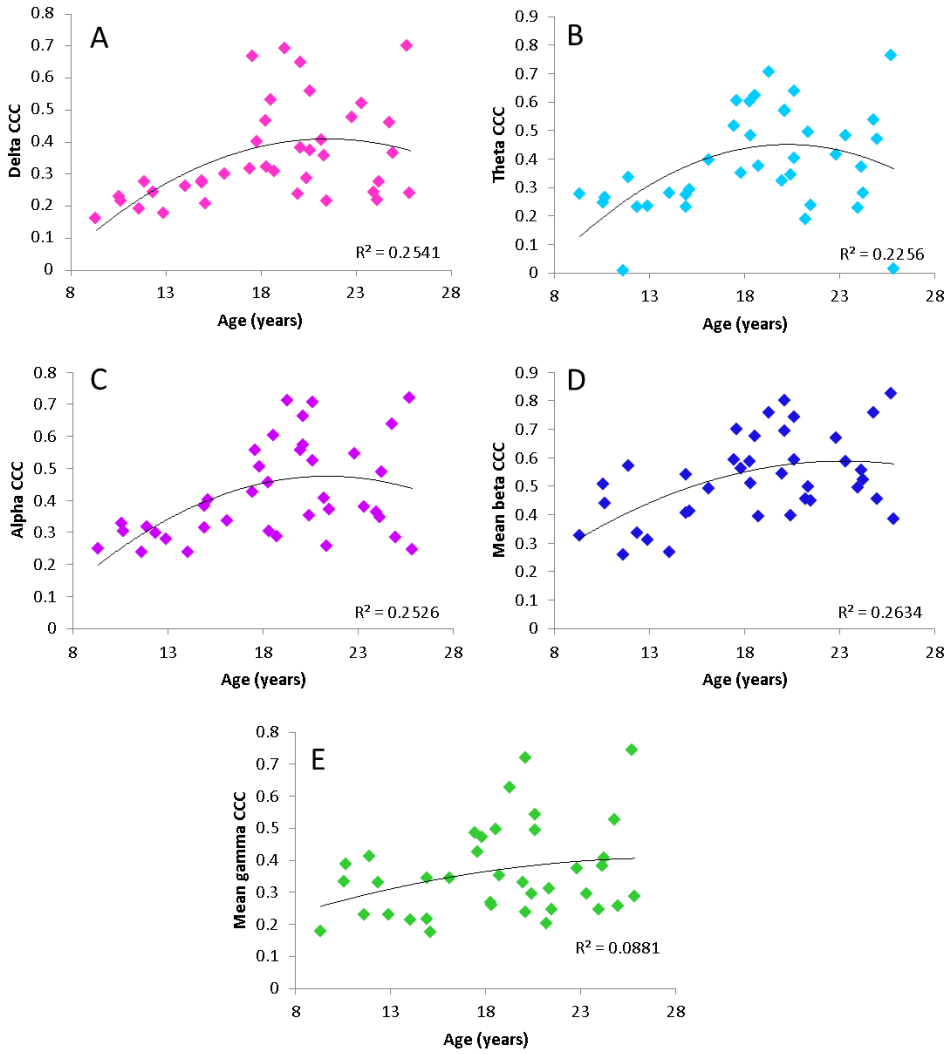


Figure 7.9: Developmental profiles of FC between the sensorimotor network region pair under resting state conditions; (A) Delta; 1-4 Hz (B) Theta; 4-8 Hz (C) Alpha; 8-13 Hz (D) Beta; 13-30 Hz (E) Gamma; 30-60 Hz.

Appendix 22. The left dorsal attention network region pair

Figure 7.10 depicts the FC profiles of the left DAN region pair for different age groups under the different task conditions studied in this thesis.

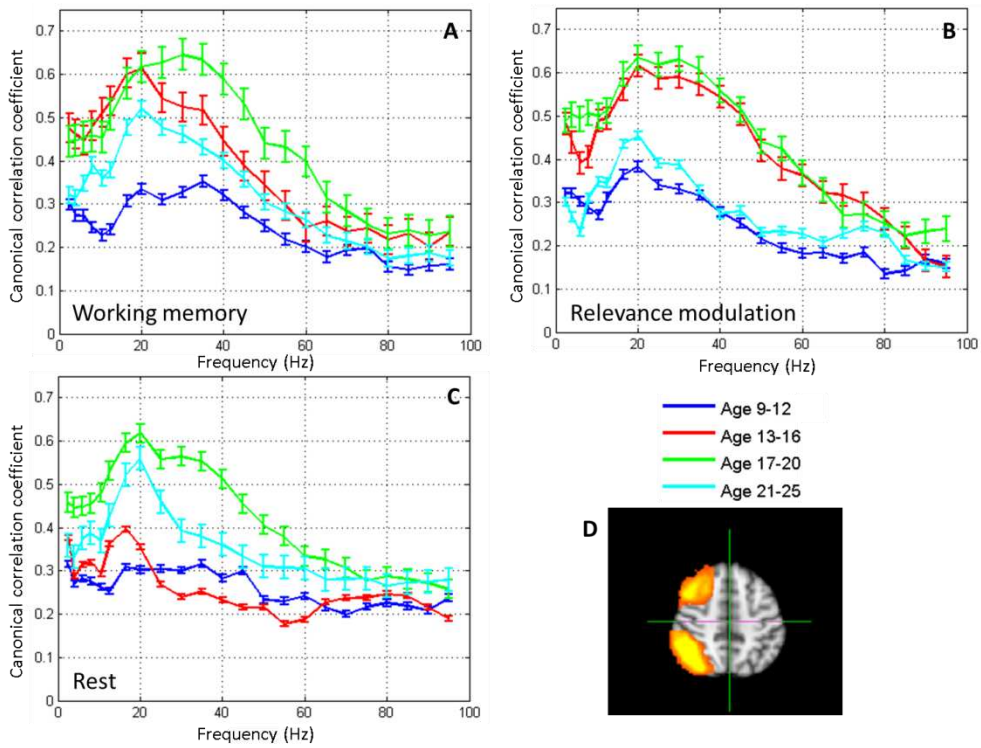


Figure 7.10: (A) The profiles of FC under different conditions from age 9-25 within the left dorsal attention network (B) The left dorsal attention network region pair used for the canonical correlation analysis.

FC appears to peak in strength broadly over the beta band. The youngest age group (age 9-12) consistently demonstrates the weakest FC profile in this network across the task conditions (working memory and relevance modulation), however, age 13-16 show similarly weak FC to age 9-12 under the resting state condition. The oldest age group (age 21-25) does not demonstrate the strongest FC profile, as it appears the younger and older adolescents (aged 13-16 and 17-20) show the strongest FC under task conditions, and the older adolescents (age 17-20) show the strongest FC under the resting state condition.

Figure 7.11 shows the developmental profiles of FC over specific frequency bands for the left DAN region pair under working memory task conditions. All are second order polynomial correlations. The trajectory of beta FC development appears to correlate most strongly with age in the left DAN under working memory task conditions. All trajectories appear to peak aged 18-20.

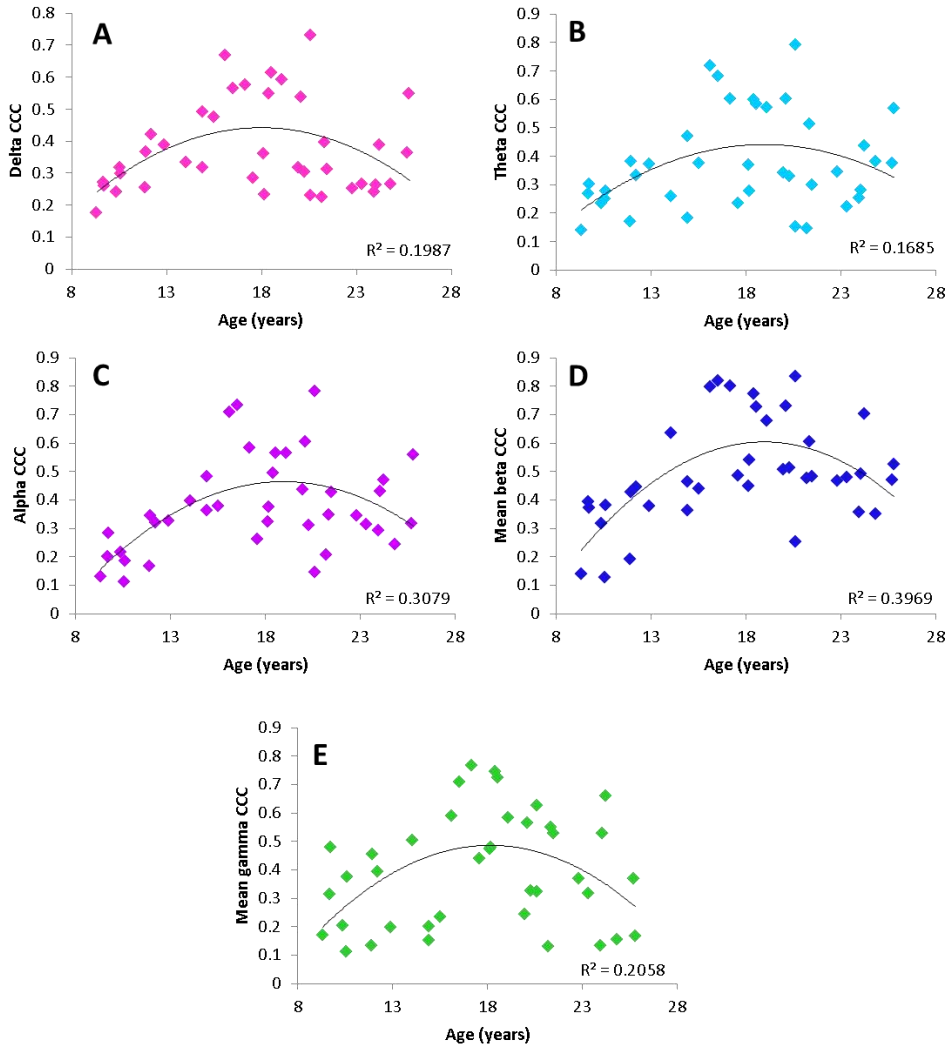


Figure 7.11: Developmental profiles of FC between the left DAN region pair under working memory conditions; (A) Delta; 1-4 Hz (B) Theta; 4-8 Hz (C) Alpha; 8-13 Hz (D) Beta; 13-30 Hz (E) Gamma; 30-60 Hz.

Figure 7.12 shows the developmental profiles of FC over specific frequency bands for the left DAN region pair under relevance modulation task conditions. All are second order polynomial correlations. The trajectory of delta FC change appears to correlate most strongly with age in the left DAN under relevance modulation task conditions. All developmental trajectories appear to peak aged 16-18.

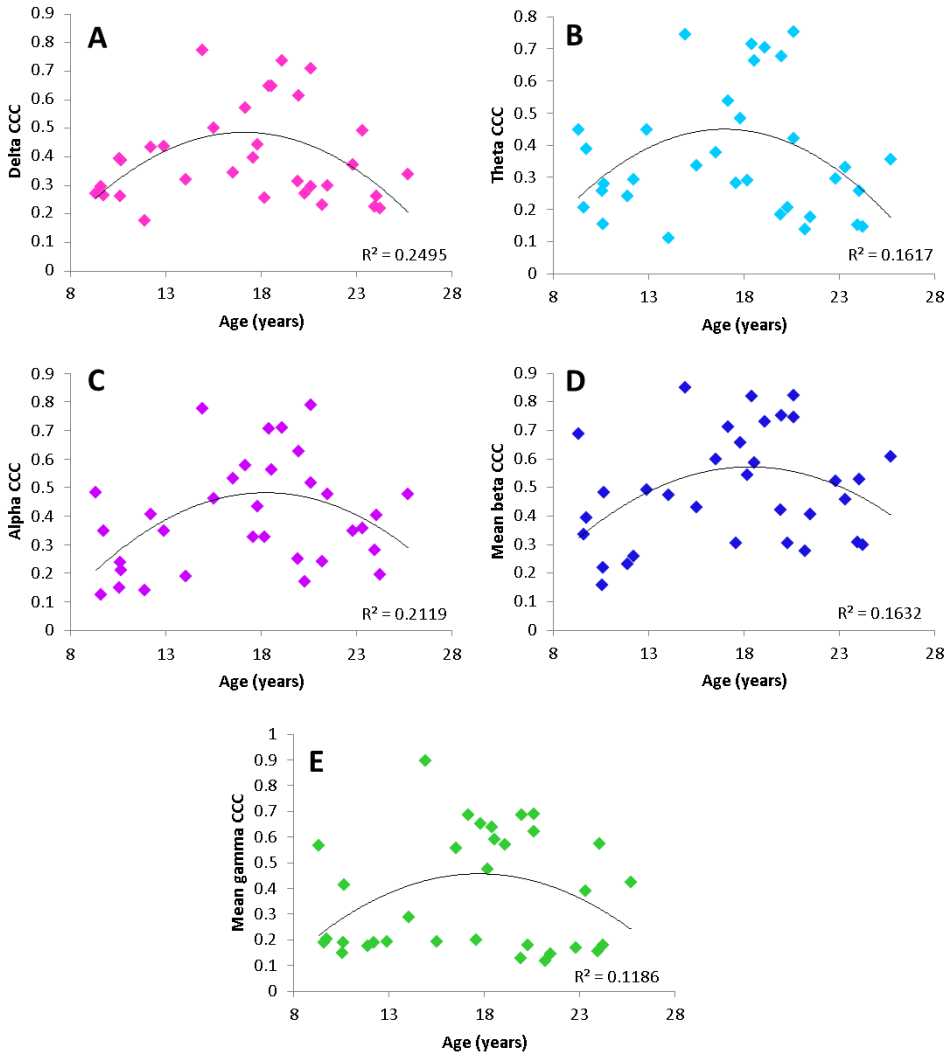


Figure 7.12: Developmental profiles of FC between the left DAN region pair under relevance modulation conditions; (A) Delta; 1-4 Hz (B) Theta; 4-8 Hz (C) Alpha; 8-13 Hz (D) Beta; 13-30 Hz (E) Gamma; 30-60 Hz.

Figure 7.13 shows the developmental profiles of FC over specific frequency bands for the left DAN region pair under resting state conditions. All are second order polynomial correlations. The trajectory of beta FC change appears to correlate most strongly with age in the left DAN under resting state conditions. The developmental trajectories of delta and alpha appear to peak around age 19, with theta peaking around age 22 and beta and gamma peaking around our upper age limit of 23-25 years.

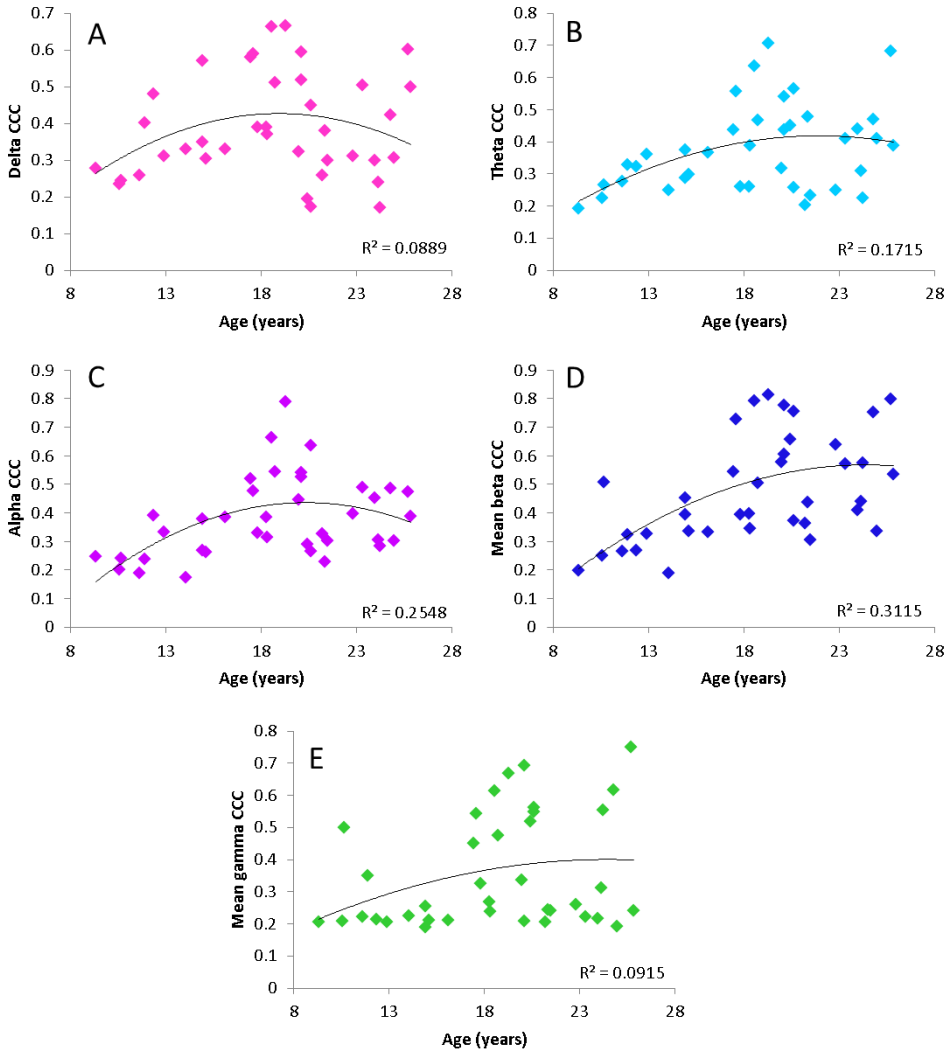


Figure 7.13: Developmental profiles of FC between the left DAN region pair under resting state conditions; (A) Delta; 1-4 Hz (B) Theta; 4-8 Hz (C) Alpha; 8-13 Hz (D) Beta; 13-30 Hz (E) Gamma; 30-60 Hz.

Appendix 23. The right dorsal attention network region pair

Figure 7.14 depicts the FC profiles of the right DAN region pair for different age groups under the different task conditions studied in this thesis.

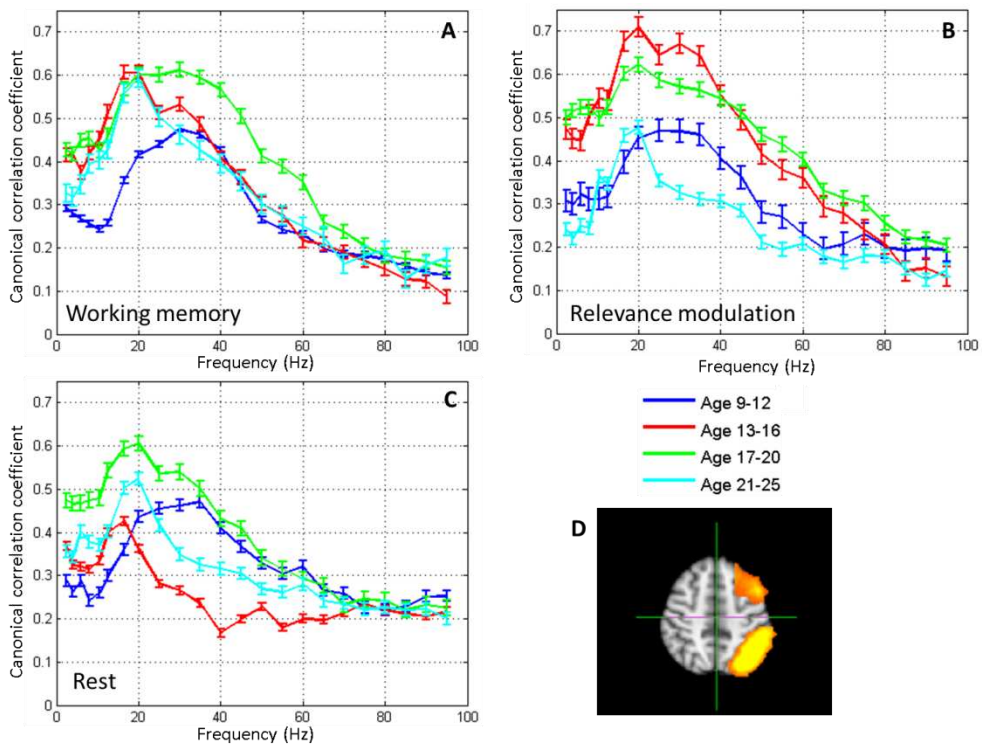


Figure 7.14: (A) The profiles of FC under different conditions from age 9-25 within the right dorsal attention network (B) The right dorsal attention network region pair used for the canonical correlation analysis.

FC appears to peak in strength broadly over the beta band however the peak in FC for the youngest age group (age 9-12) appears to be shifted to a slightly higher frequency. Again, we see that the oldest age group (age 21-25) does not demonstrate the strongest FC profile. It appears the younger and older adolescents (aged 13-16 and 17-20) show the strongest FC under the task conditions (this is particularly striking under the relevance modulation condition and much less apparent under the working memory condition), and the older adolescents (age 17-20) show the strongest FC under the resting state condition.

Figure 7.15 shows the developmental profiles of FC over specific frequency bands for the right DAN region pair under working memory task conditions. All are second order polynomial correlations. The trajectory of alpha FC development appears to correlate most strongly with age in the right DAN under working memory task conditions. The trajectories of delta, theta and gamma appear to peak aged 18-20 whilst the trajectories of alpha and beta appear to peak a little later, around age 20-22.

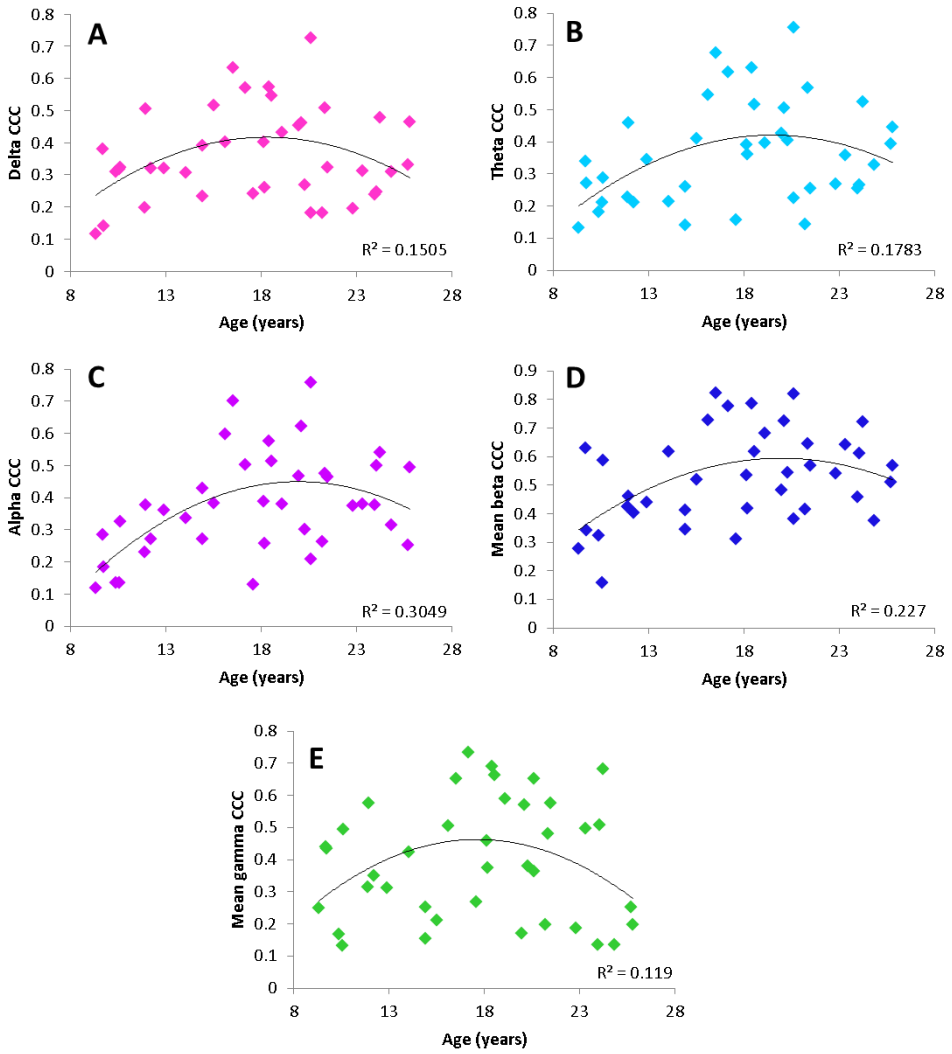


Figure 7.15: Developmental profiles of FC between the right DAN region pair under working memory conditions; (A) Delta; 1-4 Hz (B) Theta; 4-8 Hz (C) Alpha; 8-13 Hz (D) Beta; 13-30 Hz (E) Gamma; 30-60 Hz.

Figure 7.16 shows the developmental profiles of FC over specific frequency bands for the right DAN region pair under relevance modulation task conditions. All are second order polynomial correlations. The trajectory of delta FC development appears to correlate most strongly with age in the right DAN under relevance modulation task conditions. All developmental trajectories appear to peak aged 16-18.

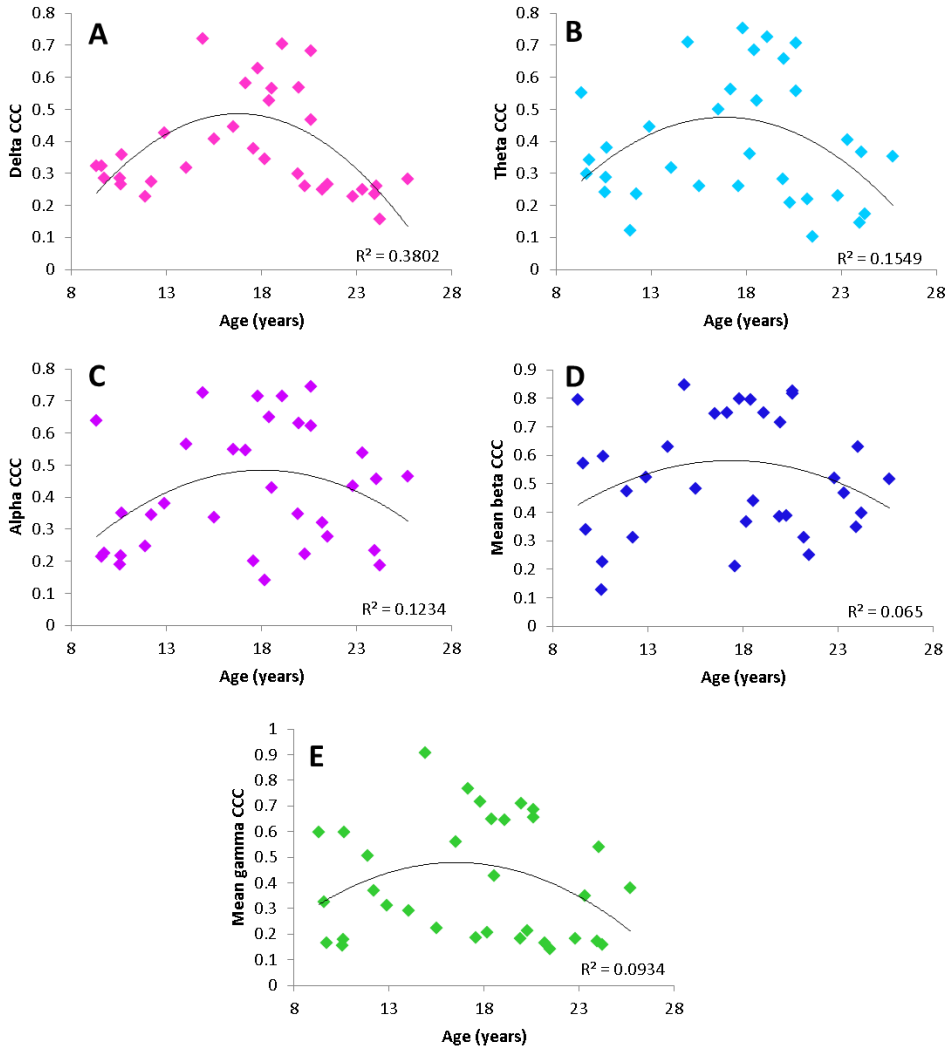


Figure 7.16: Developmental profiles of FC between the right DAN region pair under relevance modulation conditions; (A) Delta; 1-4 Hz (B) Theta; 4-8 Hz (C) Alpha; 8-13 Hz (D) Beta; 13-30 Hz (E) Gamma; 30-60 Hz.

Figure 7.17 shows the developmental profiles of FC over specific frequency bands for the right DAN region pair under resting state conditions. All are second order polynomial correlations. The trajectory of theta FC change appears to correlate most strongly with age in the right DAN under resting state conditions. The developmental trajectories of delta, theta and alpha appear to peak around age 19-20, with beta peaking around age 22 and gamma not exhibiting any peak in FC over the age range studied here.

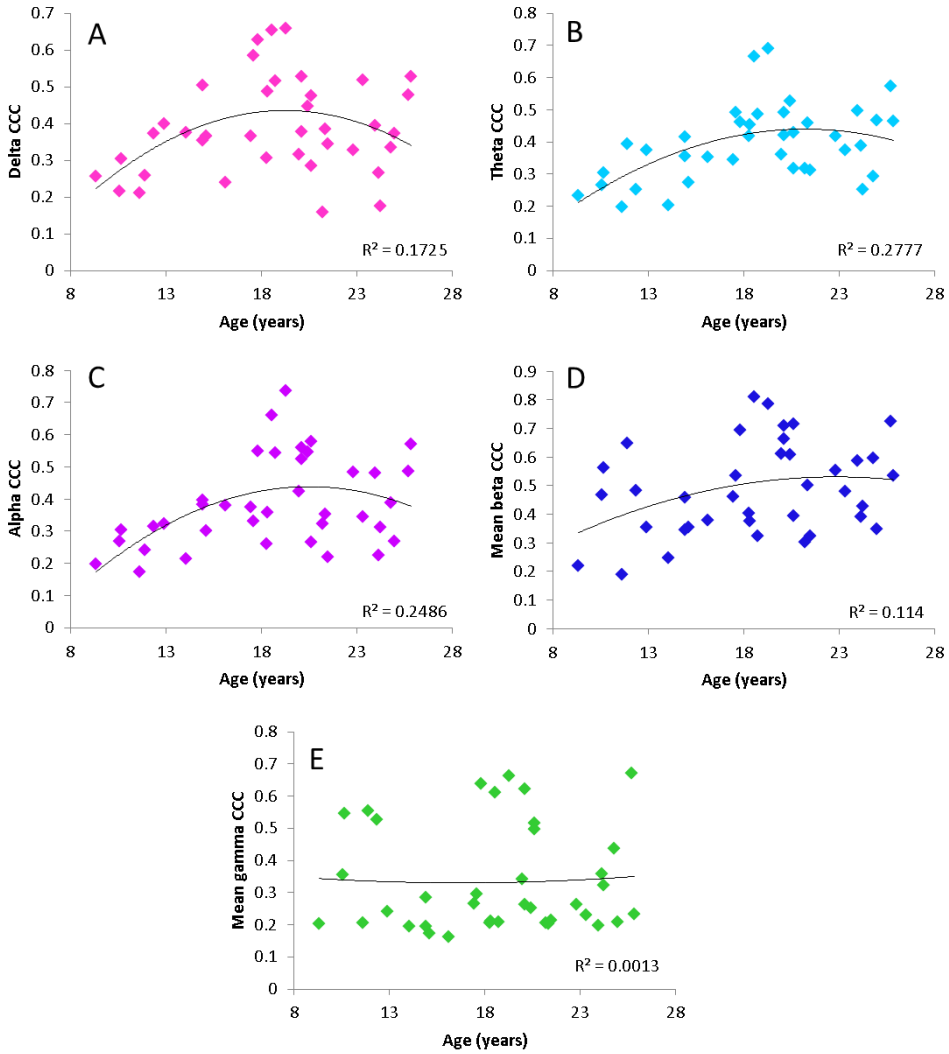


Figure 7.17: Developmental profiles of FC between the right DAN region pair under resting state conditions; (A) Delta; 1-4 Hz (B) Theta; 4-8 Hz (C) Alpha; 8-13 Hz (D) Beta; 13-30 Hz (E) Gamma; 30-60 Hz.

Appendix 24. The posterior dorsal attention network region pair

Figure 7.18 depicts the FC profiles of the posterior DAN region pair for different age groups under the different task conditions studied in this thesis.

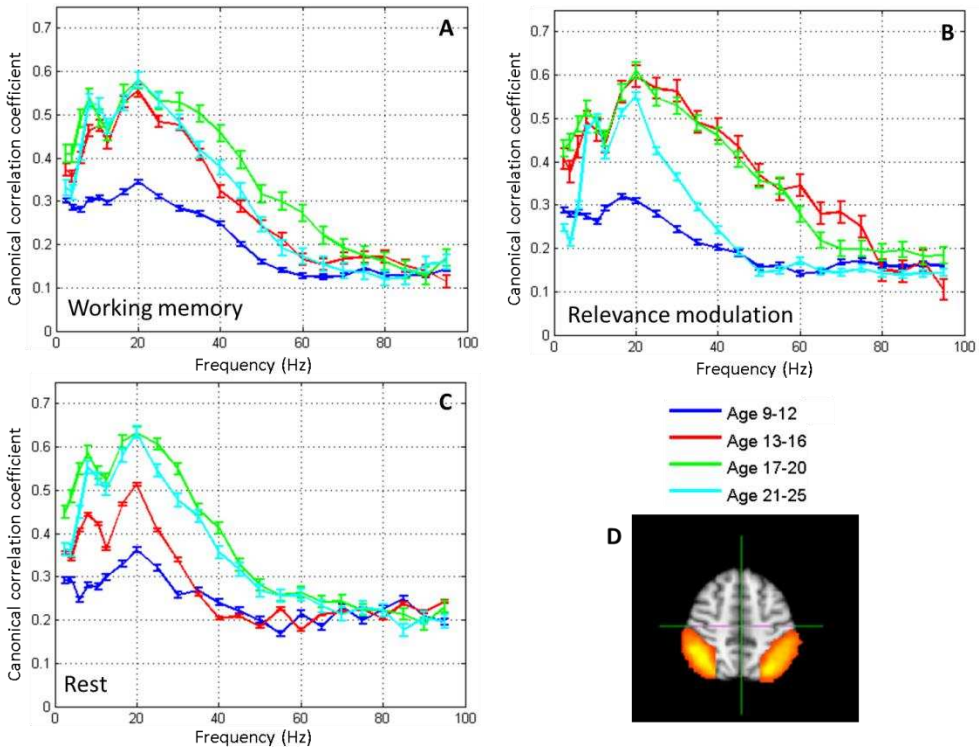


Figure 7.18: (A) The profiles of FC under different conditions from age 9-25 within the posterior dorsal attention network (B) The posterior dorsal attention network region pair used for the canonical correlation analysis.

FC appears to peak in strength over the theta and beta bands, and the youngest age group (age 9-12) consistently demonstrates the weakest FC profile in this network across all conditions (working memory, relevance modulation and resting state).

Figure 7.19 shows the developmental profiles of FC over specific frequency bands for the posterior DAN region pair under working memory task conditions. All are second order polynomial correlations. The trajectories of alpha and beta FC development appear to correlate most strongly with age in the posterior DAN under working memory task conditions. The trajectories of delta and gamma appear to peak aged 18-19 whilst the trajectories of theta, alpha and beta appear to peak a little later, around age 21-22.

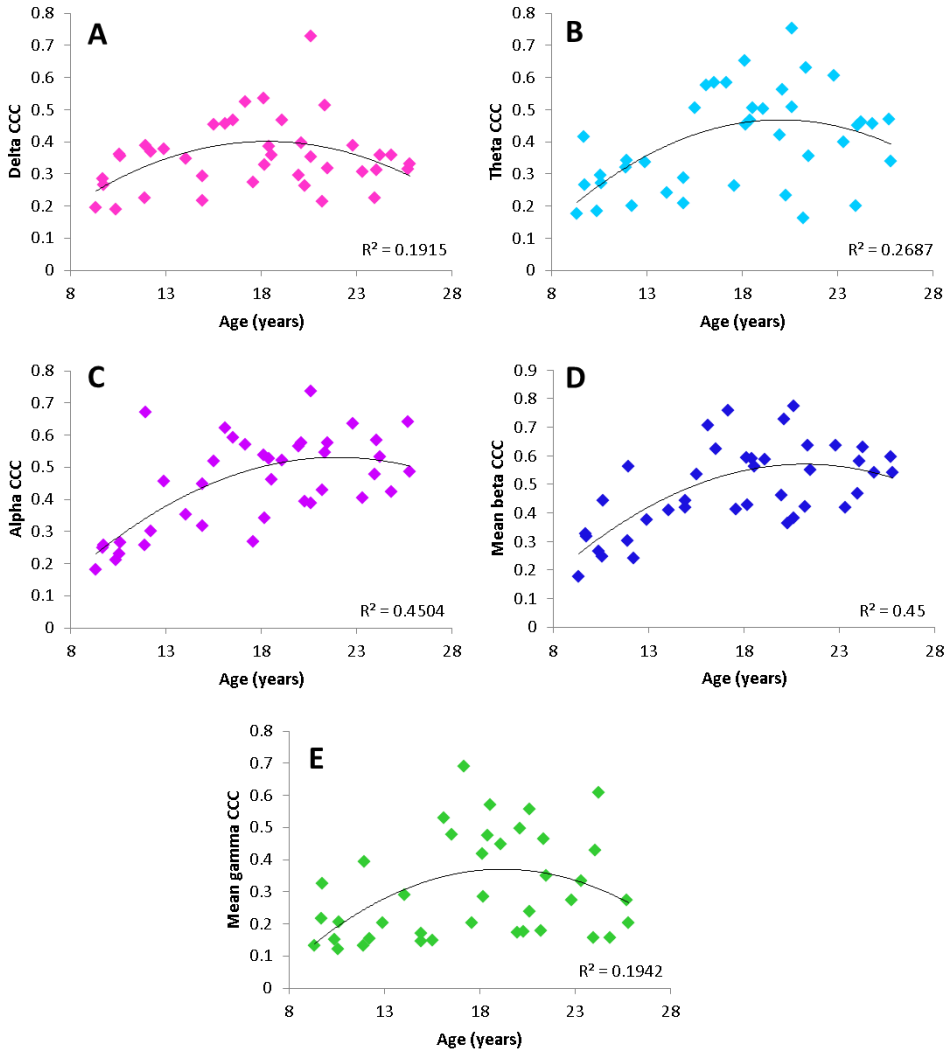


Figure 7.19: Developmental profiles of FC between the posterior DAN region pair under working memory conditions; (A) Delta; 1-4 Hz (B) Theta; 4-8 Hz (C) Alpha; 8-13 Hz (D) Beta; 13-30 Hz (E) Gamma; 30-60 Hz.

Figure 7.20 shows the developmental profiles of FC over specific frequency bands for the posterior DAN region pair under relevance modulation task conditions. All are second order polynomial correlations. The trajectory of alpha FC change appears to correlate most strongly with age in the posterior DAN under relevance modulation task conditions. Whilst the developmental trajectories of delta, theta and gamma FC appear to peak aged 17-18, the trajectories of alpha and beta appear to peak later on, approximately aged 20-21.

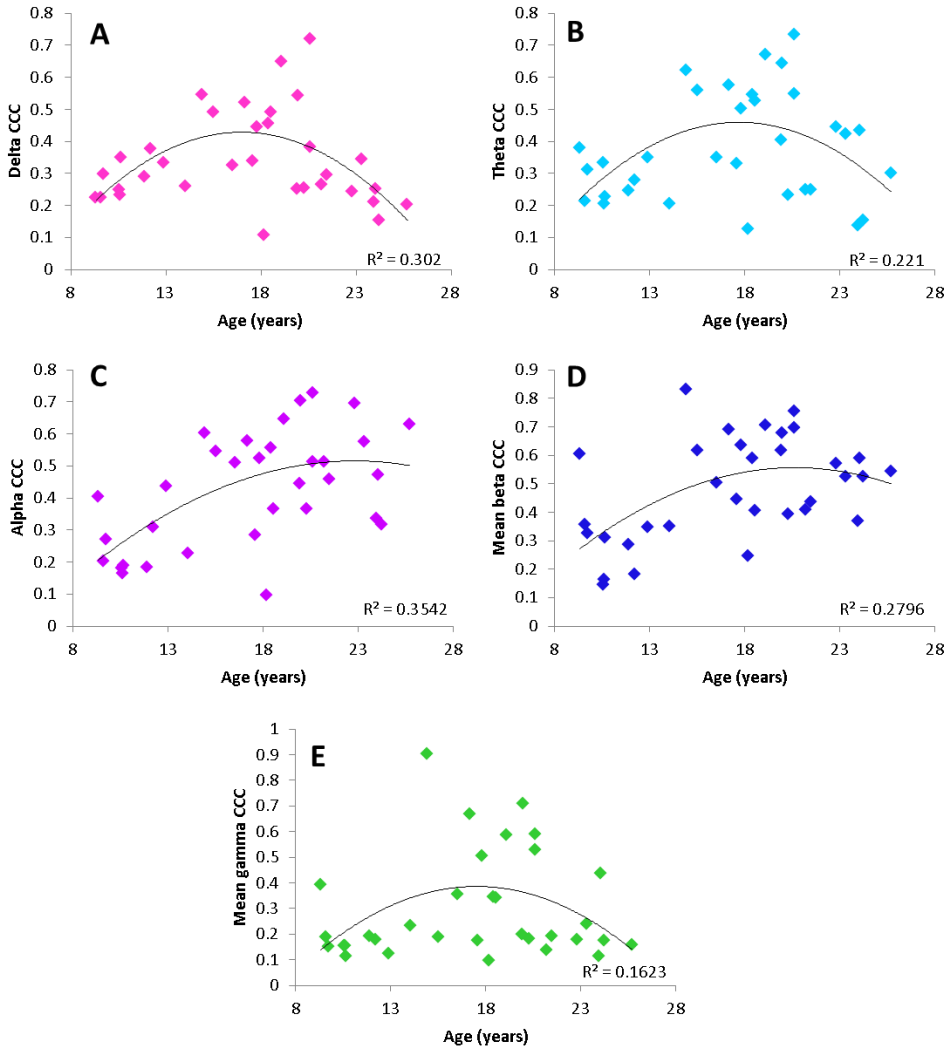


Figure 7.20: Developmental profiles of FC between the posterior DAN region pair under relevance modulation conditions; (A) Delta; 1-4 Hz (B) Theta; 4-8 Hz (C) Alpha; 8-13 Hz (D) Beta; 13-30 Hz (E) Gamma; 30-60 Hz.

Figure 7.21 shows the developmental profiles of FC over specific frequency bands for the posterior DAN region pair under resting state conditions. All are second order polynomial correlations. The trajectory of alpha FC changes appears to correlate most strongly with age in the posterior DAN under resting state conditions; theta and beta also correlate well. The developmental trajectories of delta and theta appear to peak aged 18-19, with alpha and beta peaking around age 22 and gamma possibly peaking post age 25.

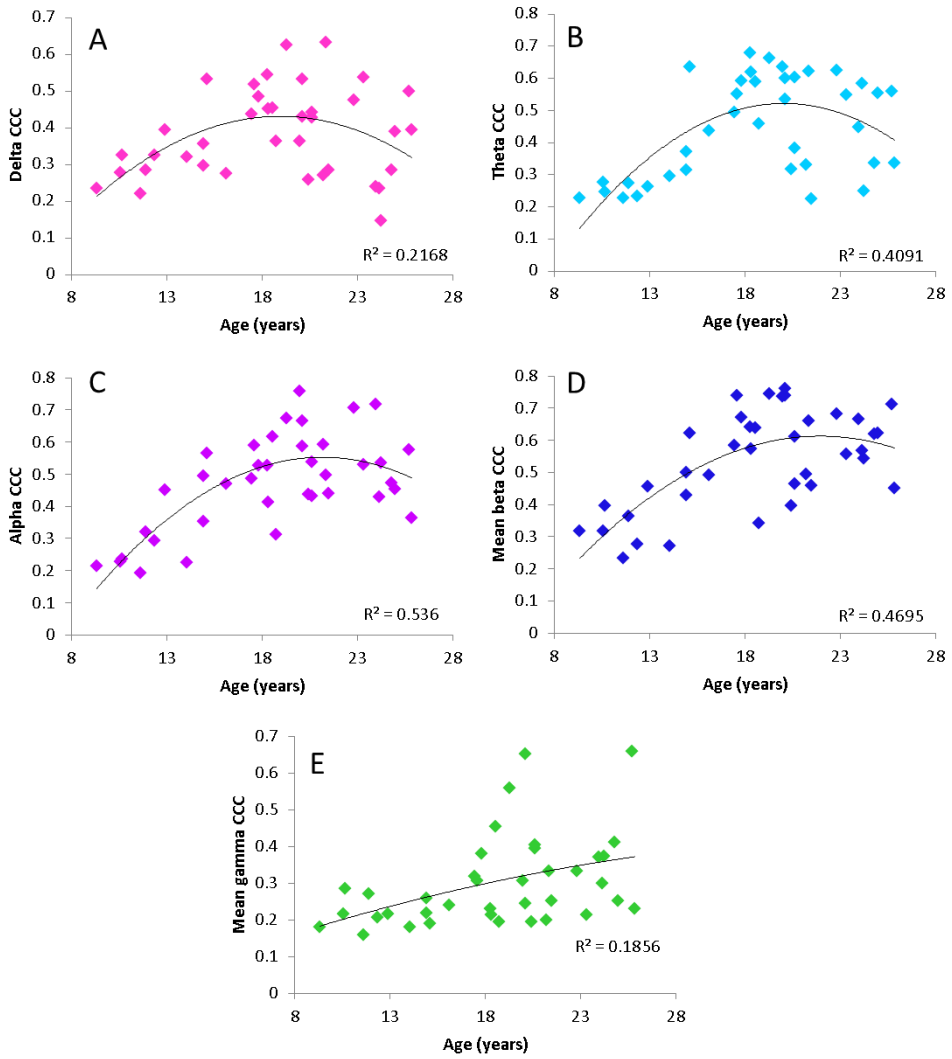


Figure 7.21: Developmental profiles of FC between the posterior DAN region pair under resting state conditions; (A) Delta; 1-4 Hz (B) Theta; 4-8 Hz (C) Alpha; 8-13 Hz (D) Beta; 13-30 Hz (E) Gamma; 30-60 Hz.

Appendix 25. The anterior dorsal attention network region pair

Figure 7.22 depicts the FC profiles of the anterior DAN region pair for different age groups under the different task conditions studied in this thesis.

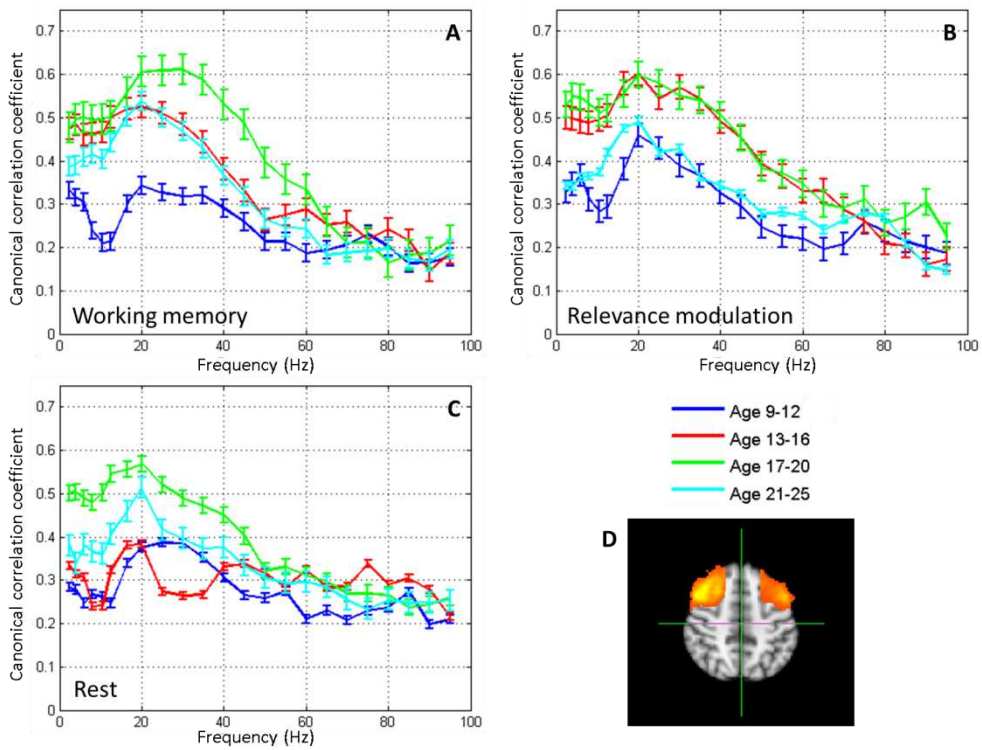


Figure 7.22: (A) The profiles of FC under different conditions from age 9-25 within the anterior dorsal attention network (B) The anterior dorsal attention network region pair used for the canonical correlation analysis.

FC appears to peak in strength broadly over the beta band. Under the working memory task condition the youngest age group (age 9-12) demonstrate the weakest FC profile, however under relevance modulation task conditions age 21-25 shows a similarly weak profile and under resting state conditions age 13-16 shows a similarly weak profile. The younger and older adolescents (aged 13-16 and 17-20) show the strongest FC profile under the relevance modulation task condition, and the older adolescents (age 17-20) show the strongest profile under both the working memory and resting state task conditions.

Figure 7.23 shows the developmental profiles of FC over specific frequency bands for the anterior DAN region pair under working memory task conditions. All are second order polynomial correlations. The trajectory of alpha FC development appears to correlate most strongly with age in the anterior DAN under working memory task conditions. The trajectory of gamma development appears to peak aged 18 whilst the trajectories of delta, theta, alpha and beta appear to peak a little later at ages 19-20.

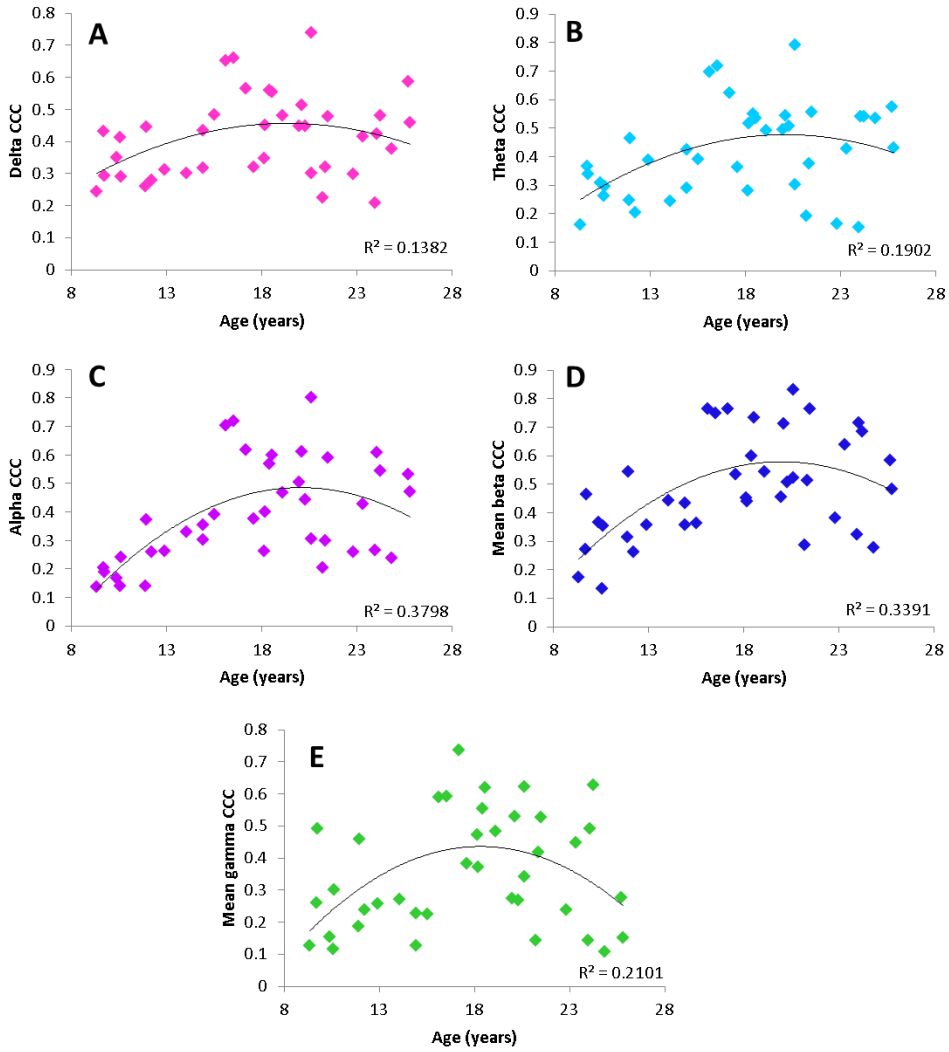


Figure 7.23: Developmental profiles of FC between the anterior DAN region pair under working memory conditions; (A) Delta; 1-4 Hz (B) Theta; 4-8 Hz (C) Alpha; 8-13 Hz (D) Beta; 13-30 Hz (E) Gamma; 30-60 Hz.

Figure 7.24 shows the developmental profiles of FC over specific frequency bands for the DAN region pair number 5 under relevance modulation task conditions. All are second order polynomial correlations. The trajectory of delta FC change appears to correlate most strongly with age in the anterior DAN under relevance modulation task conditions. All developmental trajectories appear to peak at age 16-19.

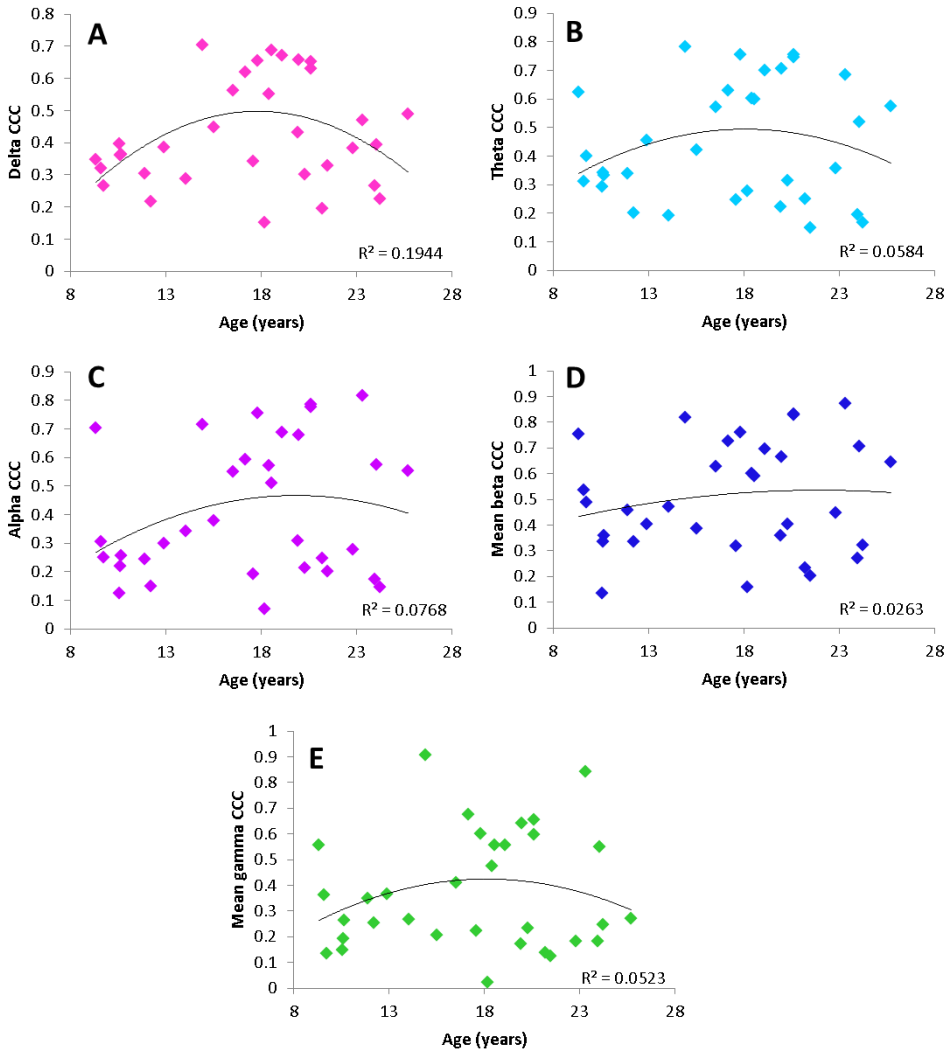


Figure 7.24: Developmental profiles of FC between the anterior DAN region pair under relevance modulation conditions; (A) Delta; 1-4 Hz (B) Theta; 4-8 Hz (C) Alpha; 8-13 Hz (D) Beta; 13-30 Hz (E) Gamma; 30-60 Hz.

Figure 7.25 shows the developmental profiles of FC over specific frequency bands for the anterior DAN under resting state conditions. All are second order polynomial correlations. The trajectory of theta FC changes appears to correlate most strongly with age in the anterior DAN under resting state conditions. The developmental trajectories of delta, theta and alpha appear to peak around age 19-21, with beta and gamma possibly peaking outside of the limits of the age range studied here.

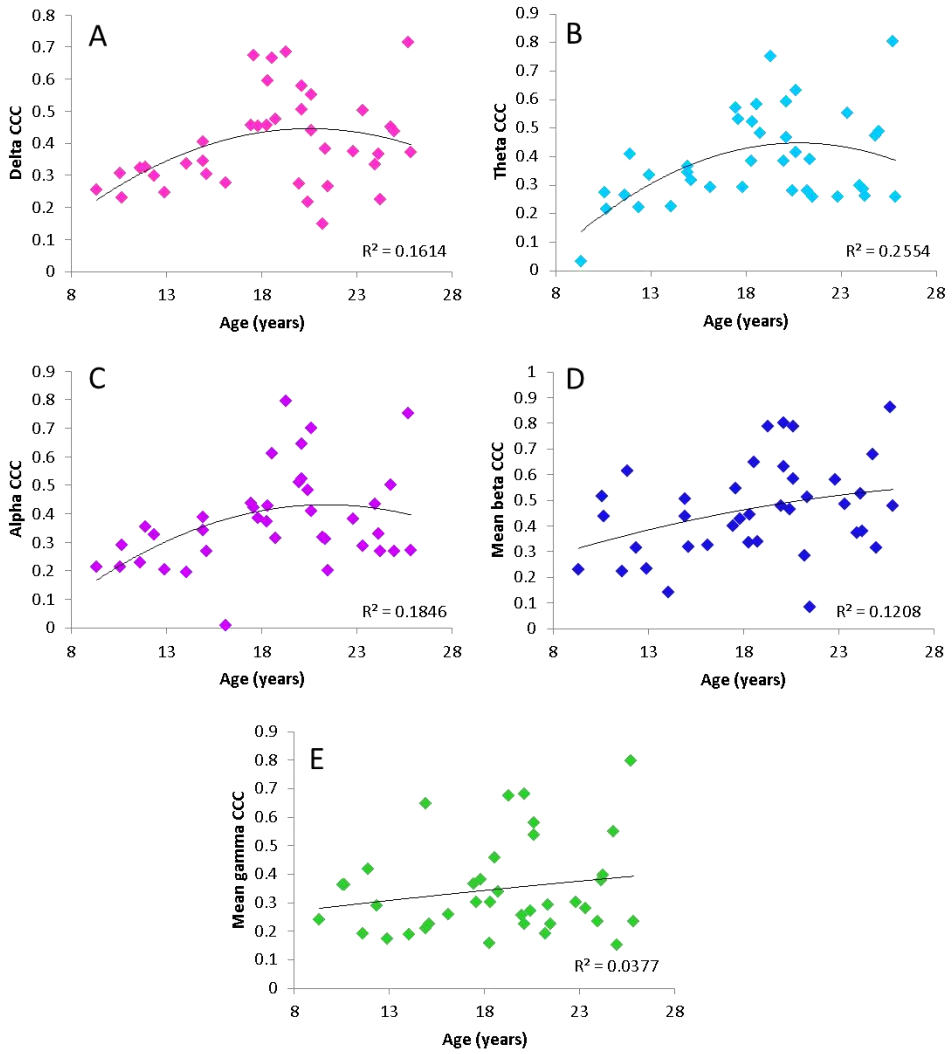


Figure 7.25: Developmental profiles of FC between the anterior DAN region pair under resting state conditions; (A) Delta; 1-4 Hz (B) Theta; 4-8 Hz (C) Alpha; 8-13 Hz (D) Beta; 13-30 Hz (E) Gamma; 30-60 Hz.

Appendix 26. The dorsal attention network region pair number 4

Figure 7.26 depicts the FC profiles of the DAN4 region pair (the left PPC and right DLPFC), for different age groups under the different task conditions studied in this thesis.

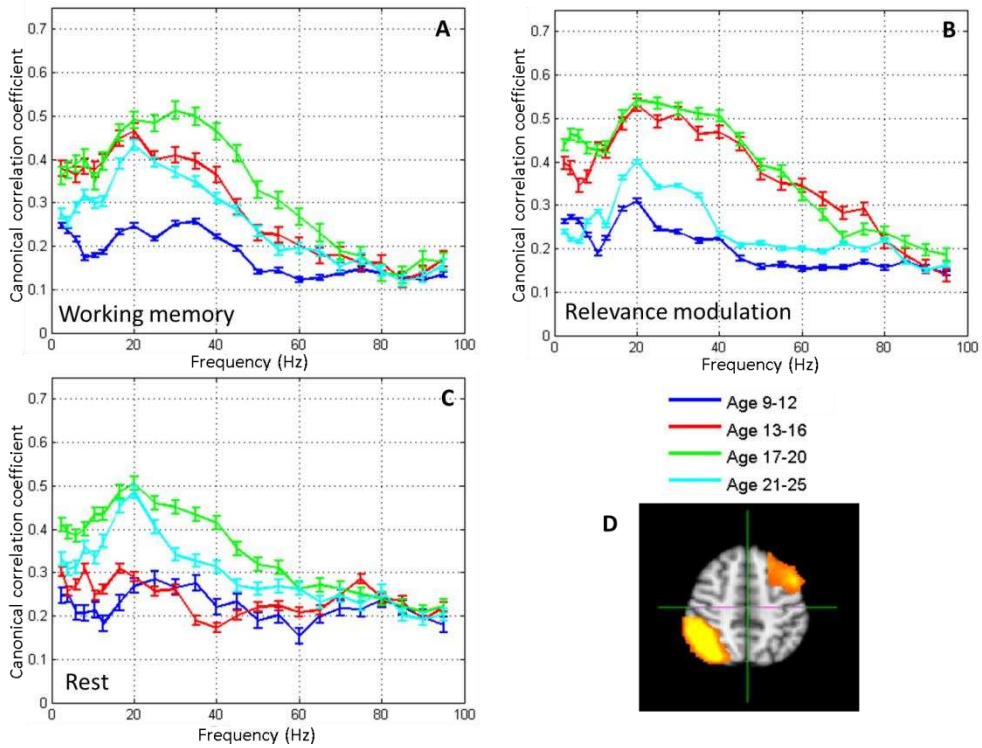


Figure 7.26: (A) The profiles of FC under different conditions from age 9-25 within the dorsal attention network region pair number 4 (B) The dorsal attention network region pair number 4 used for the canonical correlation analysis.

FC appears to peak in strength broadly over the beta band. The youngest age group (age 9-12) consistently demonstrates the weakest FC profile in this network across the task conditions (working memory and relevance modulation), however, age 13-16 show a similarly weak profile under the resting state condition. Again, the oldest age group (age 21-25) does not demonstrate the strongest FC profile, it appears the younger and older adolescents (aged 13-16 and 17-20) show the strongest FC under task conditions, and the older adolescents (age 17-20) show the strongest FC under the resting state condition.

Figure 7.27 shows the developmental profiles of FC over specific frequency bands for the DAN4 region pair under working memory task conditions. All are second order polynomial correlations. The trajectory of beta FC development appears to correlate most strongly with age in the DAN4 region pair under working memory task conditions. The trajectories of theta, delta and gamma appear to peak aged 18-19 whilst the trajectories of alpha and beta appear to peak a little later, around age 20-21.

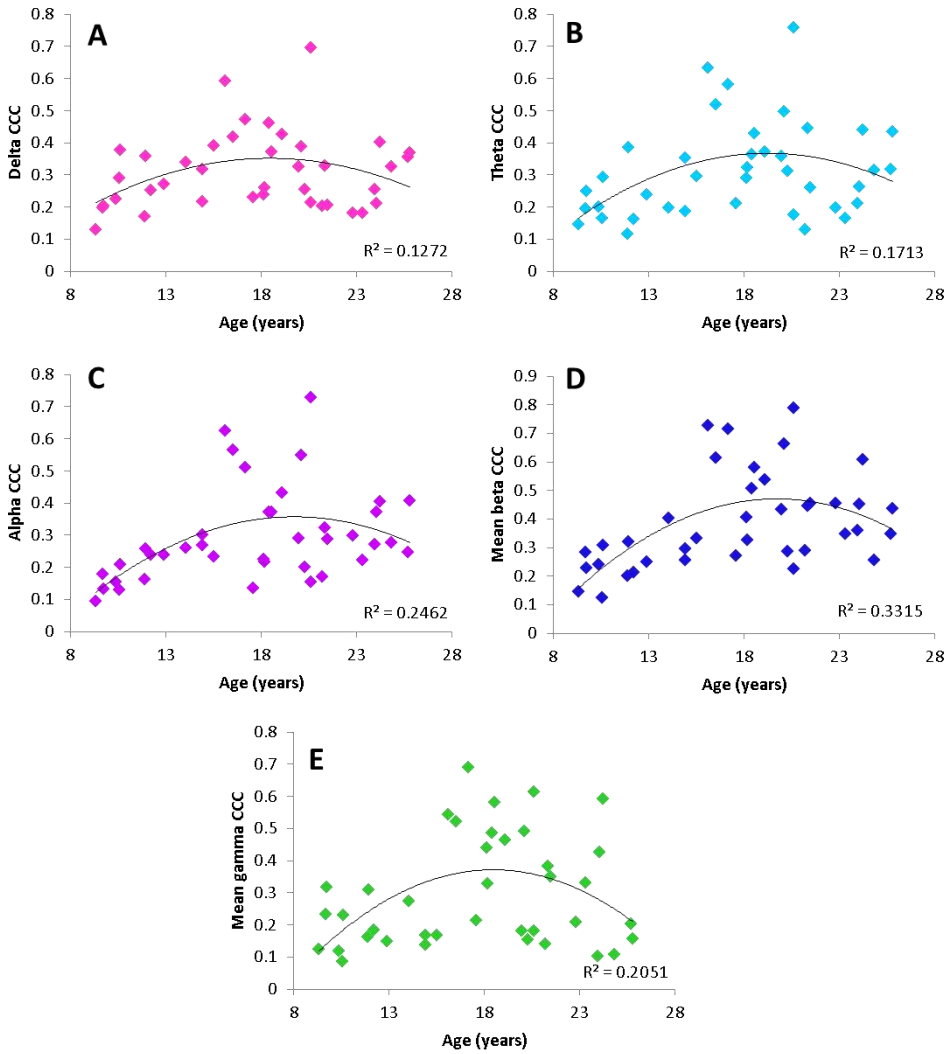


Figure 7.27: Developmental profiles of FC between the DAN4 region pair under working memory conditions; (A) Delta; 1-4 Hz (B) Theta; 4-8 Hz (C) Alpha; 8-13 Hz (D) Beta; 13-30 Hz (E) Gamma; 30-60 Hz.

Figure 7.28 shows the developmental profiles of FC over specific frequency bands for the DAN4 region pair under relevance modulation task conditions. All are second order polynomial correlations. The trajectory of delta FC change appears to correlate most strongly with age in the DAN4 region pair under relevance modulation task conditions. All developmental trajectories appear to peak aged 16-19.

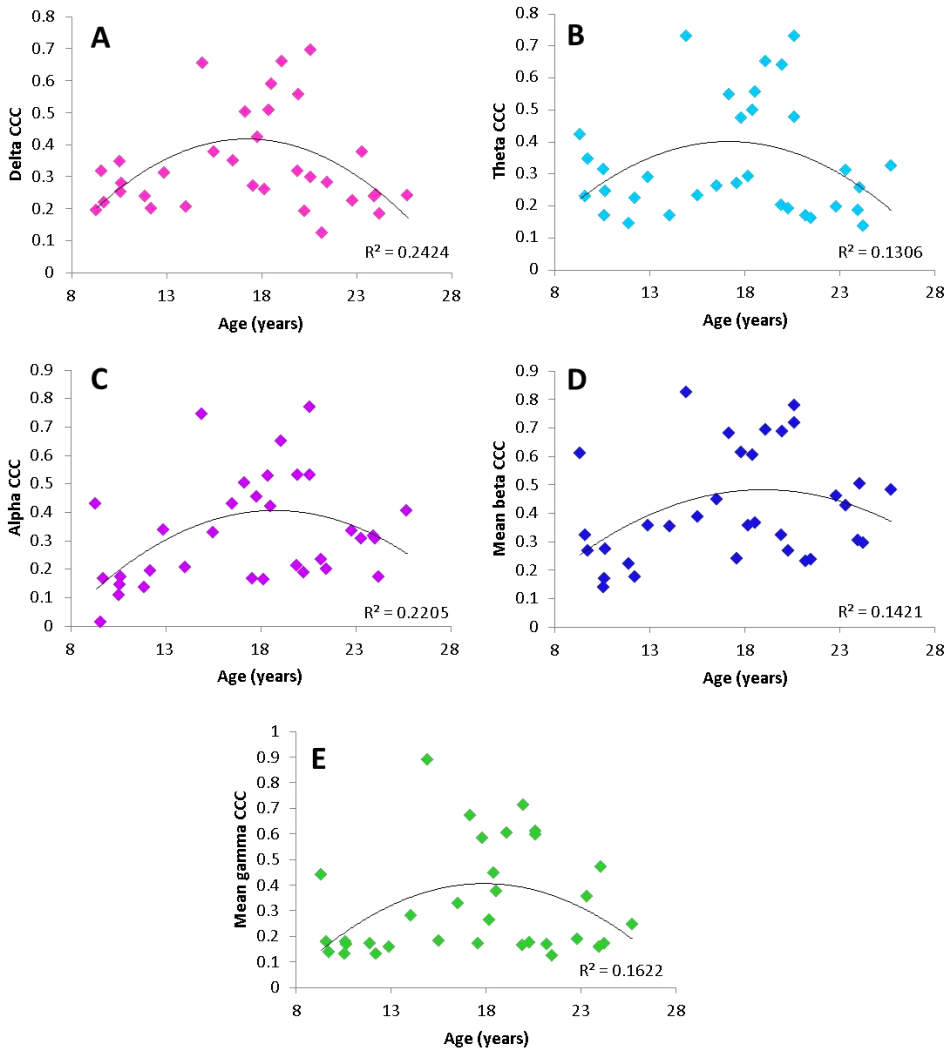


Figure 7.28: Developmental profiles of FC between the DAN4 region pair under resting state conditions; (A) Delta; 1-4 Hz (B) Theta; 4-8 Hz (C) Alpha; 8-13 Hz (D) Beta; 13-30 Hz (E) Gamma; 30-60 Hz.

Figure 7.29 shows the developmental profiles of FC over specific frequency bands for the DAN4 region pair under resting state conditions. All are second order polynomial correlations. The trajectory of beta FC change appears to correlate most strongly with age in the DAN4 region pair under resting state conditions. The developmental trajectories of delta, theta and alpha appear to peak around age 19-21, with beta and gamma possibly peaking outside of the limits of the age range studied here.

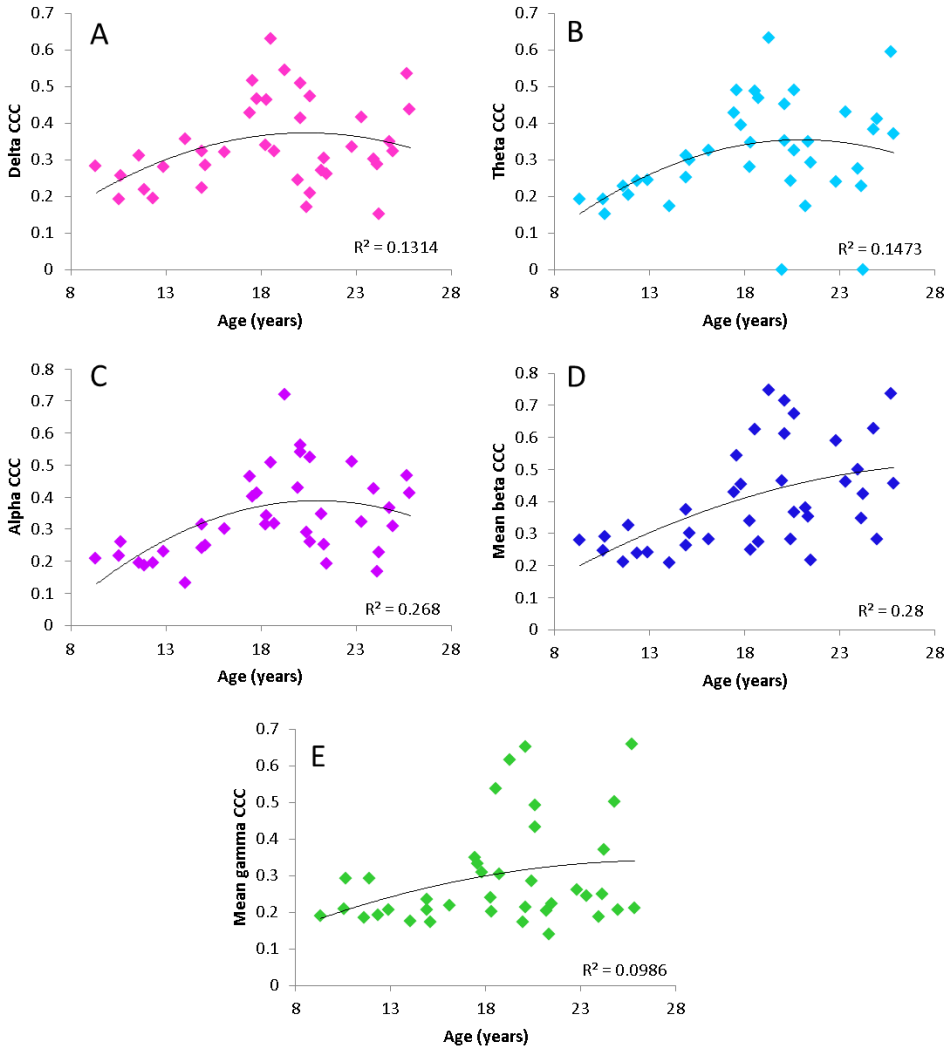


Figure 7.29: Developmental profiles of FC between the DAN4 region pair under resting state conditions; (A) Delta; 1-4 Hz (B) Theta; 4-8 Hz (C) Alpha; 8-13 Hz (D) Beta; 13-30 Hz (E) Gamma; 30-60 Hz.

Appendix 27. The dorsal attention network region pair number 5

Figure 7.30 depicts the FC profiles of the DAN5 region pair (the right PPC and left DLPFC), for different age groups under the different task conditions studied in this thesis.

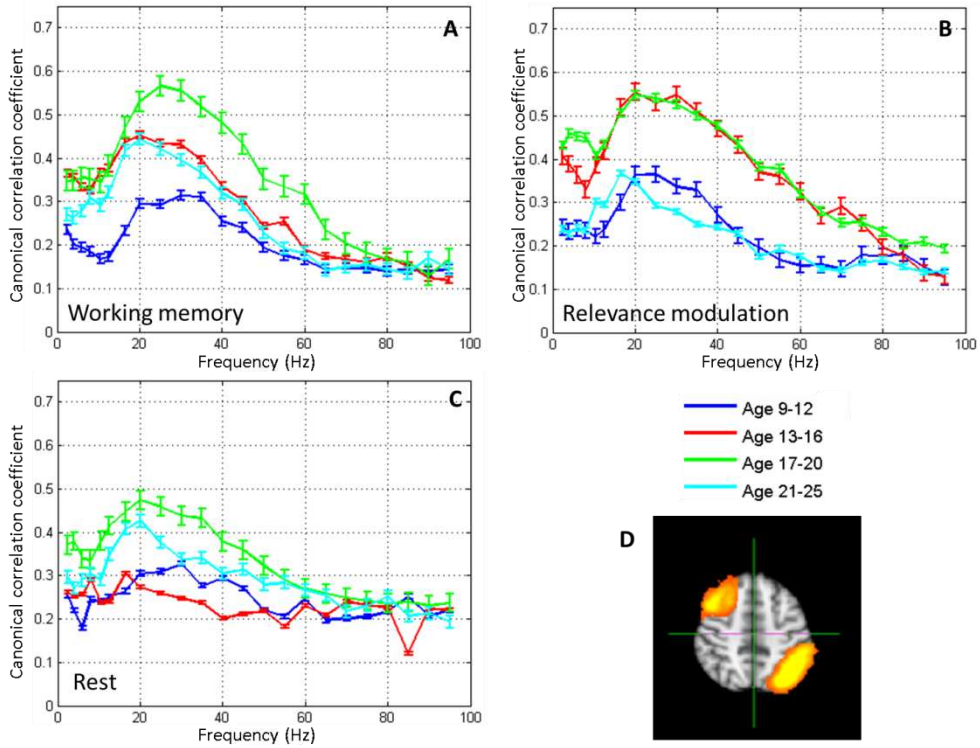


Figure 7.30: (A) The profiles of FC under different conditions from age 9-25 within the dorsal attention network region pair number 5 (B) The dorsal attention network region pair number 5 used for the canonical correlation analysis

FC appears to peak in strength broadly over the beta band. Under the working memory task condition the youngest age group (age 9-12) demonstrate the weakest FC profile, however under relevance modulation task conditions age 21-25 show a similarly weak profile and under resting state conditions age 13-16 show a similarly weak profile. The younger and older adolescents (aged 13-16 and 17-20) show the strongest FC profile under the relevance modulation task condition, and the older adolescents (age 17-20) show the strongest profile under both the working memory and resting state task conditions.

Figure 7.31 shows the developmental profiles of FC over specific frequency bands for the DAN5 region pair under working memory task conditions. All are second order polynomial correlations. The trajectory of beta FC development appears to correlate most strongly with age in the DAN region pair number 5 under working memory task conditions. The trajectories of delta and gamma appear to peak aged 18 whilst the trajectories of theta, alpha and beta appear to peak a little later, around age 19-20.

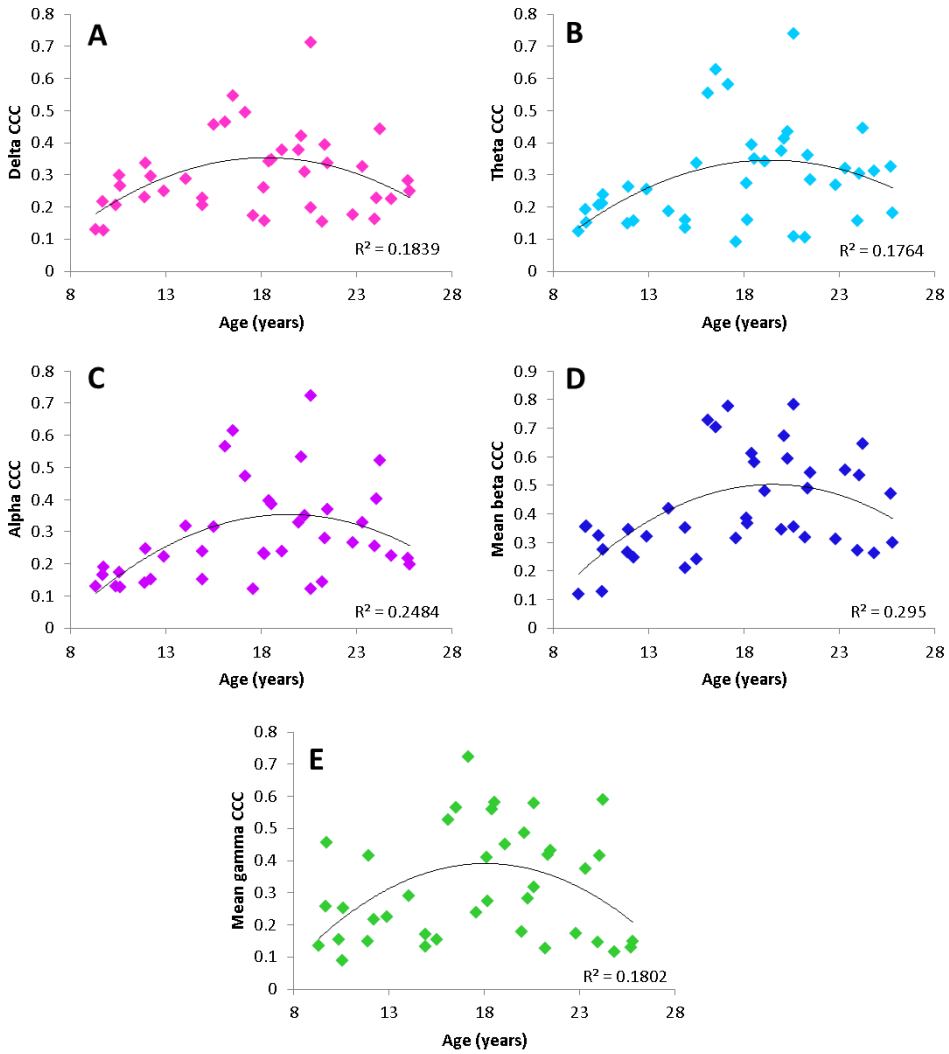


Figure 7.31: Developmental profiles of FC between the DAN5 region pair under working memory conditions; (A) Delta; 1-4 Hz (B) Theta; 4-8 Hz (C) Alpha; 8-13 Hz (D) Beta; 13-30 Hz (E) Gamma; 30-60 Hz.

Figure 7.32 shows the developmental profiles of FC over specific frequency bands for the DAN region pair number 5 under relevance modulation task conditions. All are second order polynomial correlations. The trajectory of delta FC change appears to correlate most strongly with age in the DAN region pair number 5 under relevance modulation task conditions. All developmental trajectories appear to peak aged 16-19.

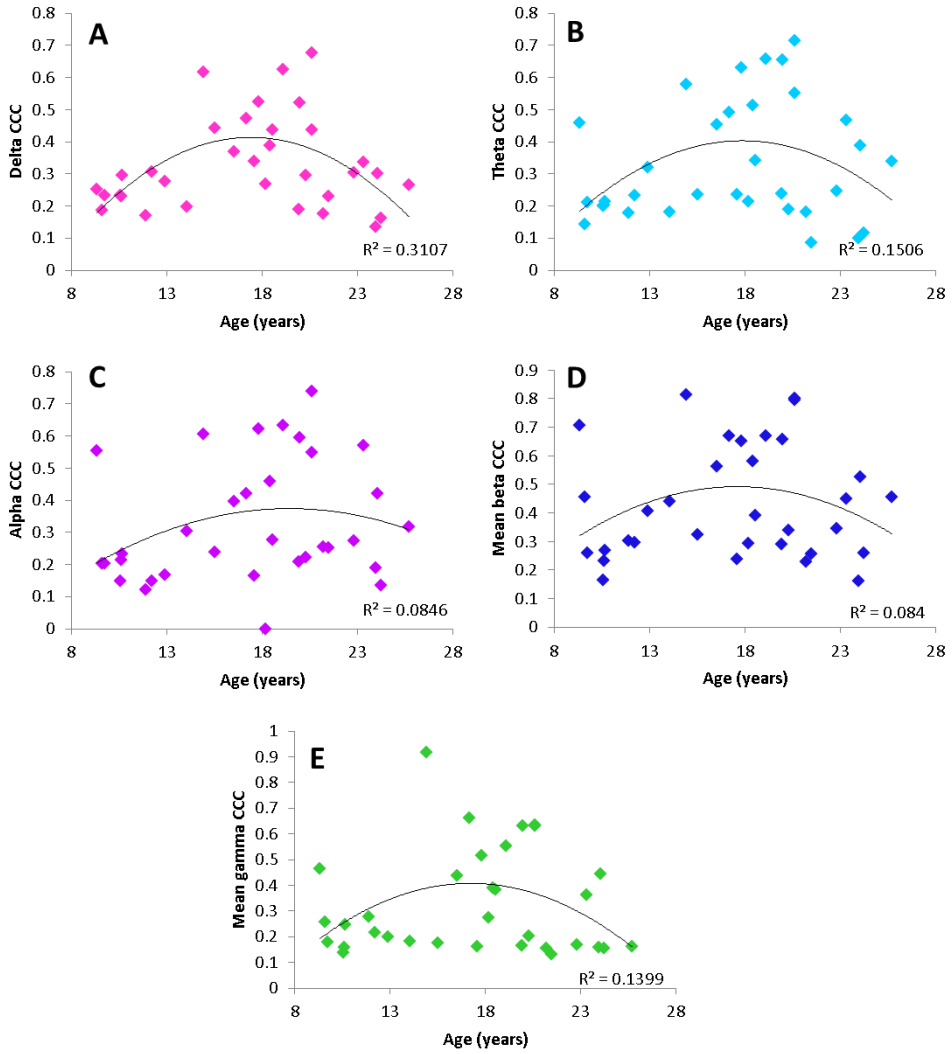


Figure 7.32: Developmental profiles of FC between the DAN5 region pair under resting state conditions; (A) Delta; 1-4 Hz (B) Theta; 4-8 Hz (C) Alpha; 8-13 Hz (D) Beta; 13-30 Hz (E) Gamma; 30-60 Hz.

Figure 7.33 shows the developmental profiles of FC over specific frequency bands for the DAN5 region pair under resting state conditions. All are second order polynomial correlations. The trajectory of theta FC changes appears to correlate most strongly with age in the DAN region pair number 5 under resting state conditions. The developmental trajectories of delta, theta and alpha appear to peak around age 19-21, with beta and gamma possibly peaking outside of the limits of the age range studied here.

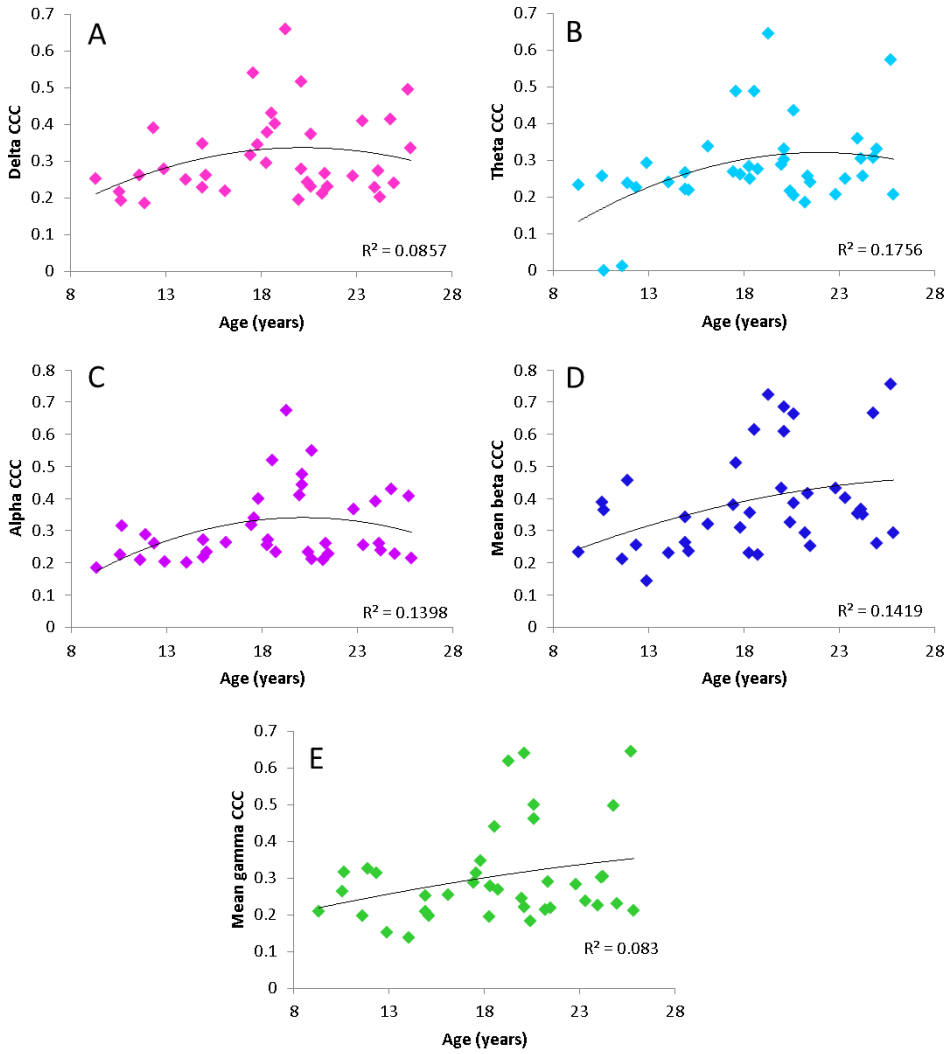


Figure 7.33: Developmental profiles of FC between the DAN5 region pair under resting state conditions; (A) Delta; 1-4 Hz (B) Theta; 4-8 Hz (C) Alpha; 8-13 Hz (D) Beta; 13-30 Hz (E) Gamma; 30-60 Hz.

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