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Modelling Whole-brain Structural Connectivity in Preterm Infants

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Abstract

Premature birth is a major issue for neurological health. Advances in medical care have led to a decrease in neonatal mortality; however, morbidity in preterm survivors remains substantial and neurocognitive issues, particularly involving cognition, language and behaviour are prevalent. Cerebral white matter injury is common in preterm-born infants and is associated with neurocognitive impairments. By identifying the pattern of connectivity changes in the brain following premature birth a more comprehensive understanding of the neurobiology underlying these deficits could be obtained.

Whole-brain macrostructural connectivity was characterised in a group of preterm-born children, and the influence of age and prematurity was explored using a data-driven analysis of diffusion magnetic resonance imaging data. An age-adapted framework, combining anatomical and tissue segmentations with probabilistic diffusion tractography, was used to derive connectivity matrices, weighted by mean tract anisotropy: a measure of connective certainty and strength. In a sparsified, group-consistent connectivity matrix, a novel feature selection method comprising Lasso regression and stability selection was used to identify connections whose mean anisotropy was related to age at imaging or delivery.

As hypothesised, older children were found to have greater connectivity in tracts involving frontal or temporal lobe structures. Increasing prematurity at birth was related to widespread, bilateral reductions in connectivity in all cortical lobes and several sub-cortical structures, consistent with previous histological and neuroimaging data. Results were robust to both increasing sparsity and the removal of subjects with serial imaging in the cohort. The pattern of white matter damage elicited in these results may be responsible for the neurocognitive impairments observed.

This thesis presents a scalable, data-driven method, which could detect contrasting effects of development and prematurity in a sparse model of infant whole-brain structural connectivity. The approach can be used to evaluate connectivity in ever-increasing detail and undertake iterative discovery of the macroconnectome with increasing precision.

Declaration of Originality

The work presented in this thesis is my own and presents research conducted at the MRC Clinical Sciences Centre and Imperial College between July 2010 and July 2013. All sources in this thesis have been appropriately cited and permissions for reproduced images have been given.

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And last but not least, to my nephew Rohan.

Dedication

For Gurudev.

For Him.

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Publications

Journal articles

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In Pediatric Academic Societies and International Society for Magnetic Resonance in Medicine

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In Pediatric Academic Societies and International Society for Magnetic Resonance in Medicine

¹ Material from this paper was integrated into work in Chapters 4 and 5 and is highlighted as ○

² Material from this paper was integrated into work in Chapters 1, 2 and 3 and is highlighted as ■

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Abbreviations

Imaging

ADC	apparent diffusion coefficient
BET	Brain Extraction Tool
BOLD	blood oxygenation level dependent
c-US	cranial ultrasound
dMRI	diffusion magnetic resonance imaging
dODF	diffusion orientation distribution function
DOF	degrees of freedom
DSI	diffusion spectrum imaging
DTI	diffusion tensor imaging
EM	expectation-maximisation
FA	fractional anisotropy
FAST	Fast Automated Segmentation Tool
fODF	fibre orientation distribution function
FOV	field of view
FSL	FMRIB Software Library
GE	gradient-echo
IRTK	Image Registration Toolkit
MCMC	Markov chain Monte Carlo
MD	mean diffusivity
MRI	Magnetic Resonance Imaging
NMR	nuclear magnetic resonance
ODF	orientation distribution
PGSE	pulsed-gradient spin echo
RF	radio-frequency pulse
ROI	region of interest
SE	spin-echo
SPM	Statistical Parametric Mapping
TBSS	Tract-Based Spatial Statistics
TE	time to echo
TR	repetition time
TSE	turbo spin-echo
uODF	uncertainty orientation distribution function
VBM	Voxel Based Morphometry

Statistics

LASSO	least absolute shrinkage and selection operator
OLS	ordinary least square
PLS	penalised least square

ADHD	attention deficit hyperactivity disorder
ASD	autism spectrum disorder
BSID	Bayley's Scales of Infant Development
CeP	cerebral palsy
CNS	central nervous system
CP	cortical plate
CSF	cerebro-spinal fluid
GA	gestational age
GM	grey-matter
IQ	intelligent quotient
IUGR	intra-uterine growth restriction
IZ	intermediate zone
OL	oligodendrocyte
OPC	oligodendrocyte precursor
preOL	premyelinating oligodendrocyte
PROM	premature rupture of membranes
PVL	periventricular leukomalacia
ROP	retinopathy of prematurity
SP	subplate
SVZ	subventricular zone
TEA	term equivalent age
VZ	ventricular zone
WM	white-matter
WMA	white matter abnormality
WMI	white matter injury

Chapter 1

Introduction

1.1 Premature birth and white matter connectivity

Premature birth is a major issue for neurological health. Despite improvements in neonatal intensive care that have led to a decrease in mortality, morbidity still remains substantial. Neurocognitive deficits, in particular, are increasingly prevalent, persistent, and worse with more extreme prematurity (Bhutta et al. 2002; Johnson 2007; Moster et al. 2008; Saigal & Doyle 2008; Kerr-Wilson et al. 2011). In recent years, the incidence of severe neurological disabilities such as cerebral palsy has decreased (van Haastert MA et al. 2011), but subtle impairments involving attention, memory, language, and behaviour have become increasingly common (Delobel-Ayoub et al. 2009; Moore et al. 2012). These impairments are complex, can overlap, and can impart substantial distress upon the preterm individual.

The pathophysiology underlying neurological impairments in preterm individuals is unclear. Neurocognitive abilities are likely to be subserved by distributed cortical–subcortical networks (Mesulam 2000; Choi et al. 2008), with efficient processing dependent on the communication between brain regions and therefore the integrity of the connecting white matter tracts (Tamnes et al. 2010). White matter injury is central to the pathogenesis of a wide range of neurological diseases and can cause disconnection of neuro-axonal circuits subserving neurological functions (Filley 1998; Schmahmann et al. 2008). Cerebral white matter injury is common after preterm birth and is at least partially responsible for the frequent neurological deficits observed (Volpe 2003; Woodward et al. 2006; Khwaja & Volpe 2007). Thus, a highly desirable clinical goal is the accurate characterisation of premature white matter connectivity damage *in vivo*. Achieving this would help in predicting the nature of neurocognitive deficits.

Neuroimaging methods, such as magnetic resonance imaging (MRI), can be used to detect white matter alterations in preterm-born individuals. Using MRI, reductions in normal white matter volume are observable from the neonatal period through to adolescence and have been associated with impairments in domains encompassing cognition, language, and behaviour (Nosarti 2004; Yung et al. 2007; Nosarti et al. 2007; Narberhaus et al. 2008; Northam et al. 2011; Thompson et al. 2011). Still, MR-detected volumetric changes are likely to be secondary effects following microstructural white matter injury.

By estimating and analysing the profile of water diffusion at discrete points throughout the brain, diffusion MRI (dMRI) can be used to infer properties of the underlying tissue microstructure and can

better approximate white matter pathology. The presence of axonal barriers in cerebral white matter restricts diffusion orthogonal to the main fibre orientation but permits it along the fibre. This directional dependence of diffusion is termed anisotropy and, when quantified, it can be used as a biomarker of the organisation of cellular elements in the connective microstructure. Decreases in anisotropy can reflect pathological white matter changes such as myelin loss, axonal damage, and disruption to the packing of axonal fibres. Numerous studies of preterm-born subjects find anisotropic reductions in cortical and subcortical white matter as compared to term-born controls, and these are persistent across the life span (Hüppi et al. 1998; Anjari et al. 2007; Skranes et al. 2007; Eikenes et al. 2011). Despite this evidence, the pattern and extent of preterm white matter injury remains poorly defined. Whereas specific changes in structures such as the internal capsule and corpus callosum have been widely emphasised, changes in the rest of the white matter appear more variable, possibly due to methodological differences.

By following the direction of maximal diffusion anisotropy in dMRI data, tractography algorithms are able to trace fibre pathways that are anatomically consistent with known white matter tracts (Catani et al. 2002; Dyrby et al. 2007). In the study of prematurity, tractography offers a significantly increased understanding of the relationship between brain connectivity, disease, and neurocognitive outcome. Previous reports demonstrate that lower anisotropy in specific fibre pathways is associated with greater prematurity (Dudink et al. 2007; Hasegawa et al. 2011; Bassi et al. 2011; Michela Groppo et al. 2012) and worse performance in relevant functional domains (Bassi et al. 2008; Kontis et al. 2009; Glass et al. 2010; Mullen et al. 2011). However, preterm tractography studies are restricted, in that assumptions need to be made about the location of affected connections. As preterm white matter injury is likely to be diffuse, widespread, and involve multiple fibre types (Judas et al. 2005; Haynes et al. 2005; Benjak et al. 2008; Verney et al. 2012), existing approaches that only study specific connections may inadequately characterise the pattern and extent of the injury. By contrast, mapping tractography for the whole brain (Hagmann et al. 2008; Robinson et al. 2010) would avoid the requirement for prior hypotheses and enable comparison of all tracts across a population simultaneously.

1.2 Motivations

A number of steps are typically required to generate whole-brain tractography or *structural macroconnectome*[‡] data from diffusion MR images. Parcellation methods first divide the brain into a number of discrete regions from which tractography is seeded. Tractography is then performed between each pair of regions and a symmetrical connectivity matrix is constructed that is unweighted, reflecting the absence or presence of a connection, or weighted according to a specific measure of connectivity.

[‡] The terms ‘whole-brain tractography’, ‘structural connectivity’ and ‘structural macroconnectome’ are used interchangeably in the thesis.

Despite the growing use of whole-brain tractography in the study of brain development and disease (Guye et al. 2010; Yap et al. 2011), no work thus far has applied whole-brain tractography in preterm-born infants and children. This subject group represents a particularly appealing population for research. After 2 to 3 years of development, the white matter begins to approximate an adult pattern of appearance. At this stage, microstructural white matter differences may appear more distinguishable between preterm and term-born individuals, since the gap between premature and normal white matter maturation would have sufficiently widened. This age is also characterised by the increasing use of neurocognitive abilities which are amenable for testing. However, a number of non-trivial issues limit the generation of macroconnectome in this population, including the requirement for age-appropriate image preprocessing and parcellation techniques.

Mathematical methods are able to analyse variability in whole-brain connectivity across populations and define connectivity patterns according to clinical and functional information. Graph theory describes the macroconnectome as a brain network, where nodes (brain regions) are interconnected by edges (tractographic connections) (Sporns et al. 2005; Bullmore & Sporns 2009). Networks typically possess an efficient, small-world architecture (Watts and Strogatz, 1998) that can be perturbed by a variety of disease states in which a breakdown of the connective microstructure occurs (Guye et al. 2010). Networks are commonly compared on the basis of general descriptors of their nodal elements and their overall global topology. These measurements offer a summary of network organisation that is related to neurocognitive function and dysfunction (Lo et al. 2010; Bai et al. 2012). Importantly however, these measures cannot compare individual tracts, or identify those which are associated with relevant parameters.

Alternative approaches involve the use of computational and statistical techniques where each subject's macroconnectome is essentially represented as a high-dimensional set of variables or feature vectors. In classification methods, this data is parsed through a machine learning algorithm, which uses the information to find a hyperplane, separating the population according to a relevant outcome of interest. The hyperplane can then be mapped back to the data domain, allowing the features to be selected that differ most between groups. Classification methods have been previously used on human macroconnectome data to categorise populations on the basis of age and gender (Robinson et al. 2008; Robinson et al. 2010). In regression analysis, the relationship between the vector of input variables and outcome variables is estimated. Commonly used methods such as ordinary least squares (OLS) linear regression can become problematic when applied in high-dimensional, limited subject data sets. OLS estimates typically have low bias but large variance and low prediction accuracy (Friedman 2011). With a large number of input variables, it is often desirable to determine a smaller subset which exhibits the

strongest effects. Feature or variable selection methods, such as penalised least squares regression, are suitable for achieving this goal, but as yet have not been applied in whole-brain tractography data sets.

1.3 Aim and contributions

This thesis seeks to develop a model of whole-brain structural connectivity adapted for preterm infants and children, using clinically-acquired diffusion MR images. Using this data, mathematical techniques are sought which can characterise the influence of prematurity on white matter connectivity.

In order to address these aims, this thesis makes two main contributions:

1. The construction of a data-driven, semi-automated framework to extract models of whole-brain probabilistic tractography data using diffusion MRI, that is feasible in clinical scanning conditions and is adapted for the infant population.
2. The development of a robust, sensitive feature selection method that can detect relevant biological connections of interest in sparse macroconnectome data.

1.4 Thesis outline

The thesis is laid out as follows:

- Chapter 2 reviews the concepts and acquisition methods underlying magnetic resonance and diffusion-weighted imaging. Applications of diffusion MRI, such as the tensor model and diffusion tractography, and strategies for dMRI analysis are discussed.
- Chapter 3 provides the neurobiological background for the thesis and is divided into four sections. First, the key processes which occur during early brain development are described and current MRI methods used to assess them are identified. Second, the epidemiology and neurodevelopmental outcomes of preterm birth are outlined. Third, the pathogenesis of white matter injury following preterm birth is discussed, and fourth, the role of diffusion MRI in the study of both early brain development and preterm birth is considered.
- Chapter 4 outlines the steps required to map whole-brain structural connectivity in preterm infants and children. It describes in detail: how the data is preprocessed; how the brain is parcellated in order to define regions of interest; the method by which tractography is delineated between pairs of regions; and the means by which connectivity strength is estimated. A critical appraisal of this framework is provided at the end of the chapter.

- Chapter 5 evaluates existing feature selection methods, before presenting a novel approach that combines Lasso regression (Tibshirani 1996) and stability selection (Meinshausen & Bühlmann 2010). This approach is employed on the sparse macroconnectome of a population of infants born preterm, in order to explore the influence of two factors known to influence observed connectivity: the age at which the scan was obtained and the post-conceptual age at delivery. The age of examination transiently reflects current brain development while the age at birth reflects more lasting effects of premature extrauterine life.
- Chapter 6 provides a summary of the work presented in this thesis and examines potential pathways for future study.

Chapter 2

Magnetic Resonance and Diffusion Imaging

2.1 Magnetic Resonance Imaging

Magnetic resonance imaging (MRI) is a non-invasive technique that can acquire three-dimensional anatomical images of the body with high spatial resolution. MRI provides exquisite contrast between soft tissues of the body and, unlike computed tomography or X-rays, does not use ionising radiation. For these reasons, MRI has become an important medical and research tool, offering unparalleled insight into the architecture of normal, developing, and diseased brains.

2.1.1 Nuclear magnetic resonance

MRI is based on the principles of nuclear magnetic resonance (NMR), a phenomenon which describes the quantum mechanical spin properties of atomic nuclei within a magnetic field (Bloch 1946; Purcell et al. 1946). Nuclei with an odd mass number have a non-zero spin and therefore possess an angular momentum that induces a magnetic moment along their rotational axis. While a number of nuclei have this characteristic and are thus amenable for use in MRI, in the clinical setting hydrogen nuclei (^1H) are most frequently used. ^1H is abundant in the human body and its single proton generates a relatively large magnetic moment, thereby offering the maximum available amount of magnetisation (Westbrook & Roth 2005).

In the presence of an external magnetic field (B_0), the magnetic moments of ^1H nuclei shift from a randomly oriented state to an ordered one (Figure 2.1A-B). The magnetic moments may align in parallel (low energy) or anti-parallel (high energy) to the direction of the field. At room temperature or at thermal equilibrium, there are a greater number of ^1H nuclei in the low-energy state as compared to the high-energy state. This small excess of nuclei results in a net magnetisation parallel to the applied field, termed *longitudinal magnetisation*, as shown in Figure 2.1B. In addition, the external magnetic field causes ^1H nuclei to rotate about the B_0 axis (*precession*), as shown in Figure 2.1C.

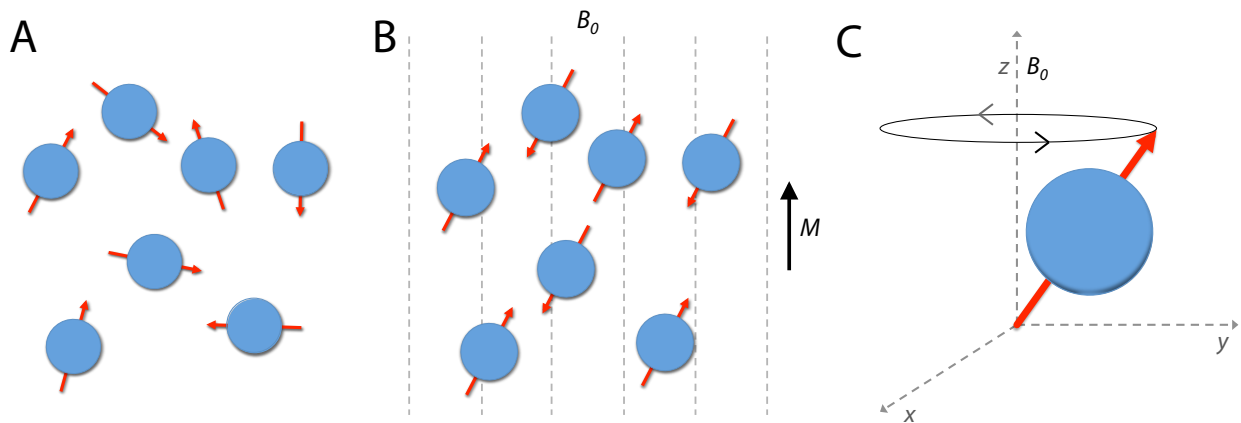


Figure 2.1: Protons in an external magnetic field. ¹H nuclei are naturally found in an unordered state (A). In (B), with the application of a strong external magnetic field, B_0 , magnetic moments align in parallel (arrows pointing up) or anti-parallel (arrows pointing down). As more nuclei are in the parallel state as compared to the anti-parallel state, a net longitudinal magnetisation vector ' M ' is induced. In (C), the B_0 field also causes the ¹H nuclei to precess around the B_0 axes.

The frequency of precession can be determined by the Larmor equation, given in Equation 2.1:

$$\omega_0 = \gamma B_0$$

[2.1]

where ω_0 is the Larmor resonant precession frequency and γ is the specific gyromagnetic ratio for the nuclei in the external magnetic field, which for ¹H nuclei is 42.57 MHz/Tesla. Thus, as the strength of the magnetic field B_0 increases, so too does the precession frequency.

2.1.2 Radio frequency pulse

Applying an oscillating perturbation in the form of a radio frequency (RF) pulse to the nuclei at their Larmor frequency and transverse to the B_0 field induces two important effects. First, the energy from the RF pulse is exchanged into the ¹H nuclei, causing some to excite and enter the high-energy, anti-parallel state, reducing longitudinal magnetisation. Second, the RF pulse causes the precession of ¹H nuclei to become synchronised or have 'phase coherence', increasing transverse magnetisation. These effects are depicted in Figure 2.2A-D.

The angle of the RF pulse determines the extent to which the longitudinal magnetisation decreases and transverse magnetisation increases. Collectively these two magnetisation vectors can be termed the net magnetisation vector, and the degree to which the net magnetisation vector is rotated away from the z -axis and towards the x - y plane is termed the flip angle (Figure 2.2C). The rotating

transverse magnetisation vector induces a current in a receiver coil placed perpendicular to B_0 and is recorded as an oscillating MR signal.

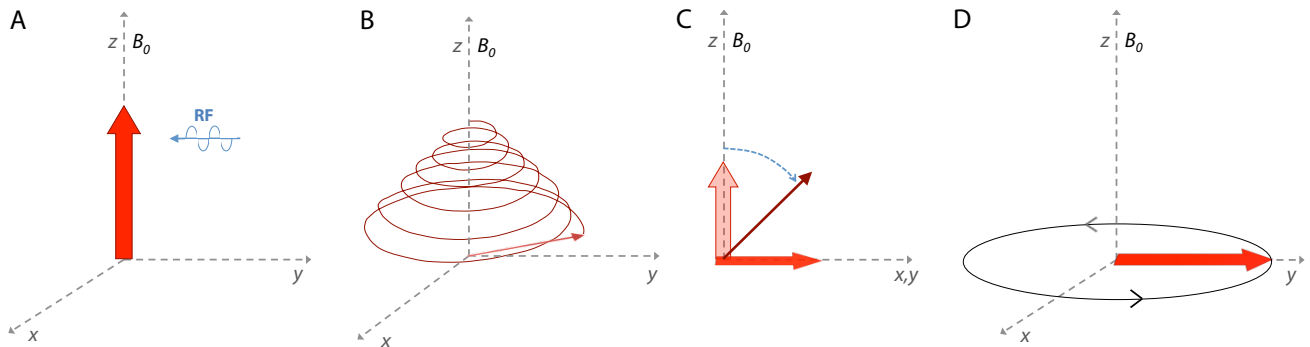


Figure 2.2: The radio frequency pulse and transverse magnetisation. An oscillating RF pulse is applied transverse to the B_0 field (A), causing the net magnetisation vector to spiral downwards towards the transverse or x,y plane (B). In a rotating frame of reference (C), the magnetisation vector (dark red thin arrow) is flipped toward the x,y axes and longitudinal magnetisation (pale red thick arrow) decreases. Concurrently, the RF pulse causes the precession of the ^1H nuclei to be phase-coherent, increasing transverse magnetisation (bright red thick arrow) in (C) and (D).

2.1.3 T_1 and T_2 relaxation

Cessation of the RF pulse causes the ^1H nuclei to revert to their initial state (Figure 2.3). This reversion is characterised by two simultaneous, exponential relaxation times: T_1 and T_2 .[§] These times are not constant and vary depending on the medium in which they are measured, thus offering a means to differentiate tissue types.

[§] T_1 is time taken for 63% of longitudinal relaxation to occur; T_2 is time for 63% of transverse magnetisation to be lost.

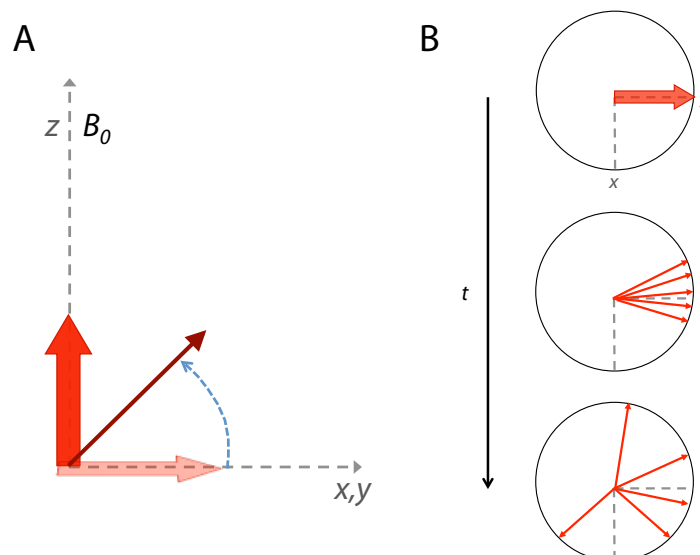


Figure 2.3: T_1 and T_2 relaxation. Increase in longitudinal relaxation (red thick arrow) as net magnetisation vector (dark red arrow) spirals back to the z-axis corresponding to T_1 relaxation (A). T_2 relaxation occurs due to the loss of uniform precession frequency and loss of transverse magnetisation and is shown in the x,y plane in (B). The thin light red arrows represent the magnetic moments of individual ^1H nuclei, which become out of phase over time due to field inhomogeneities.

T_1 , or 'spin-lattice' relaxation, reflects the increase in longitudinal magnetisation brought about by ^1H nuclei reverting to their low-energy state, and increasing nuclei becoming parallel to the B_0 field (Figure 2.3A). During this time, thermal energy is exchanged from the nuclei to the surrounding environment or lattice. The frequency of the magnetic field of the surrounding lattice determines the speed at which energy is exchanged and the T_1 time. An environment predominantly composed of high frequency molecules such as water results in a longer time in energy exchange and a longer T_1 . In contrast, an environment which is close to the Larmor frequency, such as fat, has a rapid transfer of energy and a shorter T_1 .

T_2 , or 'spin-spin' relaxation, reflects the decrease in transverse magnetisation due to ^1H nuclei losing phase coherence (Figure 2.3B). There are two main causes for this effect: external inhomogeneities in the B_0 field (T_2^* -effects) and internal inhomogeneities in the local magnetic fields of the studied sample (T_2 -effects). The extent of molecular movement in the surrounding tissue determines the extent to which local magnetic field variations are averaged. Water molecules move very fast and enable local magnetic field variations to be cancelled, maintaining the phase coherence of nuclei and thereby increasing T_2 . In contrast, tissues with a high density of macromolecules have slow, restricted movement. The local magnetic field variation fails to be cancelled, and phase coherence is easily lost, therefore T_2 is shorter.

2.1.4 Spatial encoding

The spatial location of the MRI signal is determined by several encoding gradients which are superimposed upon the main B_0 field. At different locations in the sample these encoding gradients add to, or subtract from, the B_0 magnetic field, causing position-dependent precession of the ^1H nuclei.

Three encoding gradients (x, y, z) are used in a standard MRI sequence.** The first gradient, G_z , is applied with the excitation RF pulse along the axis of the B_0 field and is used to define a slice of interest. The thickness of the slice is determined by the range of frequencies (*bandwidth*) in the RF pulse and the strength of G_z . Gradients G_x and G_y are then used to locate individual positions in the selected slice. The G_y , or *phase-encoding* gradient, is applied immediately after the RF pulse when the nuclei are phase-coherent, and is turned off prior to signal read-out. G_y causes position-dependent phase shifts in the nuclei, which are retained after it is turned off. As the signal is received, the G_x or *frequency-encoding* gradient is applied, and slice nuclei in the remaining dimension are encoded. The slope of the frequency-encoding gradient determines the field of view (FOV) along the frequency-encoding axis. Position is therefore encoded in a two-dimensional plane according to both the phase and frequency of the received signal. As it is only possible to read out with one gradient active at a time, phase encoding is achieved in a stepped fashion. After each excitation, a specific phase encode gradient is applied, then turned off before the signal is recorded during the action of the frequency encode gradient. The amplitude of the phase encoding determines the remaining dimension of the FOV. These data are stored in *K-space*, a two-dimensional matrix in the spatial frequency domain, where one row is filled after each phase-encoding step and from which the MR image can be calculated by two-dimensional Fourier transform. The spatial encoding gradients control the dimensions of the sampled grid of 3D volume elements (voxels) in the MR image.

** Directions of the encoding gradients in this section are described as part of a standard axial head scan.

2.1.5 T_1 and T_2 weighting

Several parameters can affect the T_1 and T_2 weightings of a MR image. Repetition time (TR) is the time between one RF excitation pulse and the next in a sequence (Figure 2.4). Having a short TR typically exploits the variation in T_1 recovery between tissues. Greater recovery of longitudinal magnetisation can be detected as increased transverse magnetisation when the sum vector is flipped into the transverse plane with the next RF pulse. Long TRs permit a full recovery of the longitudinal magnetisation across tissues, leaving an inadequate T_1 contrast between them. In this case, the remaining differences are predominantly due to variations in *proton density*. The time to echo (TE) is the time between the RF excitation pulse and the collection of the signal, and controls how much transverse magnetisation has been allowed to decay when the signal is read. Short TEs do not permit full dephasing between tissues, thus their coherent transverse components are similar. Long TEs allow dephasing of the transverse component and enable a contrast difference due to the difference in T_2 decay times.

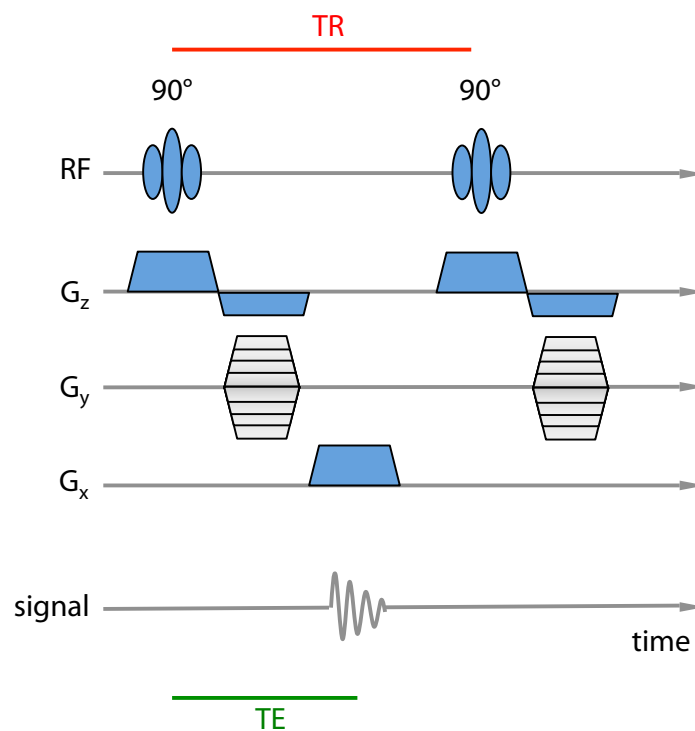


Figure 2.4: Standard pulse sequence illustrating encoding gradients and TR and TE intervals. The slice-select gradient (G_z) is applied simultaneously with the 90° RF pulse, ensuring nuclei are only selected within a specific slice through the object. Positions in y and x are identified using phase-encoding (G_y) and frequency-encoding (G_x) gradients respectively. Repetition time (TR) refers to the period between excitation pulses, while time to echo (TE) refers to the period between the excitation pulse and signal read-out.

T₁-weighted images accentuate the differences between T₁ relaxation times and minimise T₂ effects, and achieve this by using a short TR and short TE (Figure 2.5A). Tissues containing a high proportion of fat appear as hyperintense areas, while water-dense areas such as CSF are hypointense. T₂-weighted images typically have a long TE in order to emphasise differences in T₂ decay times and a long TR in order to minimise T₁ effects and avoid mixed weighting. In T₂-weighted images, CSF is hyperintense, while white matter (WM) is relatively hypointense. Other factors which can externally influence the T₁ and T₂ contrasts include the choice of pulse sequence and flip angle.

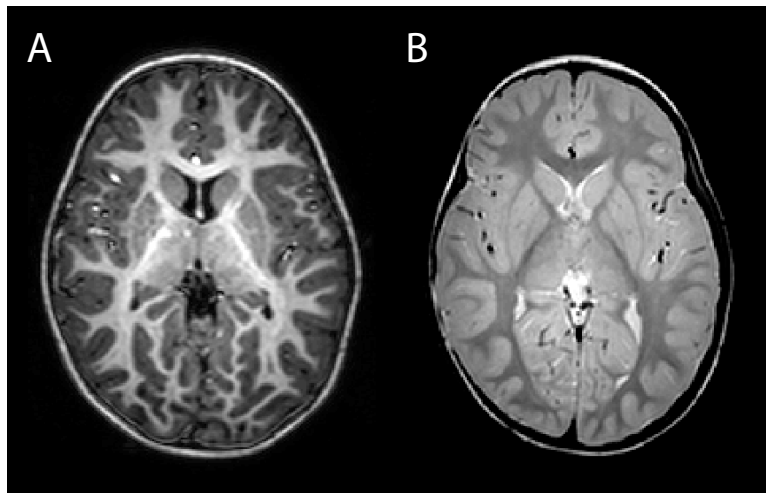


Figure 2.5: A T₁-weighted (A) and T₂-weighted image (B) in the same subject

2.1.6 Pulse sequences

Pulse sequences are defined as a series of RF pulses, gradient applications and intervening time periods that are used to manipulate TR and TE and produce different contrast types (Westbrook & Roth 2005). An important function of some sequences is their ability to remove certain inhomogeneities through rephasing in order to isolate the characteristics of T₂ decay (Westbrook & Roth 2005). Two common rephasing pulse sequences are discussed: *spin echo* and *gradient echo*.

Spin echo sequence

In a spin echo (SE) acquisition, a 90° RF excitation pulse is followed by a 180° RF pulse, midway between excitation and signal collection, that rephases the nuclei (Figure 2.6). Nuclei typically lose their precessional coherence some time after RF excitation, leading to a decay in magnetisation in the transverse plane. The 180° RF pulse flips the magnetic moments of dephased nuclei through 180°. The

nuclei then rephase and a coherent signal or echo is detected. Spin echo sequences are versatile and can be used to generate T_1 -weighted (single spin echo) and T_2 - or proton-density-weighted (dual echo) images.

Fast or turbo spin echo (TSE) is a modification of the SE sequence which uses a much lower acquisition time by performing more than one phase-encoding per TR. In each TR, the TSE sequence employs a chain of 180° RF rephasing pulses, generating a signal *echo train*. After the rephasing pulse, a phase-encoding gradient is applied and the signal from each echo is stored in K-space. Thus, multiple lines of K-space can be filled in each TR.

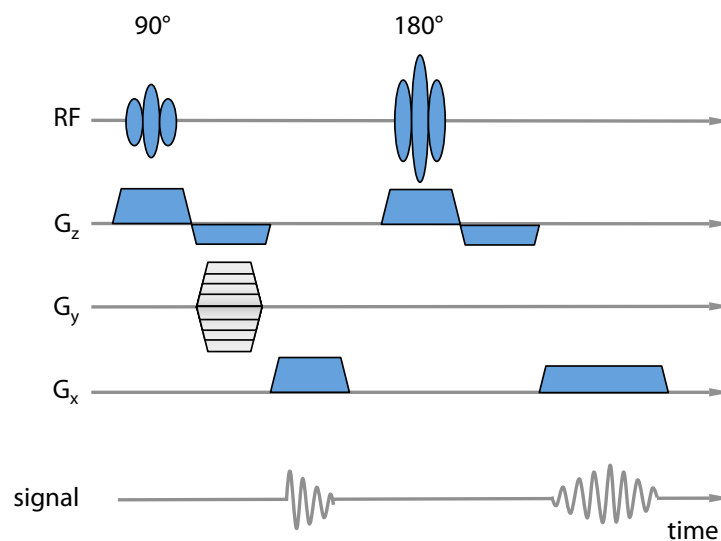


Figure 2.6: Spin-echo sequence

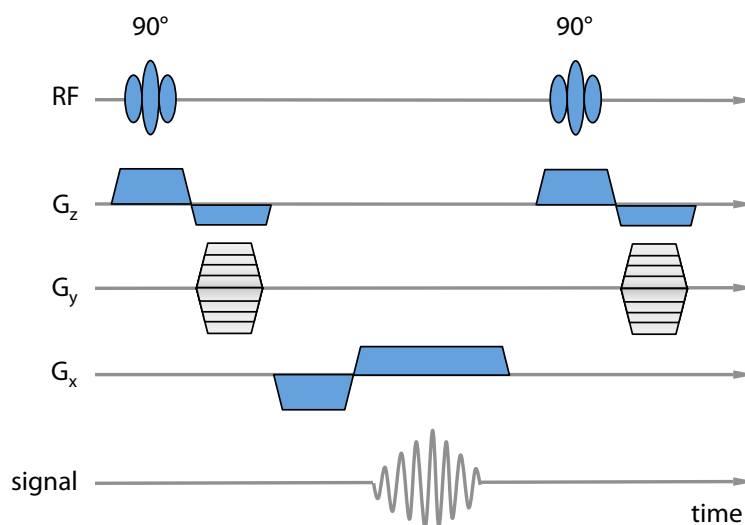


Figure 2.7: Gradient-echo sequence

Gradient-echo sequence

The gradient echo (GE) sequence is similar to SE but uses a gradient polarity reversal rather than a 180° RF pulse to rephase the nuclei (Figure 2.7). Brief negative G_x gradients are applied to accelerate the dephasing process, after which a positive G_x gradient reverses the process, producing a 'gradient' echo. Since gradient rephasing is faster than RF rephasing, conventional GE sequences have a shorter acquisition time as compared to SE. However, gradient rephasing is less efficient as it does not reverse nuclei which have been dephased due to inhomogeneities in the external B_0 field. As such, GE sequences contain T_2^* effects. T_1 -weighted images are generated using a short TR and TE and large flip angle, while T_2^* -weighted images are produced using (relatively) long TR and TE with a small flip angle.

2.2 Diffusion Magnetic Resonance Imaging[■]

With the addition of two extra magnetic field gradients, MRI can be made sensitive to the microscopic movement of water molecules. In the brain, the diffusion of water molecules is frequently hindered or restricted by various cellular or microstructural obstacles. This results in specific patterns of water diffusion which can reveal details about the underlying tissue architecture. As developmental or pathological processes have a propensity to affect cerebral tissue microstructure (Chapter 3), diffusion MRI (dMRI) offers a means of detecting these biological changes through observable and quantifiable alterations in the diffusion pattern. This section outlines the key concepts regarding dMRI, including its derivation and relevant neurological applications.

2.2.1 Diffusion

Diffusion is the process which describes the random, thermally driven movement, or Brownian motion, of molecules over time. The trajectory of a self-diffusing water molecule can be described as a 'random walk', wherein a molecule stays in a particular location for a fixed time before randomly moving to a different spatial location. While the destination of an individual molecule is impossible to predict, the bulk-averaged diffusion of molecules can be modelled mathematically. In 1905, Einstein demonstrated that the mean displacement of a sufficiently large sample of freely-diffusing particles at a fixed temperature is found to be dependent upon the time permitted and the diffusivity of the medium. The expected value of displacement is described in Equation 2.2 (Einstein 1905):

$$r^2 = 6Dt$$

[2.2]

where r , a Gaussian distributed random variable, describes displacement of the water molecules over time t , and D is the diffusion coefficient of the medium, which for water at regular body temperature equals $3 \times 10^{-3} \text{ mm}^2/\text{s}$. The distribution of displacements is modelled as a Gaussian distribution, with a peak at zero displacement and equal probability of displacement at any given distance and direction from the origin (Jones, 2011).

In the cerebro-spinal fluid (CSF) of the brain, water molecules are allowed to move relatively freely. Diffusion is described here as being *isotropic*: that is, the average displacement of water molecules in a given time is equal in all directions. In other parts of the brain parenchyma, complex cellular structures and compartments create barriers to diffusion which reduce the mean displacement of water molecules over time (Beaulieu 2002). In the white matter, water displacement is restricted in certain directions by the presence of cellular architecture, causing diffusion to be *anisotropic* (Figure 2.8).

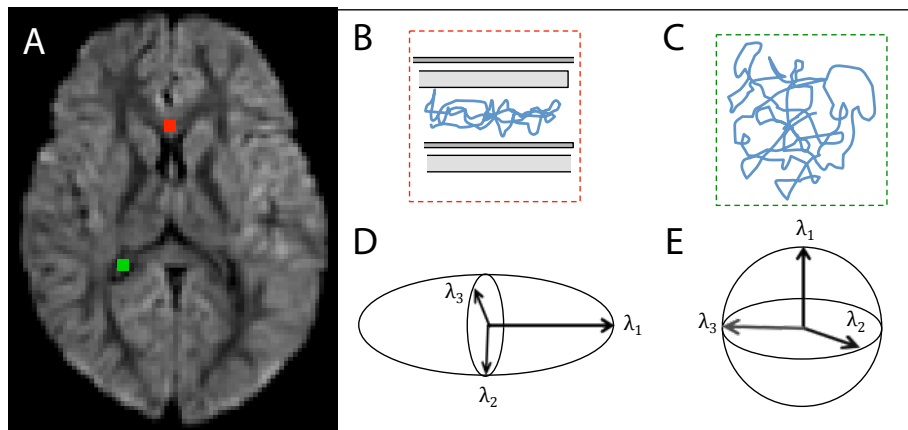


Figure 2.8: Isotropic and anisotropic diffusion in the human brain. Diffusion-weighted image of a preterm-born child reveals the nature of water diffusion within different brain tissue compartments (A). In the white matter of the corpus callosum (red square), diffusion occurs preferentially along the axonal fibres, resulting in anisotropic diffusion (B). In the ventricular cerebro-spinal fluid (CSF; green square), diffusion is unhindered and can be described as isotropic (panel C). Diffusion tensor ellipsoids representing anisotropic and isotropic diffusion are shown in panels (D) and (E) respectively. Each tensor is expressed by three eigenvectors with values λ_1 , λ_2 and λ_3 . In isotropic diffusion $\lambda_1 = \lambda_2 = \lambda_3$, whereas in anisotropic diffusion the long axis of the ellipsoid aligns with the underlying white matter and λ_1 is greater than λ_2 and λ_3 .

2.2.2 Diffusion-weighted imaging

The pulsed gradient spin echo experiment (PGSE) by Stejskal and Tanner is a common acquisition scheme that applies a pulsed gradient either side of the 180° RF pulse (Figure 2.9) (Stejskal 1965).

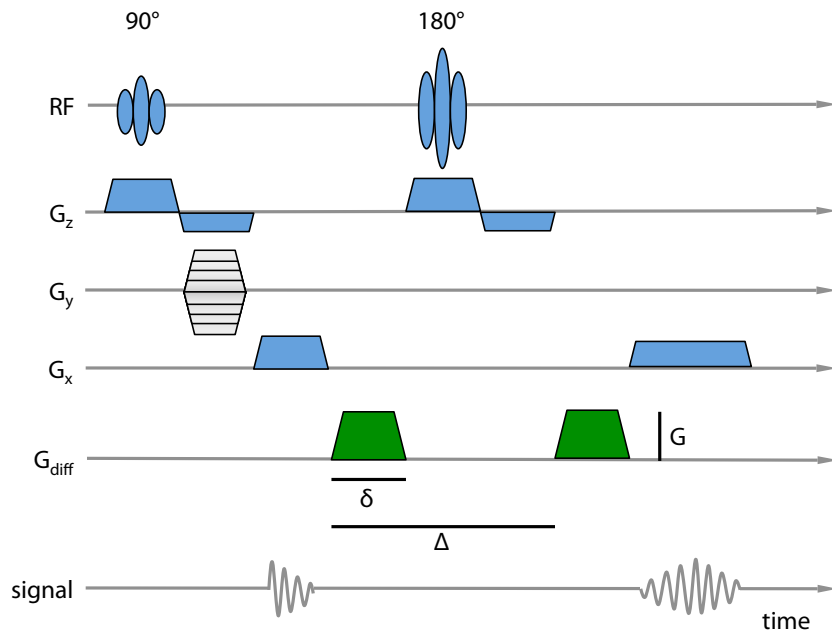


Figure 2.9: Pulsed gradient spin echo sequence. Two spatially varying diffusion gradients of strength G and duration δ are placed at an interval Δ either side of the 180° RF pulse. Stationary nuclei are refocused after the second diffusion gradient, while moving nuclei are not completely refocused, leading to MR signal attenuation

Similar to spatial encoding gradients, the first diffusion gradient imposes a position-dependent precessional frequency on the ^1H nuclei. Following their precession, the nuclei acquire a given phase which accrues over the sample volume and is dependent upon the magnitude of the gradient. After the 180° RF pulse inverts the nuclei, the second pulse, which is typically of the same amplitude and duration as the first, attempts to reverse the phase accrual, bringing the nuclei into coherence to produce the MR signal. If the water molecules (^1H nuclei in water) remain stationary between pulsed gradients, there would be no net phase difference, no loss of phase coherence, and therefore no loss in MR signal amplitude. If the water molecules move randomly with unequal displacement, there would be a distribution of phase shifts, a loss of phase coherence, and a concomitant loss of signal amplitude. As the diffusion coefficient and the spread of displacements increases, there is greater loss of phase coherence and signal amplitude. The extent to which phase dispersion occurs is dependent upon the strength and duration of the diffusion gradients, and the time interval between their application. Collectively these factors characterise the degree of diffusion-weighted variable b . This relationship is expressed in Equation 2.3 (Stejskal 1965):

$$b = \gamma^2 G^2 \delta^2 (\Delta - \frac{\delta}{3})$$

[2.3]

where γ is the gyromagnetic ratio for ^1H nuclei in water and G , δ , and Δ are the strength, duration, and time between diffusion gradients. The diffusion coefficient can now be estimated using Equation 2.4:

$$D = -\frac{1}{b} \ln \left(\frac{S}{S_0} \right)$$

[2.4]

Here S and S_0 are the signal intensity values in the diffusion-weighted and reference (with no or minimal weighting) images respectively, obtained from the pulsed gradient spin echo (PGSE) sequence, and the diffusion coefficient D is calculated on a voxel-by-voxel basis. The calculated diffusion coefficient typically appears lower when compared to values observed for free water (Jones 2009). This difference is due, at least partially, to the effects of cell structures which impede diffusion, and other potential sources such as active transport and physiological pressure gradients. As the diffusion coefficient also addresses these factors, it is termed *apparent* (ADC) (Le Bihan, 1986).

2.2.3 The diffusion tensor

In 1990, Moseley et al. found that in parts of the cat brain ADC values were strongly dependent upon the direction of the encoding gradient. In the cortical and deep white matter, greater signal attenuation was observed when the relative orientation of white matter tracts to the diffusion-sensitising gradient was parallel, as compared to that obtained with a perpendicular alignment (Moseley et al. 1990). This directional dependence of ADC was also reported in human white matter (Doran & Bydder 1990) and these results demonstrated that the organisation of axonal fibres in cerebral white matter tracts preferentially inhibited water diffusion across the tract, but permitted it when in parallel. The anisotropic signal could, therefore, reveal the ordered nature of the underlying white matter structure (Figure 2.8).

However, measuring diffusion with a single gradient direction is inadequate. ADC is rotationally variant and ineffectually captures the behaviour of water molecules within an ordered tissue such as white matter. With a minimum of six gradient directions, the *diffusion tensor* offers a more appropriate model to portray Gaussian diffusion within a voxel, and is shown in Equation 2.5:

$$D = \begin{pmatrix} D_{xx} & D_{xy} & D_{xz} \\ D_{yx} & D_{yy} & D_{yz} \\ D_{zx} & D_{zy} & D_{zz} \end{pmatrix}$$

[2.5]

In this 3×3 symmetrical matrix, the diagonal elements of the tensor (D_{xx} D_{yy} D_{zz}) correspond to diffusivities along the orthogonal axes of the scanner (x y z), and the off-diagonal elements correspond to the correlation between orthogonal displacements. In graphical terms, the diffusion tensor can be represented as an ellipsoid where the surface represents the distance that a molecule will diffuse to with equal probability from the origin. Isotropic diffusion (Figure 2.8C and E) can be modelled as a sphere in which molecular displacement is equal in all directions. Anisotropic diffusion is depicted with a skewed ellipsoid where the longest axis denotes the direction in which diffusion is greatest and is assumed to align with the underlying, principal fibre orientation (Figure 2.8B and D).

The values along each axis of the ellipsoid can be separated into directional and scalar components. The average diffusion distance along an axis is represented by a scalar magnitude known as the *eigenvalue*. The axes with the longest, middle, and shortest magnitudes are denoted by λ_1 , λ_2 and λ_3 eigenvalues respectively and *eigenvectors* v_1 , v_2 , and v_3 are their corresponding directional components. Since the eigenvalues are rotationally invariant, they can be used to summarise voxel-wise diffusion properties independently, without a frame of reference. The average diffusivity across all three directions is known as the 'total ADC' or *mean diffusivity* (MD).^{††} This is demonstrated in Equation 2.6:

$$MD = \bar{\lambda} = \frac{\lambda_1 + \lambda_2 + \lambda_3}{3}$$

[2.6]

Diffusivity along the principal axis is known as principal or *axial diffusivity* ($\lambda_{||}$), while the average of λ_2 and λ_3 is known as perpendicular or *radial diffusivity* ($\lambda_{\perp}$). *Fractional anisotropy* (FA) captures the degree to which the tensor ellipsoid is isotropic or anisotropic (Basser & Pierpaoli 1996). The calculation of FA is performed as shown in Equation 2.7, and is normalised such that it takes values from zero (purely isotropic) to one (purely anisotropic).

^{††} For the remainder of the thesis MD or total ADC will be referred to as ADC

$$FA = \sqrt{\frac{3}{2} \frac{\sqrt{(\lambda_1 - \bar{\lambda})^2 + (\lambda_2 - \bar{\lambda})^2 + (\lambda_3 - \bar{\lambda})^2}}{\sqrt{(\lambda_1)^2 + (\lambda_2)^2 + (\lambda_3)^2}}}$$

[2.7]

2.2.4 Alternatives to the diffusion tensor and multi-fibre modelling

The tensor model represents one way of characterising diffusion among many, albeit still the most frequently used. The diffusion tensor models the dispersion of water molecules as a Gaussian distribution. In voxels where the axonal fibres follow a single dominant direction (Figure 2.10A), water dispersion is typically Gaussian (Figure 2.10B). In this situation, the fibre orientation distribution function (fODF) (see Figure 2.10C and caption) can be easily resolved since the principal direction of the tensor is oriented parallel with the fibres (Figure 2.10D). However, a single voxel of 1–3mm³ contains hundreds of thousands of fibres with a wide range of complex configurations. In voxels, where the fibres fan or bend, or when there is more than one dominant fibre direction (Figure 2.10F), the molecular dispersion may not be Gaussian (Figure 2.10G). In this situation, the diffusion tensor fails to estimate the true fibre orientation (Figure 2.10H) and the fitted ellipsoid of the diffusion tensor is depicted as oblate or an isotropic sphere (Figure 2.10I).

Other approaches are better able to resolve the contribution of each fibre population to the anisotropic signal, estimate the fODF, and account for uncertainty in the data (Seunarine & Alexander 2009). These approaches can be divided into those which model the underlying diffusion profile in order to estimate distinct fibre populations (Behrens et al. 2003; Assaf et al. 2004) and others which employ non-parametric techniques and infer the fibre profile directly (Tuch 2004; Tournier et al. 2007).

A simple extension of the diffusion tensor is the multi-tensor model. Here, a mixture of n discrete Gaussian densities are modelled which correspond to n distinct fibre populations, with each being defined as a separate tensor in a voxel. Although this model offers a better portrayal of voxel-wise diffusion as compared to the single tensor, it is limited as it requires prior information regarding the number of fibre populations in each voxel (Seunarine & Alexander 2009). In the composite hindered and restricted model of diffusion (Assaf et al. 2004), diffusion measurements are taken across a range of b -values and the measured diffusion signal is decomposed into two processes which have a greater correspondence with the underlying mode of diffusion in biological tissue (Clark & Le Bihan 2000). Hindered (or free) diffusion follows a Gaussian displacement distribution, therefore it is modelled as a diffusion tensor. Diffusion within axons is restricted; the displacement here follows a non-Gaussian distribution within ‘impermeable cylinders’ (Assaf et al. 2004). The “ball and stick” model by Behrens et al.

is a simplified, partial volume representation of local diffusion with free and restricted compartments (Behrens et al. 2003) (Figure 2.10E). Restricted diffusion in and around axonal fibres is modelled using a Gaussian distribution, with only one non-zero eigenvalue representing the anisotropic signal. The remaining free diffusion population, which does not interact with the axonal fibres, is represented using an isotropic Gaussian distribution (Behrens et al. 2003). Using a Bayesian, data-driven strategy which decides the number of fibre directions, the model can be extended for multiple fibre voxels by including multiple “sticks” in the model (Behrens et al. 2007) (Figure 2.10J).

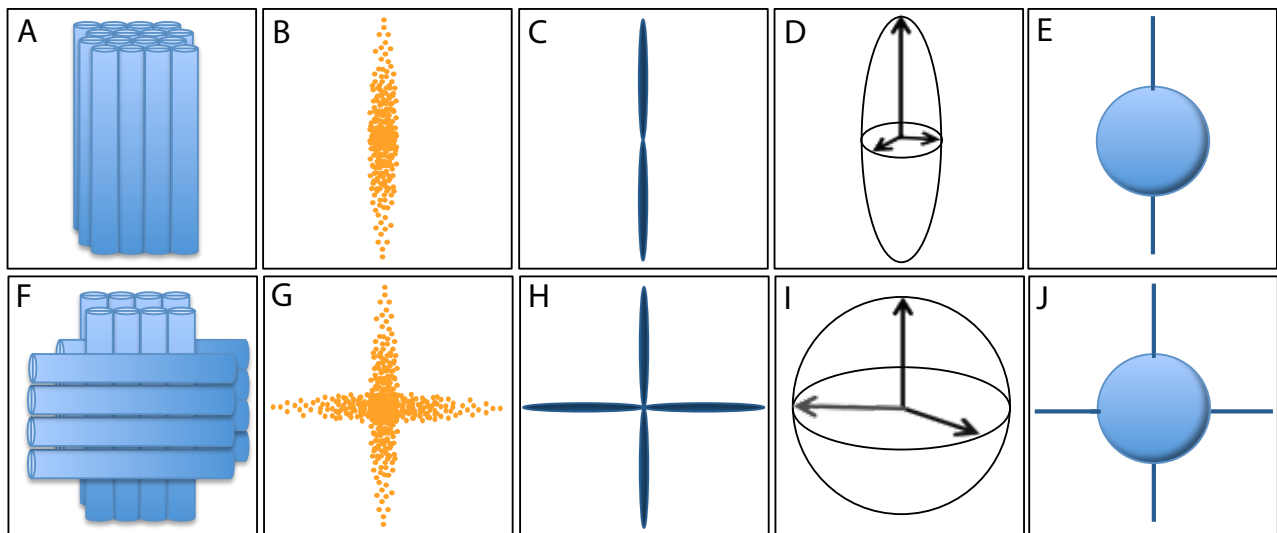


Figure 2.10 Limitations of the diffusion tensor. Voxel-wise diffusion in a single ordered direction and its respective models are shown in (A) to (E), and a voxel with two diffusion directions and its respective models appear in (F) to (J). (A) and (F) depict ordered axonal arrangement in a voxel; (B) and (G) show the water molecular dispersion from the centre of the voxel in 2 dimensions; (C) and (H) show the fibre ODFs (which quantifies the fraction of fibre orientations within a voxel where each point on the sphere corresponds to the probability of a unique orientation); (D) and (I) show the fitted ellipsoid in each voxel; (E) and (J) show the ball and stick model from (Behrens et al. 2003) which are better able to characterise diffusion in complex fibre voxels.

Non-parametric approaches, such as diffusion spectrum imaging (DSI) and QBall imaging, attempt to reconstruct the fibre ODF without placing modelling constraints (Tuch 2004; Wedeen et al. 2005). Using high b-values and a large number of gradient directions, these techniques reconstruct a function called the diffusion orientation distribution function (dODF), which attempts a more detailed characterisation of water movement within the voxel. The peaks of the dODF largely approximate the maxima of the fODF, allowing complex arrangements of fibre populations to be resolved with high accuracy. Despite their advantages, the acquisition parameters required in these approaches currently remain unachievable in the clinical setting.

2.2.5 Neurobiological correlates of diffusion measurements

The correlation between diffusion measures and the underlying neurobiology of white matter is complex. Evidence suggests that diffusion measures are a sensitive but unspecific marker of the neurobiological environment of white matter. Offering examples from processes during WM development and disease, this section attempts to relate microstructural components with selected diffusion measures

Apparent Diffusion Coefficient

ADC decreases exponentially during WM development, remains stable in adulthood, and then gradually increases during senescence (Watanabe et al. 2013). The developmental decrease in total water diffusivity is mainly due to loss of water, reduction in extracellular volume, and an increase in concentration of macromolecules such as myelin (Mukherjee et al. 2002). The increase in ADC in senescence is suggestive of demyelination, loss of axonal integrity, and a corresponding increase in extracellular volume (Watanabe et al. 2013).

White matter anisotropy

Several intra- and extracellular factors appear responsible for the anisotropy observed in the white matter. The axonal membrane is considered to be the primary and necessary determinant of anisotropy. Axons that are normally non-myelinated, in a state prior to myelination, or are not forming myelin due to genetic mutations, all show anisotropy (Song et al. 2002; Takahashi et al. 2002; Partridge et al. 2004). It is likely that myelin modulates existing anisotropy: significant reductions in anisotropy are present in animal models of dysmyelination (Gulani et al. 2001; Song et al. 2002) and elevated anisotropy corresponds to increased myelination in the developing postnatal WM (Hüppi et al. 1998; Schneider et al. 2004). Pre-myelination processes such as the recruitment of oligodendrocyte precursors and the production of proteins required in myelination are also associated with elevated anisotropy (Wimberger et al. 1995). Equally, the size of axons and their degree of packing can influence diffusion. Increasing axonal size (which can be due to myelination), causes axons to be more tightly packed together, reducing intercellular water, restricting diffusion and resulting in an increase in FA. Regarding specific diffusivities, myelin associated changes in fractional anisotropy are principally due to alterations of $\lambda_{\perp}$ rather than $\lambda_{\parallel}$. In contrast, changes to axonal integrity generally influence $\lambda_{\parallel}$. Acute axonal damage is typically characterised by axonal degeneration with comparative myelin preservation and is related to reductions in $\lambda_{\parallel}$ but not in $\lambda_{\perp}$ (Sun et al. 2008) and decreased axonal area is associated with reductions in $\lambda_{\parallel}$ (Wu et al. 2007).

2.2.6 Analysis of dMRI data

There are several analysis methods which can be used to compare diffusion MRI measures between different groups or in the same group over time. A key consideration in all these methods is to ensure that a 'like for like' comparison is made (i.e. that the examined areas are anatomically correspondent). Diffusion MR studies may investigate differences in one or more well-defined regions or fibre tracts, or across the entire white matter. This section discusses the main approaches which have been applied in diffusion studies of clinical cohorts and highlights the major advantages and limitations of each.

Region of interest

Region of interest (ROI) approaches compare diffusion measures in specific anatomical areas, defined *a priori*. Regions are usually delineated manually by an expert and can serve as a gold standard for comparison against other methods. ROI approaches are widely available, applicable for use in individual patients and avoid a multiple comparison problem when studying a limited number of regions. In spite of these benefits, manual delineation can be very time consuming in large samples, and can suffer from low repeatability and high variability (Bonekamp et al. 2007; Bisdas et al. 2008), the latter being a salient concern in samples of developing or injured subjects (Lepomaki et al. 2011). Automated segmentation methods overcome some of these limitations. ROIs are delineated by mapping the anatomical information from an *atlas*, or previously manually segmented brain, onto a *target* or subject brain. Underpinning this mapping procedure are *registration* algorithms, which attempt to align the anatomy of the atlas with the target, and the propagation of label information from the atlas to the target image (see Appendix A). By combining the information of multiple atlases to segment each subject, ROIs created by automated approaches can show high degrees of similarity to manual gold standards (Cabezas et al. 2011).

Voxel-based morphometry

Applying voxel-based morphometry (VBM) to diffusion data permits a global survey of white matter, where diffusion parameters in homologous voxels are compared across subject images (Ashburner & Friston 2000). Applying registration methods, images are first spatially normalised to a common stereotactic space. Normalised images are then partitioned according to tissue class, and the cerebral white matter is extracted (Ashburner & Friston 2005). This area is subsequently thresholded to ensure that grey matter or CSF do not cause partial voluming errors and is corrected for registration errors by *smoothing*; a process which averages the values from a single voxel with its nearest neighbours. Statistical analyses are performed at a voxel-wise level to localise and make inferences about group

differences or detect associations with particular effects under study. Given the number of white matter voxels and therefore the large number of tests, multiple comparison correction is needed to find areas with significant differences and reduce Type I error. VBM is effective in exploring local WM differences, which are not hypothesised a priori, and, like other global methods, it can be automated and is time-efficient. However, this approach is susceptible to artefacts and errors of normalisation and lacks a principled way of choosing the degree of smoothing (Jones & Cercignani 2010).

Tract-based spatial statistics

Tract-based spatial statistics (TBSS) shares some key features of VBM: it studies white matter across the whole brain and detects differences at a voxel-wise level (Smith et al. 2006). In TBSS, the FA images of group individuals are aligned to a common space and averaged. A WM skeleton is created which contains voxels at the centre of fibre tracts, common to all group members. This common skeleton is thresholded such that regions with low mean FA and high inter-subject variability are excluded. An FA skeleton for each subject is produced by performing a perpendicular search for the maximum FA value and local tract centre and projecting it onto the common skeleton. Voxel-wise statistics are then performed across subjects on the skeleton space FA data. TBSS shares many of the advantages that VBM offers, and also demonstrates low variability and high confidence that FA values are taken from relevant voxels. TBSS is not dependent upon precise spatial alignment and does not require smoothing (Smith et al. 2006). Examining a sparse number of voxels in a tract skeleton as compared to the entire white matter allows TBSS to have increased statistical power, but comes at the cost of restricting the examination to the major WM pathways.

Combined analysis

Analysis methods may be combined together. For instance, VBM or TBSS may first be employed to survey the white matter for areas of significant change. These areas in turn may be further examined using ROI methods, or one may wish to perform tractography (see below) in order to accurately identify the affected tracts which pass through this area.

2.2.7 Diffusion tractography

Diffusion tractography estimates and visualises the trajectory of WM fibres. By inferring the fibre orientation from directional information at individual voxels, a 'tract' can be sequentially pieced together. Tractography algorithms start tracing from a set of voxels known as a *seed* region, and arrest upon contact with a *target* set of voxels or by meeting a stopping criterion such as contact with GM or CSF. Tractography enables the comparison of corresponding fibre populations between individuals, even if

the precise location of a tract varies. Clinical diffusion studies typically perform tractography using either a deterministic or probabilistic tracking algorithm. Each of these approaches is next discussed.

Deterministic tractography

Deterministic algorithms propagate streamlines from seed regions along the main estimated fibre orientation in a voxel-by-voxel manner (Conturo et al. 1999) [Figure 2.11A]. A streamline can be described as a three-dimensional space-curve, where the tangent to the curve is in parallel with the voxel fibre orientation (Basser et al. 2000). The location of the streamline, $\mathbf{r}$, is a function of arc length s , which describes the distance along the streamline from the start. The tangent to the streamline at s is taken to be the local estimate of fibre orientation, which, using the tensor model, is eigenvector $\mathbf{v}_1$. This is described in Equation 2.8:

$$\mathbf{t}(s) = \mathbf{v}_1(\mathbf{r}(s)) \quad [2.8]$$

where $\mathbf{t}$ is the tangent and $\mathbf{r}(s)$ is the $[x, y, z]$ location at distance s along the streamline. The evolution of the streamline through the data is described by the differential equation below (Basser et al. 2000):

$$\frac{d\mathbf{r}(s)}{ds} = \mathbf{v}_1(\mathbf{r}(s)) \quad [2.9]$$

Since only one fibre orientation measurement is taken in each voxel, methods are required to extend discrete measurements into continuous space. In fibre assessment by continuous tracking (FACT), the trajectory is continued along the same direction until it encounters the boundary of a neighbouring voxel (Mori et al. 1999). In contrast, interpolation methods such as Runge-Kutta (Press 1992) use contributions from neighbouring points in order to facilitate a smooth streamline between grid points. Streamlines terminate upon reaching a point where fractional anisotropy is too low or when the angle created by the principal orientations of adjacent voxels in the path of the streamline voxels exceeds a critical threshold. These stopping criteria minimise the chance of a streamline encountering and accumulating large errors along its path.

Streamline tractography can be used to reconstruct white matter pathways and 'dissect' major fasciculi with high accuracy and fidelity (Catani et al. 2002) (see Figure 2.13A), and it has been successfully employed in the study of a wide range of neurological and psychiatric diseases (Ciccarelli et al. 2008). However, the technique has some important limitations. Tracking is dependent upon the resolution of

the main fibre direction in each voxel. Poor spatial resolution, low signal-to-noise ratio, and multiple and crossing fibre populations may all lead to areas of high uncertainty and low anisotropy and cause streamlines to terminate or deviate erroneously (Ciccarelli et al. 2008). In addition, deterministic tractography is subject to integration errors, with some techniques accumulating greater errors along the streamline than others (Lazar & Alexander 2003). Finally, from a practical perspective, while deterministic algorithms are computationally efficient, they typically require manual input during *in vivo* dissection, the parameters of which may be variable in and between research centres.

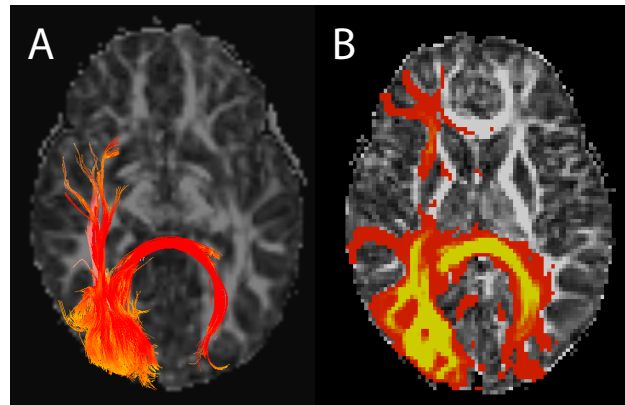


Figure 2.11: Deterministic and streamline tractography. Multiple streamlines are initialised from voxels in the right occipital region in the same subject of Figure 2.5. In (A), deterministic streamlines were drawn using TrackVis (Wang et al. 2007) whereas in (B), probabilistic streamlines were propagated using FSL ProtrackX (Behrens et al. 2007), generating a spatial probability function on the most likely trajectory of fibres from the seed region. Areas of higher probability have higher intensity in the image

Probabilistic tractography

Probabilistic algorithms attempt to define the confidence or certainty in a path from a seed point to a target region (Figure 2.11B). In the first step of the probabilistic approach, local fibre orientations are estimated in each voxel and a function is built that can characterise the uncertainty in the measurement. This function comprises the noise and uncertainty in the fitting of a model to the data signal and is referred to as the uncertainty ODF (uODF). The uODF is illustrated as a cone of uncertainty around the principal diffusion direction, where a larger cone reflects increased uncertainty in the estimate. There are a number of methods which can calculate the uODF including bootstrapping, Bayesian, and calibration approaches (Behrens & Jbabdi 2009). In bootstrapping, multiple permutations of the original data are used to define bootstrap data sets, each of which estimates the principal fibre direction. These estimates are treated as samples of the uODF, from which confidence intervals can be calculated.

Bayesian methods attempt to calculate the uODF as a posterior probability density function, which is proportional to a likelihood function multiplied with a prior distribution. The likelihood function

includes parametric assumptions about the relationship between quantities of interest and the data and noise structure, whereas the prior distribution is used to impose physical constraints on the estimation process, such as positivity of diffusion coefficients (Behrens et al. 2003). Several techniques are able to solve the Bayes' equation and recover the posterior distribution, of which the most common is the Markov Chain Monte Carlo estimation process (Gilks et al. 1993; Behrens et al. 2003).

Having modelled the uODF in each voxel, the probabilistic tracking process then proceeds as follows. The probabilistic streamline starts from a seed region A and draws a sample orientation at random from the voxel's uODF. The streamline front then moves a distance along the selected orientation to the next voxel. This continues until a target region B or stopping criterion is met. Every iteration of this procedure generates a sample streamline from the final distribution. By drawing many samples, a spatial probability density function on the path leading from A is built (see Figure 2.13B). The probability of a connection passing between A and B given the data, is equal to the number of streamlines which connect to B ($n_{A \rightarrow B}$), divided by the total number of streamlines seeded from A (n_A) [Equation 2.10].

$$P(\exists A \rightarrow B|Y) = \frac{n_{A \rightarrow B}}{n_A}$$

[2.10]

The spatial probability distribution function represents the confidence bounds on the location of the most probable single connection. The advantage of this approach is that a pathway probability can be obtained which can be used to reliably quantify connections, often spatially remote or variable across subject brains (Behrens et al. 2003; Counsell et al. 2007). Complex fibre orientation models (Behrens et al. 2007) permit pathways to be traced in the presence of low anisotropy and certainty (Figure 2.10J). In addition, few termination criteria are required. In this approach, anisotropy constraints are typically zero because probabilistic approaches are not trying to avoid low anisotropy areas, but to seek a distribution of permissible connections. Curvature constraints are also lenient, and are only used to prevent samples tracking back on themselves (Behrens & Jbabdi 2009).

Estimating connectivity

It is often important to assign weights to a connection, as this provides a means of comparing it with others linking different regions and enables comparison of homologous connections across a population. As demonstrated in Equation 2.10, probabilistic algorithms assess connectivity by estimating the probability of the target connected to the seed (Parker et al. 2002; Behrens et al. 2003). This affords a measure of certainty of the connection, but it cannot be used to derive its strength. In addition, the

measure may be length-biased and cross-sectional area invariant (Zalesky & Fornito 2009). Other studies threshold and parameterise the space occupied by the streamline distribution and an average of a particular measure, such as FA, is taken across the voxels. Although this may offer an insight into the microstructural integrity of the white matter in individual voxels and is straightforward, the integrity through the tract itself is not considered.

Metrics have been proposed based on the evolution of fast-marching wavefronts (Jackowski et al. 2005), which involves the growth of a volume from a seed region and is related to the information in the eigenvector v_1 field. In this method, connectivity metrics are based on the 'speed' at which the front proceeds toward the target. Other approaches base their connectivity scoring on principles of *information flow*. Here, the capacity or cross-sectional area of fibre bundles connecting discrete cortical regions correspond to the degree to which information can be transferred (Iturria-Medina et al. 2007; Zalesky & Fornito 2009). In (Zalesky & Fornito 2009), a white matter 'graph' (Appendix B) is first constructed from a three-dimensional lattice scaffold, and is then used to measure connectivity by calculating the maximum number of link-disjoint paths (i.e. those which share no edges) between region pairs.

Iturria-Medina employs a similar approach, whereby a graph representation (see Appendix B) is first produced and edges between nodes are weighted according to their probability of belonging to the white matter and the degree to which they are oriented in the direction of underlying axonal fibres (Iturria-Medina et al. 2007). The latter variable gauges the probability of diffusion between voxels (and fibre coherence) by calculating the proportion of the diffusion ODF in the direction of an edge connecting the centre of a voxel to an adjacent one. A pathway between nodes of different regions is then determined by solving a 'most probable path' problem in the graph which maximises fibre coherence and penalising path curvature. Inter-regional anatomical connectivity is subsequently quantified in three ways: connectivity strength is calculated by combining measures of maximum edge weight along the path with an estimation of the tract cross-section (estimated from the number of connecting seed and target voxels); connectivity density is the connectivity strength as a proportion of the total seed and target voxels; and connectivity probability is the chance that two regions are linked by at least one connection, with the value equal to the maximum connectivity between nodes of these areas.

In (Robinson et al. 2010) some of these aforementioned approaches are combined, namely the use of probabilistic tracking, from (Behrens et al. 2003), with an estimation of connectivity, similar in part to (Iturria-Medina et al. 2007). An anisotropy value for a tract is gained by taking an estimate of the inter-voxel diffusive exchange from ODFs on the partial volume model of diffusion (Behrens et al. 2003; Melie-García et al. 2008), weighted by the number of times the voxels are sampled during tracking and

integrated across all voxels in the tract. This approach represents an essential component of the macroconnectome framework outlined in this thesis and is discussed in greater detail in Chapter 4.

Chapter 3

The Early Developing Brain and Preterm Birth

3.1 Normal brain development

3.1.1 Early gestational events

The central nervous system is initially derived from the neural tube, an embryonic structure formed in approximately the 3rd to 4th week of gestation (Ladher & Schoenwolf 2005). In the most rostral section of the neural tube, two vesicles are formed: the telencephalon – later giving rise to the cerebral cortex, and the diencephalon – giving rise to the thalamus and hypothalamus. The early gestational period is chiefly characterised by neuronal proliferation and migration (Figure 3.1).

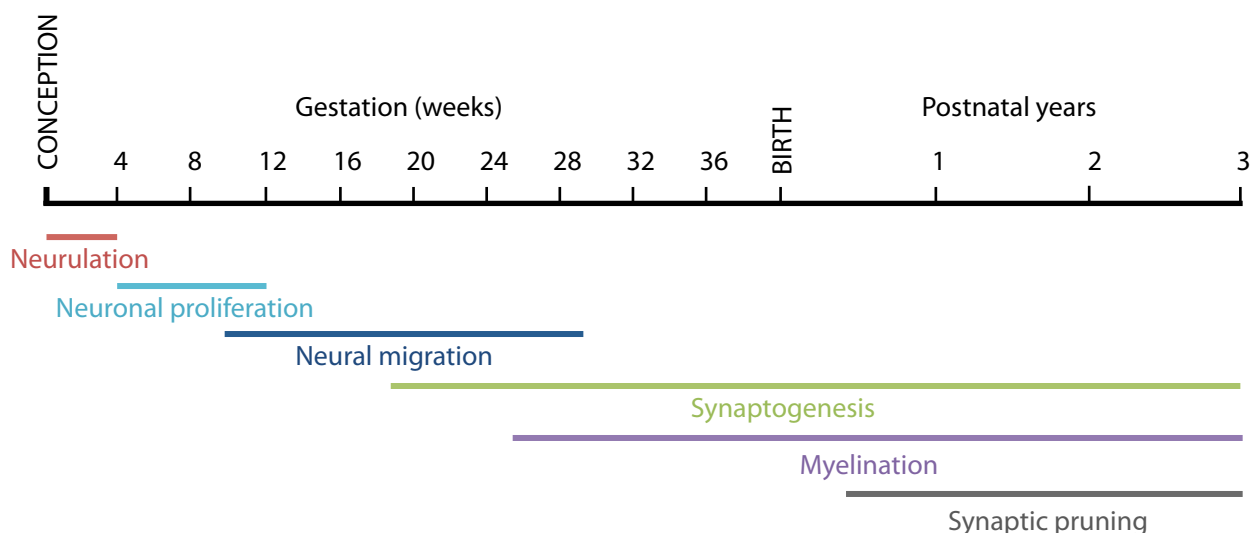


Figure 3.1: Sequence of developmental processes in the fetal and early postnatal brain. Coloured lines represent the approximate periods during which processes occur. Processes may, however, proceed at lower rates before and after.

The ventricular zone (VZ) is a proliferative or germinal area formed by epithelial cells lining the ventricular cavity (Mrzljak et al. 1992; Bystron et al. 2008) [Figure 3.2A]. The onset of neurogenesis occurs in the VZ in the 5th week of gestation, while neuroblast differentiation occurs by the 8th (Bystron et al. 2008). The neural fate of differentiating neuroblasts is dependent upon spatial position and exposure to several genetically determined molecular gradients. Early proliferation also gives rise to radial glia, which form a specialised scaffold spanning the width of the cerebrum from the VZ to the pial surface (Figure

3.2B–C) and facilitate the movement of post-mitotic neurons (Rakic et al. 1994; Rakic 1990). The first wave of post-mitotic neurons leaves the VZ to form the preplate (Marin-Padilla 1971). Later, cohorts of neurons migrate into the preplate to create the cortical plate (CP), splitting the former structure into two new layers: the marginal zone above and subplate (SP) beneath (Figure 3.2D). The initial formation of cortical layers in the CP is dependent upon the birthdate of migrating neuronal populations. With every successive wave, younger-born neurons migrate past previously formed layers toward the pial surface, forming an ‘inside-out’ protocortex (Hatten 1999; Rakic 1974). A smaller set of neurons, which will later give rise to the GABAergic interneuron population, adopt a different migration pattern by first moving tangentially from the subcortical ganglionic eminence and subventricular zone (SVZ) towards targets in the cortex and thalamus (Marín & Rubenstein 2001). Neuronal migration reaches its peak between 12 and 20 weeks gestation and is mostly complete by weeks 26 to 29 (de Graaf-Peters & Hadders-Algra 2006).

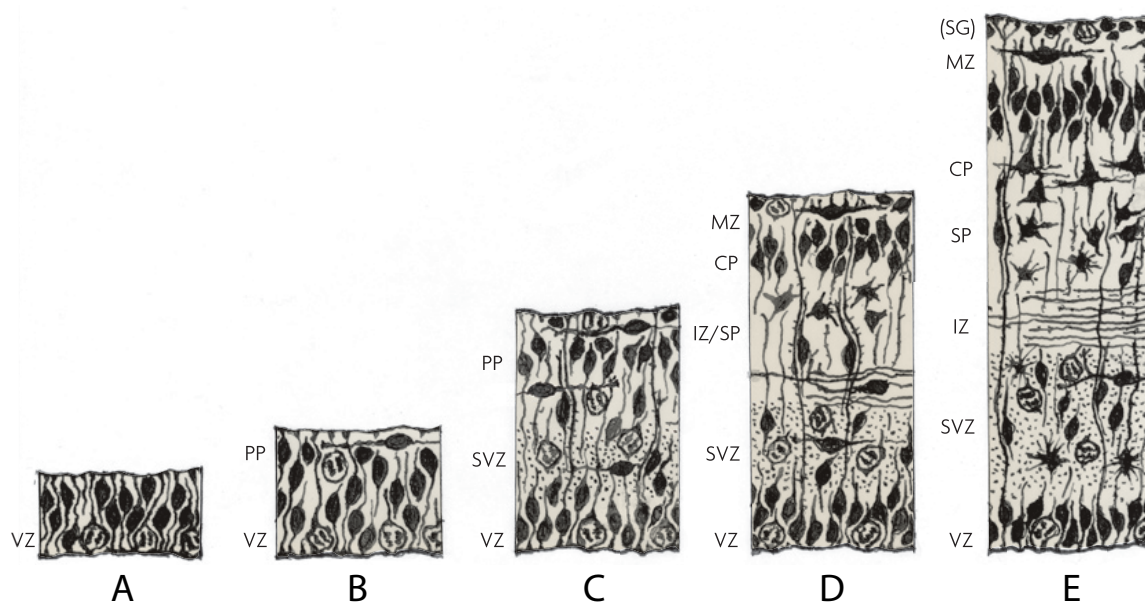


Figure 3.2: Schematic model of neocortical development. Letters correspond to the following gestational ages: (A) 4 weeks, 2 days; (B) 4 weeks, 3-4 days; (C) 6 weeks, 3 days; (D) 7 weeks, 6 days; (E) 14 weeks. Reproduced and modified from Bryston et al. (2008).

The intermediate zone (IZ) lies in the area between the SVZ and the subplate (Figure 3.2D-E), and, by the end of the first trimester, long-range interstitial neurons are found to course through it (Kostovic & Rakic 1980). In conjunction with the subplate, the IZ corresponds to the early cortical white matter. Axonal bundles of major white matter pathways appear as early as 8 weeks gestation (Vasung et al. 2010). The extension of neurons during development is critical to the assembly of the early white matter

architecture. This is made possible with the use of specialised structures at the end of the axon known as growth cones, which interact with guidance cues in the extracellular environment in order to navigate the growing axon front. Guidance cues can either be substrate-bound or diffusible, include semaphorins, netrins, Slits, and ephrins, and may act as chemoattractants or repellents (reviewed in O'Donnell et al. 2009). Thus, the interaction of these guidance cues and growth cone receptors facilitates or inhibits axonal growth and directs the axon to its correct target.

3.1.2 Development of key cellular populations and structures

Several overlapping processes dominate the remainder of gestation and the early postnatal period (Figure 3.1). These include subplate mediated interactions and glial cell proliferation during the late gestational period, and axonal growth, synaptogenesis, and myelination in the early postnatal period. In this section, these processes are discussed and the roles of key cell populations are considered. These cell populations are critical to the normal assembly of cortical and subcortical circuits. Importantly, they also represent key players in the pathogenesis of white matter injury associated with premature birth (Chapter 3.3).

3.1.2.1 Neuronal development

The subplate and early neuronal circuitry

The SP reaches its thickest size at around 20 weeks gestational age (GA) (Tau & Peterson 2009). It comprises a large, synaptically rich, extracellular matrix containing neuronal migratory afferents and glial cells. In its primary function, the SP serves as a transient waiting station for both subcortical and cortical afferents, integrating inputs from the thalamus, basal forebrain, brainstem, and, later, the ipsi-lateral and contra-lateral cortex. Glutamatergic and GABAergic afferents initially synapse with intermediary SP neurons (Kostovic & Rakic 1980), which themselves connect with the cortical plate and the marginal zone of the neocortex (Ghosh & Shatz 1992; Allendoerfer & Shatz 1994). During the waiting phase, SP neurons mediate early cortical processing and functional maturation of excitatory and inhibitory circuits (Kanold & Luhmann 2010). Afferent neurons then make more permanent connections with targets in the cortex with the aid of SP neuron guidance (Ghosh et al. 1990).

As inputs arrive in the cortex, differentiation of the CP becomes increasingly clear. Sensorimotor cortices laminate first at 25 weeks gestation (Tau & Peterson 2009), and by 32 weeks gestation the complete developing cortex (CP) is divisible into six layers (Kostović et al. 1995). Each cortical layer contains a distinct array of cell types and has a specific pattern of connections with other cortical or subcortical targets. One of the earliest cortical loops, responsible for sensory-driven function, is created when thalamic axons project to cortical layer IV and pyramidal neurons in layer V and VI project

reciprocal efferents back to the thalamic nuclei, with a number of relay circuits in between (Marin-Padilla 1970; Clascá et al. 1995). Cells in laminae which are vertically adjacent are organised into radial columns which are defined, at least partially, by the subplate (Aboitiz 1999). Radial columns each have different cellular characteristics (Rakic 1988), and can be combined to form cortical divisions with distinct cytoarchitectural and functional properties (Brodmann 1909).

As relocation of afferent fibres to the cortical plate continues, the subplate begins to regress and ingrowth of callosal and long cortico-cortical pathways occurs into the cortex (Kostovic & Rakic 1990). While the majority of subplate neurons die between 34 to 41 weeks GA (Kostovic & Rakic 1990), in key cortical regions the subplate is known to persist. This protracted dissolution of the subplate in the prefrontal and associative cortices likely underlies the prolonged maturation, delayed postnatal synaptogenesis, and myelination in these cortical areas (see later) (Kostović et al. 2012). After resolution of the subplate and loss of its growth-related molecules, axonal retraction begins, and arborisation within the cortical layers and growth of shorter cortico-cortical connections continues to increase into the early postnatal months (Kostović & Jovanov-Milosević 2006).

Synaptogenesis

Synaptic density is low at the onset of neurogenesis, with synaptic contacts mainly being formed in the nascent marginal zone and subplate layer (Bourgeois 1997) [Figure 3.3: phase I]. Approaching mid-gestation (Figure 3.3: phase II), neurogenesis and neuronal migration reach their peak. At this point, synaptic density continues to increase, with contacts forming mainly in the subplate before moving to the cortical plate as afferent projections penetrate it. In the CP, synaptogenesis begins earliest in the deeper layers and later in superficial layers, corresponding with the inside-out sequence of cortical lamination (Huttenlocher 1990). Synaptogenesis accelerates rapidly from the third trimester onwards due to a surge in dendritic sprouting and arborisation (Bourgeois 1997; Huttenlocher & Dabholkar 1997), and by week 34 approximately 40,000 synapses are formed every second (Tau & Peterson 2009) [Figure 3.3: phase III]. Synaptic density continues to increase momentarily after birth, in parallel with the sustained dendritic development and arborisation of both inhibitory and excitatory circuits (Andersen 2003; Tau & Peterson 2009). Although it has been disputed, synaptogenesis has been found to be regionally dependent, with increases in synaptic density being protracted in anterior regions such as the prefrontal cortex (Huttenlocher & Dabholkar 1997), reaching maximum density there in the 2nd to 4th postnatal years and coinciding with a regional distribution of cerebral metabolic activity (Chugani et al. 1987). Recent and surprising evidence has found that the period of overproduction and remodelling of dendritic spines on pyramidal neurons in the prefrontal cortex is in fact much longer than expected, extending to the 3rd decade of life (Petanjek et al. 2011).

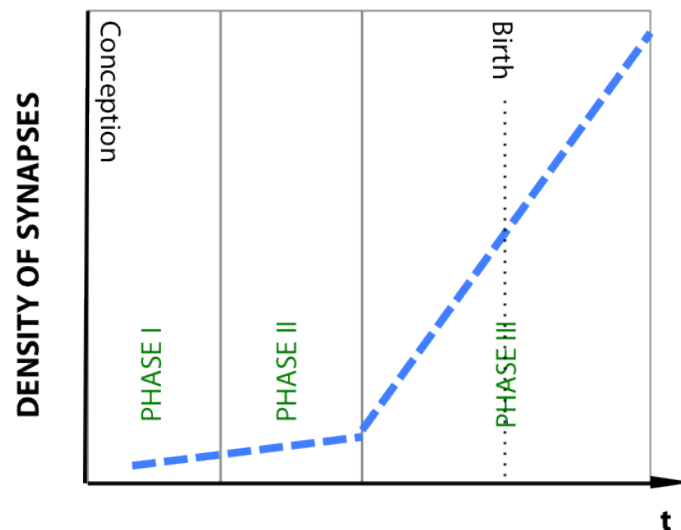


Figure 3.3: Changes in the relative density of synapses in early brain development. Synaptic density, represented by blue dashed line, as a log function of days after conception (t). Reproduced and modified from Bourgeois et al. (1997).

3.1.2.2 Glial development

Oligodendrocytes and myelination

Oligodendrocytes (OLs) are central nervous system (CNS) glial cells that generate and ensheath axons with myelin, a lipid-enriched membrane that enables the saltatory conduction of action potentials and supports axonal survival (Lappe-Siefke et al. 2003). Myelination is important because it greatly increases axonal conduction velocity and energy efficiency, and is a necessity for normal neurocognitive processing (Nave 2010). OLs are originally derived from PDGFR α +, NG2+ OL progenitor cells (OPCs) from multiple sites in the neocortex. These sites include the ventral and dorsal ventricular epithelium and the migratory stream between the ganglionic eminence and the cortical subventricular zone (Jakovcevski 2009). OLs derived from different sources may have different roles and patterns of myelination (S. Rakic & Zecevic 2002; Tripathi et al. 2011). OPCs are detected earliest between 9 to 10 weeks GA (Jakovcevski 2009), and from mid-gestation can be found in the cerebral cortex, closely apposed with radial glia fibres (Jakovcevski & Zecevic 2005). OPCs develop into late OL precursors with O4+ immunoreactivity, and by 19 to 22 weeks GA this population penetrates the dorsal telencephalon and the cortical plate (Jakovcevski & Zecevic 2005). Late OL precursors constitute approximately 90% of the total premyelinating OL population^{**} between 18 and 27 weeks (Back et al. 2001), after which the immature

^{**} Premyelinating oligodendrocytes consist of both late oligodendrocyte progenitors and immature oligodendrocytes

O1+ OL population expands rapidly. From 30 weeks GA the mature MBP+ myelinating OL is detected in sparse white matter regions, and by 40 weeks it is detected extensively throughout the cerebral white matter (Back et al. 2001).

Initial cellular events which occur prior to myelination include the increase in number of OLs in the myelinating area, transformation of the pre-myelinating OL into the mature myelin-producing OL (Pfeiffer et al. 1993), and the shift of myelin proteins such as proteolipid protein and myelin basic protein from the cell somata to its processes in order to form the myelin sheath (Hardy & Reynolds 1991). Initial axonal events which occur prior to myelination include the increase in axon diameter (Hildebrand & Waxman 1984) and membrane-specific changes such as the increase in sodium channels (Kaplan et al. 1997). Axon ensheathment and myelin growth occur in a regulated sequence, starting first with axonal selection and initiation of cellular contacts; second, the extension of myelin processes around the axon and formation of the nodes of Ranvier; third, the regulation of myelination thickness; and, finally, the longitudinal extension of myelin segments in response to axon length (reviewed in Sherman & Brophy 2005).

Immunohistological studies find that myelin is present in the posterior limb of the internal capsule and the lateral cerebellar white matter by term (Kinney et al. 1988), and major myelin proteins are present as early as 20 weeks in pallidothalamic fibres and the globus pallidus (Iai et al. 1997). As development progresses, myelination progresses in a well-established spatio-temporal sequence, typically occurring in a “caudal to rostral, proximal to distal and central to peripheral pattern, with sensory pathways myelinating before motor pathways and association fibers and fronto-temporal regions being the last to mature” (Yakovlev & Lecours 1967; Kinney et al. 1988). By the end of the second postnatal year, mature myelin is detected across all sites in the telencephalon (Brody et al. 1987), although myelogenetic cycles are likely to persist in subcortical fronto-temporal areas.

Microglia

Microglia are the resident mononuclear phagocytes of the CNS. In the adult brain, they are distributed ubiquitously (Kettenmann et al. 2011). Their primary function is the maintenance of immunity and protection of the brain parenchyma. Although there has been debate concerning the source of the microglial population in the CNS (Perry et al. 1985; Alliot et al. 1999), recent evidence has confirmed that microglia are derived from the macrophage lineage, originating from the yolk-sac and

then later colonising the brain (Ginhoux et al. 2010). Microglia enter the brain parenchyma through several routes (Monier et al. 2006; Monier et al. 2007) and have been detected in the telencephalon in limited numbers from 5 weeks GA (Rezaie 2003), reaching peak abundance in the fetal white matter in the 3rd trimester (Billiards et al. 2006). With increasing gestation, the microglia undergo morphological maturation and disperse through the brain, clustering in certain areas, such as in or near white matter tracts, from 8 weeks GA, at the cortical plate-subplate junction at 10 to 12 weeks GA, and in the centrum semi-ovale and axonal cross-road areas between 19-30 weeks GA (Verney et al. 2010). The differences in microglial location may be attributable to their variety in non-pathological functions during development. Several studies have established a developmental role for microglia in programmed cell death and in angiogenesis (reviewed in Arnold & Betsholtz 2011 and Pont-Lezica et al. 2011), and increasing evidence has shown that microglia are important in axonal remodelling and synaptic pruning (Paolicelli et al. 2011), particularly in the early postnatal period.

3.1.3 Exploring early brain development with magnetic resonance imaging

Over the last two decades, conventional MRI has served as a valuable tool for capturing complex developmental changes in the early postnatal brain. MRI studies recapitulate existing imaging and immunohistochemical knowledge regarding development, and provide additional information brought about by the increased resolution of the technique and the distinct sensitivity of T₁- and T₂-weighted contrasts. Yet the current potential of MRI use in this field lies in the fact that image information can be combined with state-of-the-art computational techniques, thus granting a superior insight into the emerging cerebral architecture. Some key developmental applications of MRI are outlined in this section.

Conventional T₁- and T₂-weighted images

Standard T₁- and T₂-weighted imaging permits the qualitative, visual assessment of brain maturation *in vivo*. T₁ and T₂ contrasts are sensitive to maturational changes in both grey and white matter signal intensities, allowing age-linked milestones to be identified, including the development of the subplate layer and myelination. The subplate layer is visible on conventional imaging from mid-fetal age due to the relatively high water content in its extracellular matrix (Judas et al. 2005). It appears as an area of hyperintensity on T₂-weighted images, and as hypointensity on T₁-weighted images, relative to the cortical plate (Huang et al. 2006). Myelination is visible as a hyperintense T₁ and hypointense T₂ signal, due to the decrease in tissue water content and the increase in macromolecular constituents and myelin (Ballesteros et al. 1993; Barkovich 2005). Myelination can be observed as early as 25 weeks gestation in the brainstem and cerebellar peduncles on T₁-weighted imaging, and by term it is apparent in the posterior limb of the internal capsule. Changes of MR signal are delayed on T₂-weighted imaging,

possibly due to different mechanisms underlying the change of signal. At 2 years, an adult pattern of appearance is present on MRI (Barkovich 2005).

Volumetric MRI

Post-acquisition segmentation techniques can be applied to high-resolution MR images in order to derive volumetric measurements of cerebral tissues, primarily on the basis of signal intensity differences. Estimation of the total brain volume and global volumes of grey matter, white matter, and cerebrospinal fluid is made possible through a variety of software packages (see Chapter 4). The total brain volume doubles between 28 and 40 weeks of gestation (Hüppi et al. 1998) and again in the first postnatal year, reaching 70% of adult size (Dekaban 1978; Knickmeyer et al. 2008). In the second year, growth is much slower, with brain volume only increasing by 15% (Knickmeyer et al. 2008). Most of the volumetric increase is driven by grey matter growth, (mainly through glial proliferation, dendritic arborisation, and synaptogenesis). In contrast, white matter volume increases linearly, at a much slower rate. With the use of manual segmentation or anatomical templates (see Chapter 4), regional volumetric changes can also be derived, allowing local patterns of maturation to be estimated (Shi 2011; Gilmore et al. 2011).

Cortical folding

Cortical folding can be assessed through computational morphological approaches which quantify surface area and gyration from a 3D reconstruction of the interface between the developing cortex and white matter (Dubois et al. 2008). Cortical sulcation starts from 14 weeks of gestation and increases dramatically throughout fetal development (Figure 3.4). The medial surface folds before the lateral surface, and morphological differentiation of sulci begins in the central region and progresses in an occipito-rostral direction (Dubois et al. 2008; Hill et al. 2010; Habas et al. 2012). The location and shape of sulci may be determined by the elastic tension from white matter fibres connecting cortical areas (Van Essen 1997), and a sulcation index can be calculated which characterises the proportion of a sulci according to the whole brain size (Dubois et al. 2008).

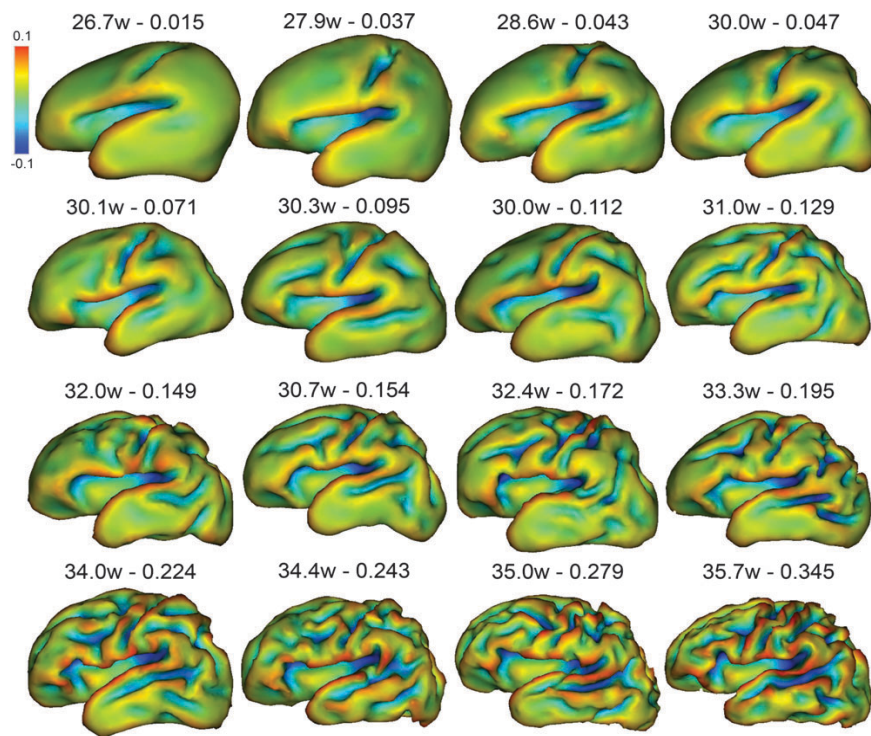


Figure 3.4: Cortical surface reconstructions in the developing brain. Images (not to scale) are examples of the 3D interface between the cortex and white matter zone for newborns of different gestational ages (in weeks; left numbers) and sulcation index (right numbers). Colors correspond to the degree of surface curvature. With increasing gestation age, sulcation index increases.

3.2 Preterm birth[■]

3.2.1 Epidemiology of preterm birth

The normal length of human gestation is between 37 to 42 weeks. Preterm birth is defined as delivery before 37 weeks, and can be further subdivided into moderately, very, or extremely preterm if the neonate is born between 32 to 36, 28 to 31, or less than 28 weeks respectively. Preterm birth is a major public health issue. With more than one in ten births currently being premature, the incidence is high and is projected to increase (World Health Organization 2012). Furthermore, the healthcare and societal burden attributed to the associated mortality and morbidity is considerable (Petrrou et al. 2011). Financial costs, including medical treatment, days spent in hospital, long-term care, and specialist education, are estimated at £3 billion per annum in the UK (Mangham et al. 2009) and \$26.2 billion in the USA (Institute of Medicine (US) Committee on Understanding Premature Birth and Assuring Healthy Outcomes et al. 2007). There are also profound negative emotional and psychosocial effects upon both the individual and the supporting family (Saigal & Doyle 2008).

The majority of preterm births have a spontaneous onset, due to premature labour, preterm premature rupture of membranes (PPROM), or vaginal bleeding (Blondel et al. 2006). The remainder are most often medically induced, typically because of pre-eclampsia (Johansson & Cnattingius 2010). The aetiology regarding preterm birth is unclear, but is likely to depend on multiple pathological mechanisms. A number of maternal and fetal risk factors have been identified which increase the likelihood of preterm birth. These include: previous history of preterm delivery (Cnattingius et al. 1999); multiple pregnancies (Blondel & Kaminski 2002); maternal age (Olausson et al. 1999); ethnic group (Kistka et al. 2007); genetic factors (Clausson et al. 2000); bacterial vaginosis and intrauterine bacterial infections (Goldenberg et al. 2000); socioeconomic status and education (L. K. Smith et al. 2007; Thompson et al. 2006); and smoking and substance abuse (Kyrklund-Blomberg & Cnattingius 1998). Biological pathways may represent an interplay of one more of these factors and comprise genetic or vascular mechanisms, inflammation, and neuroendocrine or mechanical stress (reviewed in Johansson & Cnattingius 2010). Potential preventative strategies for preterm birth have aimed to address specific risk factors. For example, strategies such as smoking cessation have resulted in a significant reduction in preterm delivery risk (Lumley et al. 2004), while others have met with little or no success (King et al. 2002; Hodnett & Fredericks 2003).

3.2.2 Neurocognitive morbidities associated with preterm birth

Advances in neonatal care, such as antenatal corticosteroid and early surfactant use (Fanaroff et al. 2003), have led to a decrease in mortality after preterm birth. However, as the survival of preterm neonates increases, (particularly of those born at younger gestational ages), the risk of adverse outcomes and neurodevelopmental impairments also become greater (Wood et al. 2000; Saigal & Doyle 2008). Neurodevelopmental impairments include severe disabilities such as developmental delay, cerebral palsy, and sensory impairments. Yet more frequent are mild neurological deficits that still confer substantial distress upon the preterm individual. These deficits are often persistent and occur across cognitive, language, motor, and behavioural domains. For example, infants born prematurely have reductions in intelligence (Kerr-Wilson et al. 2011), reduced linguistic and motor abilities (van Noort-van der Spek et al. 2012; de Kieviet et al. 2012), poor attention and social skills (Johnson et al. 2009), and a reduced likelihood of completing higher education (Moster et al. 2008). These latter problems have been demonstrated in the absence of severe disabilities and are inversely associated with gestational age at birth, although even mildly preterm individuals have shown significant neurocognitive impairment (Arpino et al. 2010; Dong et al. 2012). A fuller description of these impairments is given below.

Visual impairments are prevalent following preterm birth. Retinopathy of prematurity (ROP) is a common morbidity in extremely preterm infants and is caused by the overgrowth of blood vessels in the immature retina of the eye. ROP is more severe with increasing prematurity and can lead to scarring, detachment, and blindness in serious cases. Although the rate of blindness has reduced over the last 20 years (Saigal & Doyle 2008), myotropic, heterotropia, and the need for prescription glasses affect around a quarter of young preterm children and infants (O'Connor et al. 2002). Mild visual impairments and squints occur as high as 44% and 74% respectively in extremely preterm-born (older) children (Marlow et al. 2005). Preterm infants are also predisposed to impairments in hearing, although the prevalence rates here are generally low, ranging between 3–5% (Saigal & Doyle 2008). Infants possess auditory processing problems such as difficulty in auditory recognition and speech discrimination, which consequently may be detrimental to language acquisition and further academic ability.

Cognition, language, and behaviour

Cognitive functions encompass a number of faculties including (but not limited to) executive function, attention, and working memory. Deficits of cognition are now potentially the most common neurodevelopmental impairment in preterm children (Moore et al. 2012), and several studies have demonstrated preterm-associated differences in both global and specific cognitive abilities. A recent meta-analysis assessing general cognitive ability in preterm children demonstrated that reductions in IQ were associated with gestational age in a dose-response relationship (Kerr-Wilson et al. 2011). On average, preterm children scored 12 IQ points lower than term-born controls. Cognitive impairments persist through adolescence and adulthood, and deficits in IQ have been demonstrated in preterm-born adults (Allin et al. 2008).

Assessing cognitive function via IQ may not fully gauge its complexity during development (Aylward 2002), and is not feasible in younger age groups. Other global assessments, such as the Bayley Scales of Infant and toddler Development (BSID), define subscales that are age-appropriate and more specific to cognitive processes. The 2nd edition of the BSID yields scores for mental and psychomotor development, and several large sample studies using the BSID during early childhood have found that preterm-born children have significantly worse mental and psychomotor indices as compared to their term-born peers (Wood et al. 2000; Hintz et al. 2005). More specific cognitive deficits have also been reported in preterm-born individuals. These include abnormalities in information processing, memory, attention, and executive function (Anderson et al. 2004; Rose et al. 2011).

Preterm birth is associated with language delays and increased risk of language impairment. Preterm children attain worse scores on tests for receptive language, reading, vocabulary, expressive language, and their composites, as compared to their full-term peers (Wolke et al. 2008; Sansavini et al. 2010). Similarly, individuals born preterm are at an increased risk of psychiatric morbidity. Specific behavioural disorders known to be elevated among preterm children and adolescents include emotional impairments, attention-deficit hyperactivity disorder (ADHD), and autism spectrum disorders (ASD) (Johnson & Marlow 2011; Sansavini et al. 2011). Preterm-born children have an increased risk of developing ADHD and also frequently manifest externalising or internalising behaviours during school age (Bhutta et al. 2002; Aarnoudse-Moens et al. 2009). The cumulative burden of subtle cognitive, language, and behavioural deficits is likely to cause functional difficulties and academic underachievement in preterm individuals (Saigal & Doyle 2008; Arpino et al. 2010).

Motor

Preterm-born individuals frequently experience adverse motor outcomes (Saigal & Doyle 2008). Approximately 10% of preterm-born infants develop cerebral palsy (CP), and this group makes up 40% of those with the condition (Kirby et al. 2011). CP describes a non-progressive, heterogeneous group of disorders of movement and posture occurring in the developing fetal or infant brain (Bax et al. 2005). CP occurs in approximately 14% of extremely preterm, 6% of very preterm, and less than 1% of moderately preterm infants. More prevalent in the wider preterm population are mild gross and fine motor impairments, which can occur despite normal intelligence and absence of cerebral palsy (Davis et al. 2007). Low birth weight or preterm-born children are significantly worse as compared to normal birth weight or term-born children across a variety of motor abilities, including visuo-motor integration, balance, fine motor skills, and manual dexterity (Goyen et al. 1998; Davis et al. 2007; Potter 2007). This assorted group of motor impairments is collectively termed 'developmental co-ordination disorder' or 'minor neuromotor dysfunctions' and ranges between approximately 10% and 50% in the preterm population (Arpino et al. 2010).

3.3 Pathogenesis of white matter injury[■]

Cerebral white matter injury (WMI) is common after preterm birth and may underlie the neurological impairments observed in this population (Volpe 2003). Periventricular leukomalacia (PVL) typically consists of focal cystic necrotic lesions surrounded by diffuse abnormalities and represents a severe manifestation of WMI. PVL is associated with serious disabilities such as cerebral palsy but is uncommon, affecting less than 5% of preterm infants. In contrast, diffuse and focal non-cystic changes

are prevalent in the majority of preterm infants (Volpe 2008) and are likely to explain the more extensively observed mild to moderate neurological deficits.

A concise summary of the key pathological events underlying preterm WMI is given. For reviews on this subject, the reader is referred to (Deng 2010; and Volpe 2009). Hypoxia-ischemia and/or infection can initiate a destructive cascade (Leviton et al. 2005; Dean et al. 2011) which results in preterm exposure to excitotoxicity (Matute et al. 2007), oxidative stress (Haynes et al. 2006), and inflammation (Dammann et al. 2001; Xanthou et al. 2002). Exposure to these processes takes place in a developmentally sensitive window that would normally span the late 2nd and 3rd trimesters. In this gestational period, the brain is remarkably vulnerable to insult, specifically to the aforementioned destructive influences. The fetal cerebro-vascular system is particularly underdeveloped. Preterm neonates possess a circulation which is poorly supplied (Børch & Greisen 1998), has impaired auto-regulation (Boylan et al. 2000), is pressure-passive (Soul et al. 2007), and has low white matter vascularity (Ballabh et al. 2004). In addition, preterm neonates are at risk of hypocarbia, frequently caused by mechanical ventilation in the intensive care unit after delivery (Shankaran et al. 2006). Hyperoxia can also manifest due to the withdrawal of physiological hypoxia existing *in utero*, and has been associated with a reduction in neurogenesis (Malik et al. 2013).

3.3.1 Cellular and structural targets in white matter injury

Cell populations that are most vulnerable to these deleterious influences represent the principal targets of WMI pathogenesis. Premyelinating oligodendrocytes (preOLs), subplate neurons, and late migrating GABAergic neurons show a heightened susceptibility to mediators of pathogenicity, namely, extracellular glutamate (Matute et al. 2005; Desilva et al. 2007), free radicals (Haynes et al. 2006), and proinflammatory cytokines (Folkerth et al. 2006) potentially mediated via microglial interactions. Direct axonal damage can also occur separately from these processes. These targets are reviewed briefly in this section.

Premyelinating oligodendrocytes

PreOLs are present in very high amounts during the window of WMI vulnerability. PreOLs are susceptible to: oxidative damage due to the insufficiency of antioxidants; accumulation of iron required for differentiation during development; glutamate damage due to receptor and non-receptor mediated mechanisms; and damage by reactive nitric species (Haynes et al. 2006; Khwaja & Volpe 2007). Given its ample vulnerability to these toxic mediators, delayed maturation after insult (Buser et al. 2012), and neuropathological evidence of hypomyelination in PVL, the preOL has received much attention as the primary target for WMI (Volpe et al. 2011).

Subplate neurons

The neurons of the subplate layer are fully operational by the onset of the developmental window for WMI and are not distant from periventricular sites. The loss of subplate neurons is therefore conceivable and would potentially result in the disruption of sensory-driven thalamocortical circuitry. Subplate neurons have been found to be vulnerable to neonatal hypoxia-ischemia in an animal model similar to that of the human brain (McQuillen & Ferriero 2004), and late subplate neuron ablation has been linked with disruption in the functional maturation of visual cortical columns (Ghosh & Shatz 1992; Kanold et al. 2003). Recent *ex vivo* evidence from human PVL cases has found that granular neurons in the subplate region show specific susceptibility, distant from periventricular foci (Kinney et al. 2012).

GABAergic neurons

GABAergic neurons are found migrating throughout the developing white matter during late gestation (Xu et al. 2011) and GABA is also found to be the predominant neurotransmitter at this time (Herlenius & Lagercrantz 2004). Although there is a lack of substantial evidence implicating GABAergic neurons as a target, an immunohistochemical report of infants *ex vivo* has demonstrated a significant loss of telencephalic GABAergic neuron expression in infants with PVL white matter lesions as compared to controls (Robinson et al. 2006).

Microglia

In addition to these targets, microglia also play a key role in this pathological cascade. Indeed, animal models of WMI have observed that microglial activation is the first cellular event in and around a WM lesion following hypoxic-ischemic or infective insult (Verney et al. 2010). Microglia are found at peak density in key junctional regions of the WM anlage during the period of WMI vulnerability (Haynes et al. 2005). Microglia become activated after cytokine or glutamate exposure and, thereafter, serve to potentiate injurious processes through the secretion of toxic products (Verney et al. 2010).

Axonal damage

Direct axonal damage has also been witnessed in WMI, independent of cellular injury. Using the apoptotic marker fractin, widespread axonal degeneration was detected in the diffuse PVL component and was separate from focal necroses (Haynes et al. 2008). In a recent rodent study of the optic nerve by Alix et al., large-calibre, premyelinated axons which were radially expanding were discovered to have a heightened sensitivity to hypoxia-ischemia due to potential Ca^{2+} influx mechanisms (Alix et al. 2012). This evidence indicates that axons may also be a direct target in WMI.

3.3.2 White matter injury is persistent and affects axonal cross-road areas

The collective loss or maturational delay of the above cellular and structural targets results in hypomyelination and axonal damage. This may persist long past the initial period of injury and cause delays in neural transmission, leading to diminished neural function. A recent study which examined a rodent model using both immunohistochemical and imaging methods found that moderate systemic inflammation in the perinatal period led to persistent alterations of the regular white matter developmental program and maturational blockade of premyelinating oligodendrocytes (Favrais et al. 2011). In this report, long-lasting structural deficits such as disturbances of myelination and axonal damage were accompanied by cognitive deficits similar to those observed in preterm infants. Areas of crossing WM tracts are particularly vulnerable to WMI (Judas et al. 2005; Benjak et al. 2008; Verney et al. 2012). These areas are proximal to affected periventricular sites, and may suffer disruption of the extracellular matrix and axonal guidance cues, brought about in part by the abundant source of activated microglia present here at this time (Baud et al. 2006). A recent immunohistochemical study by Verney et al. found that very preterm infants with non-cystic periventricular white matter injury had pathological changes in multiple cross-road sites, including microglial activation and axonal injury. These changes were also found to be extensive in preterm infants who also demonstrated necrotic foci and diffuse lesions (Verney et al. 2012). Cross-road areas are composed of numerous intersecting callosal, associative, and thalamocortical axons which connect multiple cortical and subcortical targets (Figure 3.5) and subserve motor, sensory, and associative functions (Judas et al. 2005). Thus, WMI has the potential to be widespread, and to consequently affect several neurocognitive domains. Ultimately, the pattern and severity of injury to the white matter and its connecting structures will predict the profile of neurological impairment.

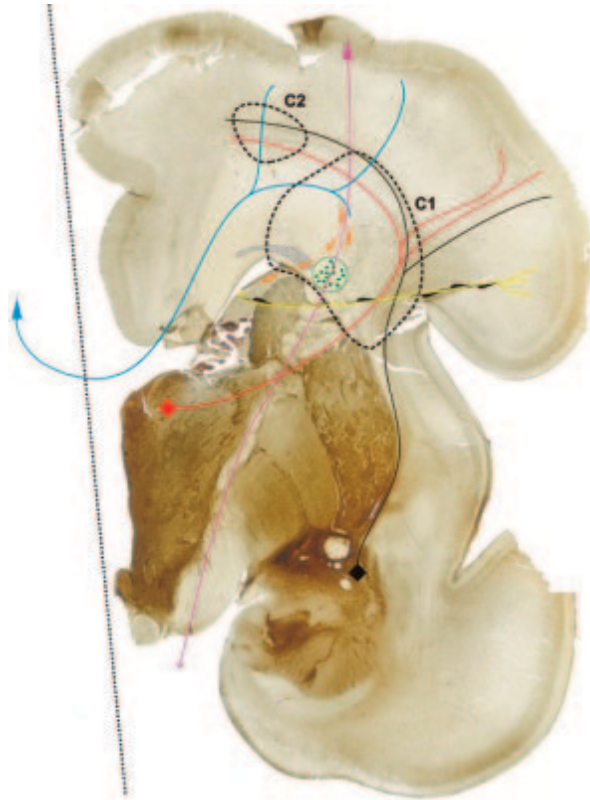


Figure 3.5: Axonal cross-road area vulnerable to preterm WMI. Acetylcholinesterase-stained coronal section through a telencephalon of 28 week gestation human fetus showing axonal cross-road areas and intersecting fibres in the frontal lobe, reproduced from Judas et al. (2005). C1 (lateral to the lateral angle of the lateral ventricle), C2 (above the dorsal angle of the anterior horn of the lateral ventricle), and black dashed lines indicate cross-road areas. Circle with green dots = developing fronto-occipital system; black filled lines = basal forebrain afferents; red = thalamocortical afferents; blue = callosal fibres; violet = corticofugal efferents; yellow with black profiles = migrating neurons on radial glial fibre; orange profiles = tangentially migrating neurons.

3.3.3 White matter injury coincides with grey matter injury

Grey matter areas are also not spared from the effects of prematurity (Dean et al, 2013) and damage here is likely to occur in conjunction with WMI. A histological study using post-mortem tissue, showed that neuronal loss was present in the thalami, basal ganglia and cerebelli of preterm infants with white matter injury, as compared to those without it (Pierson et al, 2007). In addition, significant gliotic changes were also found in the same structures in preterm infants who had diffuse WMI without focal necroses (Pierson et al, 2007). Imaging studies have found that volumetric reductions in the thalamus in preterm neonates without macrostructural pathology, occur in association with microstructural white matter damage (Boardman et al, 2006; Ball et al, 2012). Thus, WMI is further compounded by injury to the linking grey matter. It is possible that grey matter injury occurs as a result of a downstream maturational or trophic effect (Volpe et al, 2009). However, this remains to be determined and it may well be in the opposite direction.

3.3.4 Summary

In summary, white matter injury in preterm-born individuals is typified by hypomyelination and neuro-axonal damage, with a predilection for periventricular axonal cross-road areas. This is primarily caused by the engagement of destructive influences of hypoxia-ischemia and/or infection with maturation-sensitive cell populations. The delayed or arrested development of essential cell populations is likely to prolong and perpetuate white matter damage into later life.

3.3.5 Exploring premature white matter injury with neuroimaging

The high rate of neurological disability in the preterm population underscores the importance of early identification of individuals with WMI, who may benefit from intervention. Various neuroimaging modalities are currently available which can detect white matter abnormalities and predict neurodevelopmental outcomes in preterm-born neonates. In the clinical setting, established methods include cranial ultrasound (c-US) and conventional (T_1 - and T_2 -weighted) magnetic resonance imaging (MRI). c-US is routinely used and is both accessible and cost-effective. Although it can successfully identify cystic WMI, it lacks sensitivity in recognising more diffuse, non-cystic pathology (Inder et al. 2003; Debillon et al. 2003). Conventional MRI is more capable of identifying and estimating the degree of WMI.

Qualitative MRI findings include white matter abnormalities (WMA) such as focal regions of T_1 signal shortening or diffuse T_2 hyperintensity, evidence of cystic change, lateral ventricular enlargement, corpus callosum thinning, and a reduction in WM volume (Inder et al. 2003). Increasing severity of WMA in preterm neonates at term has been associated with WM microstructural disruption and poorer cognitive and motor performance at 2 years (Cheong et al. 2009; Adams et al. 2010; Bonifacio et al. 2010; Thompson et al. 2012; Liu et al. 2012; van Pul et al. 2012), and neonates with moderate-severe WMA are predicted to face serious cognitive and motor delays, including cerebral palsy (Woodward et al. 2006). However, this approach is limited by its subjective nature and lack of specificity in identifying infants with adverse outcomes.

Quantitative MRI studies of the white matter primarily examine volumetric changes across the whole brain as well as in specific regions. In preterm-born individuals, the normal trajectory of cerebral WM growth is significantly diminished and specific differences are present in bilateral frontal, temporal, and parietal regions (Ment et al. 2009). Volumetric differences have been identified in preterm infants throughout childhood and adolescence and have been related to neurodevelopmental aptitude (de Kieviet et al. 2012). Regionally, the corpus callosum has received much attention, and reductions in the cross-sectional area have been found in preterm infants at term (Thompson et al. 2011) and during adolescence (Nosarti 2004; Narberhaus et al. 2008). The splenium and other posterior regions appear to

display the greatest differences and have been associated with tests of vocabulary (Narberhaus et al. 2008) and verbal IQ (Nosarti 2004). This posterior-focused damage is likely to be due to a number of factors including its late maturation (Rakic & Yakovlev 1968) and proximity of susceptible periventricular oligodendrocytes (Volpe 2008). However, histological evidence suggests that gross WM damage in preterm infants is more widespread. Indeed, global WM volume reductions have been demonstrated in preterm children and adolescents compared to term-born controls, and have been associated with decreased intelligence and increased behavioural difficulties (Yung et al. 2007; Northam et al. 2011). In a large study of preterm adolescents, WM loss was found to be pervasive in several regions, including the brainstem, internal capsule, fronto-temporal regions, and major fasciculi, and was independently associated with cognitive and language impairments (Nosarti et al. 2008). Still, MRI volumetric WM changes are likely to be secondary, following hypomyelination and axonopathy, in preterm-associated WMI.

3.4 The role of diffusion MRI in the study of early brain development and preterm birth

3.4.1 White matter development

Diffusion MRI measures such as anisotropy and eigenvector v_1 are sensitive to microstructural changes and offer unique insight into events during development (see also Chapter 5). For example, DTI has been used to detect the radial orientation of neurons and glia in the developing cortical plate (McKinstry 2002). In a study of healthy preterm infants, anisotropy was highest in the cortex and eigenvector v_1 was orientated perpendicular to the cortical surface from 26 weeks of gestation. By 36 weeks, anisotropy values approached zero and radial orientation of diffusion was less evident, reflecting cellular changes such as increased arborisation, formation of local circuits, and loss of radial glia (McKinstry 2002). dMRI is also sensitive to changes associated with premyelination in advance of conventional MRI or histology. Wimberger et al. demonstrated anisotropy across unmyelinated cerebral white matter sites in developing rats prior to both histological staining and T_2 -weighted changes, a finding that has been replicated in later studies (Wimberger et al. 1995; Prayer et al. 2001). dMRI has been used to demonstrate the spatio-temporal sequence of early postnatal WM development (Gao et al. 2009) including the trajectory of major white matter bundles (Trivedi et al. 2010; Takahashi et al. 2012). Variability in white matter integrity has also been shown to predict functional abilities such as mental arithmetic, working memory, and visuomotor function (Tsang et al. 2009; Vestergaard et al. 2011; Madsen et al. 2011), although these correlations have been found at later ages. In sum, these findings suggest that dMRI has considerable utility in the study of early WM development.

3.4.2 White matter injury

Methods which examine the WM microstructure are also likely to gain a better approximation of the underlying neuropathology. An accurate quantification of microstructural damage may successfully predict neurological impairment. Whilst focal lesions have relatively precise anatomical and imaging correlates, diffuse WMI is more difficult to identify, at least on conventional imaging. Fortunately, diffusion MRI shows greater sensitivity for these subtle changes and has become the tool of choice for studying WM damage in the preterm brain (reviewed in Ment et al. 2009; Pandit et al. *in press*). dMRI studies demonstrate changes indicative of WM disruption in preterm-born individuals as compared to term-born controls, persistent from birth through to adulthood. These changes principally manifest as reductions in FA and increases in ADC and radial diffusivity, and are present across a wide range of study methodologies (Anjari et al. 2007; Rose et al. 2008; Hasegawa et al. 2011; Ball et al. 2010; Lee et al. 2012; Jo et al. 2012; Vangberg et al. 2006; Skranes et al. 2007; Constable et al. 2008; Eikenes et al. 2011). These findings support the role of dMRI features as biomarkers for preterm WMI.

Chapter 4

Mapping the macroconnectome in the developing preterm brain

4.1 Introduction

4.1.1 The MRI macroconnectome

The *connectome* is defined as the comprehensive mapping of neural connections in the brain, and its realisation is a highly pursued scientific goal (Sporns et al. 2005). As neurological function is predicted by patterns of neuronal interactions, an accurate mapping of the underlying substrate offers much insight into the basis of behavioural variability. Such a mapping would also provide clarity regarding the structural correlates of neurological disease.

At present, the complete human connectome remains unresolved. Very high spatial resolution imaging techniques need to be developed, accompanied with massive data storage capabilities and computational power. Several MRI studies have instead attempted a coarse reading of the human connectome *in vivo*. These so-called *macroconnectome* approaches map inter-regional connectivity non-invasively across the entire brain at macroscopic scale ($\geq 1\text{mm}^3$) and rely upon different principles of MRI. The most common approaches are briefly described here. dMRI tractography (Chapter 2) delineates white matter structural connections by examining the anisotropic diffusion of water from one voxel to another, allowing the trajectory and integrity of axonal bundles to be inferred. Conventional MRI is used to infer structural connectivity by correlating inter-regional cortical thickness or volume. Functional MRI measures blood oxygenation level-dependent (BOLD) signals across the brain. By detecting regions with statistically dependent BOLD signals, ‘functional’ connections can be delineated. Macroconnectome data from any of these approaches are normally summarised as an adjacency matrix where the axis labels and matrix elements refer to regions of interest in the brain and measures of connectivity respectively.

4.1.2 Classifying structural macroconnectome approaches

Macroconnectome approaches which apply whole-brain dMRI tractography (termed ‘structural macroconnectome’) are the primary interest of this chapter. These can be classified by four properties: the parcellation scheme that divides the brain into cortical and subcortical regions; the dMRI acquisition scheme; the tractography algorithm; and the metric used to quantify the tract. In this section, the impact

of all four properties on the structural macroconnectome are described. As the different types of tractography algorithm and methods of estimating connectivity were discussed in some detail in Chapter 2, the main focus of this section is the remaining two properties.

Parcellation schemes are required to partition the brain and delineate source and target regions of interest (ROIs) from which connections start and end respectively. Parcellations generally include cortical grey matter ROIs and subcortical structures. When these correspond across individuals, homologous connections between ROIs can be compared, even despite variable trajectories across an individual connection. Two types of parcellation scheme are recognised.

Anatomically-dependent parcellations are generated by registering an atlas to the brain of a subject of interest and then propagating the corresponding label information. An atlas is an expertly divided anatomical template of the brain that takes into account one or more sources of data and enables parcellation of a subject's brain on the basis of an MRI scan (de Reus & van den Heuvel 2013). Parcellations are based on natural divisions of the cortex and sub-cortex, which can be derived from studies of cytoarchitecture (Brodmann 1909), myelin content (Glasser & Van Essen 2011), cortical surfaces and gyri (Van Essen et al. 2012), and, more recently, patterns of functional or structural connectivity (Johansen-Berg et al. 2004; Cloutman 2012). By having parcellations which demarcate regions based on prior neuroanatomical and biological knowledge, one can easily identify the connections which pass between these regions and compare them with results from previous literature. However, variations between parcellation schemes in the boundary, size, and number of ROIs can have significant effects upon further connectome analysis (Bassett et al. 2011). Therefore their selection is an important consideration.

Anatomically-independent parcellation schemes overcome this issue by performing an unbiased parcellation of the brain into small, equally sized ROIs. For example, Hagmann et al. randomly divided the brain into 500 to 4000 ROIs at the white-grey matter interface of the cortex by a method of region growing (Hagmann et al. 2007), while Zalesky et al. randomly subsampled the whole brain (excluding CSF) into $5 \times 5 \times 5$ mm voxel 'cubes', each of which encapsulates a distinct volume of grey or white matter tissue (Zalesky et al. 2012). With an anatomically independent method the resolution of the macroconnectome can be greatly increased as the upper limit on number regions is, in effect, the number of possible seeding voxels (a voxel-wise parcellation). However, having more ROIs increases the computational cost as the total number of tracking simulations increases. Furthermore, the scope for registration errors also increases as many small ROIs need to be mapped between subjects.

Diffusion MRI acquisition schemes vary widely, and their selection can have a significant impact upon the macroconnectome. Gigandet et al. (2010) compared the effects of several acquisition schemes

including DTI, Q-ball imaging, and DSI (Gigandet et al. 2010). Connectome density was found to be lowest when using DTI and highest when using high b-value DSI, with differences being most apparent for medium- to long-distance connections. DSI appears to produce the most complete and biologically meaningful results, but can suffer from long acquisition times, which may not be acceptable for clinical routine scans. Similarly, choices in tractography algorithm and its parameters have a large effect upon both macroconnectome data and subsequent network analysis. For example, a recent study found that multiple rather than single diffusion directions and probabilistic rather than deterministic algorithms yield macroconnectome with increased density and network efficiency and reduced small-worldness (Appendix B) (Bastiani et al. 2012).

Macroconnectome studies use a variety of metrics to quantify connections between pair-wise ROIs. These include conventionally tractography-derived measures such as FA, ADC, streamline count, and tract volume, each potentially characterising a different aspect of white matter connectivity. Other connectivity measures include those based on principles of information flow such as 'anatomical connectivity strength' (Iturria-Medina et al. 2007) (Chapter 2) and mean anisotropy (Robinson et al. 2010) (see 4.2.6). All metrics may be used in their weighted form or can be thresholded and binarised to indicate whether connections are present or absent.

4.1.3 Structural macroconnectome methods for the developing preterm brain

Structural macroconnectome approaches are well placed to explore the extent of white matter change in the preterm brain (Chapter 3). Two studies have assessed the partial structural macroconnectome in preterm neonates (Ball et al. 2012a; Pannek et al. 2013). Ball et al. performed probabilistic tractography between the thalamus and the cortex, randomly parcellated into approximately 500 small regions (Ball et al. 2012a). By taking several iterations of the parcellation and averaging the tractography results in each, a voxel-wise map of connectivity was produced. Pannek et al. parcellated the cortex by propagating regions from a normal neonatal atlas, which each subject's FA map was registered to (Pannek et al. 2013). Maps of cortico-cortical connectivity were generated by performing probabilistic tractography between pair-wise ROIs.

However, in spite of these preliminary reports, no approach currently exists which reads the preterm structural macroconnectome across the whole brain *per se*. In addition, there is no study which assesses the preterm macroconnectome in infancy or early childhood. This is a period when axonal development and myelination reach maturity, and may represent a critical time in the evolution of preterm WMI.

4.1.4 Chapter aim

In this chapter a data-driven, semi-automated method of mapping the developing macroconnectome in infants using dMRI is proposed, which is feasible in clinical scanning conditions. This method is based on the work of Robinson et al., which combines an anatomically-dependent parcellation, a modified probabilistic tracking algorithm, and a novel anisotropy measure in order to model whole-brain structural connectivity (Robinson et al. 2010) . This framework has been successfully used to map connections in young and old-age adults (Robinson et al. 2010), but is inappropriate for use in the infant population. Several modifications are presented which adapt the framework for infants and younger children, including adjustments in image preprocessing, registration, and parcellation scheme.

4.2 Framework to map the structural macroconnectome in the developing preterm brain^o

4.2.1 Outline of framework

The framework required to delineate whole-brain structural connectivity is summarised graphically in Figure 4.1 and the individual steps are described now in greater detail.

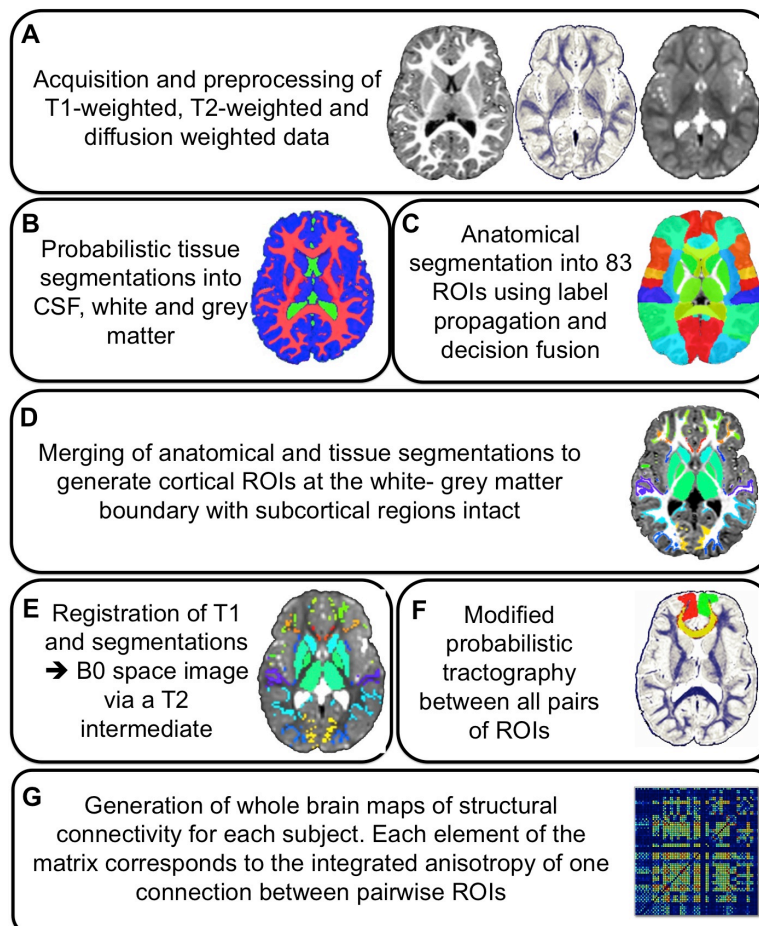


Figure 4.1: Overall framework to map the developing macroconnectome. Graphical pipeline of processing steps required to transform raw MR data to macroconnectome matrices.

4.2.2 Subjects

Data from 49 preterm-born children were analysed. Subjects had a median post-conceptual age at birth of 28.3 weeks [range 24.6–34.7] and median birth-weight of 0.99 kilograms [0.56–3.71]. Nineteen subjects were scanned with a median corrected age of 13 months [11–16], and 37 were scanned with a median corrected age of 25 months [23.5–31.5]. Seven infants had paired imaging data from both scanning sessions. Subjects were included if high quality T_1 , T_2 , and 32-direction diffusion tensor imaging (DTI) data were all present. Subjects were excluded if focal, destructive cerebral lesions

(cystic periventricular leukomalacia, haemorrhagic parenchymal infarction, or post-haemorrhagic ventriculomegaly) were present on T₁-weighted or T₂-weighted MRI scans. Twenty-six subjects (53.1%) required mechanical ventilation in the early neonatal period. Forty-eight subjects had neonatal imaging data, of which: 5 (10.4%) subjects had germinal matrix haemorrhage; 4 (8.3%) had punctate white matter lesions, mainly located in the posterior periventricular white matter; and 11 (22.9%) had enlarged ventricles (mild: 10, moderate: 1; left: 2, right: 2, bilateral: 7). The demographic data are outlined in Table 5.1.

Table 4.1: Infant demographic data. Respiratory co-morbidity is defined as the number of infants with chronic lung disease – as requiring oxygen at 36 weeks post-menstrual age. IUGR is defined here as being less than 10th centile weight. PROM is defined here as greater than 18 hours.

Characteristics	Total (n=49)
Gestational age, median in weeks (range)	28.29 (24.56–34.71)
Birth Weight, median in kilograms (range)	0.986 (0.56–3.71)
Male, No. (%)	23 (46.9)
Singleton/twins, No. (%)	28/21 (57/43)
Premature rupture of membrane [PROM], No. (%)	11 (22.4)
Late-onset sepsis positive blood culture, No. (%)	9 (18.4)
Respiratory co-morbidity, No. (%)	11 (22.4)
Patent ductus arteriosus [PDA], No. (%)	6 (12.2)
Intra-uterine growth restriction [IUGR], No. (%)	11 (22.4)

4.2.2 Data acquisition

T₁-weighted and T₂-weighted data were acquired according to a protocol as defined previously (Counsell et al. 2008). Single shot, echo planar DTI was acquired on a Phillips 3-T system in 32 non-collinear directions using the following parameters: TR 9000ms, TE 49ms, slice thickness 2mm, field of view 224mm, matrix 128 × 128, voxel size 1.75 × 1.75 × 2 mm³, b-value = 750 s/mm², a SENSE factor of 2, and scanning time for the sequence of 7 minutes. A paediatrician trained in MRI procedure was in attendance at all times. An adult head coil was used (Vade et al. 1995), because brain size at this age is not considerably smaller as compared to adults (Giedd et al. 1996; Knickmeyer et al. 2008).

Children are typically less compliant than adult subjects, and adequate data is more difficult to obtain. Facing the prospect of an unknown, uncomfortable environment of the MR scanner, children understandably may suffer substantial anxiety and are also likely to move during scanning (Poldrack et al. 2002). For these reasons, children were sedated using oral chloral hydrate: (100 mg/kg up to a maximum of 1g) prior to scanning. Pulse oximetry, temperature, and electrocardiography data were monitored throughout. Ear protection was used for each infant, comprising earplugs moulded from a silicone-based putty (President Putty, Coltene Whaledent, Mahwah, NJ) placed in the external ear and neonatal earmuffs (MiniMuffs, Natus Medical Inc, San Carlos, CA).

4.2.3 Data preprocessing

Brain extraction

Initial raw data (T_2 -weighted and 32 direction diffusion-weighted images) were acquired and brain extracted using the FSL (FMRIB Software Library) Brain Extraction Tool (BET) (Smith 2002). BET (**bet2**)^{ss} robustly removes non-brain tissue in the head and neck, including skin, fat, skull, and eyeballs. An accurate brain extraction can improve the fidelity of later steps, particularly inter-subject image registration. BET determines the centre of gravity of the head and defines an initial sphere based on this centre. A tessellated mesh is used to model a brain surface, and is allowed to deform according to various dynamic controlling terms until it reaches the brain edge. The fractional intensity threshold (f) sets the brain/non-brain intensity level, with lower thresholds generating larger brain outlines. In some cohorts, default f values have been found to give inaccurate results (Popescu et al. 2012). Using the above acquisition sequence for the preterm infant brain, default f values were found to remove brain tissue in T_2 -weighted images, so lower f levels ($f = 0.35-4$) were used for brain extraction.

In infant T_1 -weighted images, the above brain extraction procedure frequently failed (Figure 4.2A), therefore several additional steps were added. The binary mask of the previously brain-extracted T_2 -weighted image was transformed to T_1 space after an initial low degrees of freedom (DOF) registration was made between the mask and the T_1 image. This new mask was slightly dilated and multiplied with the T_1 -image in order to remove neck tissue. The dilation and multiplication of images was performed using **fslmaths**. The new T_1 image was then brain extracted with very low f values (0.05) to make an accurate brain-extracted T_1 image (Figure 4.2B).

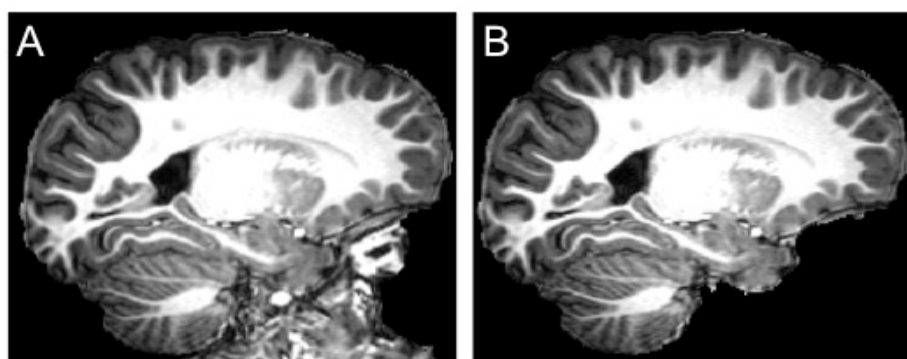


Figure 4.2: Brain extraction of T_1 -weighted images. FSL BET, $f = 0.4$, leaves considerable neck tissue with the brain (A) while brain extraction using a transformed T_2 brain mask in T_1 space (see text) removes non-brain tissue effectively (B)

^{ss} Bold font indicates UNIX command line executable.

Bias field correction

Spatial intensity variations arise due to the distance of voxels from the image coil. These are known as intensity non-uniformities or bias, and can be modelled in the process of segmenting brain tissues. Here, FSL Fast Automated Segmentation Tool (FAST, **fast**) was used in order to bias-correct the data (Smith et al. 2004).

DWI processing

Diffusion-weighted images were inspected before further processing. Individual gradient directions were assessed and directions that were artefactual due to subject motion were discarded (up to a maximum of 3 gradient directions per subject). A conservative threshold of 3 was chosen to maintain the quality of diffusion-weighted images for further processing and tractography. Eddy currents are induced when the diffusion-encoding gradient pulses are rapidly switched on and off, and can lead to distortions such as stretches and shears in the reconstructed image (Le Bihan et al. 2006). In order to minimise these and other geometric distortions, diffusion-weighted images were affinely registered to their respective b_0 image. This was performed using FSL FDT (FMRIB's Diffusion Toolbox), **eddy_correct**.

Using FSL FDT, **dtifit**, a diffusion tensor was fitted at each voxel of the eddy current corrected data and maps of diffusion parameters were generated. v_1 and FA maps were checked for imaging artefacts. The v_1 map and its false colour gradient allows the voxel-wise fibre direction to be examined. In this way, the architecture of major WM bundles, the corpus callosum (including forceps major and minor), anterior, and posterior limbs of the internal capsule and cingulum bundles was inspected.

4.2.4 Parcellation scheme

The composite parcellation scheme used to map the developing macroconnectome is described here. It combines anatomical and tissue segmentation of T_1 -weighted images, optimised for infants and young children, and generates a parcellation with cortical ROIs at the white-grey matter (WGM) boundary and full subcortical ROIs.

Probabilistic tissue segmentations were obtained using statistical parametric mapping (SPM) (Ashburner & Friston 2005). In the SPM model, each voxel of a target MR image can be classified according to its tissue type: cerebro-spinal fluid, grey matter, and white matter, based on its signal intensity and the information from a prior probabilistic image (Figure 4.3). A brief explanation of this procedure is now given.

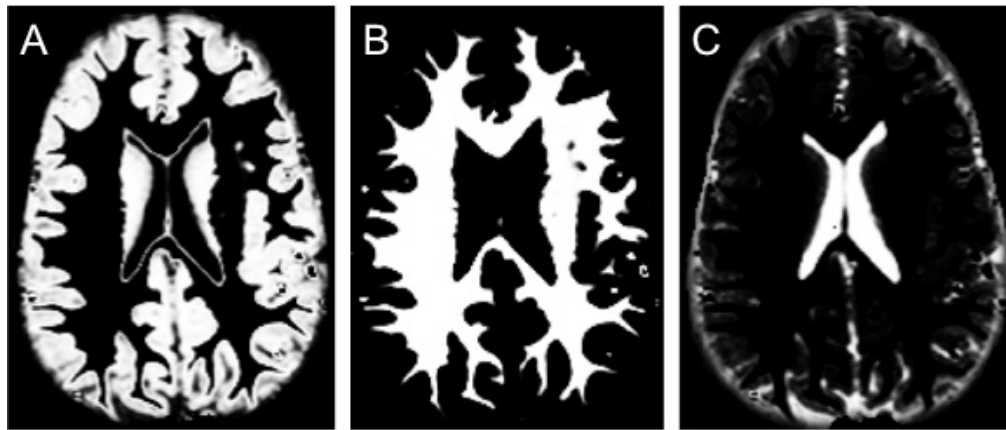


Figure 4.3: Probabilistic tissue segmentation using SPM. (A) grey matter (B) white matter (C) cerebro-spinal fluid

The distribution of voxel intensities within a tissue class can be described by the number of voxels, the mean intensity, and the variance. This intensity distribution is generally assumed to be Gaussian and, since there is overlap between tissue intensity distributions, tissue types are collectively modelled as a mixture of Gaussians. The likelihood of a voxel with a particular intensity belonging to a tissue class can be initially estimated from this model. A spatially registered, prior probability image is generated by combining several previously tissue-segmented brains. This generates a map for the prior probability estimate of each voxel location containing white or grey matter or CSF. The problem of classifying voxels in a target image (missing data) is solved iteratively using an expectation-maximisation (EM) approach. Here, the missing data is estimated based on the observed voxel intensity and an estimation of the likelihood of it belonging to each class. For every voxel, the probability of each tissue class is estimated from the spatially correspondent voxel of the prior, multiplied with the likelihood from the intensity distribution model of voxels in the target image (expectation step). With new estimates of voxel tissue class probabilities, the parameters of the tissue intensity distribution model are re-estimated (maximisation step). With new estimates for the model parameters, the process is repeated until a convergence criterion is met.

In the developing infant brain, the use of adult tissue priors is not well suited. Children's brains differ significantly from adult brains in size and shape, and this is particularly apparent in central, subcortical structures (Wilke et al. 2003). Thus, with an affine registration, the alignment of adult priors with infant images may not be successful. Moreover, there are differences in the tissue intensity distribution between the adult and developing brain. Performing tissue segmentation in infants with SPM and a default adult prior, the WM in central brain structures such as the thalamus are overestimated, whereas in other areas the WM segmentation is deficient (Figures 4.4 and 4.5). In order to optimise the tissue segmentation, an age-appropriate prior was obtained (Murgasova et al. 2007). This was created by transforming the manual segmentation of a single 2-year-old subject to 37 other 2-year-olds with non-rigid registration. A probability map was then made after affine-aligning all 37 segmented subjects with a reference image and averaging. This map was then used in place of the adult prior. As an example, Figures 4.4 and 4.5 demonstrate the difference in using adult and 2-year-old priors in a preterm child (male, 26.5 months).

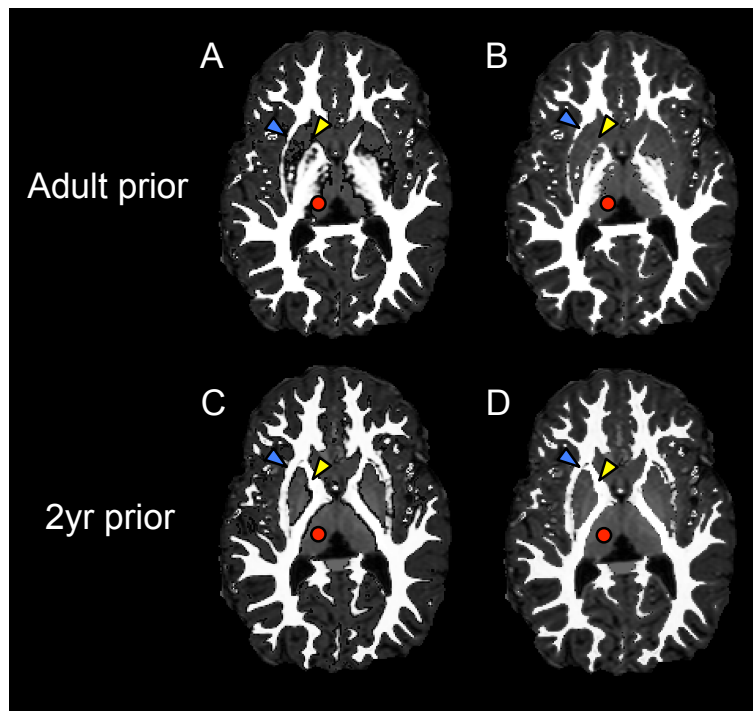


Figure 4.4: Comparison between adult and 2-year old priors in SPM tissue segmentation (axial). Using an adult probalistic prior (A,B), WM intensity cannot be differentiated from GM in the thalamus (red dot) and WM cannot be detected in the external capsule (blue arrow head) and anterior limb of the internal capsule (yellow arrow head) as compared to a 2-year old prior (C,D). Differences are persistent at WM intensity threshold of 0.01 (A,C) and 0.5 (B,D). All images shown in the same plane.

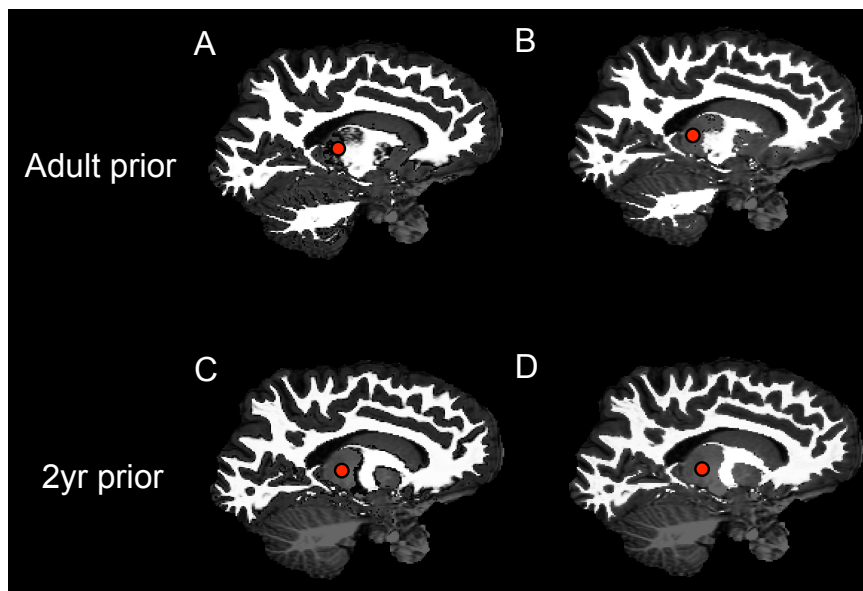


Figure 4.5: Comparison between adult and 2-year old priors in SPM tissue segmentation (sagittal). Using an adult probalistic prior (A,B), WM intensity cannot be differentiated from GM in the thalamus (red dot) as compared to a 2-year old prior (C,D). Differences are persistent at WM intensity threshold of 0.01 (A,C) and 0.5 (B,D). All images are shown in the same plane

Anatomical segmentation was performed by dividing each subject's T₁-weighted image into 83 cortical and subcortical regions of interest (outlined in Table 4.1). This was achieved using a multi-atlas label-propagation/decision fusion approach that has been previously implemented in adults (Heckemann et al. 2006) and infants of the same age as the subjects of this thesis (Gousias et al. 2008). The segmentation proceeded in two stages. In Gousias et al. a set of 30 manually segmented adult template images was mapped to the T₁-weighted images of a group of 33 two-year-old subjects (Gousias et al. 2008). Every two-year-old was paired with every adult subject for image registration, resulting in 990 registration sets. All pairs were registered in three steps: rigid, affine, and non-rigid (Appendix A). Each registration produced a separate 'classifier' by propagation of the atlas labels and created 30 classifiers for each subject. For each voxel in the target image, a decision fusion step was then applied, which takes a consensus vote of all corresponding classifier voxels (Heckemann et al. 2006; Aljabar et al. 2009). This enabled retention and fusion of labels in such a way that every voxel gets classified according to the brain region it has the greatest probability of belonging to. The second stage proceeded in a similar fashion to the first, again using label-propagation and decision fusion. Initial atlases were the automatically segmented two-year-old brains described above, and the target images were the images of a new group of one- and two-year-old subjects (used in this thesis). Figure 4.6 illustrates the value of decision fusion in increasing the accuracy of label propagation.

Table 4.2: List of regions of interest (ROIs) as delineated by anatomical segmentation. ROIs in italics were not used as seed/target regions in analysis. These include: CSF areas (45 – 49) from where no tracking is anatomically possible, the corpus callosum (44) to allow tracking of inter-hemispheric connections, and infra-tentorial structures (17-19; 74-75), which suffered from signal artefact in diffusion imaging and so were removed (see 4.3.1).

Number	Abbreviation	Label
1	<i>r Hip</i>	Hippocampus, right
2	<i>l Hip</i>	Hippocampus, left
3	<i>r Amg</i>	Amygdala, right
4	<i>l Amg</i>	Amygdala, left
5	<i>r aTm</i>	Anterior temporal lobe, medial part right
6	<i>l aTm</i>	Anterior temporal lobe, medial part left
7	<i>r aTl</i>	Anterior temporal lobe, lateral part right
8	<i>l aTl</i>	Anterior temporal lobe, lateral part left
9	<i>r PHipG</i>	Parahippocampal gyrus, right
10	<i>l PHipG</i>	Parahippocampal gyrus, left
11	<i>r psTG</i>	Superior temporal gyrus, posterior part right
12	<i>l psTG</i>	Superior temporal gyrus, posterior part left
13	<i>r m/iTG</i>	Medial and inferior temporal gyri, right
14	<i>l m/iTG</i>	Medial and inferior temporal gyri, left
15	<i>r lOTG</i>	Lateral occipitotemporal gyrus, gyrus fusiformis right
16	<i>l lOTG</i>	Lateral occipitotemporal gyrus, gyrus fusiformis left
17	/	<i>Cerebellum, right</i>
18	/	<i>Cerebellum, left</i>
19	/	<i>Brainstem, spans the midline</i>
20	<i>l Ins</i>	Insula, left

21	<i>rIns</i>	Insula, right
22	<i>lOcc</i>	Occipital lobe, left
23	<i>rOcc</i>	Occipital lobe, right
24	<i>laCgG</i>	Cingulate gyrus, anterior part left
25	<i>raCgG</i>	Cingulate gyrus, anterior part right
26	<i>lpCgG</i>	Cingulate gyrus, posterior part left
27	<i>rpCgG</i>	Cingulate gyrus, posterior part right
28	<i>lmFG</i>	Middle frontal gyrus, left
29	<i>rmFG</i>	Middle frontal gyrus, right
30	<i>lpTem</i>	Posterior temporal lobe, left
31	<i>rpTem</i>	Posterior temporal lobe, right
32	<i>lPar</i>	Parietal lobe, left
33	<i>rPar</i>	Parietal lobe, right
34	<i>lCauN</i>	Caudate nucleus, left
35	<i>rCauN</i>	Caudate nucleus, right
36	<i>lNacc</i>	Nucleus accumbens, left
37	<i>rNacc</i>	Nucleus accumbens, right
38	<i>lPut</i>	Putamen, left
39	<i>rPut</i>	Putamen, right
40	<i>lThal</i>	Thalamus, left
41	<i>rThal</i>	Thalamus, right
42	<i>lGP</i>	Globus Pallidus, left
43	<i>rGP</i>	Globus Pallidus, right
44	/	<i>Corpus callosum</i>
45	/	<i>Lateral ventricle, frontal horn, central part and occipital horn right</i>
46	/	<i>Lateral ventricle, frontal horn, central part and occipital horn left</i>
47	/	<i>Lateral ventricle, temporal horn right</i>
48	/	<i>Lateral ventricle, temporal horn left</i>
49	/	<i>Third ventricle</i>
50	<i>lPrecG</i>	Pre-central gyrus, left
51	<i>rPrecG</i>	Pre-central gyrus, right
52	<i>lStrG</i>	Straight gyrus, left
53	<i>rStrG</i>	Straight gyrus, right
54	<i>laOG</i>	Anterior orbital gyrus, left
55	<i>raOG</i>	Anterior orbital gyrus, right
56	<i>liFG</i>	Inferior frontal gyrus, left
57	<i>riFG</i>	Inferior frontal gyrus, right
58	<i>lsFG</i>	Superior frontal gyrus, left
59	<i>rsFG</i>	Superior frontal gyrus, right
60	<i>lPoscG</i>	Post-central gyrus, left
61	<i>rPoscG</i>	Post-central gyrus, right
62	<i>lSPG</i>	Superior parietal gyrus, left
63	<i>rSPG</i>	Superior parietal gyrus, right
64	<i>lLin</i>	Lingual gyrus, left
65	<i>rLin</i>	Lingual gyrus, right
66	<i>lCun</i>	Cuneus, left
67	<i>rCun</i>	Cuneus, right
68	<i>lmOrG</i>	Medial orbital gyrus, left
69	<i>rmOrG</i>	Medial orbital gyrus, right
70	<i>lIOrg</i>	Lateral orbital gyrus, left
71	<i>rIOrg</i>	Lateral orbital gyrus, right
72	<i>lpOrg</i>	Posterior orbital gyrus, left
73	<i>rpOrg</i>	Posterior orbital gyrus, right
74	/	<i>Substantia nigra, left</i>
75	/	<i>Substantia nigra, right</i>
76	<i>lSubgF</i>	Subgenual frontal cortex, left
77	<i>rSubgF</i>	Subgenual frontal cortex, right
78	<i>lSubCa</i>	Subcallosal area, left

79	<i>rSubCa</i>	Subcallosal area, right
80	<i>lpreSubgF</i>	Pre-subgenual frontal cortex, left
81	<i>rpreSubgF</i>	Pre-subgenual frontal cortex, right
82	<i>lasTG</i>	Superior temporal gyrus, anterior part left
83	<i>rasTG</i>	Superior temporal gyrus, anterior part right

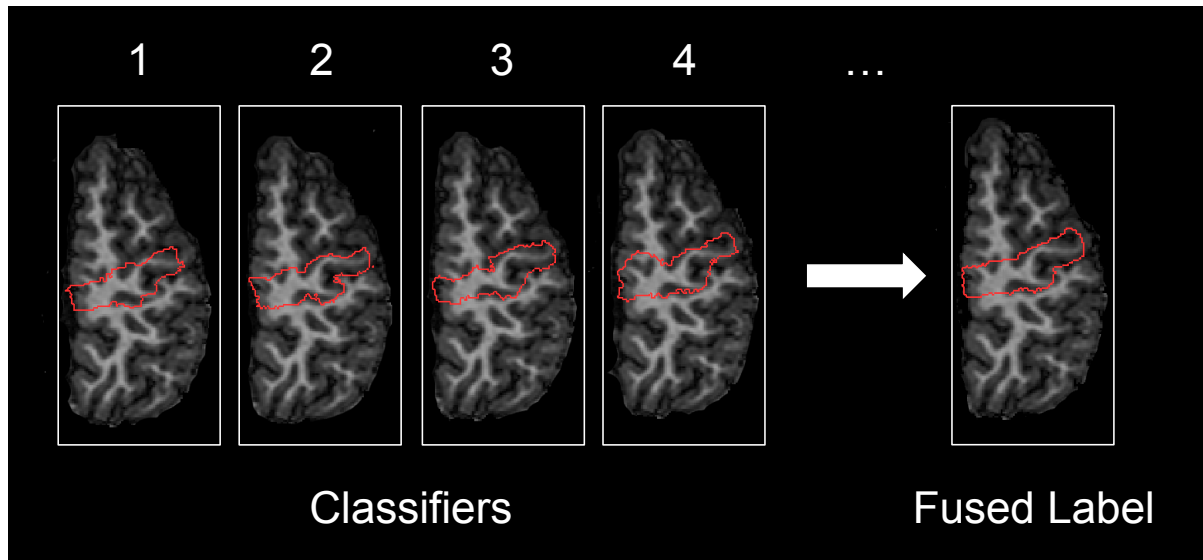


Figure 4.6: Decision label-fusion. Multiple manual-propagated labels (classifiers, left) are fused together using majority voting to form the final fused label (right) which, on average, is more accurate than each individual classifier. ROI shown here is the left precentral gyrus.

Segmentation processing

Anatomical ROIs were initially eroded at the junction between regions in order to reduce the effects of partial voluming, atlasing and/or registration errors. For each cortical ROI, the anatomical label and tissue segmentations were first compared and then merged to redefine the ROI at the WGM boundary. Although individual axons terminate at various levels in the cortical grey mater, voxel-wise resolution of anisotropy and tracking certainty reduces dramatically past the cortical WGM boundary. Therefore, in this approach cortical ROIs were retained, on average, as a one-voxel thickness ribbon at the WGM boundary and subcortical ROIs were left intact. Regions which were not felt to be constructive in delineating whole-brain structural connectivity were removed from the analysis. These included CSF spaces such as the 3rd and lateral ventricles, and the corpus callosum (in order to permit the passage of inter-hemispheric connections during tracking). The cerebellum and brainstem were also removed as they consistently showed signal artefacts across subjects in dMRI. Thus, from an original set of 83 regions, 72 cortical and subcortical ROIs were retained.

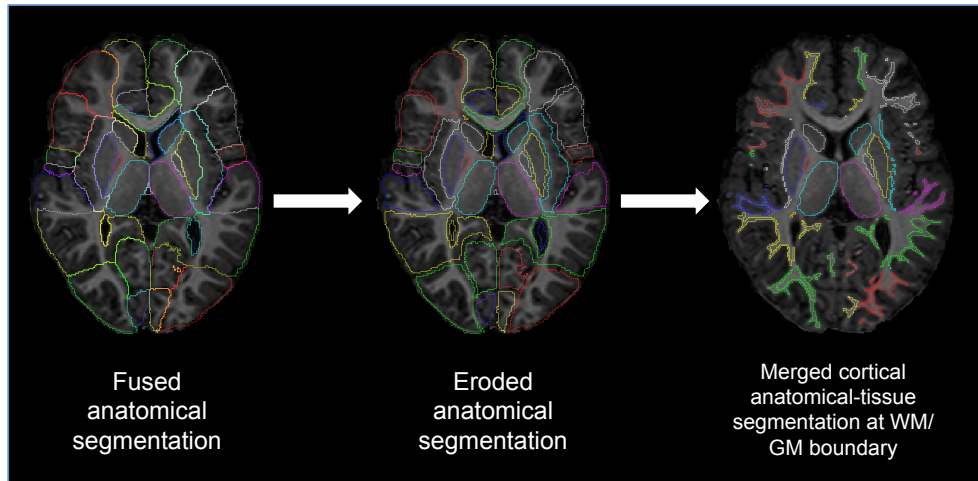


Figure 4.7: Segmentation processing. Fused anatomical segmentations (left) are first eroded by 1 voxel (centre) before merging with tissue segmentations to create cortical ROIs at the cortical white-grey matter boundary (right).

4.2.5 Image registration

ROIs were transformed from T_1 to diffusion space (b_0) via an intermediate T_1 – T_2 registration. An intermediate T_2 -weighted image was used to improve the registration as the tissue intensity profile is similar to diffusion-weighted images. Image alignment was achieved using voxel-based registration in three steps: rigid (**rreg**), affine (**areg**), and, finally, non-rigid (**nreg**) registration using the image registration toolkit (IRTk; (Rueckert et al. 1999); available via www.doc.ic.ac.uk/~dr/software/). Non-rigid registrations were performed due to geometric distortions present in the EPI-acquired b_0 images and to keep registration-based error at a minimum. All affine registrations applied default parameters. Non-rigid transformations, required for both intra-subject T_2 – b_0 registrations and inter-subject T_1 – T_1 atlas registrations, were optimised for the infant brain using parameters developed by Gousias et al. (Gousias et al. 2008; Gousias et al. 2010). These parameters are shown in Table 4.2 and are explained in Appendix A. Briefly, the non-rigid registration was refined in a multi-resolution fashion by stepwise reduction of the control point spacing from 20mm to 10mm, 5mm, and finally 2.5mm and this approach has been validated for use in both one- and two-year-old infants (Gousias et al. 2008; Gousias et al. 2010). Non-rigid registration, although precise, can lead to implausible transformations which are anatomically invalid (Crum 2004). In order to ensure plausible warping between images, while still allowing for accurate alignment of infant images, the weighting of the regularisation (λ) was set to 1×10^{-6} (Ball et al. 2012b). To improve the accuracy of the non-linear registration, all images were padded with a halo of voxels of intensity -1. All voxels in the target image with a value less than or equal to this padding value were ignored. As such, the registration was restricted to an area consisting of unpadded voxels. Affine and non-rigid registrations were mediated with parallel processing on a cluster of ~ 400 Linux PCs, controlled by Condor software (<http://www.cs.wisc.edu/condor/>).

Table 4.3: List of parameters used in non-rigid registration of infant images

Parameter	Value
Number of resolution levels	4
Number of bins	64
Padding value	-1
Similarity measure	NMI
Interpolation mode	Linear
Control point spacing (mm)	Level 1: 20 × 20 × 20 Level 2: 10 × 10 × 10 Level 3: 5 × 5 × 5 Level 4: 2.5 × 2.5 × 2.5
$\lambda_1, \lambda_2, \lambda_3$	$\lambda_1 = 0.000001, \lambda_2 = 0, \lambda_3 = 0$
Optimisation method	Gradient descent
Target blurring (mm)	Level 1: no blurring Level 2: 1 Level 3: 2 Level 4: 2
Target resolution (mm)	Level 1: no resampling Level 2: 1 × 1 × 1 Level 3: 2 × 2 × 2 Level 4: 4 × 4 × 4
Source blurring (mm)	Level 1: no blurring Level 2: 1 Level 3: 2 Level 4: 2
Source resolution (mm)	Level 1: no resampling Level 2: no resampling Level 3: no resampling Level 4: no resampling
No. of iterations	Level 1: 20 Level 2: 20 Level 3: 20 Level 4: 20
No. of steps	Level 1: 4 Level 2: 4 Level 3: 4 Level 4: 4
Length of steps	Level 1: 1 Level 2: 2 Level 3: 4 Level 4: 8

4.2.6 Whole-brain tractography

A modified form of a widely used probabilistic tracking algorithm was implemented to delineate tracts between all pairs of ROIs as described in Robinson et al. (Robinson et al. 2010).

Bayesian estimation was used to fit the parameters of a two-compartment partial volume model of diffusion, fitting up to two fibres per voxel, and confidence distributions on the principal direction of diffusion were generated based on Markov Chain Monte Carlo (MCMC) sampling of the diffusion data using FSL BedpostX (Behrens et al. 2003). 5000 streamlines were propagated from each seed ROI voxel for

a total of 2000 steps of 0.5 mm in length. Tracking was terminated if streamlines reached any other ROI, breached a critical turning threshold of 60° , or exceeded the territory of a conservative WM mask (created at the tissue segmentation step by thresholding and removing voxels with less than 50% probability of being WM and then transforming to b_0 space). Forcing the tracking algorithm to halt at the first target ensured that only direct connections were retained, and connections that passed through other ROIs to reach the target were not counted. This was necessary for a graph theory representation (Chapters 5), where a single, direct connection (edge) between any pair of ROIs (nodes) is assumed not to bypass through any other nodes.

Whilst the standard model of probabilistic tractography offers a measurement of tract probability, Robinson et al. also integrated a measure of anisotropy for each tract. In this modification, orientation distribution functions (ODFs – ψ) were calculated directly from the partial volume fraction of diffusion at the MCMC sampling stage (Melie-García et al. 2008; Robinson et al. 2010) and could be used to estimate diffusive exchange along the tract. The value of the ODF in any particular direction summarises the proportion of volume fraction of diffusion found in that direction ($f_l^{(\theta,\phi)}$).

$$\psi(\theta, \phi) = \frac{1}{N_s} \sum_{l=1}^{N_s} f_l^{(\theta,\phi)}$$

[4.1]

where N_s is the total number of MCMC samples, $f_l^{(\theta,\phi)}$ is the volume fraction of diffusion for iteration l , in direction (θ, ϕ) . The diffusive exchange between adjacent voxels, E , was taken by calculating the proportion of the ODF contained within a solid angle (β) surrounding a unit vector joining one voxel ($\vec{r}_i$) to the centres of neighbouring voxels ($\vec{r}_j$):

$$E^j(\vec{r}_i, \Delta\vec{r}_{ij}) = \frac{1}{N_s} \sum_{l=1, (\theta,\phi) \in \beta}^{N_s} f_l^{(\theta,\phi)}$$

[4.2]

There are 26 neighbouring voxels and therefore there are 26 components of the diffusive exchange per voxel. The total exchange overall all of these components is normalised to 1. Estimates of anisotropy ($a_{i \rightarrow j}$) between voxels was calculated by averaging the exchanged proportions in both directions:

$$a_{i \rightarrow j} = \frac{E^j(\vec{r}_i, \Delta \vec{r}_{ij}) + E^{-j}(\vec{r}_i, \Delta \vec{r}_{ij})}{2}$$

[4.3]

The mean anisotropy along connective tracts was then calculated as a weighted average of anisotropy ($a_{i \rightarrow j}$) for all pairs of adjacent voxels sampled during tractography. Weighting was derived from the number of times each voxel was sampled during tracking (Robinson et al. 2010). This differs from fractional anisotropy, which is most commonly reported in the literature. The anisotropy measure used in this thesis (henceforward known as *mean anisotropy*) ranges from 0 to 1, with 0 representing no connection between ROIs and 1 representing a connection with maximal coherence and certainty.

Important microstructural differences between WM fibre types have been detected using existing connectivity metrics in both the developing and adult brain. At this point in maturation, commissural bundles (that interconnect homologous cortical regions between hemispheres) are compact and have relatively high axonal integrity and FA values. In contrast, association fibres (connecting different cortical regions in the same hemisphere) lack the same degree of integrity and are associated with low FA values (Partridge et al. 2005; Geng et al. 2012). In Figure 4.8 we show that mean anisotropy, calculated in this approach, is also sensitive to microstructural differences between fibre types. Three connections, classically representing each type of tract, were compared.

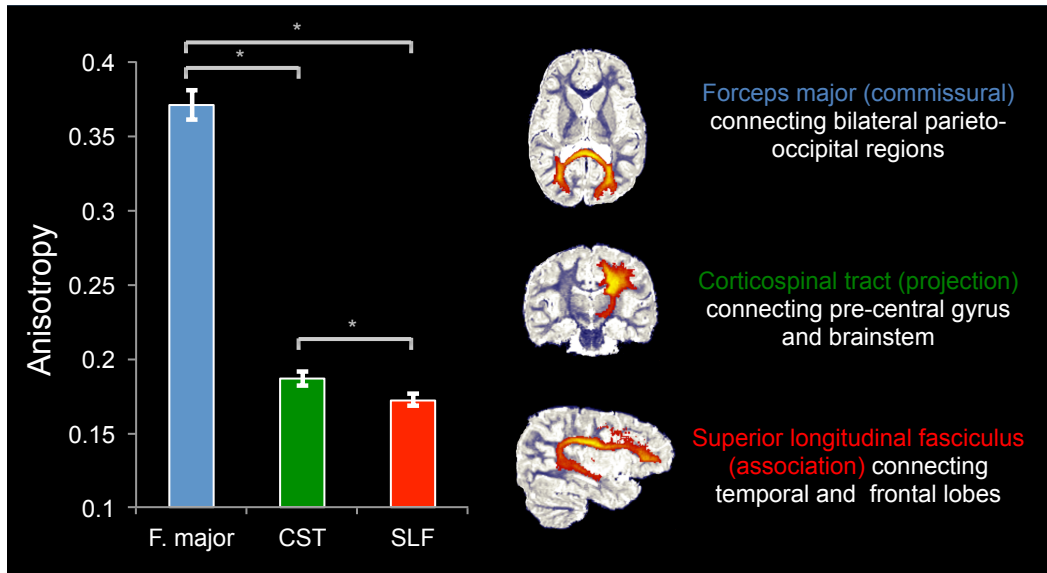


Figure 4.8: Mean anisotropy differences between fibre types in the developing preterm brain. In a group of preterm children ($n = 59$), 3 probabilistic WM connections representing commissural (F. major), projection (CST), and association (SLF) fibres were compared. F.major had the highest anisotropy, which was significantly higher than CST and SLF, while anisotropy of the CST was significantly higher than the SLF ($*p < 0.0001$). Images on the right show the probabilistic tracking of each connection. F. major, seed = left occipital lobe; target = right occipital lobe; CST, seed = putamen, target = pre-central gyrus; SLF, seed = insula, target = middle frontal gyrus.

4.2.7 Macroconnectome processing

A connectivity matrix was generated for each subject where each element within the matrix corresponded to the mean anisotropy of a particular 'tract'. Initial data reduction was achieved by removing self-connections along the diagonal and anatomically impossible connections (such as connections seeding or terminating from the CSF). As it is not possible to determine the direction of the tract, symmetry was enforced to ensure each pair of tracts connecting two regions were the same, at the largest value of the pair. A minimal anisotropy threshold of 0.01 was applied to remove erroneous and false positive connections. Since the dynamic range of this anisotropy measure is not known, nor is the exact relationship with cerebral WM microstructure well defined, matrix thresholding was kept to a minimum. If a high threshold was set, it is possible that important connections with low strength but high certainty might be discarded.

4.3 Discussion

4.3.1 Critical discussion of the framework

This chapter has proposed a framework for modelling the structural macroconnectome in the developing infant brain from clinically acquired diffusion data. The quality of this data ultimately depends upon the quality of the framework steps taken to produce it. A brief critical appraisal of each step is offered in this section.

dMRI acquisition scheme

The acquisition scheme used in this framework was felt to be clinically feasible. 32-direction dMRI data was obtained with a low b-value of 750s/mm^2 in less than 7 minutes. Although a high diffusion gradient imaging acquisition scheme such as DSI would allow more optimal reconstruction of the data, it lacks clinical feasibility and was therefore not used in this framework. With this scanning sequence, signal artefacts were present in multiple gradient directions in the cerebellum and brainstem. These were possibly caused by pulsation artefacts in the region, and consequently resulted in the removal of cerebellar and brainstem ROIs from whole-brain tractography and further analyses.

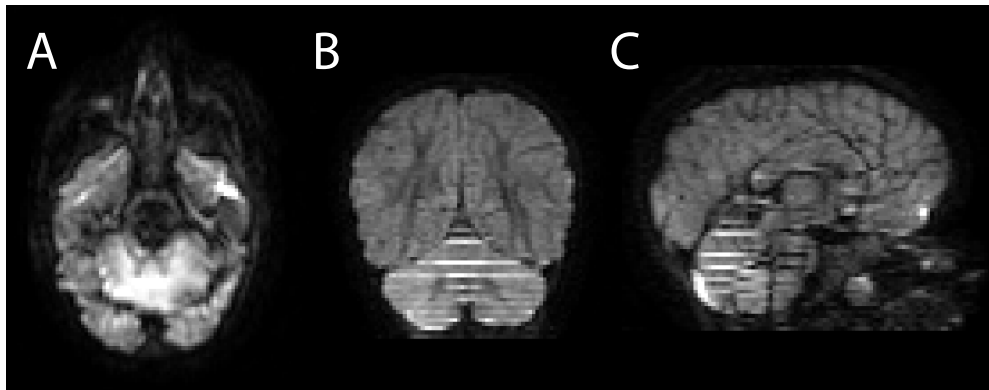


Figure 4.9: Pulsation artefacts in the cerebellum and brainstem. Artefacts appear as high signal intensity in the cerebellum in axial view (A) and as slices penetrating the cerebellum and brainstem in coronal (B) and sagittal views (C).

Parcellation scheme

When performing tissue segmentation in children, age-matched priors are well suited because they have correspondence in size, shape, and tissue intensity to subject brains. Whilst this framework was optimised for the 2-year-old brain, it is not fully validated in 1-year-olds. Significant differences in the level of WM development are apparent between 1 and 2 years (Knickmeyer et al. 2008; Geng et al. 2012). In this thesis, 1-year-old preterm infants formed a significant part of the study cohort (approximately one third). If minor but consistent inaccuracies were present in the location of cortical ROIs or in the extent of the WM tractography mask in these subjects, then a systematic bias might result. In addition, alternative tissue segmentation approaches were not explored. Implementations such as FSL FAST or FreeSurfer may have offered a more accurate tissue demarcation in the developing brain. Nonetheless, SPM has shown evidence of outperforming other implementations in adults (On Tsang 2008), has been previously used in young children (Maria Murgasova 2006), and in the framework from which this work was developed (Robinson et al. 2010). Tissue-segmented images obtained by the presented framework also appeared to be anatomically accurate upon visual inspection (Figure 4.3), although this was not quantitatively assessed.

An atlas-based parcellation was selected for this framework to ensure that connections could be identified accurately and efficiently, and easily compared with existing data. Manually defining anatomical segmentations in each subject's image would have improved the parcellation as it would have given an unmatched level of spatial correctness; however, this method is expert-dependent, observer-demanding, and time-consuming, and was thus felt inappropriate for this thesis. Whilst the presented framework does not attain the accuracy of individual manual segmentations, the use of fused labels from multiple templates is significantly better than the propagation of labels from a single template (Heckemann et al. 2006). An important limitation in this approach is that comparability with

connectome studies using different atlases is limited as differences in size and region boundaries in the anatomical template significantly affect further analyses (Bassett et al. 2011) .

The location of the seeding voxels also impacts upon whole-brain tractography (Li et al. 2011). If, for example, probabilistic tractography is initiated from the grey matter, it can suffer distance-related artefacts due to the progressive dispersion of uncertainty along the tracking path and result in preferential weighting of short-distance connections in the connectome (Li et al. 2011). This framework attempted to avoid this issue by defining regions at the cortical WGM interface, thereby limiting both the total connection distance and tracking through low anisotropy or high uncertainty cortical grey matter. The use of a single-voxel thick, cortical ROI significantly reduced the number of seeding voxels for tractography. As tracking through cortical GM was unlikely to improve the estimation of the tract, having a sparse ROI maintained a high representation of the region at a lower computational cost.

Diffusion tractography and measure of connectivity

The general advantages of using a probabilistic tractography algorithm have been discussed previously (Chapter 2). In the developing infant brain, the cerebral white matter is underdeveloped and has a lower diffusion anisotropy as compared to adults. Furthermore, a preterm population with known neurological and behavioural deficits is likely to have significant variability in white matter connectivity. Therefore, a probabilistic algorithm which can track through areas of low anisotropy and can also gauge the confidence in the tracking process is particularly suited to these infants. FSL ProbtrackX (the specific algorithm this method is inherently based upon) has previously been used in macroconnectome studies (Gong et al. 2009; Owen et al. 2013) and numerous studies of preterm neonates and infants (Counsell et al. 2008; Bassi et al. 2008; Aeby et al. 2009).

Mean anisotropy approximates the diffusive exchange between voxels and is more closely related to 'connectivity' as compared to a scalar diffusion measure such as FA, which only offers a pointwise estimate of anisotropy diffusion in the tract and not along it. FA is also inappropriate for use here as it is based on a tensor model of the diffusion data which this framework does not adopt, and does not account for the certainty or coherence of a tract in its measurement. For these same reasons, the comparability of mean anisotropy with studies which use FA is unclear and the dynamic range of the measure is undetermined (a particular level of mean anisotropy does not necessarily imply the same degree of FA). In spite of these differences, mean anisotropy can still detect predictable connectivity variations between fibre types in the developing preterm brain (Figure 4.8) and has been used reliably in both young and old-age adults (Robinson et al. 2010) and preterm neonates (Ball, et al. 2012a). Alternative connectivity metrics, such as streamline count or volume, were not evaluated for use in this framework.

4.3.2 Approaches to macroconnectome analysis

A number of mathematical techniques are available for the analysis of connectomic data (Figure 4.10). These range from system-level models to those which interpret the structural macroconnectome as a ‘bag of connections’ (Craddock et al. 2013). *Graph* theory is used in many of these techniques and describes the macroconnectome as a network (Bullmore & Sporns 2009). Here, cortical and subcortical ROIs are defined as *nodes*, and *edges* are generally represented by tractographic connections. Normal structural brain networks have an efficient, small-world architecture and this can be disturbed by a variety of disease states in which disruption of the brain connective microstructure is present (Guye et al. 2010)

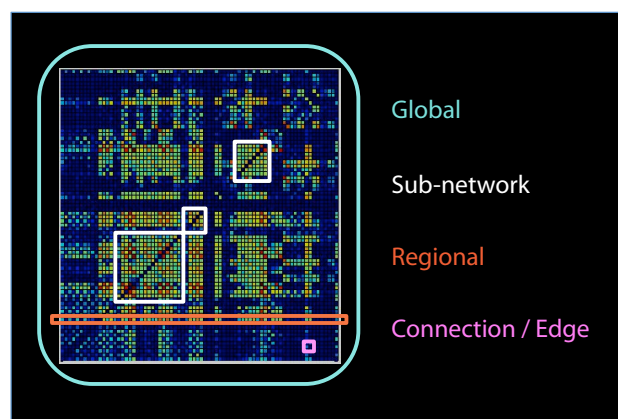


Figure 4.10: Spatial levels of macroconnectome analysis. Central square shows a symmetrical connectivity matrix (dark blue) with rows and columns corresponding to ROIs and matrix elements and colour corresponding to individual connections and their values respectively. Coloured outline specify the level of macroconnectome analysis. Connectivity can be studied at a global level (turquoise), within a sub-network (white), at regional or nodal level (orange), or as a set of connections (pink).

Global measures attempt to summarise the entire macroconnectome by using a single parameter for each subject’s data matrix. In network approaches, global measures describe various topological aspects of the macroconnectome, such as the total number of connections, efficiency and small-worldness (Appendix B). Regional metrics attempt to calculate the centrality of a node within the network, by assessing the number and strength of its connections (degree and weight) or its role in connecting other pairs of nodes (betweenness) (Craddock et al. 2013). Alternatively, by choosing regions based on prior knowledge, subnetworks can be investigated within the macroconnectome, using an approximate summary statistic that has contextual interpretation (Meskaldji et al. 2011). Finally, the simplest and most direct method of analysis involves modelling the macroconnectome as a high-dimensional set of variables which can be parsed through a machine learning algorithm in order to classify subjects into groups, or identify biologically relevant connections that are associated with an outcome of interest. Chapter 5 adopts this approach and uses penalised regression and stability selection

to explore a sparsified preterm macroconnectome and detect white matter connections associated with development or degree of prematurity.

4.3.3 Key contributions

The key contributions of this chapter have been: (1) the construction of a data-driven, semi-automated framework to map the developing macroconnectome using dMRI that is feasible in clinical scanning conditions, and (2) the adaptation of this framework for the infant and early childhood brain.

Chapter 5

The Influence Of Development And Prematurity On The Sparse Infant Macroconnectome

5.1 Introduction^o

5.1.1 Chapter aim

Having methodically outlined a framework for generating age-appropriate macroconnectome data in Chapter 4, this chapter seeks to investigate factors suspected to influence whole-brain structural connectivity in a group of 49 preterm children ranging in prematurity between 25 and 35 weeks post-conceptual age with scans being obtained between 11 and 31 months (see Materials and methods). Thus, the two significant biological factors thought to affect this group are the level of brain development and the degree of prematurity. By characterising the connectome as a high-dimensional set of predictors (connections), the aim of this chapter is to find a sparse set of tractographic connections which are most associated with each factor. A parsimonious solution is sought in order to obtain a predictive model that is interpretable within a clinical context and enable a search for focal points for intervention.

In order to solve this feature selection problem, a novel method combining sparse penalised regression (Tibshirani 1996) and stability selection (Meinshausen & Bühlmann 2010), has been employed. The first part of this introduction offers the background and rationale for choosing this method over others, while the second part briefly discusses the relevance of each biological factor.

5.1.2 Feature selection methods

By treating each structural connection from the macroconnectome as a predictor variable, one could apply statistical regression tools in order to find a set of features which best predict outcome. However, standard linear regression tools such as ordinary least squares (OLS) regression are not appropriate for solving this problem. In the first instance, the number of potential predictor variables (P) is far greater than the number of observations or subjects (n). This compromises the assumption that $n \geq P$. In the second, the interaction between predictors variables is unknown, and in structural connectome data it is likely that there is significant co-linearity between them. Finally, since a sparse solution is sought, an OLS regression approach which accepts *all* the predictors into the model is not suitable.

An alternative approach would be to first implement a dimension reduction technique such as principal components analysis. Here, 'components' are selected which explain most of the variance, and then a regression model is fitted on the response of these components. While this may seem acceptable, it is unsuitable for this type of neuroimaging data for two reasons: first, the initial components are selected without regard for the dependent variable and may not necessarily be predictive, and second, the derived principal components are not easily interpretable since they are linear combinations of all the original variables (Bunea et al. 2011). Stepwise selection strategies such as forward or backward selection are another common approach by which to perform predictive modelling (Bystron et al. 2008; Efroymson 1960). Forward selection starts with no predictor variables, and in each step it tests whether adding one variable into the model leads to an improvement with respect to a pre-specified criterion, such as the Akaike Information Criterion and Mallows's Cp. If no variable can be added that improves the model, the process stops. Otherwise, the variable yielding the largest improvement is included into the model and the process continues. Backward selection occurs through a similar process, except that it starts with all variables, and in each step tests whether dropping a variable leads to an improvement. Stepwise selections are limited in that they optimise the selection criterion only in the final step, with no guarantee that the selected model is the overall best possible candidate model (Roecker 1991). Highly variable results may be also obtained with different selection criteria (Bendel 1977). All-subset regression computes all possible models, but is considered unfeasible here due to its computational complexity (in the present study, more than 10^{70} regression models would have been generated). An alternative and more traditional approach to univariate variable selection would consist of performing hypothesis testing, e.g. a t-test on each regression coefficient. However, this approach would operate under the assumptions that all of the covariates are entirely independent, which is both statistically restrictive and biologically unrealistic. Corrections would also be required to control either the family-wise error rate or false discovery rate due to the massive multiple testing problem (Benjamini 2010).

Penalised least square (PLS) regression models are efficient feature selection methods which are able to overcome many of the aforementioned difficulties, and which have recently found application in multivariate neuroimaging data (Carroll et al. 2009; Vounou et al. 2010; Bunea et al. 2011). Lasso^{***} regression (Tibshirani 1996) is one such PLS model, capable of handling data in which $P > N$ and sparse solutions can be generated. Lasso regression attempts to minimise the residual sum of squares plus a penalty term (see Materials and methods). To determine the appropriate level of regularisation, a recently proposed stability-selection procedure was used which is based on data sub-sampling with repeated regression (Meinshausen & Bühlmann 2010). This approach enables an easily interpretable ranking of

^{***} least absolute shrinkage and selection operator

variables (connections) in decreasing order of predictive importance, whilst taking into account small sample variability.

5.1.3 Development and prematurity

Development

Brain development in the 2nd and 3rd postnatal year represents a highly dynamic period encompassing major structural and functional changes. Functionally, this period is characterised by the increasing use of language, self-awareness, and capacity for inference (Herschkowitz 2000). Structurally, this period is characterised by a number of processes, including axonal growth, myelination, and synaptogenesis, that lay out and strengthen the connective framework required for efficient impulse propagation and neurological function (Chapter 3.1.2). White matter development occurs in an established spatio-temporal sequence that reaches adult patterning by the 2nd postnatal year (Sampaio & Truwit 2001) and roughly corresponds to the developmental range of study in this work. White matter in the frontal and temporal cortices and associated areas are typically last to mature. Myelination and synaptic modelling are also protracted in these areas, and are detected up to the 2nd or 3rd decade of life (Chapter 3.1.2).

Prematurity

A large body of histological and neuroimaging evidence clearly demonstrates that premature birth has detrimental effects on white matter connectivity and that these effects are persistent up to adult age (see Chapter 3.3). However, a consensus pattern of white matter microstructural damage remains poorly defined, and it is unclear how initial white matter injury evolves with increasing age. Diffusion MR imaging in the assessment of preterm birth has identified specific areas consistent with WM damage, particularly in the internal capsule and corpus callosum (Hasegawa et al. 2011; Jo et al. 2012). Others studies, particularly those at adolescent or adult age, suggest that damage is widespread (Constable et al. 2008; Eikenes et al. 2011). This variation in results may perhaps be due to methodological and group differences. This experimental chapter is suitably arranged so that the extent of preterm white matter damage in childhood age can be effectively explored. The use of a structural macroconnectome approach permits unrestricted exploration of the extent of WM pathology without the need for *a priori* hypotheses of anticipated WM change. The feature selection method described in this chapter attributes a selection probability to connections that are predictive or prematurity, thereby giving an estimation of certainty in the result.

5.1.4 Hypothesis

In this chapter, the dMRI macroconnectome of preterm children in their 1st and 3rd year after birth will be interrogated with a novel feature selection approach in order to find a sparse set of connections predictive of degree of development or prematurity. Rapid development of fronto-temporal brain structures is anticipated in this age range, therefore it is hypothesised that the strength of connections linking these regions would positively predict age at scan. As development is well-studied, this experiment also represents a test of feasibility in the novel feature selection method described.

The influence of increasing prematurity is then estimated by determining whether younger post-conceptual age at birth is related to reduced connectivity strength and ascertaining which white matter connections most consistently express this relationship.

5.2 Materials and methods^o

Ethics

The Hammersmith and Queen Charlotte's and Chelsea Hospitals Research Ethics Committee approved this work, and written parental consent was obtained prior to scanning (Approval 07/H0707/101).

Data acquisition and processing

T₁- and T₂-weighted and diffusion MRI data were acquired and processed according to the framework described in Chapter 4. Macroconnectome data were generated for each subject following the protocols in Chapter 4, and are summarised in Figure 4.1. A single but important difference in the framework used in this chapter was that adult priors were employed in the tissue segmentation step, rather than the 2-year-old priors used previously (see Discussion).

Statistical analysis and feature stability selection

Each subject matrix was converted into a vector and then vertically concatenated to create the group-wise data matrix. Connections that were traced consistently across the group were retained, producing a new, robust $n \times p$ data matrix, where n was the number of subjects and p was the number of retained connections. Each row in the matrix represents the set of retained connection strengths for a specific individual. The matrix was used in two analyses: in the first, corrected age at scan was the response variable and post-conceptual age at birth was treated as a confounder variable; in the second, post-conceptual age at birth was the response and age at scan was the confounder. In the first part of

each analysis, confounders were removed using an ordinary least squares (OLS) regression model. This was done for each connection variable across the group, with the confounder treated as the response variable, and is described in Equation 5.1.

$$X_j = Z\alpha_j + c_j + \varepsilon_j \quad [5.1]$$

Here, the X vector represents a connection variable, across the group and X_j refers to the j -th column of X . Z is the confounder, which, in this OLS step, is the response variable and α_j is its coefficient. c_j is the intercept term and ε is the error term, representing the residuals in the model, $\tilde{X}_j$, as shown in Equation 5.2:

$$\tilde{X}_j = X_j - (Z\hat{\alpha}_j + \hat{c}_j) \quad [5.2]$$

where $\hat{\alpha}_j$ and $\hat{c}_j$ are the estimates of α_j and c_j . $\tilde{X}_j$ was then implemented as the 'new' input variable for the Lasso regression, representing the second part of the analysis [Equation 5.3]:

$$\{\frac{1}{2}\|Y - \tilde{X}\beta\|^2 + \lambda\|\beta\|_1\} \quad [5.3]$$

Here $\lambda \in \mathbb{R}^+$ is the penalty term, and Lasso regression operates by minimising a penalised sum of squares subject to the penalty imposed on the L_1 norm of the regression coefficients. λ controls the degree of sparsity in the solution: when λ is equal to zero, no sparsity is imposed. In this case, the regression estimates are equivalent to those obtained by OLS regression. As λ increases, fewer non-zero covariates are included in the model, until it reaches a maximum value such that no variable is selected. The OLS estimate often has low bias yet large variability, and the Lasso estimate sacrifices lower bias to decrease the variance. The means by which certain variables are shrunk, exactly to zero, are explained in Figure 5.1.

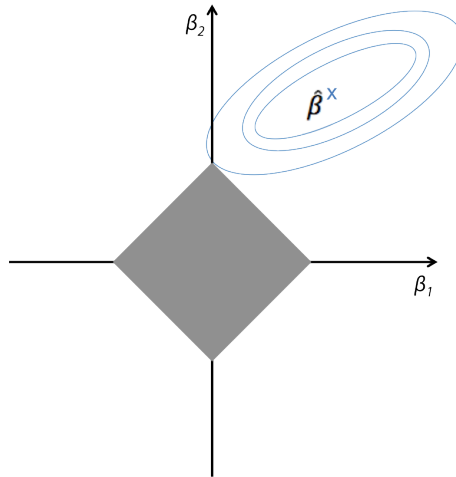


Figure 5.1: Lasso coefficient shrinkage. When $p = 2$, the sum of square errors loss can be plotted as a quadratic function shown as the symmetrical, elliptical contours, of which the centre $\hat{\beta}$ represents the OLS estimate; and on each contour, the squared loss obtains the same value. The rotated square represents the constrained region. Here, $\lambda \|\beta\|_1 \equiv \|\beta\|_1 \leq t$, where t is a tuning parameter which defines the level of λ . When $p = 2$, $|\beta_1| + |\beta_2| \leq t$, and the size of t determines the size of the square. The Lasso solution is equivalent to the first place that the contours touch the square. If they contact at a singularity point (a corner of the square), this corresponds to a zero coefficient, and the variable is eliminated from the solution.

In the third part of the analysis, the appropriate level of regularisation was determined using the stability-selection procedure, which is based on data sub-sampling with repeated regression and examines a range of regularisation parameter values (Meinshausen & Bühlmann 2010). Briefly, stability selection performs repeated random sub-sampling from the data and fits a Lasso regression model on each random sub-sample. To be more specific, $B = 1,000$ random sub-samples were extracted from the n subjects, without replacement, and with a fixed size of $m = 2n/3$ subjects. For each sub-sample, and for a fixed λ value, a Lasso regression model was fitted and the regression coefficients were estimated. For each predictor variable, a probability of selection was calculated by taking the ratio between the number of times that the variable was selected and the total B model fits. Despite the variability introduced by the sub-sampling procedure, important predictors should be selected more frequently, and therefore their selection probability provides a measure of predictive importance. This procedure was repeated for different values of the regularisation parameter on a grid of points from a maximum value of λ , for which about 10% of all covariates were selected, down to a minimum value of λ , for which about 30% of all covariates were selected.

Two important points concerning the definition of this range are raised here. First, these values were selected under the assumption that only a relatively small number of connections are truly important in predicting the outcome. This is in accordance with previous methodological work, which gives guidance on the choice of maximum and minimum λ for the purposes of error control (Meinshausen & Bühlmann 2010; Yen 2010). Very low values for the λ minimum may lead to an increase in the entry of noise variables in the model. Second, the initial minimal and maximal values are

approximate. It has been found that the choice of the initial set of regularisation parameters typically does not have a strong influence on results if the range is varied within reason (Meinshausen & Bühlmann 2010) (Figure 5.2).

In the fourth part, the final estimate of the selection probability for each predictor variable was derived by taking the maximum probability obtained across the range of λ values described above. A list of selection probabilities for the predictor variables was produced, representing the importance of each in predicting the response. In order to provide information regarding the direction of association between predictor variables and the response variable, an average coefficient was obtained by repeated univariate linear regression on all the sub-samples computed for each connection.

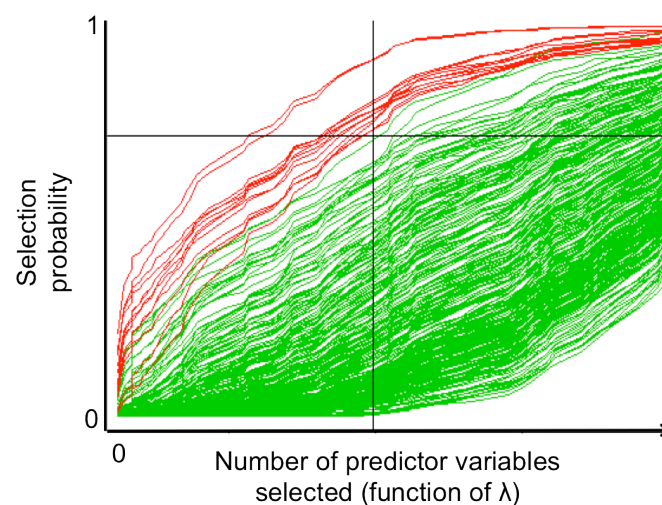


Figure 5.2: Stability selection paths over a range of λ values following Lasso regression with repeated subsampling. Over the λ value range, important variables (red) are selected consistently. The graph illustrates the ability of stability selection to separate relevant variables and irrelevant (green) variables – even when the noise level is unknown. Cross hairs indicate the lambda value at which relevant variables are best separated from irrelevant variables. Stability selection graphs were used in this method to confirm that the range of λ was appropriate.

Sparse macroconnectome analysis

As a preliminary, the unweighted, sparse consensus macroconnectome of the coherent connections was described and visualised using network analysis. As this connectome was heavily sparsified (<10% of total possible connections were retained) a bias may have been introduced into the results. For example, it is possible that connections in the sparse macroconnectome may have had limited anatomical coverage. To exclude the possibility of this bias, coherent connections were assessed topologically (Figure 5.3) and anatomically (Figure 5.5A). In addition, graph theoretical metrics were used to estimate measures for typical global network characteristics, including small-world architecture (see Appendix B).

To examine the effects of further sparsification on the network, the weighted connectome was thresholded at different levels of selection probability, up to a maximum sparsity at which ~10% of the original connections existed. The proportion of connections of different types were investigated, including inter-hemispheric, intralobar, and (intra-hemispheric) interlobar connections (Figure 5.4). This was carried out in order to see if the network structure was robust and if differences between development and premature-associated macroconnectome were maintained after further sparsification.

Visualisation

In order to emphasise different aspects of the results, several types of visualisation were employed. These visualisation methods and the techniques used to produce them are outlined below:

1. The network representation of the sparse connectome (Figure 5.3) was visualised with Cytoscape (Cline et al. 2007).
2. Connectograms (Figures 5.4A–C) were constructed using the Circos visualisation tool (Krzywinski et al. 2009). This tool is capable of displaying the topological variation between whole-brain structural networks and was used here to compare the macroconnectome weighted by connection selection probability with either development (age at scan) or prematurity (post-conceptual age at birth) as the outcome.
3. A neuroanatomical representation of the effects of development and prematurity on white matter was created (Figure 5.5) using a sequence of steps with various FSL tools (Smith et al. 2004). Every connection was binarised in individual subject space and then transformed to a common T_2 -weighted space. To achieve this warping, two sets of affine and non-linear registrations were used (Appendix A). The first set of registrations transposed connections from individual b_0 to individual T_2 space, and the second from individual to a common T_2 space. Each connection was then iteratively merged across the group, thresholded, and binarised to define the approximate common spatial extent of the connection (now referred to as the 'group connection'). Group connections were subsequently weighted by their selection probability, with respect to either response variable. The new set of weighted group connections were thresholded at 0.1 (selection probability) as this was found to be the optimal threshold to elicit voxel-wise white matter differences between 'development' and 'prematurity'. A second round of merging was then performed on the thresholded, group connections. This generated a voxel-wise selection-probability map¹⁰ in which voxels with higher intensity had a greater probability of being associated with the response variable.
4. Finally, a minimal set of connections were defined that were most stable with respect to each response variable. To this end, the ten strongest connections above a 0.5 selection probability threshold were visualised (i.e. those connections which were selected across regression model more than 50% of the time). Ten is an arbitrary number and only represents a small portion of the total connections studied; however, in order to gain an anatomical perspective of the most salient predictor variables associated with development or prematurity, this number was felt to be

¹⁰ Voxels themselves were not used as predictor variables, therefore this is not a *true* voxel-wise probability map. A voxel with high intensity actually signifies that the *connections running through that voxel* were highly associated with the response.

adequate. If ten connections were not available, the maximum number equal to or above the threshold were obtained. Population density maps were generated for each of these connections, using a sequence of registration steps to a common T₂-weighted space, similar to point 3. Subset connections were visualised as population density maps using MRICron (Rorden et al. 2007) and FSLView (Smith et al. 2004). Putative WM territories and, where possible, specific WM tracts directly connecting pairwise ROIs were suggested. Neuroanatomical information from the atlases of Wakana et al. (Wakana et al. 2004), Mori et al. (Mori et al. 2005), and Catani et al. (Catani et al. 2012) were used for labelling specific WM tracts and fibre bundles.

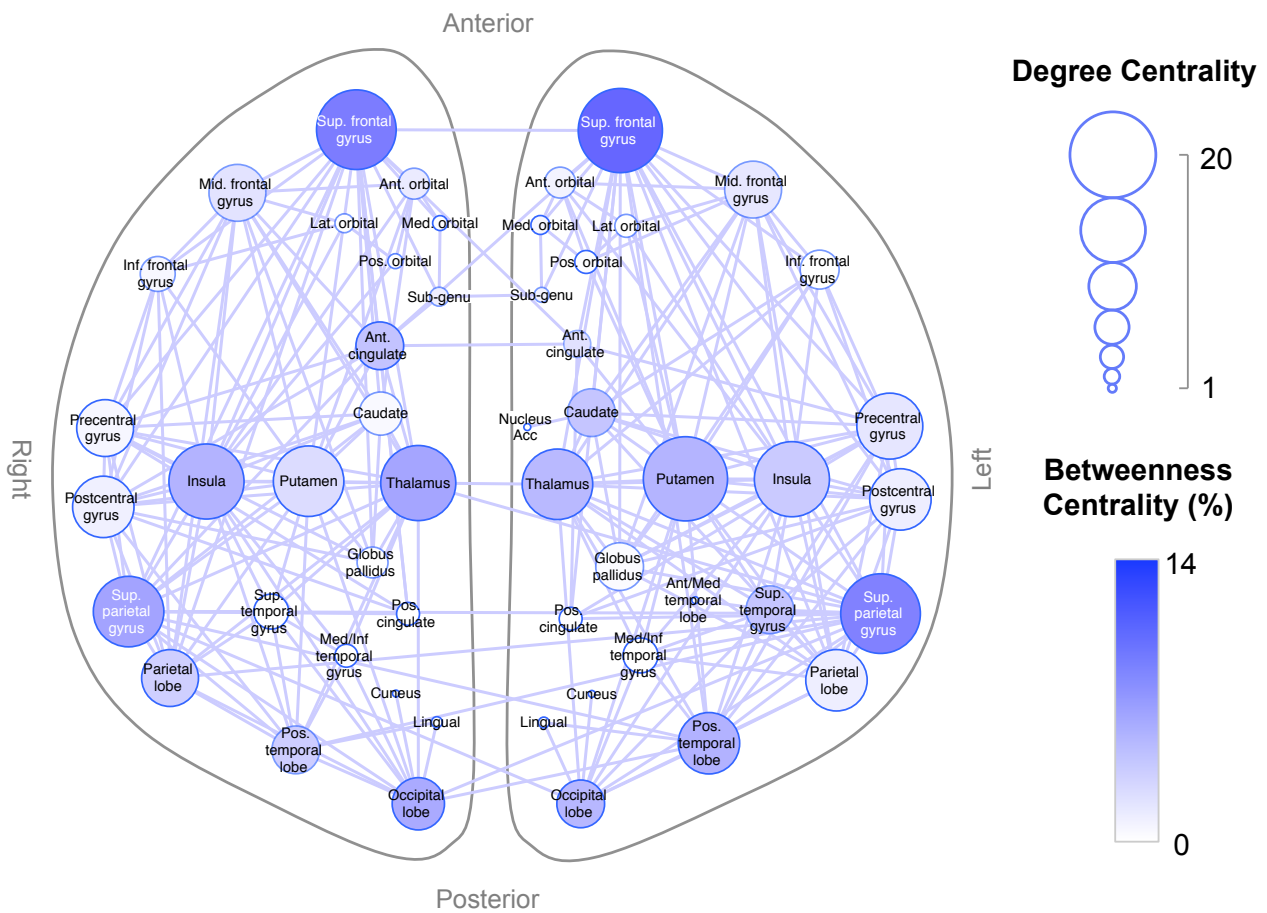


Figure 5.3: Consensus macroconnectome of preterm-born children. Figure illustrates the group-wise data matrix in network format. Regions of interest are displayed as circles where the size of the circle corresponds to the degree and shading corresponds to the local betweenness centrality. Blue lines represent robust tractographic connections between regions and are shown here unweighted. Sup = superior, Inf = inferior, Pos = posterior, Lat = lateral, Med = medial, Ant = anterior.

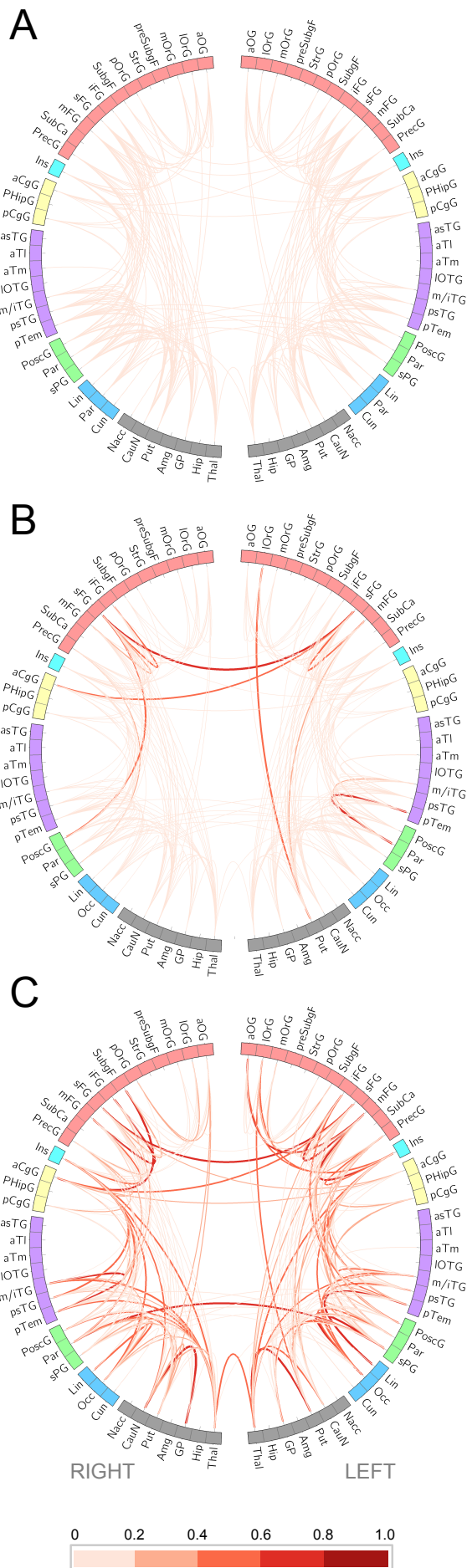


Figure 5.4: Connectogram rendering of group structural connectivity. Connections are unweighted (A) or weighted by their probability of selection with respect to corrected age at scan (B) or post-conceptual age at birth (C). Increased thickness and colouration of lines (legend) indicates higher selection probability. Regions of interest (ROIs) are divided in blocks according to lobe (red = frontal, cyan = insula, yellow = limbic, purple = temporal, green = parietal, blue = occipital, grey = subcortical structures) and shown in relative anatomical location (anterior and posterior correspond to the top and bottom of each connectogram). A full list of abbreviations for individual ROIs is provided in Table 4.1.

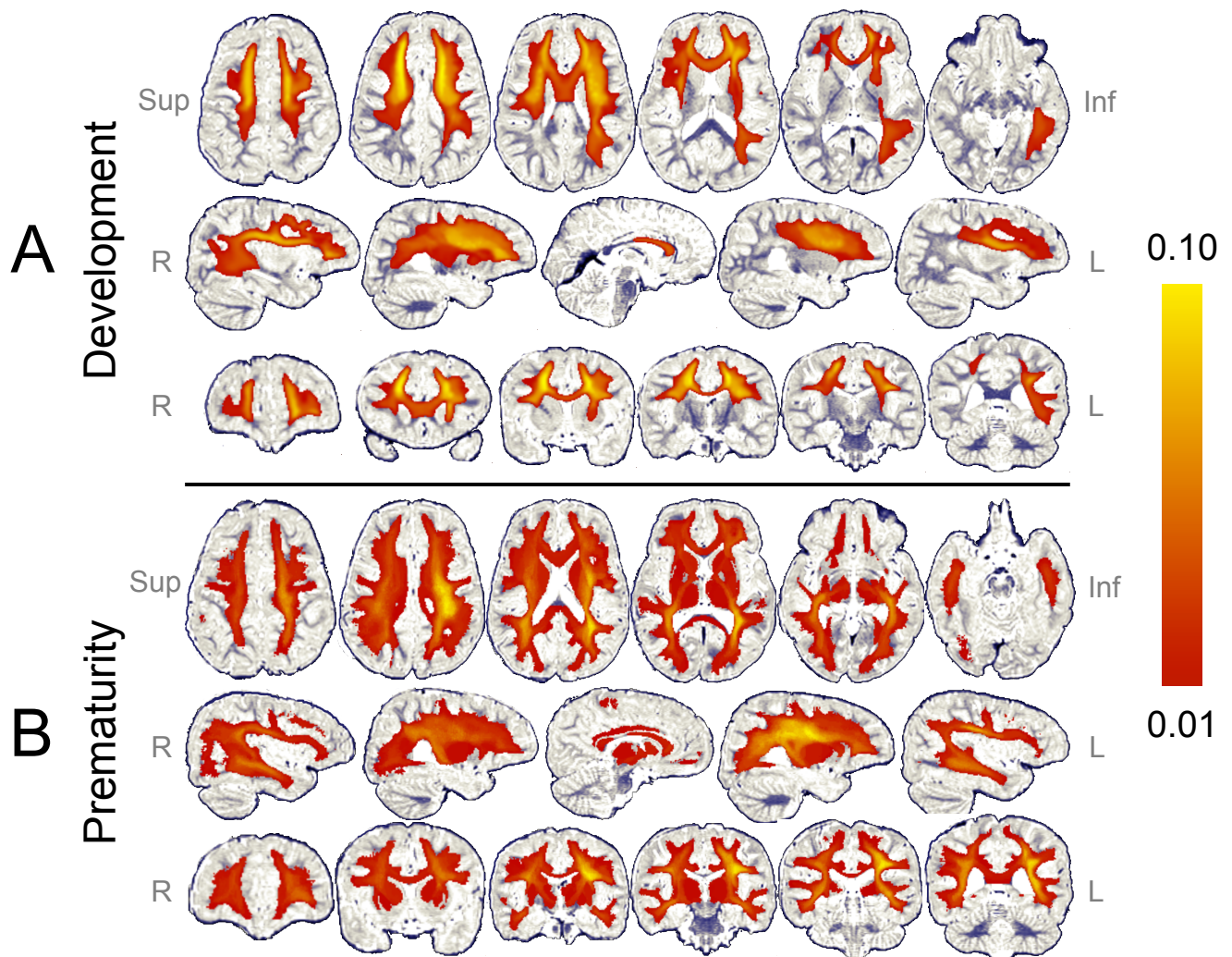


Figure 5.5: Anatomical white matter selection probability map. Merged connectivity map showing white matter regions through which run tracts associated with age at scan (A) and post-conceptual age at birth (B). The colour bar indicates level of voxel-wise selection probability: white matter regions with the highest intensity have the highest probability of selection following regression. In (A), these include anterior aspects of the centrum semiovale, corona radiata, and corpus callosum and involve the medial and lateral frontal lobes on both sides. In (B), these regions also include posterior and superior aspects of the corona radiata and sagittal striatum and particularly involve the parietal and occipital lobes bilaterally.

Table 5.1: Selected connections associated with age-at-scan. Selection probability is calculated by estimating the frequency of a connection being selected across a range of regularisation parameters and iterations of the regression. Mean anisotropy represents the average value of integrated anisotropy for a particular tract across the group (see Materials and methods for further information).

Connection	Region 1	Region 2	Selection Probability	Average Regression Coefficient	Mean Anisotropy [range]
A	Superior frontal gyrus, left	Superior frontal gyrus, right	0.908	1.443	0.2837 [0.2135–0.3346]
B	Posterior temporal lobe, right	Parietal lobe, left	0.851	1.113	0.2096 [0.1462–0.2761]
C	Putamen, left	Lateral orbital gyrus, left	0.749	1.152	0.1805 [0.1331–0.2273]
D	Frontal lobe, left, becomes middle frontal gyrus	Superior frontal gyrus, left	0.748	1.036	0.1557 [0.134–0.2155]
E	Superior frontal gyrus, right	Post-central gyrus, right	0.684	0.932	0.1761 [0.1483–0.2152]
F	Cingulate gyrus, anterior part, right	Superior frontal gyrus, left	0.660	0.884	0.2757 [0.199–0.344]
G	Inferior frontal gyrus, right	Superior frontal gyrus, right	0.546	0.814	0.1759 [0.1391–0.215]

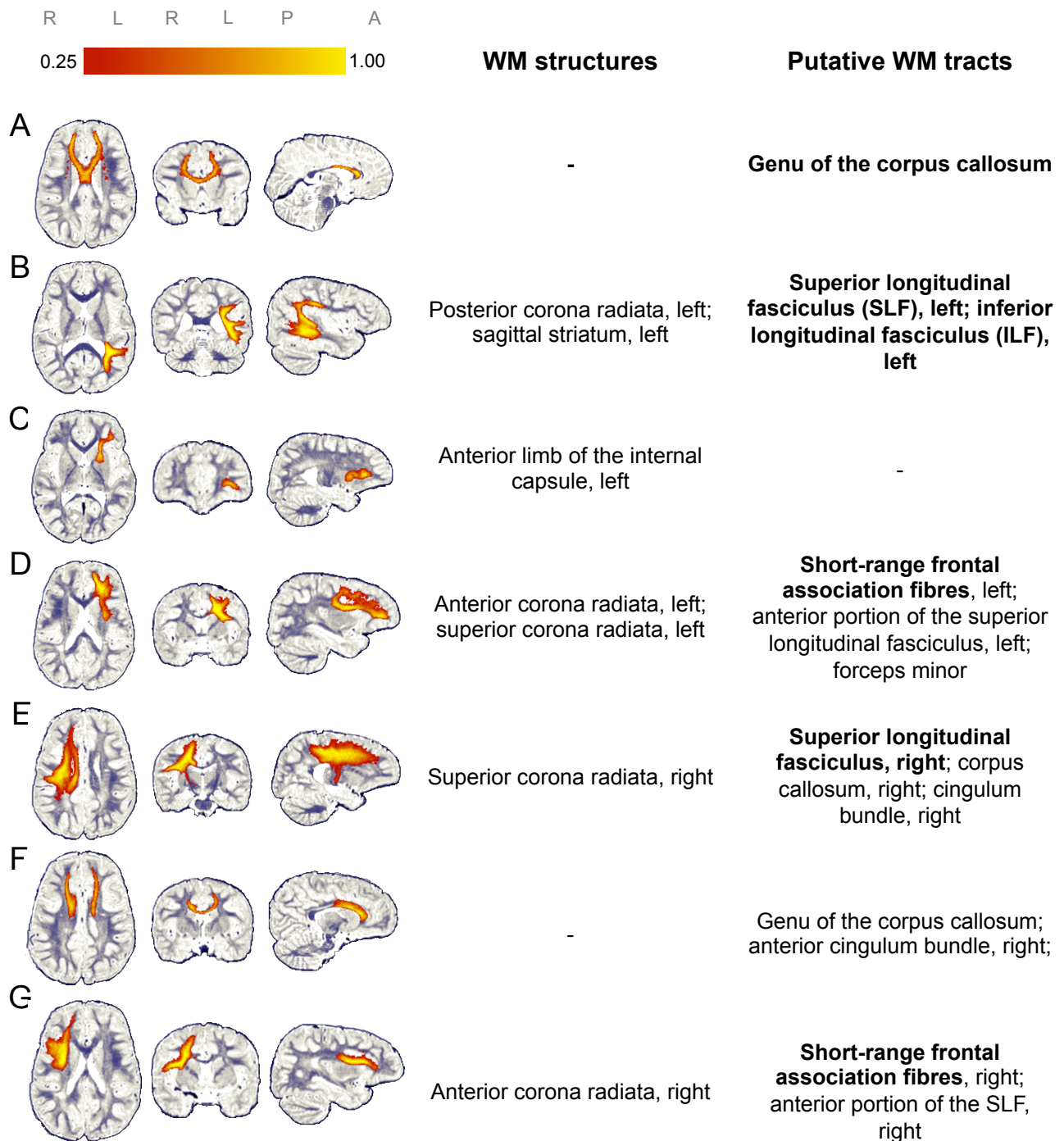


Figure 5.6: Population density maps for a subset of age-associated connections with the highest selection probability. Connections are displayed in axial, coronal, and sagittal sections (left to right) with putative white matter tracts involved in each. The colour bar indicates the proportion of the group present in each voxel of a connection, where lighter areas reveal parts of the connection with the most consistent anatomy across the group. In the description, **bold** white matter tracts represent known direct anatomical fibres between seed and target regions (see Figure 5.7). Tracts in regular font represent fibres which are indirect and/or may be encompassed as part of each probabilistic connection.

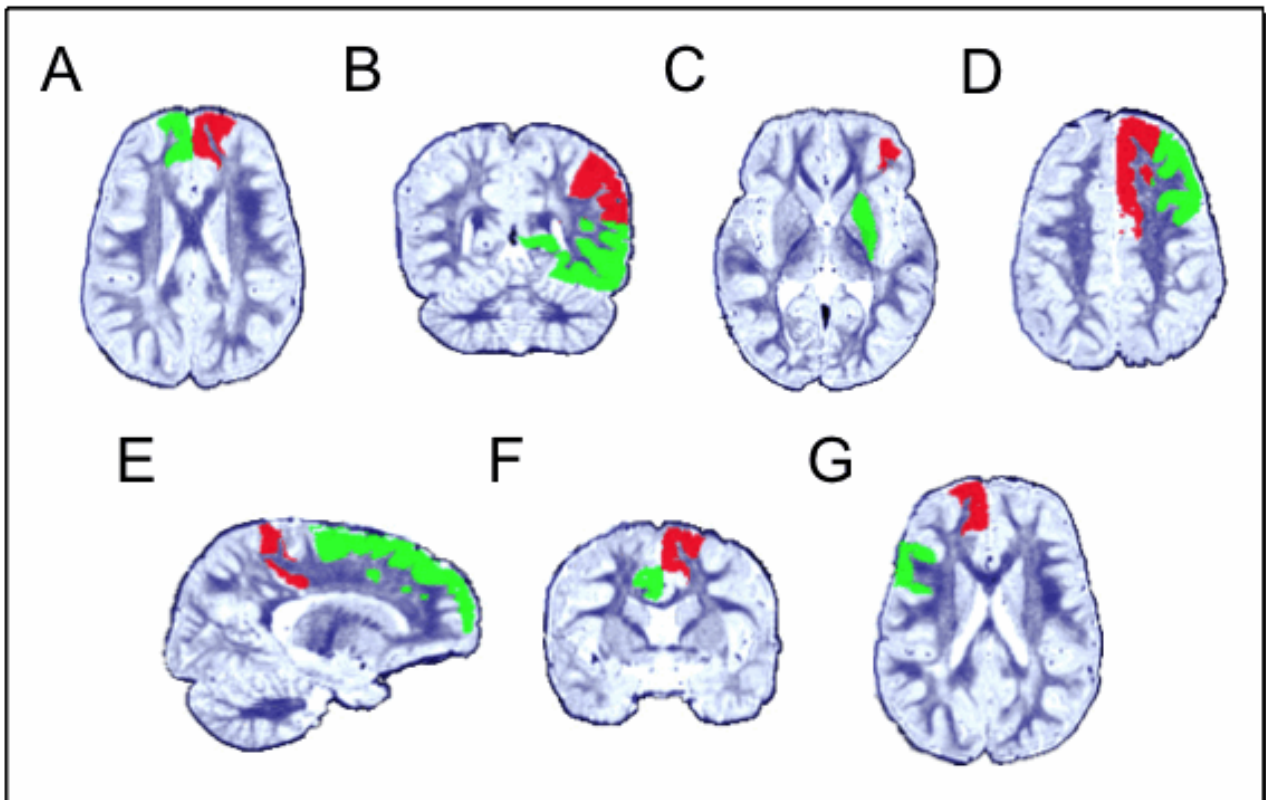


Figure 5.7: Seed and target regions for each connection associated with age-at-scan. For ease of visualisation, region pairs are shown in axial (A, C, D, G), coronal (B, F), and sagittal (E) sections.

Table 5.2. Selected connections associated with post-conceptual age at birth.

Connection	Region 1	Region 2	Selection Probability	Average Regression Coefficient	Mean Anisotropy [range]
A	Caudate nucleus, right	Globus Pallidus, right	0.95	0.072	0.1755 [0.1135–0.2749]
B	Cingulate gyrus, anterior part right	Subgenual frontal cortex right	0.912	0.046	0.1468 [0.0898–0.2281]
C	Occipital lobe, left	Superior parietal gyrus, left	0.856	0.095	0.2034 [0.1436–0.2597]
D	Putamen, left	Thalamus, left	0.854	0.024	0.2202 [0.1659–0.3176]
E	Insula, left	Frontal lobe, left, becomes middle frontal gyrus	0.832	0.041	0.1716 [0.1209–0.2113]
F	Parietal lobe, right	Superior parietal gyrus, left	0.817	0.060	0.3188 [0.2567–0.4076]
G	Medial and inferior temporal gyri, right	Posterior temporal lobe, right	0.779	0.043	0.1978 [0.1234–0.2733]
H	Superior frontal gyrus, left	Superior frontal gyrus, right	0.773	0.067	0.2837 [0.2135–0.3346]
I	Frontal lobe, left, becomes middle frontal gyrus	Inferior frontal gyrus, left	0.772	0.036	0.1589 [0.1269–0.2131]
J	Frontal lobe, right, becomes middle frontal gyrus	Inferior frontal gyrus, right	0.77	0.013	0.1630 [0.1373–0.2027]

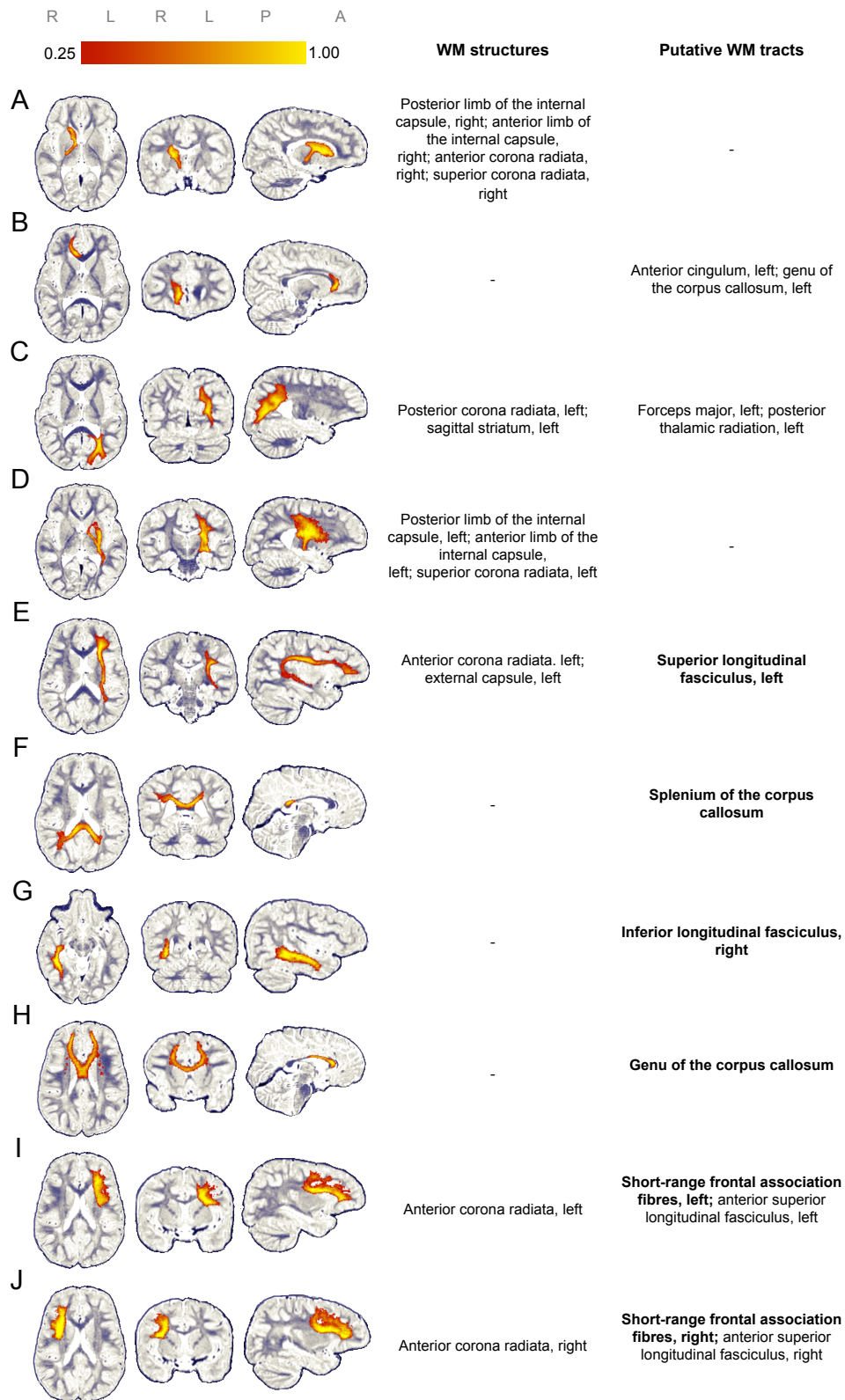


Figure 5.8: Population density maps for a subset of prematurity-associated connections with the highest selection probability. Connections are displayed in axial, coronal, and sagittal sections (left to right) with putative white matter tracts involved in each. The colour bar indicates the proportion of the group present in each voxel of a connection: seed and target regions are shown in Figure 5.9. Fibre populations in this subset include commissural (F, H), projection (A, D), and associative (E, G, I, J) white matter connections.

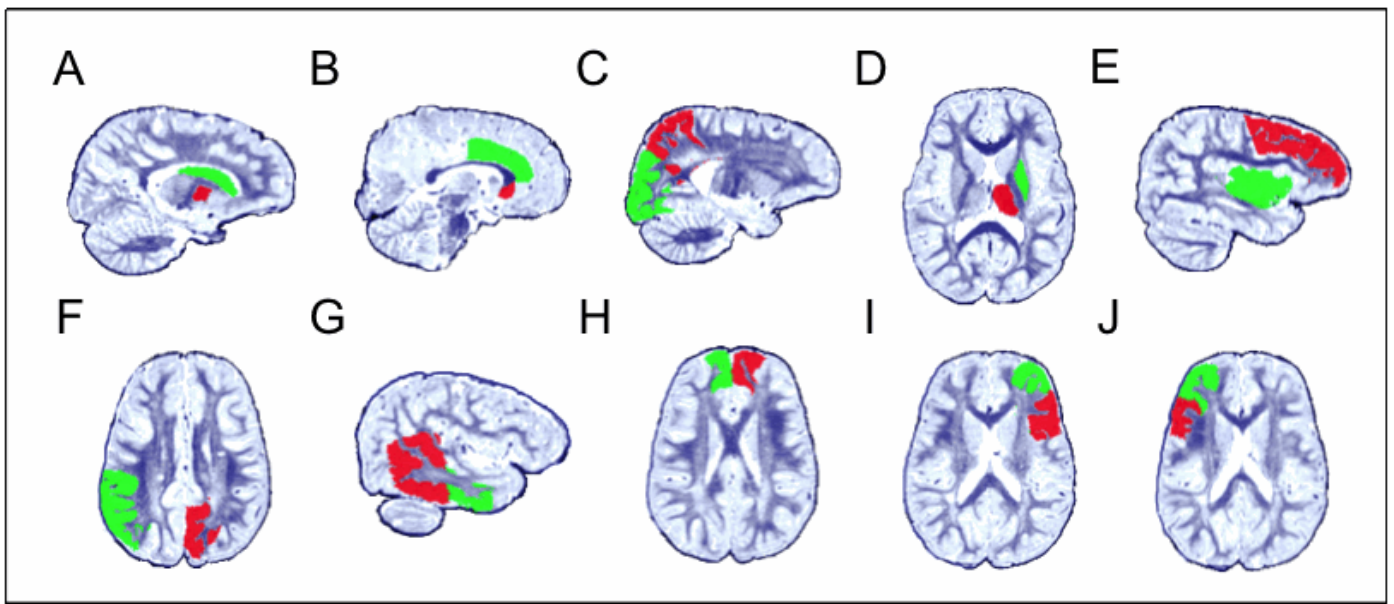


Figure 5.9: Seed and target regions for each connection associated with post-conceptual age at birth.
For ease of visualisation, region pairs are shown in axial (D, F, H, I, J) and sagittal (A, B, C, E, G) sections.

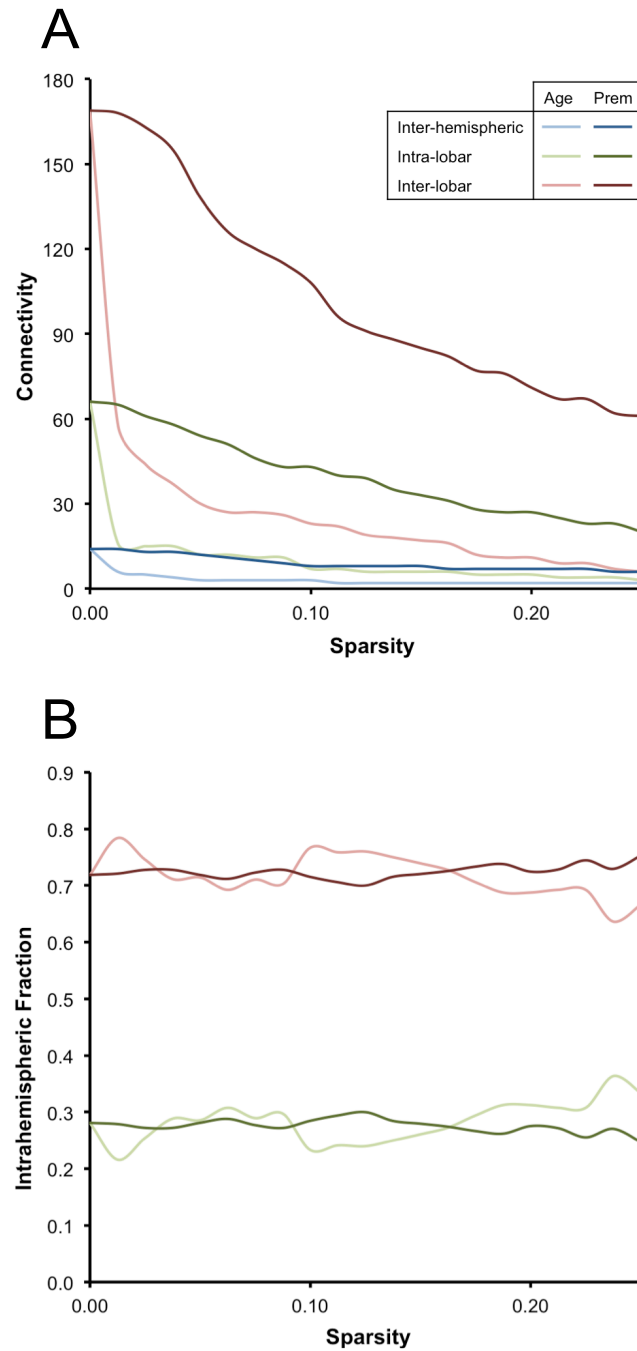


Figure 5.10: Topological differences in the premature infant connectome. Weighting by the selection probability in relation to corrected age at scan (legend: *Age*, lighter lines) or post-conceptual age at birth (legend: *Prem*, darker lines). Differences are shown in the number of connections (A) categorised by type: interhemispheric (blue), intra-lobar (green) and inter-lobar (red) or in the proportion of inter- and intra-lobar connections (B). Sparsity axis indicates selection probability threshold level, below which connections are ignored. Maximum sparsity taken as the threshold level at which <10% of the consistent macro-connectome is retained.

5.3 Results^o

5.3.1 Results I

The sparse macroconnectome has typical network topology but atypical anatomy

A non-subjective, topological representation of the sparse consensus macroconnectome in this cohort of preterm-born infants is given in Figure 5.3 while Figure 5.4A displays all connections and their cortical and subcortical targets.

From Figure 5.4A, one can observe that all connection types exist (inter-hemispheric and intra-hemispheric intralobar and interlobar) and that these involve all lobes and several subcortical structures. However, of the 72 potential regions of interest, 20 (28%) remained unconnected. These include the bilateral pre-subgenual frontal gyri, straight gyri, sub-callosal area, parahippocampal gyri, anterior superior temporal gyrus, lateral anterior temporal lobe, lateral occipitotemporal gyri, amygdala, and hippocampi. Thus, certain potentially significant connections to the frontal and temporal lobes and subcortical structures appear to be absent.

Network metrics are next considered. In Figure 5.3, the size of each node is proportional to its degree and the shading is proportional to its betweenness centrality. Nodes with the highest degree or betweenness centrality are considered network ‘hubs’ and are important in facilitating processing in the network. Hubs in this sparse macroconnectome, according to both degree and betweenness, include bilateral superior frontal gyri, superior parietal gyri and insulae in the cortex, and bilateral thalami and putamen in the subcortex. Global metrics such as small-world topology were also assessed (Appendix B). Using the unweighted connectome data: mean clustering coefficient (C) = 0.4576, characteristic path length (L) = 2.350, and small-worldness (S) = 1.9624 after comparing the ratio of C/C_{rand} and L/L_{rand} . Using the weighted connectome for each subject, average values for the 3 global metrics given above were: C = 0.0854 (range = 0.0741–0.0963), L = 0.4517 (0.3810–0.5236), S = 1.7270 (1.5960–1.8771). These results demonstrated typical properties of anatomical brain networks, including small-world architecture (Watts & Strogatz 1998), and were found to be stable with further sparsification.

5.3.2 Results II

Figure 5.4 shows the macroconnectome with connections unweighted (Fig 5.4A) or weighted by their selection probability in predicting development (Fig 5.4B) or prematurity (Fig 5.4C). Appendix C, Tables C.1 and C.2 present the complete list of connections for each response variable and their respective selection probabilities and coefficients. Figure 5.5 merges the set of connections to create a white matter selection-probability map for each response variable in anatomical space. Figures 5.6 and

5.8 depict, for development and prematurity respectively, a set of population density maps for the most selected connections. Respective seed and target regions for each population density map are illustrated in Figures 5.7 and 5.9, and their statistical information is presented in Tables 5.2 and 5.3. Finally, Figure 5.10 provides additional information regarding connection types and their differences in the developmental or premature-associated connectome.

Postnatal age is associated with increasing frontal and temporal connectivity

Figure 5.4B weights topographical connections by selection probability using corrected age at scan as the response variable. Of a possible 249 connections, 131 were selected as predicting age at scan. However, the majority of these non-zero connections (>86%) had a selection probability below 0.2 and close to zero (Figure 5.4B, Appendix C: Table C.1). Connections with the highest level of association connected fronto-temporal areas (Fig 5.4B) and encompassed white matter structures including the anterior centrum semiovale, corona radiata, and the genu of the corpus callosum (Figure 5.5A). These connections mainly coupled regions in the frontal lobe: one connection paired homologous regions between hemispheres (connection A, Figures 5.6 and 5.7), while one linked temporal and parietal cortices (connection B). The strength of connection was positively associated with development in all cases (Table 5.2). Connections involving commissural tracts (A, F, Figure 5.6 and Table 5.2) had, on average, a greater mean anisotropy than connections predominated by associative tracts (B, D, E, G).

More severe prematurity is associated with decreasing connectivity throughout the cortex and subcortex

The influence of prematurity on white matter connections was widespread and involved bilateral cortical and subcortical structures (Figures 5.4C and 5.5). All of the connections in the sparse, consensus macroconnectome were selected as predicting post-conceptual age at birth and, in contrast to the connections associated with development, approximately half of these had a selection probability above 0.2 (Figure 5.4C, Appendix C, Table C.2).

The ten connections with the highest selection probability were retained for further visualisation and analysis (Figures 5.8 and 5.9). Identified connections involved: frontal, parietal, temporal, and occipital cortices; subcortical structures (connections A and D, Figure 5.8 and 5.9); and homologous (H) or symmetrical connections between hemispheres (I, J). Selected white matter connections included bilateral commissural, projection, and associative tracts (Figure 5.7). Across all subset connections, higher connection strength positively correlated with greater post-conceptual age at birth (Table 5.3). Connections involving commissural tracts (B, F, H, Figure 5.8 and Table 5.3) had, on average, a greater mean anisotropy than connections involving projection fibre bundles (A, D), which in turn had larger anisotropy values compared to connections predominated by associative tracts (E, G, I, J).

Figure 5.10 provides additional information regarding the types of connection and their proportion in the connectome. For all connection types and for additional increasing levels of sparsity, the number of connections associated with prematurity (with non-zero selection probability) was greater than those associated with development (Fig 5.10A). The relative proportions of intra- and interlobar intra-hemispheric connections were maintained across additional increasing levels of sparsity and were similar for both premature and development-associated connectomes (Fig 5.10B).

5.3.3 Results III

Serial imaging data

In order to account for the 7 subjects with serial imaging, further analyses were performed after removing these individuals' earlier or later scan data. Statistical regression analysis was repeated with either the earlier (analysis A) or later (analysis B) imaging data removed for the group of 7 subjects who had serial scans. Results were compared between the original and new regressions by 2 methods. First, the standard deviation of the selection probability of each connection/covariate across all three analyses was calculated and a mean over all connections was taken for each dependent variable. On average, connections associated with corrected age at scan (development) had a standard deviation of ± 0.04 in selection probability, whereas connections associated with post-conceptual age at birth (prematurity) had a standard deviation of ± 0.09 . Second, in Results II a minimal, unique set of connections (7 for development and 10 for prematurity) were highlighted that were most associated with either dependent variable. It was thus examined whether these connections would also be retained as the most selected in the subsequent analyses. In analysis A 7/10 connections associated with prematurity and 6/7 connections associated with development were retained, and in analysis B 5/10 connections associated with prematurity and 6/7 connections associated with development were retained. That so many connections are retained despite stringent inclusion criteria and that the variance of each connection is low indicates that analyses with and without serial imaging subjects are highly comparable.

5.4 Discussion^o

5.4.1 Overview and summary

In this chapter, the influence of development and prematurity on a sparse preterm infant macroconnectome was analysed using a novel, data-driven feature selection strategy. It identified connections related to development, showing as anticipated that connections in frontal and temporal regions increased with age. It also detected widespread reductions in connectivity in all cortical lobes and several subcortical structures bilaterally associated with preterm birth.

5.4.2 Influence of development on the macroconnectome

As a feasibility test for this approach, a pattern of selected connections associated with age was first identified. Recalling from Chapter 3, it is known that early postnatal white matter development is typically characterised by processes of axonal growth, myelination, and synaptogenesis (Yakovlev & Lecours 1967; Haynes et al. 2005; Sampaio & Truwit 2001). From mid-gestation, axons undergo rapid elongation and maturation, and by late infancy reach relative completion (Haynes et al. 2005). Neuro-axonal development is extended in the frontal and associative cortices and appears to be related to the persistence and reorganisation of the sub-plate layer (Kostović et al. 2012). Dendritic arborisation and synaptogenesis are also protracted in the frontal cortex (Webb et al. 2001; Huttenlocher & Dabholkar 1997; Petanjek et al. 2011) and coincide with changes in cerebral metabolic activity (Chugani et al. 1987). Histological evidence suggests that myelination typically occurs in a caudal to rostral, proximal to distal and central to peripheral pattern, with sensory pathways myelinating before motor pathways and association fibers and fronto-temporal regions being the last to mature (Yakovlev & Lecours 1967; Kinney et al. 1988). The findings of conventional MRI studies correspond with this sequence and indicate that myelination reaches an adult pattern of appearance between the 2nd and 3rd postnatal years, with frontal areas last to show the presence of mature myelin (Barkovich 2000; Barkovich 2005).

As the developmental range of this subject group occupied the latter part of this maturation sequence, one would expect that age-selected connections would encompass tracts connecting the rostral and fronto-temporal areas. Indeed, the results of the macroconnectome analysis agree with this prediction, with selected connections that had greater mean anisotropy with increasing age predominantly identified in hypothesised areas.

DTI data have also suggested analogous changes in local microstructural integrity in relevant white matter regions, despite differences in methodological approach. DTI changes include increased FA and reduced ADC and radial diffusivity, indicative of axon bundle maturation and myelination (Dubois et al. 2006). Regions in which these diffusion changes have been found include the anterior corona radiata or frontal white matter (Hermoye et al. 2006; Gao et al. 2009; Löbel et al. 2009; Schneider et al. 2004), the genu of the corpus callosum (Gao et al. 2009; Löbel et al. 2009), the internal capsule (Hermoye et al. 2006; Gao et al. 2009; McGraw et al. 2002; Schneider et al. 2004) and the superior longitudinal fasciculus (Zhang et al. 2007). A recent study of subjectively selected white-matter pathways, including the genu of the corpus callosum, anterior limb of the internal capsule, and arcuate fasciculi, showed significant increases in FA from birth up to two years in a large cohort of normal infants (Geng et al. 2012).

5.4.3 Influence of prematurity on the macroconnectome

In contrast, the effect of prematurity on structural connectivity involved all major brain regions. Prematurity-associated connections were widespread, bilateral, and affected all cortical lobes and several subcortical structures, including projection, commissural, and association fibres. As infants with macrostructural pathology were excluded, these results are probably due to the effects of premature extra-uterine life and, more specifically, the diffuse white matter abnormalities common in this population (Volpe 2003).

The pathophysiology of preterm white matter injury (WMI) involves destructive influences such as perinatal hypoxia-ischemia, hyperoxia, and infection and/or inflammation, concentrating on several maturation-sensitive cell populations, and is part of a spectrum of developmental abnormality that includes reduced grey and white matter volumes, reduced cell density, and hypomyelination (Volpe 2009)(Chapter 3.3). Premyelinating oligodendrocytes (preOLs) are present in very high numbers in the prenatal period and are highly susceptible to these aforementioned destructive influences, making them an exceptional cellular target during WMI. Maturation blockade of the oligodendrocyte lineage (Buser et al. 2012) and interruption of the normal white matter developmental program would result in persistent structural deficits (Favrais et al. 2011) that would perpetuate white matter damage into childhood and beyond. The pervasive character of WMI may be at least partially due to the variety of cell populations found to be affected. In addition to preOLs, sub-plate and GABAergic neurons have also shown evidence of involvement in WMI pathogenesis (Chapter 3.3.1). As each susceptible neuronal population corresponds with a unique distribution of cortical and subcortical white matter circuits, often at a distance from periventricular foci (Kinney et al. 2012), collective damage to these populations may lead to widespread disruption to distributed cerebral targets and their connections. The susceptibility of axonal cross-road areas may also be a contributing factor. These areas are located near periventricular foci of WMI and are therefore vulnerable to damage (Verney et al. 2012)(Chapter 3.3.2). Injury to these areas would result in disruption of numerous intersecting projection, commissural, and association axons, in addition to linking cortical and subcortical targets, and would therefore promote the widespread extent of WMI.

Previous diffusion MRI studies have found microstructural changes across white matter sites, despite differences in methodology. A whole-brain study of preterm neonates after birth found reduced fractional anisotropy in the thalami, posterior, and anterior limbs of the internal capsule (ALIC, PLIC), centrum semiovale (CSO), and optic radiations with decreasing gestational age (Aeby et al. 2009). At term-equivalent age (TEA), reduced FA was found in the CSO, frontal white matter, sagittal striatum, and corpus callosum (CC) as compared to term-born controls (Anjari et al. 2007; Rose et al. 2008; Hasegawa et

al. 2011) and extended to the PLIC, external capsule (EC), and posterior aspects of the CC with greater prematurity (Anjari et al. 2007). Region and tract-specific approaches were consistent with these findings (Hüppi et al. 1998; Dudink et al. 2007; Hasegawa et al. 2011) and previous connectome-based analyses also demonstrated disruption to connections involving frontal and motor cortices and thalamocortical pathways following premature birth (Ball, et al. 2012a; Pannek et al. 2013).

Studies have found that prematurity impacts upon the tempo of white matter development and that preterm neonates and infants show a diminishing rate of FA increase as compared to term controls (Hüppi et al. 1998; Miller et al. 2002; Adams et al. 2010). After term, the gap between normal and preterm WM developmental trajectories is likely to widen, with a greater proportion of white matter regions showing statistically significant changes as compared to normal controls. WM microstructural changes are apparent from childhood (Jo et al. 2012; Yung et al. 2007) and persist up to adolescence and adulthood (Mullen et al. 2011; Constable et al. 2008; Vangberg et al. 2006; Eikenes et al. 2011). As predicted, with increasing age wider reductions in FA are revealed across the whole brain (Yung et al. 2007) and in a range of additional pathways including the corona radiata, uncinate fasciculus, superior and inferior longitudinal fasciculi, superior and inferior fronto-occipital fasciculi, corticospinal tract, and cingulum bundle (Mullen et al. 2011; Constable et al. 2008; Vangberg et al. 2006; Eikenes et al. 2011). Collectively, these areas of reduced microstructural integrity largely approximated the regions in this study through which prematurity-associated connections ran. The specific reductions in connectivity strength attributable to increasing prematurity demonstrated in this work are thus consistent with previous measures of local tissue integrity. The sensitivity of this approach (see later) and imaging during early childhood together with the wide range of post-conceptual age at birth in this dataset may account for certain differences in the results.

5.4.4 Advantages and limitations of the approach

The sparse infant macroconnectome

A critical discussion of the framework used to generate infant macroconnectome data has been previously outlined (Chapter 4.3.1). As mentioned in the Methods section, a crucial difference in this chapter's framework was that default adult probabilistic tissue priors were used instead of two-year-old priors. Admittedly, this was a clear oversight. In Chapter 4.2.3 it was discussed that age-appropriate atlases may better reflect the grey-white matter differences and better estimate white matter boundaries. Using adult priors may have led to a bias in the macroconnectome, but arguably the effects of this bias are limited. For instance, a potential issue is that adult tissue priors have been shown to overestimate white matter in and around central brain structures (see Chapter 4.2.3). However, the probabilistic tracking process terminates on contact with any region of interest, therefore any part of the WM

segmentation that may have erroneously overlapped into a grey matter structure would have been ignored. This would have prevented any spurious tracking around the subcortical structures. Another issue with using adult priors is that white matter structures such as the external capsule and anterior limb of the internal capsule would be underestimated. This would have limited the streamlines passing through these structures and caused a bias in the results. Still, this bias would likely have been equal across the group and, importantly, the feature selection strategy used in this approach still obtained connections involving these structures that demonstrated normal anatomy (development: Figure 5.6 connection C; prematurity: Figure 5.8 connection A)

A further limitation in this approach was the substantial amount of data discarded during the process of mapping group whole-brain connectivity. In order to make robust group-wise comparisons, only consistent connections that were detected in all subjects were used. Given the significant heterogeneity in the brain structure of the groups studied, expected data loss would be great. High fidelity in registration and segmentation steps minimised this problem, but out of a possible 2556 connections only ~250 were retained across the group. These results thus represented a robust but coarse summary of structural connectivity at the cost of probable loss of sub-group effects. Observing Figure 5.3, there appears to be a bias towards measurement of within-hemisphere connections and commissural connections between structures that lie along the midline. Conversely, there is underestimation of connectivity of lateral regions with their opposite-hemisphere analogues. In spite of this, the preterm macroconnectome maintained a typical brain network architecture including small-world topology, and differences in connectivity related either to development or prematurity were maintained over increasing sparsity (Figure 5.3 and 5.10).

In this chapter, the influence of development or prematurity on a sparse preterm infant macroconnectome were modelled. With regards to development, this model is limited as it not clear how effective the initial regression step was in removal of the prematurity confounder effects in the first part of each analysis. Comparing this data with the development of normal children may not be completely valid, as they suffer no influence of prematurity nor its associated conditions. With regards to prematurity, this model is limited in that it neither accounts for grey matter injury nor the effect of macroscopic white matter injury (since these subjects were excluded). Both types of injury may independently lead to impaired structural and functional development (Volpe 2009; Ment et al. 2009; de Kieviet et al. 2012). Other factors such as intra-uterine growth restriction (IUGR) and extra-uterine factors such as respiratory co-morbidity were also unaccounted for, and are known to influence white matter. Respiratory disease has shown a propensity to affect white matter microstructure in immature neonates, independent of prematurity (Ball et al. 2010; Anjari et al. 2009) and IUGR has been associated with global

and local white matter changes in infants (Batalle et al. 2012). Thus, an avenue for future work would be to extend this approach to subjects with macroscopic pathology and account for the above confounders.

Feature selection

Lasso regression and stability selection provided an objective approach to feature selection in the sparse connectivity matrix, and useful results were obtained using a relatively small sample of subjects. Features selected with respect to postnatal age were remarkably consistent with prior histological and neuroimaging knowledge and offer some degree of credibility in the feasibility of the approach.

Lasso regression was ideally suited for this work since it was capable of handling data which had $P > N$; it was computationally efficient and sparse solutions could be generated (Tibshirani 1996). Ordinarily, Lasso regression has certain limitations, specifically its high dependency on λ , difficulty in choosing λ , and the failure to exclude noisy data variables. However, with the use of stability selection these issues could be overcome. With stability selection, repeated sub-sampling and regression could deduce the frequency of variables in the model, therefore their coefficient was not strictly important. The selection of λ did not need to be highly accurate since the highest selection probability appeared to be most frequently selected across a range of λ .

By selecting variables on account of their importance rather than on their association has been shown here to be sensitive to relevant biological patterns in the connectivity matrix. Impeding the wider use of this method is the fact that stability-selection metrics are not directly comparable to linear regression-based methods. Furthermore, the use of the average coefficient in this work is highly unstable and is based on a finite number of sub-samples. One cannot reliably say to what extent the response variable is dependent on each predictor, only that the predictor is important.

In order to rectify this limitation, a line of further research would be to take the set of predictor variables with the highest selection probability and model their association with the response using supplementary techniques. With postnatal age as the response variable, and a limited number of a predictor variables with large effects, linear regression or all-subset selection may be appropriate to deduce the degree of association. With prematurity as the response variable and a large number of predictor variables with small effects, ridge regression may be useful to model the relationship (Tibshirani 1996). An alternative method which could have been used in place of the Lasso is the elastic net (Hastie 2005). Here, an additional penalty term is added which compensates for the correlation between predictor variables and is particularly useful when predictor variables are highly correlated (as would be anticipated in this type of data). However, sub-sampling two tuning parameters would make the model much more computationally complex.

5.4.5 Conclusion and key contribution

In conclusion, this chapter sought to investigate the influence of prematurity and development on the sparse preterm infant macroconnectome. Connections in frontal and temporal regions were found to increase with age, while widespread reductions in connectivity in all cortical lobes and several subcortical structures were associated with preterm birth. This global decrease in connectivity after preterm birth is likely to have consequences for neurodevelopmental outcomes in this population.

The key contribution of this chapter was the development and integration of a robust, yet sensitive, feature selection method that could detect relevant biological patterns of interest in the sparse structural connectivity matrix.

Chapter 6

Conclusion

6.1 Summary

An accurate characterisation of cerebral white matter injury after preterm birth is a highly sought-after goal within neonatal medicine. Neurocognitive impairments are common in preterm-born children (Bhutta et al. 2002; Saigal & Doyle 2008) and white matter injury is thought to be an important mechanism for their occurrence (Volpe 2003). Previous histological and neuroimaging evidence has suggested that white matter injury is pervasive and leads to the persistent microstructural disruption of connections linking functionally important cortical and subcortical targets (Judas et al. 2005; Verney et al. 2012; Skranes et al. 2007; Eikenes et al. 2011; Ball et al. 2012a). However, the extent of white matter disconnectivity has been inadequately defined using existing methods, and it is unclear how this pattern of injury evolves during development. In order to address these issues, this thesis aimed to model whole-brain structural connectivity in preterm infants and children using clinically acquired MR images. A feature selection method was proposed which could characterise the influence of prematurity on white matter connectivity without the need for *a priori* hypotheses.

Models of whole-brain structural connectivity or structural macroconnectome methods enable a comprehensive mapping of the wiring in the brain, albeit at a macroscopic resolution (Sporns et al. 2005). These models provide a crude, unrestricted survey of the connective substrate that underlies effective neurological function and is altered in states of development or disease (Yap et al. 2011; Griffa et al. 2013). Underpinning the construction of a typical structural macroconnectome are a number of essential components, including the parcellation scheme used to delineate brain regions and the diffusion MR tractography algorithm which infers white matter connections between these regions. The choice of these components can have a significant impact upon connectivity estimates in the resulting data matrix (Gigandet et al. 2010; Zalesky et al. 2010; Bassett et al. 2011; Bastiani et al. 2012) and may be inappropriate for use with the developing brain.

In Chapter 4, a data-driven, semi-automated framework of modelling whole-brain structural connectivity in infants and young children was proposed which uses clinically acquired diffusion MRI data. An age-appropriate, anatomically dependent parcellation scheme incorporating tissue class information was used to generate cortical regions of interest at the white-grey matter boundary and subcortical regions that were intact. Diffusion tractography was performed between these regions using

a modified version of a commonly used probabilistic tracking algorithm (Behrens et al. 2003; Behrens et al. 2007), which was able to weight connections by both their certainty and integrity (Robinson et al. 2010). Although this structural macroconnectome framework had certain limitations, such as the elimination of cerebellum and brainstem regions of interest, it was felt to be appropriate for use in the study of preterm infants.

A growing number of techniques are available which are capable of exploring the effects of pathology on the structural macroconnectome. By representing the connectome as a brain network using graph theoretical principles (Bullmore & Sporns 2009), one can first define summary metrics of connectivity at a regional or global level (Rubinov & Sporns 2010), before attempting to predict relevant disease outcomes (Griffa et al. 2013). However, network metrics fail to consider malign influences at the level of individual connections. Alternative statistical approaches model the structural macroconnectome as a set of variables that are amenable to the application of supervised feature selection techniques. In neurological terms, these can be used to identify a pattern of white matter connectivity that is associated with disease characteristics.

In Chapter 5, a novel feature selection method was applied on a sparsified, consistent macroconnectome. This method combined sparse penalised regression (Tibshirani 1996) with stability selection (Meinshausen & Bühlmann 2010) in order to detect biologically relevant connections in a group of preterm-born children. As a test of feasibility, connections were initially sought that predicted corrected age at scan, a transient indicator of brain development. As hypothesised, connections linking frontal and temporal structures were found to be most predictive in this range of development. The influence of prematurity was then studied by exploring connections associated with post-conceptual age at delivery. The effects of prematurity were widespread, affecting connectivity in all cortical lobes and several subcortical structures.

The pattern of white matter damage elicited in these results may have been responsible for the neurocognitive impairments present in this group. One might expect that the neuro-axonal circuits which engage these connections would be disrupted with greater prematurity, causing the neurocognitive functions transmitted by these circuits, to worsen. Due to a number of factors, including time constraints and the lack of functional outcome data in several subjects, this was not tested in this work. Other limitations are also present in the approach employed in this thesis. Grey matter injury (Pierson et al. 2007) and macrostructural white matter pathology (Volpe 2009), are often present after preterm birth and contribute to neurological dysfunction (Nosarti et al. 2008; de Kieviet et al. 2012; Woodward et al. 2006). However, these injuries were not accounted for.

Testing only the consistent connections in the structural macroconnectome resulted in a substantial (>90%) loss of data and possibly much of the structural variance within the group. This method is therefore extremely sensitive to effects of individual subjects. If just one subject has pervasive disconnectivity due to pathology or from acquired noise; then the entire analysis would be restricted to the residual connections present in this subject. Remaining connections in this study showed anatomical bias, with connectivity to important regions such as the hippocampus, amygdala and various cortical gyri absent in the sparsified macroconnectome, and therefore not explored. It is possible that these missing connections may have an entirely different relationship with outcomes of interest as compared to the results shown here.

6.2 Future Work

In this next section, potential avenues for future study are described, which would extend the work presented in this thesis.

Improved modelling of whole-brain structural connectivity

Modelling whole-brain structural connectivity is an iterative process which aims, ultimately, to define a complete map of neural connections within the brain. Using current *in vivo* neuroimaging techniques and processing methods, this is impossible to achieve. However, as advances in both MR imaging and technology continue to occur in the field, connectivity data are likely to be obtained with higher quality, greater resolution and in shorter acquisition and processing times. Improvements in macroconnectome construction that are designed for the normal adult brain should, over time, be distilled for use in developing or diseased populations. Some of the imminent or available improvements that would allow the methodology pipeline of this thesis to be upgraded are described below.

Increased image spatial and angular resolution would help define complex white matter architecture. Sequences with high b-values and more gradient directions, which generate high angular resolution diffusion imaging (HARDI) MR data, could soon be employed within clinically feasible time frames for preterm cohorts.

Precise modelling of the voxel-wise diffusion profile is also important. The diffusion model employed in this thesis was capable of dealing with crossing fibres (Behrens et al. 2007), but lacked the ability to model other complex configurations such as fanning, kissing, and bending fibres. These configurations are ubiquitous within the cerebral white matter and should be accounted for in order to reduce erroneous streamlines (Jbabdi & Johansen-Berg 2011). Available models such as constrained

spherical deconvolution (CSD) have greater ability to resolve intra-voxel geometry and improve diffusion tractography (Tournier et al. 2007; Jeurissen et al. 2011).

The presented macroconnectome framework cannot be easily extended to other preterm subjects (e.g. those with macrostructural white matter lesions). These subjects often have coinciding ventriculomegaly (Leviton & Gilles 1996), which is not captured in the source atlas database and would cause the current parcellation method (Chapter 4.2.3) to fail. Methods such as multi-atlas propagation with enhanced registration (MAPER) incorporate tissue information into the atlas-registration process (Heckemann et al. 2010), enabling the parcellation approach to be extended to these subjects.

Further improvements in brain parcellation may also be possible through the use of connectivity-based approaches (Gigandet et al. 2010; Cloutman 2012; Zalesky et al. 2010; Bassett et al. 2011; Bastiani et al. 2012). The functional ability of a region can be determined by its connectivity pattern. By identifying the similarities and differences in connection patterns it is possible to segregate functionally discrete regions. Using structural connectivity data, clustering techniques can be used to combine a region (or fixed-size area) with neighbouring regions that have similar patterns in connectivity (Johansen-Berg et al. 2004). Using functional MRI data, seeding regions for tractography can be drawn from statistically dependent, task-based, or intrinsic resting-state functional MRI activations (Beckmann et al. 2005). Connectivity-based parcellations may be more accurate in defining regional borders, do not require the prior use of an atlas, and may be better suited for groups with significant anatomical variability, such as in developing or diseased populations.

Improved analysis of whole-brain structural connectivity

What is the most appropriate method of characterising patterns of connectivity? As macroconnectome resolution becomes finer and we head toward data-sets with a massive number of connections, this question becomes increasingly pertinent. In future exploratory work, given the wide array of analysis techniques (Figure 4.10), one may wish to take a hierarchical approach and analyse the macroconnectome in a data-driven sequence: first characterising the data as a global system, then examining important sub-networks, before proceeding to dissect specific regions or connections (as performed in this thesis) which carry the greatest influence. Thus, the effects of a specific factor, can be analysed at multiple levels.

With respect to analysing only consistent connections across the group (see previous), protocols should be implemented which can detect and remove outlier subjects with exceptionally aberrant connectivity matrices. This would limit the level of forced sparsification on the macroconnectome, enabling a greater proportion of connections to be studied.

Predicting neurological impairment

Preterm survivors are at increased risk of developing neurocognitive deficits. Therefore, *in vivo* biomarkers which can predict the occurrence and nature of these deficits are in demand. These would allow parents to be warned of future adverse outcomes and allow targets for possible interventional therapies to be identified. Previous diffusion MR studies of preterm infants have confirmed that alterations to the connective microstructure are associated with functional deficits. White matter damage has been demonstrated in connections underlying visual (Bassi et al. 2008), hearing (Reiman et al. 2009), cognitive (Skranes et al. 2009), language (Northam et al. 2012), and behavioural (Rogers et al. 2012) domains. Macroconnectome approaches offer a number of advantages in predicting neurological functions. As a whole-brain technique, it avoids the requirement for specific regional hypotheses and can detect areas which show compensatory or plastic changes.

Predictive modelling can assess the ability of one, some, or all of the connections within the macroconnectome to forecast neurocognitive phenotype. Summary network measures, at global or regional level, (4.3.2) can also be used as predictor variables, allowing topological information to be retained (Craddock et al. 2013). Given the results of this thesis, one might envisage that connections most associated with prematurity (Figure 5.8) could be used in a multivariate regression or classification model with the aim of predicting neurocognitive ability. Additional variables which account for regional grey matter volume, the presence of perinatal co-morbidities, or genetic information might also be added to attain a more holistic model that would predict impairments with greater accuracy. Such models would also help ascertain exactly which pathological components hold greater influence in the manifestation of later impairments. Advanced machine learning techniques that can logically combine multivariate information will undoubtedly prove useful in this endeavour (Wolz et al. 2012) and will be an important focus for further research.

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Appendix A

MR Image registration

A.1 Introduction

The goal of registration is to achieve spatial correspondence between a pair of images, facilitating their comparison in a common stereotactic space. Registration can be performed between images that are acquired from different subjects, or are acquired in the same subject from different modalities or at different time-points. Image registration is an important part of this thesis, and has been used in several steps including: eddy current correction of diffusion-weighted images; parcellation of T_1 -weighted images; transformation of parcellations from T_1 to B_0 space (all Chapter 4); and transformation of important probabilistic connections to a common T_2 space for visualisation (Chapter 5).

Registration can typically be separated into five components: transformation, similarity measurement, optimisation, interpolation and regularisation. Briefly, the process can be described as follows: the transformation model estimates the mapping between a source image and a target. The similarity between the transformed source and target is then measured, and the process is optimised iteratively by varying the parameters in the transformation model and finding the transformation which induces the greatest similarity. Interpolation is needed to resample the voxel grid of the transformed source to that of the target and regularisation controls the degree to which the transformation is smooth and invertible.

A.2 Transformation

There are three main types of image transformation: rigid, affine and non-rigid. Each transformation is characterised by their degrees of freedom (DOF): the number of parameters which are used to describe the mapping of features from source to target images. Rigid and affine transformations have low DOF and model global differences such as orientation, size and shape between images. Non-rigid approaches have high DOF and can account for local differences in anatomy, enabling more precise transformations. A higher number of parameters and increased complexity in the transformation accrues a greater computational cost. Rigid and affine transformations can usually be estimated in minutes, while non-rigid algorithms can take hours to process on a single unit (Crum 2004).

A.2.1 Rigid

In a rigid transform, a source image is mapped to a target through a set of translations and rotations over 3 dimensions, resulting in a total of 6 DOF. This is used for intra-subject registrations (e.g. when mapping a T_1 -weighted to a T_2 -weighted image) where size and shape are preserved, but positional differences may occur between acquisition sequences due to subject movement or an altered field of view.

A.2.2 Affine

Affine transforms add shear and stretch parameters over 3 dimensions to the rigid deformation and can be used for the correction of scanner calibration errors such as eddy currents in diffusion-weighted (Chapter 4) and echo-planar images. Affine transformations are often used to approximate global inter-subject alignment, and generate a mapping estimate before non-rigid registration.

A.2.3 Non-rigid

Linear (rigid and affine) transforms are often insufficient to describe the spatial relationship between two images. Non-rigid registrations are better suited in situations where soft tissues may deform in a single subject over time or when registrations are required to accommodate substantial anatomical variability between individuals. Non-rigid transformations extend the affine model by incorporating localised deformations which can follow complex functions and quadratic and higher order polynomial curves (Hajnal et al. 2010). Some non-rigid algorithms approximate physical processes, including the elastic properties of solids (Bajcsy & Kovačič 1989) and the dynamics of viscous fluids (Christensen et al. 1996). Others such as spline-based transformations, place a set of control points on both source and target images and apply a spline function to map the location of the control point in the target image into its corresponding counterpart in the source image (Hajnal et al. 2010). Control points are placed on the basis of anatomical landmarks or via a grid system and the transformation typically appears as a smoothly varying displacement field between them.

Freeform deformations

Freeform deformation (FFD) is a type of spline-based transformation which relies on the manipulation of an underlying mesh of uniformly spaced control points using B-splines (Rueckert et al, 1999). FFD registrations are widely applicable and computationally efficient and offer more precise transformations as compared to other spline-based functions (Crum 2004). Whereas in other functions, the manipulation of a control point has a global influence on the transformation (Bookstein 1989); in FFDs, B-splines are only defined in the vicinity of each control point. Thus perturbations in the position of

a control point only affect the transformation in the local neighbourhood of that point rather than the entire grid.

The degree of achievable deformation is determined by the spacing of control points. Large spacing induces global deformations, whereas small spacing defines local deformations. A study applying FFD, would normally optimise registration over several resolution levels. As the level increases, the number of control points increases, and the spacing between them decreases (Schnabel et al. 2001). Transformation is then calculated from the sum of the deformations at each level.

Non-rigid FFD transformations have been used extensively in this thesis, both in the parcellation of subject brains and visualisation of important connections. All have been performed using IRTK (Image Registration Toolkit; <http://www.doc.ic.ac.uk/~dr/software/>; (Rueckert et al. 1999)).

A.3 Similarity Measurement

Measurements of similarity attempt to quantify the extent to which images are aligned after transformation. Similarity measurements can be defined on the basis of geometry or correspondence of voxel intensities.

Geometry

In geometrical approaches, landmarks are chosen that can be reliably identified in source and target images (Beil et al. 1997; Rohr et al. 2001). In the mammalian brain, landmarks include manually placed points and surface-based markers.

Approaches which utilise manually placed points, generally compute an average or 'centroid' of the point set in source and target images. Displacements between the centroids of each image are calculated in three dimensions, providing a translation which can be used for mapping the centroid and its respective set of points to the other image. Once in a common space, the point set is rotated about its translated centroid until the sum of squared distances between corresponding point pairs are reduced to a minimum. The square root of the mean of the square distance (RMS) is used to assess the error in the registration (Hajnal et al. 2010). In order to reduce errors in the transformation, manual placement of landmarks using this approach have to be precise. Anatomical surfaces such as ventricular borders and cortical sulci and gyri can also be used as markers for transformation (Audette et al. 2000). Surfaces are often extracted automatically, can be used to define many possible points to guide mapping, and can accurately map complex and variable cortical convolutions between subjects (Rademacher et al. 1993;

Joshi et al. 2010). The minimal distance between points of a corresponding surface between images, is typically used to measure similarity after transformation.

Voxel similarity

In voxel similarity based approaches, signal intensities are measured in corresponding voxels of source and target images. These methods use a large proportion or all of the data and match intensity patterns in each image using various mathematical criteria (Crum 2004). In images of the same modality, similarity metrics such as the sum of squared differences (SSD) and cross-correlation are suited for intra-modality transformations, whereas for inter-modality transformations, entropy-based metrics are commonly used. These similiary metrics are reviewed briefly below.

The signal intensity of one voxel in an image can be subtracted from its corresponding couterpart in the other image after transformation. This value is squared and summed across all points in the image to derive the SSD. This metric assumes that image intensities only vary according to Gaussian noise i.e. the intensity range of voxels of a specific tissue are alike in both images. In cross-correlation, a linear relationship is assumed to exist between intensities of corresponding points. During registration, the source is moved with respect to the target until the largest cross-correlation is attained.

Information theoretic techniques aim to maximise the amount of shared information between images, by estimating the amount of entropy. In mathematical terms, entropy measures the uncertainty associated with a particular variable. In registration terms, entropy describes the expected uncertainty of the image, specifically, the probabilistic relationship between voxel intensities. In practice, a joint histogram of intensities is produced by categorising the intensities of all corresponding pairs of voxels into discrete bins of fixed width. Dividing this by the number of voxels, results in a joint probability distribution function (PDF) which provides a number equal to the probability that intensities occur together at corresponding locations in the two images. Joint entropy can be calculated directly from the joint PDF and is found to increase with greater misregistration, thus allowing it to be a measure of image alignment. The mutual information (MI) metric compares the information in an overlapping volume of the overlaid images to the information in the original, separate images. The greater similarity between transformed source and target images, the lower their joint entropy and greater their marginal entropies. As MI can be influenced by the overlap of background areas after transformation, NMI is more generally used as a similarity metric as it is overlap-invariant (Studholme et al. 1999) and is used for the majority of registrations in this thesis.

A.4 Optimisation

Optimisation is the iterative search for the best solution of a registration, defined as the specific set of transformation parameters which maximises (or minimises) a similarity function, such as SSD, CC, MI or NMI. Optimisation approaches can be divided into global and local methods which converge on the optimal solution using a variety of techniques, such as gradient descent, least-squares and Powell's direction set method (reviewed in (Frederik Maes 1999)).

For the registrations employed in this thesis, a multi-resolution approach applying both global and local optimisation with gradient descent was employed (Rueckert et al. 1999). In this approach, optimisation is performed over several stages. In the first stage, global affine transformation parameters are optimised (Studholme et al. 1997) and in the following stage, local non-rigid transformation parameters are adjusted. After choosing a set of parameters which are within the capture range (Studholme et al. 1997), in each stage, a gradient descent technique is employed that involves the perturbation of the transformation parameters by a chosen step size. The similarity metric is estimated after the perturbation, and the parameter with the greatest improvement is chosen. The transformation is then updated, and the process continues iteratively until no further improvement in similarity is achieved. The step size is then decreased, and the process is repeated until a minimum step size is reached. As the step size is linked to the resolution of the data, with smaller step sizes, the data is sub-sampled onto lower resolution voxel grids. This permits registration to be performed in a coarse to fine sequence through resolution levels, first optimising the coarse features in the image before progressing to finer anatomical details.

A.5 Regularisation

In non-rigid registration, a transformation is sought which can balance maximum image similarity with the smoothness of the transformation. This is fitted as a cost function equation as shown in A.1. Having a deformation field is smooth and invertible (or diffeomorphic) that transformations are not implausible, e.g. containing sharp warps, folds or tears (Crum 2004). In equation A.1, C_{smooth} is the regularisation term which controls the degree of smoothness and λ_s controls the contribution of regularisation, which is set heuristically to attain the required level of constraint.

$$C = C_{similarity} + \lambda_s C_{smooth}$$

[A.1]

A.6 Interpolation

Interpolation is required in voxel similarity based registrations in order to ensure correspondence between source and target voxel grids. A variety of techniques are able to resample the transformed source image onto the same grid as the intended target, and these vary according to processing speed and accuracy. In nearest neighbour interpolation, the intensity value of a voxel in the transformed image is assigned as the intensity of the geometrically nearest voxel centre in the original image. In tri-linear interpolation, the linearly weighted average of the eight nearest voxels from the original system is taken. B-spline and sinc interpolation are more accurate but also more computationally demanding (Hajnal et al. 1995; Hajnal et al. 2010).

A.7 Choice of registration parameters

The choice of non-rigid registration parameters in this thesis is influenced by previous work (Boardman et al. 2006; Gousias et al. 2008; Ball et al. 2012). These parameters have been outlined in Table 4.12.

Appendix B

Graph theoretic concepts and measures

An anatomical brain network is composed of a set of nodes (ROIs) with interlinking edges (structural connections). Chapter 5 described a sparsified macroconnectome with edges un-weighted (as shown in Figure 5.3) or weighted according to the strength of connection. Local network measures include *degree centrality*, which is the sum of all connections from a particular node and *betweenness centrality*, which is defined as the fraction of all shortest paths, which pass through a given node. Centrality measures typically assess the importance of individual nodes and determine the major hubs in the network. At a global level, brain networks typically attempt to reconcile the opposing demands of specialised processing in local modules (segregation) and the need for integrated processing across the whole network (integration). Networks, which strike an optimal balance with these demands, are said to have *small-world* architecture (Watts and Strogatz 1998).

B.1 Unweighted measures

The *clustering coefficient* quantifies the fraction of a node's neighbours that are also neighbours of each other (Rubinov and Sporns 2010) and the *mean clustering coefficient* (C) is a commonly used measure of network segregation. Its calculation is outlined below:

$$C = \frac{1}{n} \sum_{i \in N} C_i = \frac{1}{n} \sum_{i \in N} \frac{2t_i}{k_i(k_i - 1)}$$

[B.1]

C_i is the clustering coefficient of node i , N is the total number of nodes in the network, k_i and t_i are the degree and number of triangles for node i respectively. Similarly, *characteristic path length* (L) is the most commonly used measure of network integration:

$$L = \frac{1}{n} \sum_{i \in N} L_i = \frac{1}{n} \sum_{i \in N} \frac{\sum_{j \in N, j \neq i} d_{ij}}{n - 1}$$

[B.2]

where L_i is the average distance between node i and all other nodes. d refers to the shortest (geodesic) path length between nodes i and j . The measures above have been written for un-weighted networks. For further information, and equations regarding weighted networks, the reader is referred to (Rubinov and Sporns 2010). In small world networks, the mean clustering coefficient is significantly greater than for a random network whereas characteristic path length is approximately equal to it. A typical index of network 'small-worldness' (S) (Humphries and Gurney 2008) combines these two ratios after comparing them against measures C_{rand} and L_{rand} from equivalent random networks:

$$S = \frac{C/C_{rand}}{L/L_{rand}}$$

[B.3]

Final values for C/C_{rand} and L/L_{rand} were estimated from the averages of values obtained for 500 equivalent random networks with equal numbers of nodes and edges, however the edges were randomly distributed. Small-world brain networks typically have S greater than 1 and small-worldness has been shown to vary across both development and disease. Graph theoretical metrics were calculated using the Brain Connectivity Toolbox (Rubinov and Sporns 2010) in MATLAB.

Appendix C

Lasso selected connections

Table C.1: Lasso selected connetions associated with corrected age at scan. Presented in order of selection probability. Only connections that have non-zero selection probabilities are shown. For calculation of average linear coefficient, see methods section in Chapter 5.

Region 1	Region 2	Selection probability	Average Linear Coefficient
Superior frontal gyrus, left	Superior frontal gyrus, right	0.908	1.44301368
Posterior temporal lobe, left	Parietal lobe, left	0.851	1.11265062
Putamen left	Lateral orbital gyrus, left	0.749	1.15190294
Middle frontal gyrus, left	Superior frontal gyrus, left	0.748	1.03551462
Superior frontal gyrus, right	Post-central gyrus, right	0.684	0.9316023
Cingulate gyrus, anterior part right	Superior frontal gyrus, left	0.66	0.88353249
Inferior frontal gyrus, right	Superior frontal gyrus, right	0.546	0.81364261
Middle frontal gyrus, right	Superior frontal gyrus, right	0.483	0.70937815
Middle frontal gyrus, left	Putamen left	0.424	0.63455811
Medial and inferior temporal gyri left	Parietal lobe, left	0.275	0.49162588
Pre-central gyrus, right	Superior parietal gyrus, right	0.255	0.60238101
Middle frontal gyrus, left	Caudate nucleus left	0.247	0.43363168
Middle frontal gyrus, left	Pre-central gyrus, left	0.243	0.52911125
Pre-central gyrus, left	Superior parietal gyrus, left	0.23	0.37339394
Insula right	Superior frontal gyrus, right	0.228	0.3630735
Putamen left	Inferior frontal gyrus, left	0.211	0.45154174
Anterior temporal lobe, medial part left	Superior temporal gyrus, posterior part left	0.206	0.56481023
Insula right	Inferior frontal gyrus, right	0.205	0.48351124
Middle frontal gyrus, right	Putamen right	0.185	0.36055298
Insula left	Lateral orbital gyrus, left	0.175	0.35669291
Pre-central gyrus, right	Superior frontal gyrus, right	0.172	0.50048583
Putamen left	Superior frontal gyrus, left	0.167	0.4568489
Posterior temporal lobe, left	Post-central gyrus, left	0.165	0.38492273
Medial and inferior temporal gyri left	Superior parietal gyrus, left	0.163	0.46243487
Middle frontal gyrus, left	Post-central gyrus, left	0.155	0.41850587
Caudate nucleus left	Inferior frontal gyrus, left	0.14	0.34245499
Pre-central gyrus, right	Post-central gyrus, right	0.133	0.38257549
Pre-central gyrus, left	Superior frontal gyrus, left	0.123	0.33762466
Pre-central gyrus, left	Post-central gyrus, left	0.12	0.30194008
Insula left	Middle frontal gyrus, left	0.118	0.38232334
Cingulate gyrus, posterior part right	Pre-central gyrus, right	0.115	0.37406735
Posterior temporal lobe, right	Parietal lobe, right	0.112	0.41993914
Cingulate gyrus, anterior part left	Cingulate gyrus, anterior part right	0.101	0.31696005
Putamen left	Thalamus left	0.099	0.38527814
Occipital lobe, left	Putamen left	0.098	0.38399292
Superior temporal gyrus, posterior part left	Superior parietal gyrus, left	0.098	0.43460605
Caudate nucleus right	Thalamus right	0.097	0.55619196
Parietal lobe, left	Post-central gyrus, left	0.096	0.3082764
Inferior frontal gyrus, left	Superior frontal gyrus, left	0.09	0.33868748

Medial and inferior temporal gyri left	Putamen left	0.088	0.43134794
Caudate nucleus right	Superior frontal gyrus, right	0.078	0.39991086
Anterior orbital gyrus, left	Lateral orbital gyrus, left	0.074	0.30684605
Caudate nucleus right	Pre-central gyrus, right	0.06	0.33072972
Posterior temporal lobe, left	Superior frontal gyrus, left	0.057	0.27239803
Parietal lobe, left	Putamen left	0.054	0.43456833
Posterior temporal lobe, left	Putamen left	0.047	0.39005616
Middle frontal gyrus, right	Pre-central gyrus, right	0.045	0.29038997
Insula right	Middle frontal gyrus, right	0.042	0.24837227
Posterior temporal lobe, left	Pre-central gyrus, left	0.042	0.23065304
Parietal lobe, right	Superior frontal gyrus, right	0.042	0.34198308
Parietal lobe, right	Superior parietal gyrus, left	0.041	0.47266993
Cingulate gyrus, posterior part left	Superior frontal gyrus, left	0.039	0.36848802
Middle frontal gyrus, left	Thalamus left	0.038	0.35717746
Insula left	Anterior orbital gyrus, left	0.038	0.33402159
Middle frontal gyrus, left	Inferior frontal gyrus, left	0.038	0.21175117
Anterior orbital gyrus, left	Posterior orbital gyrus, left	0.038	0.26915349
Putamen right	Superior frontal gyrus, right	0.037	0.3360912
Cingulate gyrus, posterior part right	Post-central gyrus, right	0.031	0.27807506
Occipital lobe, left	Thalamus left	0.03	0.49579307
Middle frontal gyrus, right	Globus Pallidus, right	0.029	0.22787089
Occipital lobe, right	Posterior temporal lobe, right	0.028	0.26337197
Cingulate gyrus, anterior part left	Superior frontal gyrus, left	0.028	0.19054346
Occipital lobe, right	Posterior temporal lobe, left	0.027	0.27988187
Superior temporal gyrus, posterior part left	Occipital lobe, left	0.025	0.32477076
Medial and inferior temporal gyri left	Insula left	0.024	0.19431431
Posterior temporal lobe, left	Superior parietal gyrus, right	0.023	0.49529521
Globus Pallidus, left	Pre-central gyrus, left	0.022	0.33514251
Putamen left	Pre-central gyrus, left	0.021	0.28753987
Caudate nucleus right	Post-central gyrus, right	0.021	0.35631665
Putamen right	Inferior frontal gyrus, right	0.02	0.22308614
Cingulate gyrus, anterior part right	Subgenual frontal cortex right	0.019	0.29055493
Middle frontal gyrus, right	Thalamus right	0.018	0.28818982
Insula left	Superior frontal gyrus, left	0.018	0.25925329
Parietal lobe, right	Post-central gyrus, right	0.016	0.2200355
Superior temporal gyrus, posterior part left	Parietal lobe, left	0.014	0.31280063
Posterior temporal lobe, left	Thalamus left	0.014	0.36282492
Middle frontal gyrus, right	Post-central gyrus, right	0.014	0.15478127
Insula right	Lateral orbital gyrus, right	0.014	0.10967862
Insula left	Occipital lobe, left	0.013	0.28631135
Occipital lobe, right	Putamen right	0.013	0.24315918
Posterior temporal lobe, right	Putamen right	0.012	0.21161776
Caudate nucleus right	Superior parietal gyrus, right	0.012	0.41848497
Insula left	Posterior temporal lobe, left	0.01	0.53432349
Caudate nucleus left	Superior frontal gyrus, left	0.01	0.27559088
Superior temporal gyrus, posterior part left	Insula left	0.009	0.13785037
Thalamus left	Inferior frontal gyrus, left	0.009	0.39539513
Superior frontal gyrus, left	Post-central gyrus, left	0.009	0.33973622
Cingulate gyrus, anterior part left	Superior frontal gyrus, right	0.008	0.17219017
Inferior frontal gyrus, left	Post-central gyrus, left	0.008	0.22263404
Post-central gyrus, left	Superior parietal gyrus, left	0.008	0.19214457
Medial and inferior temporal gyri left	Posterior temporal lobe, left	0.007	0.25303538
Globus Pallidus, right	Pre-central gyrus, right	0.007	0.28313033
Parietal lobe, right	Pre-central gyrus, right	0.006	0.1654383
Thalamus left	Superior frontal gyrus, left	0.006	0.37317606

Cingulate gyrus, posterior part right	Superior frontal gyrus, right	0.006	0.27022725
Anterior orbital gyrus, right	Medial orbital gyrus, right	0.006	-0.59660589
Caudate nucleus right	Putamen right	0.005	0.28818826
Posterior temporal lobe, right	Lingual gyrus, right	0.005	0.20783851
Putamen right	Thalamus right	0.004	0.27521591
Globus Pallidus, left	Inferior frontal gyrus, left	0.004	0.19175706
Parietal lobe, left	Superior parietal gyrus, left	0.004	0.21140238
Thalamus right	Superior parietal gyrus, left	0.004	0.23089984
Superior temporal gyrus, posterior part right	Superior parietal gyrus, right	0.004	0.14102132
Middle frontal gyrus, right	Caudate nucleus right	0.003	0.14853752
Parietal lobe, left	Globus Pallidus, left	0.003	0.25358243
Superior frontal gyrus, left	Superior parietal gyrus, left	0.003	0.2626846
Superior frontal gyrus, right	Superior parietal gyrus, right	0.003	0.15434424
Superior temporal gyrus, posterior part left	Posterior temporal lobe, left	0.002	0.0513889
Parietal lobe, right	Putamen right	0.002	0.20021061
Parietal lobe, left	Pre-central gyrus, left	0.002	0
Pre-central gyrus, left	Inferior frontal gyrus, left	0.002	0.16069385
Inferior frontal gyrus, right	Post-central gyrus, right	0.002	0
Posterior temporal lobe, right	Superior parietal gyrus, left	0.002	0.06629126
Putamen left	Superior parietal gyrus, left	0.002	0.03188924
Cingulate gyrus, posterior part right	Superior parietal gyrus, right	0.002	0.0263538
Superior temporal gyrus, posterior part left	Medial and inferior temporal gyri left	0.001	0
Medial and inferior temporal gyri left	Occipital lobe, left	0.001	0
Cingulate gyrus, anterior part right	Middle frontal gyrus, right	0.001	0
Middle frontal gyrus, right	Parietal lobe, right	0.001	0.01996395
Superior temporal gyrus, posterior part left	Thalamus left	0.001	0
Middle frontal gyrus, left	Globus Pallidus, left	0.001	0.24200127
Thalamus left	Globus Pallidus, left	0.001	0.02358135
Thalamus right	Pre-central gyrus, right	0.001	0.21927548
Putamen left	Anterior orbital gyrus, left	0.001	0.24445566
Middle frontal gyrus, right	Inferior frontal gyrus, right	0.001	0
Pre-central gyrus, right	Inferior frontal gyrus, right	0.001	0
Parietal lobe, left	Superior frontal gyrus, left	0.001	0.1681074
Occipital lobe, right	Superior parietal gyrus, right	0.001	0.06631352
Posterior temporal lobe, right	Superior parietal gyrus, right	0.001	0.24568501
Occipital lobe, left	Cuneus left	0.001	0.24301081
Medial orbital gyrus, left	Subgenual frontal cortex left	0.001	0

Table C.2: Lasso selected connections associated with post-conceptual age at birth in order of selection probability. Presented in order of selection probability. Only connections that have non-zero selection probabilities are shown.

Region 1	Region 2	Selection probability	Average Linear Coefficient
Caudate nucleus right	Globus Pallidus, right	0.95	0.07246205
Cingulate gyrus, anterior part right	Subgenual frontal cortex right	0.912	0.045554298
Posterior temporal lobe, left	Lingual gyrus, left	0.876	0.102878458
Occipital lobe, left	Superior parietal gyrus, left	0.856	0.095342742
Putamen left	Thalamus left	0.854	0.024357754

Insula left	Middle frontal gyrus, left	0.832	0.041274984
Parietal lobe, right	Superior parietal gyrus, left	0.817	0.059944837
Middle frontal gyrus, left	Anterior orbital gyrus, left	0.805	0.01592903
Medial and inferior temporal gyri right	Posterior temporal lobe, right	0.779	0.042693244
Superior frontal gyrus, left	Superior frontal gyrus, right	0.773	0.067358994
Middle frontal gyrus, left	Inferior frontal gyrus, left	0.772	0.036305199
Middle frontal gyrus, right	Inferior frontal gyrus, right	0.77	0.013359869
Insula right	Parietal lobe, right	0.725	0.069078497
Superior temporal gyrus, posterior part right	Insula right	0.713	0.00487453
Medial and inferior temporal gyri right	Thalamus right	0.713	-0.008937599
Posterior temporal lobe, right	Thalamus right	0.71	0.060181148
Occipital lobe, right	Lingual gyrus, right	0.71	0.064832365
Inferior frontal gyrus, left	Post-central gyrus, left	0.703	0.10830682
Globus Pallidus, left	Superior parietal gyrus, left	0.674	0.101513087
Insula left	Thalamus left	0.663	0.083508068
Insula right	Thalamus right	0.66	0.02729805
Caudate nucleus left	Thalamus left	0.646	0.050587492
Parietal lobe, right	Putamen right	0.634	0.07865896
Superior temporal gyrus, posterior part left	Occipital lobe, left	0.61	0.07975524
Posterior temporal lobe, left	Pre-central gyrus, left	0.604	0.1067258
Thalamus left	Thalamus right	0.601	0.012268131
Insula right	Occipital lobe, right	0.595	0.069185167
Cingulate gyrus, anterior part right	Superior frontal gyrus, left	0.592	0.035103233
Medial and inferior temporal gyri left	Thalamus left	0.578	0.097403289
Insula left	Anterior orbital gyrus, left	0.576	0.079320485
Putamen left	Lateral orbital gyrus, left	0.576	0.012420502
Medial and inferior temporal gyri left	Occipital lobe, left	0.575	0.08864846
Parietal lobe, left	Pre-central gyrus, left	0.568	0.110575003
Putamen left	Superior parietal gyrus, left	0.567	0.119305417
Insula left	Occipital lobe, left	0.565	0.05980465
Occipital lobe, left	Parietal lobe, left	0.559	0.100813362
Insula right	Caudate nucleus right	0.544	0.043151524
Insula left	Lateral orbital gyrus, left	0.523	0.013244284
Thalamus right	Post-central gyrus, right	0.52	0.063107011
Insula right	Middle frontal gyrus, right	0.517	0.049624821
Medial and inferior temporal gyri left	Posterior temporal lobe, left	0.505	0.079084178
Insula left	Pre-central gyrus, left	0.504	0.054520304
Inferior frontal gyrus, left	Superior frontal gyrus, left	0.49	0.055988992
Occipital lobe, right	Superior parietal gyrus, right	0.483	0.062256489
Parietal lobe, left	Superior parietal gyrus, left	0.467	0.085893318
Insula left	Parietal lobe, left	0.46	0.094669517
Caudate nucleus right	Thalamus right	0.459	0.046435798
Posterior temporal lobe, left	Superior frontal gyrus, left	0.455	0.100570575
Parietal lobe, right	Thalamus right	0.45	0.068934659
Insula left	Superior parietal gyrus, left	0.444	0.128191368
Caudate nucleus right	Putamen right	0.443	0.025638107
Middle frontal gyrus, left	Caudate nucleus left	0.441	0.032693811
Putamen right	Superior parietal gyrus, right	0.436	0.087366018
Cingulate gyrus, posterior part left	Pre-central gyrus, left	0.427	0.006733087
Superior frontal gyrus, right	Subgenual frontal cortex right	0.42	0.021230647
Cingulate gyrus, anterior part right	Pre-central gyrus, right	0.408	0.007789563
Inferior frontal gyrus, right	Post-central gyrus, right	0.405	0.05392851
Lateral orbital gyrus, right	Posterior orbital gyrus, right	0.399	0.020602367
Middle frontal gyrus, left	Thalamus left	0.395	0.035581213
Middle frontal gyrus, left	Pre-central gyrus, left	0.377	0.028935678
Occipital lobe, right	Putamen right	0.367	0.077313934
Caudate nucleus left	Pre-central gyrus, left	0.363	0.041642171
Insula left	Putamen left	0.358	0.082100459

Medial orbital gyrus, left	Subgenual frontal cortex left	0.337	-0.003246745
Pre-central gyrus, right	Superior frontal gyrus, right	0.333	0.026016086
Parietal lobe, left	Putamen left	0.332	0.100651072
Middle frontal gyrus, left	Lateral orbital gyrus, left	0.329	-0.002690602
Middle frontal gyrus, right	Post-central gyrus, right	0.327	0.060768209
Medial and inferior temporal gyri right	Occipital lobe, right	0.317	0.06924121
Caudate nucleus right	Anterior orbital gyrus, right	0.314	0.026412858
Thalamus left	Inferior frontal gyrus, left	0.307	0.063347904
Posterior temporal lobe, left	Post-central gyrus, left	0.307	0.099722971
Cingulate gyrus, posterior part left	Post-central gyrus, left	0.302	0.014965477
Insula right	Posterior temporal lobe, right	0.291	0.071464395
Superior temporal gyrus, posterior part right	Thalamus right	0.288	0.035122749
Anterior orbital gyrus, right	Posterior orbital gyrus, right	0.28	0.036044262
Putamen right	Anterior orbital gyrus, right	0.277	0.048367949
Cingulate gyrus, anterior part left	Superior frontal gyrus, right	0.27	0.036125399
Cingulate gyrus, anterior part right	Post-central gyrus, right	0.267	0.051297072
Cingulate gyrus, posterior part left	Superior parietal gyrus, left	0.264	0.0650798
Cingulate gyrus, posterior part right	Pre-central gyrus, right	0.261	0.016515668
Superior temporal gyrus, posterior part left	Pre-central gyrus, left	0.26	0.084893399
Insula right	Superior parietal gyrus, right	0.259	0.080109205
Cingulate gyrus, anterior part left	Cingulate gyrus, anterior part right	0.257	0.03176602
Pre-central gyrus, left	Superior parietal gyrus, left	0.257	0.078489033
Occipital lobe, right	Thalamus right	0.253	0.081937031
Superior frontal gyrus, left	Post-central gyrus, left	0.252	0.034989664
Lateral orbital gyrus, left	Posterior orbital gyrus, left	0.247	0.073912947
Inferior frontal gyrus, right	Lateral orbital gyrus, right	0.244	0.021772304
Cingulate gyrus, anterior part left	Putamen left	0.24	0.040591016
Anterior orbital gyrus, left	Superior frontal gyrus, left	0.239	0.052559561
Parietal lobe, left	Superior frontal gyrus, left	0.237	0.101679804
Pre-central gyrus, left	Post-central gyrus, left	0.234	0.088559593
Cingulate gyrus, posterior part right	Superior parietal gyrus, right	0.232	0.080405015
Superior parietal gyrus, left	Superior parietal gyrus, right	0.23	0.052847221
Parietal lobe, left	Thalamus left	0.227	0.077322339
Superior temporal gyrus, posterior part left	Insula left	0.226	0.073962914
Parietal lobe, right	Post-central gyrus, right	0.221	0.054045664
Middle frontal gyrus, right	Anterior orbital gyrus, right	0.22	-0.025514415
Anterior orbital gyrus, left	Lateral orbital gyrus, left	0.208	0.070179314
Middle frontal gyrus, left	Post-central gyrus, left	0.205	0.06502983
Caudate nucleus left	Superior frontal gyrus, left	0.203	0.053759985
Superior temporal gyrus, posterior part right	Posterior temporal lobe, right	0.202	0.033081982
Posterior temporal lobe, right	Lingual gyrus, right	0.202	0.038539588
Insula right	Inferior frontal gyrus, right	0.2	0.040766058
Thalamus left	Superior parietal gyrus, left	0.196	0.090488228
Insula left	Inferior frontal gyrus, left	0.193	0.055678557
Superior temporal gyrus, posterior part left	Superior parietal gyrus, left	0.189	0.081880209
Medial and inferior temporal gyri left	Superior parietal gyrus, left	0.189	0.086285267
Putamen right	Post-central gyrus, right	0.188	0.080742628
Superior frontal gyrus, left	Superior parietal gyrus, left	0.179	0.090400083
Medial orbital gyrus, right	Subgenual frontal cortex right	0.178	0.033430687
Pre-central gyrus, right	Superior parietal gyrus, right	0.174	0.077226302
Superior temporal gyrus, posterior part right	Occipital lobe, right	0.173	0.070320462
Occipital lobe, left	Cuneus left	0.171	0.083753297
Occipital lobe, right	Parietal lobe, right	0.17	0.047629354
Parietal lobe, right	Pre-central gyrus, right	0.17	0.04622191
Pre-central gyrus, left	Inferior frontal gyrus, left	0.166	0.048062455
Putamen left	Superior frontal gyrus, left	0.165	0.060092328
Occipital lobe, right	Cuneus right	0.164	0.057543214

Occipital lobe, right	Posterior temporal lobe, right	0.158	0.046713051
Middle frontal gyrus, right	Caudate nucleus right	0.154	0.078137817
Putamen right	Thalamus right	0.153	0.010758047
Posterior temporal lobe, right	Superior parietal gyrus, left	0.152	0.001303048
Anterior temporal lobe, medial part left	Superior temporal gyrus, posterior part left	0.151	0.031869659
Superior temporal gyrus, posterior part left	Putamen left	0.151	0.050872392
Superior temporal gyrus, posterior part right	Medial and inferior temporal gyri right	0.15	0.070362922
Insula left	Caudate nucleus left	0.142	0.050610439
Superior temporal gyrus, posterior part left	Medial and inferior temporal gyri left	0.139	0.063730931
Middle frontal gyrus, right	Thalamus right	0.138	0.07850963
Posterior temporal lobe, right	Post-central gyrus, right	0.138	0.029031272
Inferior frontal gyrus, right	Superior frontal gyrus, right	0.136	0.021622357
Insula right	Superior frontal gyrus, right	0.135	0.051838485
Posterior temporal lobe, right	Parietal lobe, right	0.132	0.053182901
Middle frontal gyrus, left	Posterior orbital gyrus, left	0.131	0.028393515
Superior frontal gyrus, left	Subgenual frontal cortex left	0.131	0.008045114
Caudate nucleus right	Superior frontal gyrus, right	0.128	0.05544407
Medial orbital gyrus, left	Posterior orbital gyrus, left	0.128	0.020040842
Cingulate gyrus, posterior part left	Superior frontal gyrus, left	0.123	0.073219785
Insula left	Globus Pallidus, left	0.122	0.077187003
Middle frontal gyrus, right	Pre-central gyrus, right	0.119	0.050921767
Putamen left	Posterior orbital gyrus, left	0.119	-0.003345396
Parietal lobe, right	Superior frontal gyrus, right	0.117	0.0581389
Insula left	Superior frontal gyrus, left	0.114	0.037716435
Middle frontal gyrus, right	Parietal lobe, right	0.112	0.035493132
Occipital lobe, left	Lingual gyrus, left	0.111	0.052415117
Medial and inferior temporal gyri left	Putamen left	0.11	0.063032965
Posterior temporal lobe, right	Putamen right	0.109	0.06755136
Thalamus left	Superior frontal gyrus, left	0.109	0.069246375
Thalamus left	Post-central gyrus, left	0.109	0.060302787
Cingulate gyrus, anterior part right	Middle frontal gyrus, right	0.108	0.001891405
Superior temporal gyrus, posterior part left	Post-central gyrus, left	0.108	0.055371912
Post-central gyrus, left	Superior parietal gyrus, left	0.108	0.077245104
Parietal lobe, left	Globus Pallidus, left	0.107	0.058163981
Thalamus right	Superior frontal gyrus, right	0.104	0.0929365
Middle frontal gyrus, right	Putamen right	0.103	0.052912706
Medial and inferior temporal gyri left	Parietal lobe, left	0.102	0.100761256
Caudate nucleus left	Globus Pallidus, left	0.102	0.081940937
Caudate nucleus left	Post-central gyrus, left	0.102	0.036962068
Thalamus right	Superior parietal gyrus, right	0.099	0.066175041
Globus Pallidus, left	Superior frontal gyrus, left	0.096	0.053611883
Middle frontal gyrus, left	Parietal lobe, left	0.095	0.080483233
Putamen right	Pre-central gyrus, right	0.093	0.057284739
Cingulate gyrus, posterior part right	Post-central gyrus, right	0.093	0.041975763
Posterior temporal lobe, left	Superior parietal gyrus, right	0.092	0.066063602
Putamen left	Post-central gyrus, left	0.091	0.07270314
Putamen left	Pre-central gyrus, left	0.089	0.044977282
Insula right	Anterior orbital gyrus, right	0.086	0.071911197
Occipital lobe, right	Posterior temporal lobe, left	0.084	0.013280941
Caudate nucleus left	Putamen left	0.08	0.035856754
Middle frontal gyrus, right	Superior frontal gyrus, right	0.08	0.018747851
Insula right	Post-central gyrus, right	0.08	0.06817938
Caudate nucleus left	Inferior frontal gyrus, left	0.078	0.04770984
Superior temporal gyrus, posterior part right	Parietal lobe, right	0.077	0.050283837
Putamen left	Anterior orbital gyrus, left	0.077	0.037825789

Pre-central gyrus, right	Inferior frontal gyrus, right	0.077	0.0280911
Cingulate gyrus, anterior part right	Cingulate gyrus, posterior part right	0.075	0.064564095
Superior temporal gyrus, posterior part left	Posterior temporal lobe, left	0.075	0.078546205
Globus Pallidus, left	Inferior frontal gyrus, left	0.075	0.052295669
Parietal lobe, right	Superior parietal gyrus, right	0.072	0.054779341
Thalamus right	Pre-central gyrus, right	0.07	0.048846794
Cingulate gyrus, anterior part right	Superior parietal gyrus, right	0.07	0.082604732
Occipital lobe, right	Superior parietal gyrus, left	0.069	0.060344605
Anterior orbital gyrus, right	Superior frontal gyrus, right	0.067	-0.017494905
Anterior orbital gyrus, right	Medial orbital gyrus, right	0.066	-0.022573657
Parietal lobe, right	Inferior frontal gyrus, right	0.065	0.053504874
Insula left	Posterior temporal lobe, left	0.064	0.049945685
Superior temporal gyrus, posterior part right	Putamen right	0.063	0.045272424
Posterior temporal lobe, left	Parietal lobe, left	0.062	0.069146295
Cingulate gyrus, anterior part left	Pre-central gyrus, left	0.062	0.037972558
Insula right	Pre-central gyrus, right	0.062	0.016496825
Parietal lobe, left	Caudate nucleus left	0.061	0.086361948
Thalamus left	Pre-central gyrus, left	0.061	0.037421694
Pre-central gyrus, left	Superior frontal gyrus, left	0.061	0.068223742
Occipital lobe, left	Posterior temporal lobe, left	0.06	0.086959474
Cingulate gyrus, anterior part left	Cingulate gyrus, posterior part left	0.058	0.066765103
Occipital lobe, left	Superior parietal gyrus, right	0.057	0.045068683
Cingulate gyrus, anterior part left	Superior frontal gyrus, left	0.056	0.022546661
Superior temporal gyrus, posterior part right	Superior parietal gyrus, right	0.056	0.066296053
Parietal lobe, left	Post-central gyrus, left	0.055	0.069506829
Posterior temporal lobe, right	Superior parietal gyrus, right	0.053	0.048307674
Medial and inferior temporal gyri left	Insula left	0.052	0.055386701
Middle frontal gyrus, left	Globus Pallidus, left	0.052	0.050052936
Putamen right	Inferior frontal gyrus, right	0.05	0.053976171
Insula right	Lateral orbital gyrus, right	0.048	-0.012302321
Caudate nucleus right	Superior parietal gyrus, right	0.046	0.052268575
Posterior temporal lobe, left	Putamen left	0.045	0.067867297
Insula right	Putamen right	0.045	0.033582598
Cingulate gyrus, anterior part right	Thalamus right	0.044	0.057769769
Superior temporal gyrus, posterior part left	Parietal lobe, left	0.042	0.058686108
Globus Pallidus, left	Post-central gyrus, left	0.042	0.06117743
Globus Pallidus, right	Post-central gyrus, right	0.042	0.074450197
Globus Pallidus, left	Pre-central gyrus, left	0.041	0.05356314
Putamen left	Inferior frontal gyrus, left	0.041	0.040289505
Putamen right	Superior frontal gyrus, right	0.041	0.070086381
Thalamus left	Globus Pallidus, left	0.04	0.05357603
Caudate nucleus right	Pre-central gyrus, right	0.04	0.024295694
Insula left	Post-central gyrus, left	0.04	0.057689121
Thalamus right	Superior parietal gyrus, left	0.04	0.004256992
Cingulate gyrus, anterior part right	Putamen right	0.039	0.042168022
Putamen left	Globus Pallidus, left	0.039	0.058991925
Globus Pallidus, right	Pre-central gyrus, right	0.039	0.06913984
Cingulate gyrus, anterior part right	Superior frontal gyrus, right	0.039	0.019627621
Post-central gyrus, right	Superior parietal gyrus, right	0.039	0.054352535
Anterior orbital gyrus, left	Posterior orbital gyrus, left	0.039	0.023365561
Medial and inferior temporal gyri right	Insula right	0.038	0.06069993
Middle frontal gyrus, right	Globus Pallidus, right	0.037	0.066195018
Posterior temporal lobe, left	Thalamus left	0.036	0.042965298
Putamen right	Globus Pallidus, right	0.035	0.070345243
Occipital lobe, left	Thalamus left	0.034	0.053035869
Middle frontal gyrus, right	Lateral orbital gyrus, right	0.034	0.053185541

Caudate nucleus left	Nucleus accumbens left	0.033	0.019227266
Occipital lobe, left	Putamen left	0.032	0.052181334
Cingulate gyrus, posterior part right	Superior frontal gyrus, right	0.031	0.042129874
Superior frontal gyrus, right	Post-central gyrus, right	0.031	0.063781438
Globus Pallidus, right	Superior frontal gyrus, right	0.029	0.083385914
Middle frontal gyrus, left	Putamen left	0.028	0.055352135
Superior frontal gyrus, left	Medial orbital gyrus, left	0.024	-0.021694878
Superior frontal gyrus, right	Medial orbital gyrus, right	0.024	0.010284611
Superior temporal gyrus, posterior part left	Thalamus left	0.021	0.031854975
Posterior temporal lobe, left	Superior parietal gyrus, left	0.02	0.0404045
Anterior orbital gyrus, left	Medial orbital gyrus, left	0.018	0.029248243
Pre-central gyrus, right	Post-central gyrus, right	0.017	0.047166294
Superior frontal gyrus, right	Superior parietal gyrus, right	0.015	0.034602217
Thalamus right	Globus Pallidus, right	0.014	0.016412951
Subgenual frontal cortex left	Subgenual frontal cortex right	0.014	0.028349801
Insula right	Posterior orbital gyrus, right	0.013	0.015118631
Caudate nucleus right	Post-central gyrus, right	0.009	0.055294975
Middle frontal gyrus, left	Superior frontal gyrus, left	0.006	0.010946141

Appendix D

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Figure 3.2

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Figure 5.3–5.10

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