Motor, sensory and executive function brain networks in children with unilateral cerebral palsy

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MBBS, BSc

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The University of Queensland in 2016

School of Medicine
Abstract

Background: Cerebral palsy (CP) is an umbrella term for a clinical picture caused by antenatal or perinatal insult resulting in non-progressive motor dysfunction. Secondary impairments in executive and social functioning are also evident. Unilateral spastic CP is defined by predominant motor spasticity impacting only one side of the body, and may be due to either unilateral or bilateral lesions. Underlying pathology may be lesions impacting primarily either periventricular white matter or cortical and/or deep grey matter, malformations during brain development, or be non-specific. MRI imaging has become the standard for assessing brain pathology. Advanced MRI processing techniques have been developed allowing groupwise detection of changes beyond those visible on gross assessment of structural images. These techniques include automated identification of intracerebral surfaces to allow volume estimation; or registration to a standard space to allow voxel-wise comparison between groups. In addition, diffusion MRI allows measurement of water molecule diffusion, giving insight into the integrity of organised tissue such as axonal fibres. Complex mathematical models are applied to the raw data to allow clinically relevant information; significant advances in these models has allowed improved accuracy and clinical utility of results, however, these advanced models have thus far been infrequently applied in CP research.

Method: A literature review was initially performed to summarise findings, assess methodology, and identify areas in which further research was needed. Subsequently several of the techniques described above were applied to subsets of a large cohort of children with unilateral spastic CP. Using high resolution T1 weighted images, volume of subcortical structures in children with cortical or deep grey matter lesions and periventricular white matter lesions was analysed, along with a whole brain voxel wise grey matter analysis. Subsequently using diffusion MRI with diffusion tensor imaging (DTI), a whole brain white matter analysis was performed in children with periventricular white matter lesions. In both of these studies comparisons were made between children with bilateral and unilateral lesions. Correlation between imaging parameters and motor function were also assessed. In an aim to assess the executive function network, a tractography study using constrained spherical deconvolution (CSD) was performed, seeded in the anterior cingulate cortex. Correlation between diffusion metrics in these tracts and executive function was assessed. Finally, an updated literature review was undertaken (four years following publication
of the initial review), making specific recommendations for future studies using diffusion MRI in CP.

Results: In the initial review (2012), the corticospinal tracts as well as posterior and superior thalamic radiations were the most widely studied tracts in CP, with minimal and often conflicting results in other tracts or regions. Subcortical analysis showed bilateral reduction in thalamic volume as well as unilateral reduction in volume of several basal ganglia structures across both grey matter and white matter lesions, alongside extensive cortical changes in children with grey matter lesions. Subsequent whole brain white matter analysis of children with periventricular white matter lesions showed children with unilateral lesions had focal unilateral changes in the vicinity of the corticospinal tract; while children with bilateral lesions had diffuse bilateral changes extending into all lobes. Diffusion parameters of the posterior thalamic radiations significantly correlated with hand function. Tractography seeded in the anterior cingulate cortex revealed altered diffusion parameters of the connection to the precuneus in children with periventricular white matter lesions. Additionally, performance in the flanker task significantly correlated with diffusion parameters of the connection to the superior frontal cortex. Finally, repeat review of the literature showed improved knowledge around multiple brain regions with an increased focus on prediction of clinical outcome and response to rehabilitation. To improve homogeneity of methods and avoid common study limitations in future studies a set of nine specific recommendations was produced.

Conclusions: Several advanced MRI techniques were applied to contribute to a rapidly advancing understanding of the pathophysiology underlying CP. Whole brain grey and white matter analysis allowed differentiation between unilateral and bilateral lesions, as well as identifying common features between primary grey or white matter lesions. All analyses showed evidence of changes in at least one subgroup in parts of the executive function network. Specific study of the anterior cingulate demonstrated that damage to tracts involved in the executive function network is related with clinical outcome. This rapidly expanding field holds promise for ongoing advances in the understanding of motor, sensory and executive dysfunction in this cohort, as well as prediction of outcome and response to therapy.
i. Declaration by author

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Peer Reviewed Journal Papers


Conference Abstracts


International Cerebral Palsy Conference; 2012 Oct 10-13; Pisa, Italy.  


iii. Publications included in this thesis

All of these publications have in some part been shaped by feedback from anonymous reviewers as part of the peer review process of the journal, and in some cases multiple journals.


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vii. Keywords

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<tr>
<td>ACC</td>
<td>Anterior cingulate cortex</td>
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<tr>
<td>AFD</td>
<td>Apparent fibre density</td>
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<td>BM</td>
<td>Brain malformation</td>
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<td>CDGM</td>
<td>Cortical or deep grey matter</td>
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<td>CP</td>
<td>Cerebral palsy</td>
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<td>CST</td>
<td>Corticospinal tract</td>
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<td>CTD</td>
<td>Children with typical development</td>
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<td>DTI</td>
<td>Diffusion tensor imaging</td>
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<td>FA</td>
<td>Fractional anisotropy</td>
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<td>FOD</td>
<td>Fibre orientation distribution</td>
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<td>HARDI</td>
<td>High angular resolution diffusion imaging</td>
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<td>JTHFT</td>
<td>Jebsen Taylor hand function test</td>
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<td>MD</td>
<td>Mean diffusivity</td>
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<td>MRI</td>
<td>Magnetic resonance imaging</td>
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<tr>
<td>PLIC</td>
<td>Posterior limb of the internal capsule</td>
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<td>PTR</td>
<td>Posterior thalamic radiations</td>
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<td>PWM</td>
<td>Periventricular white matter</td>
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<td>ROI</td>
<td>Region of interest</td>
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<td>SIFT</td>
<td>Spherical deconvolution informed filtering of tractograms</td>
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<td>UCP</td>
<td>Unilateral cerebral palsy</td>
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<td>VBM</td>
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<td>WM</td>
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Chapter 1. Introduction

1.1. Unilateral Spastic Cerebral Palsy

Cerebral palsy (CP) is an umbrella term encompassing several common key clinical features as a result of antenatal or perinatal insult. Guidelines published in 2000 by Surveillance of Cerebral Palsy in Europe (SCPE) acknowledged various definitions of CP, identifying three key features of a diagnostic definition: a permanent but not unchanging nature; disordered movement and/or posture; and an underlying non-progressive interference, lesion or abnormality of the immature brain. The guideline recommends further classification by the predominant motor type: spasticity (which may be bilateral or unilateral); dyskinesia (which may be dystonic or choreo-athetotic); or ataxia. Unilateral spastic CP accounts for approximately one third of CP, and represents a spectrum of mild to severe disability. Motor impairment comprises dysfunction in motor planning and execution, sensorimotor ability and bimanual coordination.

Executive Dysfunction

In addition to motor dysfunction, cognitive and social dysfunction are well described in children with CP. Impairments are evident across multiple executive function domains and have considerable impact on everyday life or these children, with up to 50% meeting criteria for an intellectual disability. In addition, there is increased prevalence of psychiatric and behavioural disorders amongst children with CP, with estimates of 25-50% having attention deficit disorder or attention deficit hyperactivity disorder. Not surprisingly, these social and cognitive impairments in childhood have a flow on effect to adulthood, with almost a third of adults with CP showing no regular involvement in social, leisure and employment activities. Unemployment rates in adults with CP cannot be explained by motor ability alone.

Pathological Features

Lesions in CP are caused by either antenatal or perinatal insult. It is hypothesised that timing of the insult bears correlation with the nature of the lesion: periventricular white matter lesions
account for 56% of children with CP (45% of UCP) and are thought to arise from early third trimester hypoxic-ischaemic insult; cortical or deep grey matter lesions account for 18% of lesions (30% of UCP) and are thought to relate to late third trimester or perinatal insult; global maldevelopments account for 9% (14% with UCP) and occur earlier in foetal development \(^{15,16}\) (the remaining 17% are non-specific [11% with UCP]). Despite unilateral clinical features, around half of children with UCP have bilateral lesions, most prevalent in periventricular white matter lesions \(^{16}\). The pathophysiology underlying these periventricular white matter lesions is hypothesised to result from hypoxic-ischaemic when oligodendrocyte progenitors are most vulnerable \(^{15,17}\). These lesions are thought to comprise focal periventricular components, which may be unilateral, and diffuse components that are more symmetrical, accounting for the bilateral component \(^{17}\).

**Epidemiology and Impact on Society**

Cerebral palsy occurs in roughly 2.1 per 1000 live births; where birthweight is <1500g this increases to 59.2 per 1000; and similarly where gestational age at delivery is less than 28 weeks this increases to 111.8 per 1000 \(^{18}\). These figures seem to have remained stable despite advances in neonatology with increasing survival rates of infants in these high risk categories. The expected lifetime cost of CP is estimated at around €800 000 \(^{19}\), with social care comprising the largest component. Unquantifiable costs are also significant, and extend to physical, social, psychological and financial wellbeing of both patients and their families or primary caregivers \(^{20}\).

**Treatment**

Treatment of CP is largely focused on rehabilitation to improve clinical function. Orthopaedic surgery is often indicated, and recent advances in medical therapy have led to common administration of medications. Rehabilitation extends from routine physiotherapy and occupational therapy to specific treatments such as constrained-induced movement therapy or intensive bimanual therapy for unilateral CP \(^{21}\). Medical therapy includes administration of medications which aim to reduce spasticity either acting peripherally such as botulinum toxin A \(^{22}\) or centrally, such as baclofen \(^{23}\). Experimental treatments such as administration of autologous cord blood are also being explored \(^{24}\).
1.2. Advanced Techniques in Neuroimaging

Structural MRI

Magnetic resonance imaging (MRI) was first implemented in medicine as a tool for detecting tumours \textit{in vivo} in 1971\textsuperscript{25}. It was highlighted at this time that the technique did not cause potential damage to tissues (in contrast to radiation emitting techniques such as X-ray). MRI utilises magnetic resonance to induce a transient magnetic “spin” in protons within tissues, which is able to be measured after the magnetic field is removed, representing physical properties of the underlying tissue, depending on the acquisition parameters. Structural MRI of the brain allows 3-dimensional identification of brain anatomy, including differentiation between grey matter, white matter and cerebrospinal fluid and subsequent detailed description of brain lesions and abnormalities such as those in CP\textsuperscript{15}.

When considering groups of subjects, automated techniques have been identified to allow detection of between group differences. These techniques typically involve either registering images to a common space to allow voxel based analysis or identifying surfaces to allow structure based analysis. Surface based analysis allows between group comparison of measurements such as cortical thickness and volume of structures (such as basal ganglia or ventricles). Examples are shown in Figure 1.1. Voxel based analysis (such as Voxel Based Morphometry\textsuperscript{26}) identifies clusters of voxels with reduced MRI signal in one group compared with another, or may identify regions where a correlation exists with a clinical measure. An example of the results of this type of analysis is shown in Figure 1.1. Both of these techniques are implemented in Chapter 3; details around methodology and limitations are described therein.
Figure 1.1. Structural MRI techniques allowing groupwise comparison.

Left: Cortical automated surface based parcellation (3-dimensinal rendering displayed). Centre: Subcortical automated surface based parcellation (displayed over T1 image). Right: Examples of results of a voxel based comparison between groups, areas with significant difference in grey matter shown in yellow/red.

Diffusion MRI

Diffusion MRI (dMRI) utilises measurement of water molecule diffusion to give information about biological tissue \(^{27,28}\). In particular, where diffusion of water molecules is restricted by biological membranes, such as axonal membranes in neural tracts, data can be measured which may give insight into underlying tissue integrity \(^{28}\). Diffusion is measured along an arbitrary number of directions for the entire brain, resulting in an array of 3-dimensional images for each scan. The specification of acquisition parameters poses a significant challenge in children with CP as increased spatial and angular resolution requires greater acquisition time, which is problematic in both children and subjects with movement disorders. We elect to avoid the use of routine sedation or anaesthesia by inviting all children to attend a mock scanner prior to the true scan. This method has been shown to allow high quality image acquisition \(^{29}\).

Mathematical models are employed to simplify acquired data and allow clinicians and researchers to extract meaningful information. The most common model is Diffusion Tensor Imaging (DTI) whereby the diffusion within each voxel is summarised by a single ellipsoid described by three eigenvectors \(^{30}\). An example is shown in the second row of Figure 1.2. This approach greatly reduces the volume of information contained within each voxel, which has both beneficial and detrimental effects. Simple scalar values can be extracted from each voxel such as fractional anisotropy (FA), measuring the uniformity of diffusion, and mean diffusivity (MD), measuring net
amount of diffusion \(^{28}\) (sample images shown in the third row of Figure 1.2). Whole brain voxel based analysis similar to that described above used in structural MRI is possible using the FA or MD images; this technique is utilised and discussed in detail in chapter 4. For tracts that contain few crossing fibres, such as the corticospinal tract (CST) at the level of the posterior limb of the internal capsule (PLIC), tensor derived fibre orientations and FA may be reliable, however, where complex WM architecture exists, multiple fibres may cross within each voxel. When a voxel contains multiple crossing fibres, FA results can be unintuitive and difficult to interpret \(^{31}\). A higher order model is therefore more appropriate \(^{32}\). The fibre orientation distribution (FOD) is one such model, computed via constrained spherical deconvolution \(^{33, 34}\). An example of FOD images is shown in the second row of Figure 1.2; this image shows where the callosal fibres cross the descending fibres that both fibre bundles are represented in the FOD while meaningful information is lost with DTI. In this thesis both models (DTI and FOD) are utilised and discussed in depth, with chapter 5 demonstrating the benefits of the higher order model.
Figure 1.2. Samples from steps of diffusion MRI processing.

The first row shows both the diffusion weighted image and a T1 weighted structural image. The second row shows a sample of diffusion tensor imaging (DTI) vs the fibre orientation distribution calculated by constrained spherical deconvolution (CSD). Note particularly the differences where the major fibre tracts cross. The third row shows the fractional anisotropy (FA) and mean diffusivity (MD) images calculated from the diffusion tensors, as well as a sample of probabilistic tractography of the corticospinal tract calculated using the FOD. Results of whole brain voxel-based between group analysis of FA also shown.
From the underlying model results may be extracted directly from voxels in regions of interest (ROIs), or a whole brain approach may be applied similar to the voxel based analysis outlined above (example shown in Figure 1.2, bottom left); alternatively tractography may be employed to model underlying fibres. Tractography is performed by repeatedly seeding in either a specified or random voxel and creating a streamline which propagates in a direction determined using the underlying model in each voxel traversed \(^{35}\). In this way the streamline is thought to approximate water diffusion, and therefore white matter tracts. This can be performed in a whole brain approach or by selecting particular ROIs to investigate tracts relating to these regions, which may be manually or automatically defined. For example, whole brain parcellation (as outlined above, see Figure 1.1) may be used to derive the corticospinal tracts by seeding tractography in the brainstem, and including only tracts reaching the precentral gyrus without passing through either the cerebellum or the thalamus – the bottom right image in Figure 1.2 shows an example of this.

Tractography is either deterministic, in which a single streamline direction is calculated for each voxel, or probabilistic, where a probability function determines the direction of each streamline at each voxel based on the underlying model \(^{32}\). As shown in Figure 1.3, probabilistic tractography allows for crossing streamlines within voxels, while deterministic tractography only accounts for the effect of the largest tract. Where multiple fibres cross within voxels, single ellipsoid tensor orientations are insufficient for tractography, particularly when used in conjunction with a deterministic approach \(^{32}\). Tractography allows 3-dimensional visual rendering of tracts, reporting of streamline numbers as a measure of tract integrity, or reporting of diffusion metrics, such as FA, from the voxels traversed by each streamline \(^{36}\).
1.3. Rationale and Hypotheses

Addressing Heterogeneity

The heterogeneity in CP poses a significant challenge to the design and interpretation of neuroimaging studies. Subjects can be grouped by clinical subtypes, although often an overlap exists (for example a child with prominent spasticity may also have features of dystonia). An alternate way to group subjects is by pathological subtype, which bears some relationship to clinical subtype as discussed below. The most widely used pathological classification system describes lesions as periventricular white matter lesions, cortical or deep grey matter lesions, malformations or non-specific lesions as described above. Each of these subdivisions is itself heterogeneous, and comprises multiple further possible sub classifications. In children with unilateral CP, it is also of interest whether the lesion appears unilateral or bilateral.

It is therefore complex to propose an appropriate study population; in this thesis the subject population included children clinically diagnosed as having unilateral spastic CP (USCP) – i.e. symptoms were limited to one side. Wherever children with multiple lesion types were included in a study (chapter 3), subjects were split into separate groups to allow between group analyses.
Subject Selection

Subjects were prospectively enrolled via the Queensland Cerebral Palsy and Rehabilitation Research Centre in Brisbane, Australia. A standard baseline scan is performed for all children enrolled for an ever increasing number of clinical studies within the centre, spanning across all health science domains including physiotherapy, occupational therapy, psychology, orthopaedics, neurology and others. The number of subjects available for each study was dynamic and increased with time. Subjects were included in multiple studies (including publications not related to this thesis by other researchers); raw data consisted of T1 and diffusion weighted MRI images, pre-processing, processing and analysis of this data differs for each study. Each study utilised a specific automated technique for brain processing, which in all cases mandated exclusion of subjects unable to be processed. As subjects were grouped according to pathological subtype, those subtypes with inadequate numbers for statistical analysis were unable to be included; as children with cortical and deep grey matter lesions have structurally more atypical brains than children with periventricular white matter lesions they were unable to be processed adequately for inclusion in the studies outlines in chapters 4 and 5. There were insufficient numbers of children with uncommon pathological subtypes of brain malformations or non-specific lesions for inclusion in any study. The subjects included are outlined in Table 1.1.

Subjects were identified through a population-based research database. Baseline scans and clinical assessments were acquired prior to any study specific intervention. Selection criteria included age 3-17 years, a confirmed diagnosis of unilateral cerebral palsy, attendance at a mainstream school, and a score of II (two) or below in both the Gross Motor Function Classification System (GMFCS) \(^{37}\) and Manual Ability Classification System (MACS) \(^{38}\) as assessed by an occupational therapist (scores are given in each study). A range of study specific clinical measures were taken, and therefore different scores were available for subsets of the overall cohort. Clinical scores were included in studies based on specific hypotheses as outlined in each chapter.
Table 1.1. Number of children with unilateral CP included in each study, by lesion type. Note that the same subjects were used in multiple studies; subject recruitment was ongoing over time accounting for difference in numbers.

<table>
<thead>
<tr>
<th>Chapter</th>
<th>CDGM</th>
<th>PWM</th>
<th>Malformation</th>
<th>Non-Specific</th>
</tr>
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</tr>
<tr>
<td>4</td>
<td>Excluded (unable to be processed n=20)</td>
<td>46</td>
<td>Excluded</td>
<td>Excluded</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td>(Excluding 11 unable to be processed)</td>
<td>(insufficient numbers n=2)</td>
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<tr>
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<td>0</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>(Excluding 1 unable to be processed)</td>
<td>(insufficient numbers n=2)</td>
</tr>
</tbody>
</table>

Hypotheses

1. Children with unilateral spastic cerebral palsy comprise a vastly heterogeneous pathological group; common pathological features may be present which contribute to the common phenotype

2. Children with lesions appearing unilateral on structural MRI may show subtle bilateral changes detectable using advanced structural MRI techniques and diffusion MRI

3. A significant correlation can be demonstrated between abnormal structural and diffusion MRI findings and motor function

4. Diffusion MRI may give insight into impact on executive function pathways; diffusion changes may bear correlation to clinical measures of executive function
5. DTI is known to have many pitfalls yet remains widely used in studies of children with CP; superior methods are available and could be readily implemented in this population with a significant and measurable benefit.
Chapter 2. Systematic Literature Review: Diffusion imaging in Cerebral Palsy

2.1. Introduction to Chapter 2

Structural MRI is a well-established tool in CP, and a standard part of the clinical work up for preterm children with suspected brain injury as well as those with motor or executive dysfunction. Several studies have systematically reviewed the literature; detailed classification systems have been devised based on these studies \(^2,^{15,39}\).

Diffusion MRI is a relatively novel technique, and since its rapid expansion into both research and clinical practice, multiple studies have explored the microstructural changes present in CP using this technique. There is marked heterogeneity in subject selection, imaging acquisition parameters and processing techniques, brain regions studied, analysis techniques and interpretation of findings. Consolidation of these results is therefore complex, particularly where conflicting results and interpretations arise.

The purpose of this review is to address this problem, and systematically identify as many studies as possible where diffusion MRI has been used in subjects with CP. Analysis of collated data includes common findings amongst studies; conflicting findings between studies; identification of brain regions and tracts requiring further study; review of methodology across studies and subsequent recommendations for future studies.

The proceeding manuscript was up to date at the time of publication in 2012. Since then there have been significant advances in diffusion MRI, and multiple subsequent publications which would meet inclusion criteria for this study. An updated review (conducted in 2016) is therefore included as chapter 6.
2.2. Chapter 2 Publication


Cerebral palsy (CP) is a heterogeneous group of non-progressive brain pathologies manifesting as motor, sensory, cognitive, and communication difficulties. It impacts an estimated 1 to 2.5 per 1000 live births in Western countries. There is no cure for CP; current research is aimed at prevention, early detection, and rehabilitation. The pathological features of brain development in this group of disorders have been repeatedly shown to correlate with functional and clinical outcomes for impaired children. Although the clinical features of CP allow some insight into the timing and aetiology of the insult, neuroimaging can provide additional information about the location and extent of injury. Multiple neuroimaging modalities are used both clinically and for research to probe the structural integrity of various parts of the brain in vivo. Conventional structural magnetic resonance imaging (sMRI) is the most widely used tool for assessing gross brain pathology in CP. Brain pathology can be described as being normal, maldeveloped, having predominately grey matter lesions, periventricular white matter lesions, or not fitting any of these descriptions, as described by Krageloh-Mann. There are limitations of the use of sMRI for diagnosis and classification of children with CP. Systematic reviews have shown 14 to 17% of children with functional impairment have no abnormality on T1- and T2-weighted MRI. This is largely due to the inability of sMRI to probe the microstructural integrity of the underlying brain tissue. There is consequently sound motivation to investigate more advanced, non-invasive imaging techniques to improve our understanding of the relationship between brain structure and function in children with CP.

Diffusion MRI (dMRI) uses imaging sequences that are sensitive to the motion of water molecules. In this approach, diffusion anisotropy is measured along several orientations, with earlier studies typically using six directions. The introduction of improved imaging hardware and image analysis technology has enabled more accurate measures of diffusion, with some studies using up to 64 diffusion-encoding directions. Although the resolution of dMRI is dependent on the acquisition sequence and scanner performance, the typical resolution of image voxels ranges from...
sizes of 2 to 2.5mm$^3$. The most common method used to study diffusion processes in the brain is diffusion tensor imaging. In this case, the diffusion of water is mathematically modelled using a tensor approach, whereby the shape of the resulting diffusion ellipsoid is described by the resulting primary eigenvectors and eigenvalues $^{30}$. From these indices, quantitative measures of diffusion anisotropy, such as the fractional anisotropy (FA), can be calculated $^{28}$. This summary measure, along with other quantitative diffusion indices such as the directionally averaged mean diffusivity (MD; also referred to as average apparent diffusion coefficient, abbreviated as mean diffusivity, $D_{av}$ or ADC$_{ave}$) have proved useful for probing the integrity and development of white matter pathways in the brain $^{49}$. The value of FA ranges between 0 and 1, which gives an indication of how uniform (anisotropic) the directionality of diffusion of water molecules is within the given voxel $^{28}$. MD is calculated by taking the mean of measured apparent diffusion coefficients from each direction measured in a voxel $^{49}$. It is therefore important to know both of these values as they confer different findings. For example, a tract may have minimal diffusivity, but still have a high anisotropy if the diffusion is unidirectional. Conversely, a tract may have high diffusivity, but a low anisotropy if the diffusion is more isotropic. Specific types of white matter pathology are known to have specific diffusion ‘signatures’: for example Wallerian degeneration shows decreased FA with little change in MD $^{31}$.

These measures are typically reported for one or more particular region of interest (ROI), normally drawn manually on sMRI or FA colour maps using standardised guidelines, or, in more recent studies, using an automated processes involving novel brain atlases $^{50,51}$. Diffusion tensor imaging can also be exploited to map the trajectories of white matter fibre tracts in the brain using tractography-based algorithms $^{35}$. Using this approach, specific white matter pathways can be independently investigated by tracking the coherent diffusion properties in adjacent voxels using a streamline framework $^{49}$ (see Figure 2.1). Currently, this imaging strategy is the only non-invasive method for mapping white matter architecture in the living brain (other methods, such as transcranial magnetic stimulation allow for mapping of cortical areas). Diffusion measures of FA and MD can then be assessed within specific white matter pathways $^{52}$. Additionally, the number of streamlines generated by the tractography algorithm can be reported as a measure of connectivity within the tract $^{53}$. More recent alternatives to the diffusion tensor model have been shown to be reliable and reproducible $^{54}$. These approaches, which use optimised acquisition schemes such as high angular resolution diffusion imaging (HARDI) $^{55}$ and higher-order modelling
of diffusion anisotropy, allow improved resolution of crossing fibres within each voxel, thus affording more accurate estimations of tract injury or plasticity within corticomotor networks.\textsuperscript{12} Several excellent review articles have been published which outline the latest techniques for resolving crossing fibres using dMRI\textsuperscript{32, 56-58}.

Figure 2.1  Anisotropy and tractography maps from a participant with right congenital hemiplegia.

Representative images showing (a) fractional anisotropy, (b) mean diffusivity, (c) colour-coded fractional anisotropy (green, tracts projecting in an anterior–posterior orientation; red, left–right; blue, superior–inferior orientation), and (d) corticospinal tracts for a participant with right hemiplegia. The corticospinal tractography map was generated using a fully automated image processing pipeline.\textsuperscript{48} R, right; L, left.

The use of dMRI and tractography in CP is a novel way to elucidate further insights into its pathogenesis. This allows specific insights into white matter anatomy, and identification of tracts with altered integrity compared to the brains of typically developing children. The corticospinal tract (CST) was classically thought to be the primary tract involved with functional motor impairment following autopsy studies in the early 1960s\textsuperscript{59}. This was challenged by Hoon et al. in 2002 with the proposal that connectivity in ascending sensory pathways may be more compromised than descending motor pathways\textsuperscript{60}. Consequently there have been many studies probing the integrity of several tracts in different types of CP, both ascending and descending, with several studies finding that sensorimotor thalamic pathways may have more influence on sensory
and motor function than descending corticomotor pathways. There has been increasing interest in correlations between tract damage and clinical outcomes, as this information may hold prognostic value, and guide specific early therapy. Clinical correlations investigated typically include one or more of global motor function, including standardized schemes such as the Gross Motor Function Classification Scale (GMFCS) and upper limb motor and sensory function.

The aim of this review is systematically to identify and integrate the evidence provided by cross-sectional cohort studies and case-control studies using diffusion imaging in children with CP, and therefore elucidate which white matter tracts are most likely to be involved with a quantitative clinical deficit. This information may be of use to guide early intervention and therapy targeted towards infants at risk of developing CP, which has been repeatedly shown to be effective, as well as improving quality of life for these children.

**Methods**

**Search terms**

A literature search was conducted of relevant databases (Pubmed, Embase, Cinahl, Scopus, and Psycinfo) on 29 February 2012 for the keywords ‘cerebral palsy’ and any of ‘tractography’, ‘diffusion imaging’, ‘diffusion magnetic resonance imaging’, ‘diffusion tensor imaging’, ‘high angular resolution diffusion imaging’, or ‘diffusion weighted imaging’ (including associated acronyms). Only peer-reviewed publications in English were considered. A full protocol is available in Appendix II.

**Inclusion and exclusion criteria**

Studies were included if they met the following criteria: (1) the study type was a cross-sectional cohort or case–control study; (2) at least one participant group were clinically diagnosed as having CP; and (3) diffusion MRI (either dMRI, diffusion tensor imaging, or newer techniques) had been performed on all included participants, with or without tractography. Consequently, studies were excluded if they (1) did not pertain solely to CP, (2) were studies of acute or traumatic brain injury, or (3) were studies of animal models. All studies meeting these criteria were included, with any potential bias due to technology, methodology, or participant selection being reported and discussed below. Single case studies were not included as methodologies, equipment, and reported
variables are extremely heterogeneous, and therefore a comparison between groups is only valid within each specific study protocol.

**Data extraction**

Data were extracted independently, with reference to imaging parameters used (magnet strength, B value, number of directions used), methodology used (diffusion tensor imaging, HARDI, tractography, tract-based spatial statistics, method of ROI placement), participants and controls (subtypes of CP, age, gender, number of participants/controls), clinical measures used, ROIs or white matter tracts included, and finally imaging parameters reported with statistical power between groups. Results were then categorized into descending corticomotor tracts, ascending sensorimotor tracts, and commissural and association tracts. As hemiplegia was studied in significantly more detail than any other subtype of CP, all asymmetry findings in hemiplegia were additionally reported. As results across different scanners, varying acquisition parameters, and heterogeneous analysis methodologies are not directly comparable, a meta-analysis was not possible. The most valuable data from each study are therefore the statistical significance of any differences noted between groups or hemispheres within each study. Statistical $p$ values have been included where reported. The method for calculation of these values varies within each study according to methodology. For instance, tract-based spatial statistics uses a cluster-based statistical analysis within the analysis software $^{63}$, whereas ROI or tract-based analysis is largely study specific, and may require corrections for multiple comparisons depending on variables analysed. As such, comparison of $p$ values between studies is a significant challenge and stated $p$ values should be interpreted cautiously.

**Results**

The initial search on 29 February 2012 returned 162 unique results. Of these, 20 studies met strict inclusion criteria. Two further peer-reviewed articles $^{48, 64}$ that did not occur in the initial search but were known to the authors also met inclusion criteria. Details of the included studies, including limitations, are outlined in Table 2.1.
Table 2.1. Details of included papers.

<table>
<thead>
<tr>
<th>Study</th>
<th>Methods</th>
<th>Subjects</th>
<th>Other</th>
<th>Limitations</th>
</tr>
</thead>
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<td></td>
<td>Group, Year</td>
<td>Journal</td>
<td>Imaging, Analysis</td>
<td>n</td>
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<td>Arzoumanian et al 2003</td>
<td>1.5T B: 1000</td>
<td>American Journal of Neuroradiology</td>
<td>FA and MD analysis of manually drawn ROIs (using FA and MD maps)</td>
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<td></td>
<td>6 dirs</td>
<td></td>
<td></td>
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<td>Pediatrics</td>
<td>Symmetry of manually drawn ROIs (using FA colour maps) in axial planes</td>
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<td>Chang et al 2012</td>
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<td>Neuroscience Letters</td>
<td>FA and MD analysis of manually drawn ROIs (using FA colour maps) and tracts (using probabilistic tractography)</td>
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<td>Faria et al 2011</td>
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<td>NeuroImage</td>
<td>Automated parcellation of CP brain anatomy using diffusion properties</td>
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<tr>
<td></td>
<td>31/34 dirs</td>
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<td>Faria et al 2010</td>
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<td>Hoon et al</td>
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<td>1.5T B: 600 32 dirs</td>
<td>FA, MD and asymmetry analysis of manually drawn ROIs (using FA colour maps) and tracts (using deterministic tractography)</td>
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**Note:** CP = Cerebral Palsy, GMFCS = Gross Motor Function Classification System, FA = Fractional Anisotropy, MD = Mean Diffusivity, UL = Upper Limb, CST = Corticospinal Tract, TBSS = Tract based spatial statistics.
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<th>Analysis</th>
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<td>2</td>
<td>Hemiplegia</td>
<td>3y-6y (4y6m)</td>
<td>1M</td>
<td>6 Ctrl (GF)</td>
<td>↓ FA and ↓ streamline count in affected CST of CP group 2-5 years prior to clinical diagnosis of CP</td>
<td>Limited Subject numbers&lt;br&gt;No ROI reliability analysis</td>
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<td>Hemiplegia</td>
<td>12y-16y (14y)</td>
<td>2F</td>
<td>5 None</td>
<td>↓ Streamline count in CST, corticobulbar tract and superior thalamic radiation in contralateral side. ↑ Streamline count in ipsilateral corticobulbar tract.</td>
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<td>?</td>
<td>9F</td>
<td>14 30M</td>
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<td>Quadriplegia</td>
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<td>10 GMFCS, Other (GF)</td>
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<td>Hemiplegia (n=8) Diplegia (n=19) Quadriplegia (n=6)</td>
<td>5m-8y (2.17y)</td>
<td>14F</td>
<td>21 20M</td>
<td>GMFCS correlated with both motor and sensory parameters</td>
<td>Heterogeneous CP group&lt;br&gt;No ROI reliability analysis&lt;br&gt;Use of GMFCS as continuous scale</td>
</tr>
</tbody>
</table>
**Imaging parameters**

Most studies (95%) reported findings using the diffusion tensor model. Only one study used high angular resolution diffusion imaging with a higher-order diffusion model. Thirteen studies (59%) used tractography and one study (5%) used tract-based spatial statistics to probe the integrity of white matter pathways. Three studies (14%) used 3T MRI scanners, whereas the remainder (86%) used 1.5T MRI scanners. Included studies used B values ranging from 500 to 3000s/mm² (median 850s/mm²; mode 1000s/mm²). Diffusion was measured in several directions ranging from six to 60 (median 23; mode 32). Of the 20 studies (91%) that used ROI placement, 17 (77%) used manual placement, whereas three (14%) used automatic parcellation. Only six (35%) of the studies using manual ROI placement included reliability analysis.

**Participants and clinical measures**

All 22 studies included at least one patient group consisting solely of participants with CP. Twenty of these studies (91%) compared this group with a typically developing comparison group. Four studies included comparisons between groups: children with athetosis versus spasticity; different GMFCS levels; and children with quadriplegia versus diplegia. The number of participants ranged from 2 to 45 (mean 17.1). Ages of participants ranged from 5 months to 29 years (only one study included participants over age 18 [Lee et al.]) with mean range being 3 years 9 months to 12 years 0 months, and overall mean age being 7 years 0 months. There were on average 1.6 males to each female. Four studies (18%) included participants with spasticity in any motor distribution; 14 studies (64%) investigated specific motor distributions of spasticity, including seven (32%) of children with spastic hemiplegia; two studies (9%) of children with spastic diplegia; three studies (14%) of children with spastic quadriplegia; one study of children with bilateral spasticity; and one study comparing spastic quadriplegia with diplegia. One study compared participants with athetosis and spasticity; the remaining three studies (14%) included all types of CP or did not specify which subtypes were included. Twelve studies (55%) assessed correlations between imaging and clinical outcomes. Of these, six (50%) assessed GMFCS, three (25%) used other clinical measures of global function, and five (42%) assessed upper limb function.
**Corticospinal Tract**

The neural network most frequently investigated was the CST. Results are summarized in Table 2.2. Eighteen studies (82%) reported diffusion properties of the CST 48, 60, 61, 64-72, 75, 77. All studies reported at least one significant difference between CP and comparison groups. Twelve studies used tractography to assess tract-based FA of the CST 64-72, 75, 77, 79, of which 10 (83%) reported significantly decreased FA in CP 64, 65, 67-72, 75, 79. Five studies used ROI-based analysis of CST at one or more locations 67, 70, 72, 78, 82, of which four (80%) reported significantly decreased FA in participants with CP 70, 72, 78, 82. No studies reported increased FA by either method. Eight studies used tractography to assess MD of the CST 64-66, 68, 70, 71, 77, 79, of which six (75%) reported increased MD in the participant group 64, 66, 68, 70, 71, 79. Three studies used ROI-based analysis to assess MD of the CST 70, 78, 82, of which one (33%) showed increased MD in CP (p<0.001) 82. No studies showed decreased MD. Three studies assessed the volume or cross-sectional area of the CST 75, 76, 81, and all reported a decrease in children with CP. Four studies also reported on the number of tracts generated using tractography 48, 69, 72, 77, and all reported a decrease in the number of tracts in CST of CP groups. Positive correlation was shown between FA of the CST and motor function as measured by GMFCS in participants with spastic diplegia (p<0.03) 73 and spastic quadriplegia (p<0.001) 71 as well as qualitative severity of spastic hemiplegia 64. Additionally, it was shown that children with quadriplegia displayed significantly lower FA and higher MD than children with diplegia (p<0.025) 79. One study looking at all motor distributions of spasticity in CP showed GMFCS correlated well with FA of the CST when assessing ROI-based FA (p<0.001) but not tract-based FA 72. Only one study, which included all types of CP, found no significant correlation between CST diffusion parameters and sensory or motor outcomes 61. This study used a qualitative scheme to assess tract integrity, whereas all conflicting studies used quantitative measures. It was shown in spastic quadriplegia that FA of the CST is increased after treatment with botulinum toxin A and physiotherapy (p<0.001) 82.
Table 2.2. Diffusion properties of the corticospinal tract.

Number of tracts, number of tracts generated using tractography; CP>comparison, significantly higher value in group with cerebral palsy; CP=comparison, no significant difference between comparison and cerebral palsy groups; CP<comparison, significantly lower value in group with cerebral palsy. CP, cerebral palsy.

<table>
<thead>
<tr>
<th>Method</th>
<th>Measure</th>
<th>CP&gt;Control</th>
<th></th>
<th></th>
<th>CP=Control</th>
<th></th>
<th></th>
<th>CP&lt;Control</th>
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<tbody>
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<td></td>
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<td>n</td>
<td>N</td>
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<td>n</td>
<td>%</td>
<td>N</td>
<td>%</td>
<td></td>
</tr>
<tr>
<td>ROI</td>
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<td>1</td>
<td>20%</td>
<td>4</td>
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<td></td>
</tr>
<tr>
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<td>MD</td>
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<td>1</td>
<td>33%</td>
<td>2</td>
<td>67%</td>
<td>0</td>
<td>0%</td>
<td></td>
</tr>
<tr>
<td>Tractography</td>
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<td>2</td>
<td>17%</td>
<td>10</td>
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<tr>
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<td>MD</td>
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<td>5</td>
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<td>25%</td>
<td>0</td>
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<tr>
<td></td>
<td># Tracts</td>
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<td>0</td>
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<td>3</td>
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</tbody>
</table>

Other descending/corticomotor tracts

Other descending tracts studied included the corticobulbar tract and the posterior limb of the internal capsule (PLIC). Three studies (18%) reported diffusion properties of the corticobulbar tract 70, 71, 76. One of these assessed volume only, reporting a decreased volume in CP 76. The other two studies both reported a reduced FA and increased MD in CP 70, 71, consistent with findings in the CST. Three studies showed significantly reduced FA in the PLIC using ROI-based analysis 65, 72, 78. One of these also reported an increased MD ($p=0.002$) 65. No studies reported any findings to the contrary; however, one study assessed qualitatively that the PLIC was relatively intact compared with other tracts 76. The FA of the PLIC was not different between participants with athetosis and spasticity; however, MD was significantly increased only in the group with athetosis 78. FA in the PLIC was shown to correlate with upper limb dexterity as measured by the Box and Blocks test ($p=0.027$) 65.
Ascending sensorimotor tracts

Two studies demonstrated significantly decreased FA in the posterior thalamic radiations (PTR) of participants with CP. In addition two studies noted the PTR to be among the most injured tracts qualitatively. One study found no difference in FA or mean diffusivity between controls and children with hemiplegia, and another showed no difference in FA, MD, or number of tracts between controls and children with diplegia. Two studies compared clinical correlations between the PTR and CST. One study showed PTR injury to be correlated with both sensory and motor outcomes including proprioception (right side only) and ambulation, while finding no correlation with CST injury. Another found ROI-based (but not tract-based) FA of both the CST and PTR to correlate with GMFCS; however, CST was found to be the stronger predictor. Two studies reported on the superior thalamic radiations, both reporting an increased MD. One study reported no change in FA, whereas the other reported decreased FA only in GMFCS level V (highest impairment: ‘transported in a manual wheelchair’) (p<0.05).

Commissural and association tracts

Five studies assessed the diffusion properties of the corpus callosum. Results were conflicting, with two studies showing no significant difference between controls and CP. (One of these studies did show a difference on ROI-based analysis but not on tract-based analysis; the other did not show a significant difference by either method.) Of the remaining studies, one reported significant qualitative damage to the corpus callosum, whereas the other two both reported reduced FA and increased MD compared with controls. Participants with athetosis had further reduced FA and increased MD in the right genu of the corpus callosum compared with spasticity. One study looked particularly at transcallosal motor fibres, and showed reduced FA and increased MD, more pronounced than in the CST. One study showed that FA of the corpus callosum decreased with increasing global impairment as measured by GMFCS. Two studies assessed association fibres; the other showed significantly reduced FA and increased MD in the superior longitudinal fasciculus, significantly more pronounced in children with athetosis than those with spasticity. The inferior longitudinal fasciculus was not significantly
different from controls, with the exception of reduced FA on the left side only in the children with athetosis ($p=0.01$).

**Spastic hemiplegia**

Four studies used diffusion tensor imaging to identify the CST and assess symmetry of properties between ipsilateral and contralateral tracts in spastic hemiplegia \(^{48, 64, 68, 81}\). Asymmetry was demonstrated in cross-sectional area \(^{81}\), FA \(^{64, 68}\), MD \(^{68}\), and number of tracts \(^{48}\). Asymmetry was shown to have significant correlation with upper limb sensory function as measured by stereognosis ($p<0.001$), dexterity ($p=0.009$), and manual ability as measured by the ABILHAND-kids rating system ($p=0.014$) \(^{81}\) as well as overall qualitative severity of hemiplegia \(^{64}\). One study showed no significant correlation between CST asymmetry and impaired limb function but did show significant correlation with sensorimotor thalamic projections ($p=0.0061$) \(^{48}\). A correlation was shown between FA in the contralateral CST and upper limb dexterity as measured by the Box and Blocks test ($p=0.007$) \(^{65}\). A compensatory hypertrophy of the ipsilateral tract was not demonstrated \(^{65}\); however, an increased FA was found in the ipsilateral tract compared with controls \(^{70}\). One study showed in two participants that decreased FA and fibre count in the CST in early childhood were present before manifestation of clinical symptoms \(^{69}\).

**Discussion**

**Affected tracts**

Our systematic review confirmed that both descending corticomotor and ascending sensorimotor tracts are involved in the pathogenesis of CP and both are clinically significant (Figure 2.2 outlines studies for and against involvement of particular tracts). It is not yet possible to say which is more significant; however, descending corticomotor tracts have been studied more comprehensively. Evidence for commissural and association fibre involvement is conflicting, most likely because of the small number of studies published so far that target these pathways. Further studies are warranted to elucidate fully how these networks are impacted upon in CP.
Figure 2.2. Evidence for and against involvement of ascending, descending, and other tracts in cerebral palsy.

The CST carries fibres from the motor cortex to the spinal cord, and was first suggested in 1962 to have involvement in spasticity. It is no surprise that with the advent of diffusion imaging the CST is the most frequently assessed tract in children with CP. Results repeatedly showed decreased FA and increased MD within this white matter pathway. Such consistent findings suggest a decrease in the integrity of the CST compared with typically developing children. Further evidence of injury or perturbed early development of this pathway, namely reduced volume and decreased fibre count, has been demonstrated by dMRI and tractography studies. The prognostic use of this information is highlighted by the abnormal results preceding the clinical outcomes, and interestingly the compensatory changes after physical therapy and peripheral muscle botulinum toxin A administration. Other important descending corticomotor tracts are...
the corticobulbar tract, which also shows reduced FA and increased MD, and regions within the PLIC which show reductions in FA, and normal or increased MD.

The findings for the ascending sensorimotor pathways were more varied. The PTR was investigated in several studies as a sensory tract. The PTR connects the thalamus to the posterior parietal and occipital cortices. The posterior parietal cortex is involved with complex upper limb function and visuospatial performance \(^{83}\), consistent with the finding that damage to the PTR correlates with GMFCS and both motor and sensory function. The STR connects the thalamus to the somatosensory cortex. This pathway was shown to have increased MD as well as decreased FA only in severe cases. One recent study also showed statistically significant correlations between this pathway and upper limb function in spastic hemiplegia \(^{48}\), which were not present in the CST. Combined, these findings highlight the importance of preservation of ascending sensorimotor networks in motor function, and provide new insight for the design of new neurorehabilitation therapies, which may enhance sensory pathways.

The evidence for involvement of the corpus callosum is conflicting, but suggests involvement with a similar pattern of reduced FA and increased MD, correlated with clinical severity. The transcallosal motor fibres in particular have been shown to be involved in participants with spastic diplegia. Further research is warranted in this area.

**Correlations with clinical measures**

Over half the included studies assessed correlations between clinical measures and imaging parameters. The most frequently used assessment to compare with imaging indices was the GMFCS. Although this was shown to correlate with corticomotor, sensorimotor, and commissural tracts, interpretation of these results is difficult, as it is a classification system based on five categories, not a continuous scale. A structure–function correlation cannot be established using this measure, rather a comparison between groups. There were fewer studies that used clinical measurements with continuous variables, and among those that did there was little consistency across studies. Use of standardized continuous clinical measures with well-documented reproducibility, such as the Melbourne Unimanual Upper Limb assessment of unimanual capacity \(^{84}\), and Assisting Hand Assessment of bimanual coordination \(^{85}\), would enhance interpretation of multiple studies and allow for meta-analyses in the future.
Differences between subgroups

Only two of the included studies have looked at differences between different subtypes of CP. Both studies have shown significant differences in both FA and MD in specific tracts between different CP groups. Children with athetosis showed similar but more exaggerated changes than those with spasticity. Within the spasticity subtype of CP, children with quadriplegia showed changes in specific parts of the CST that were not evident in children with diplegia. These studies highlight the need to reduce heterogeneity of CP groups within dMRI studies in order to allow highlighting of specific changes and locations which may correlate with pathogenesis of each individual subtype. Few studies have assessed children with athetosis, as dystonia is infrequent, and further dMRI studies looking at correlations with clinical measures are likely to reveal further insight into the pathogenesis of this condition.

Study design

Study design of diffusion imaging studies in the research domain is extremely important to allow meaningful interpretation of results. Owing to the heterogeneity of acquisition parameters and analysis techniques, results are not directly comparable between studies. For a result to be meaningful, each study must compare ‘affected’ regions with some sort of control. Most easily interpreted is a comparison with typically developing controls; however, comparisons between ipsilesional and contralesional hemispheres in children with hemiplegia, or between different subtypes of CP, have also been used.

Limitations and methodology

The manual definition of ROIs for the analysis of diffusion anisotropy indices has limitations due to the high operator dependency. Only 35% of included studies using manual ROI placement included reliability analysis. Therefore both the accuracy and repeatability of the remaining studies may be compromised. Additionally, the use of qualitative and semi-qualitative measurements for reporting used in some studies adds a second element of operator dependence. Ideally quantitative metrics, such as streamline count, FA, and MD should be reported, such that repeatability is maximized. Two included recent manuscripts have reported an automated approach to define ROIs. The major focus of these studies was to introduce an atlas-based framework for investigating brain pathology in CP. Such an approach has significant potential for more in-depth assessment of
important structure–function relationships. Automated whole-brain analyses are essential to investigate neural networks in CP other than corticomotor pathways, such as those associated with executive function, which has been shown to be impaired in children with CP in recent neuropsychological studies \(^{86-88}\). Neither automated parcellation nor manual ROI placement take individual variation in functional anatomy into account. To account for these, some recent studies (not involving CP) have seeded tractography studies using regions localised to each individual participant with functional MRI \(^{89}\) or transcranial magnetic stimulation \(^{90}\).

The conflicting findings in some corticomotor regions are likely to be a reflection of the heterogeneous natures of both the underlying pathology and the neuroimaging methodologies used. Ideally studies with large numbers of participants with specific subtypes of CP could provide more convincing evidence for specific tract involvement. The acceptance of standardized acquisition imaging protocols for dMRI would also progressively build knowledge and progress our understanding of CP pathology. Analysis methods such as those introduced by Faria et al. \(^{74, 75}\) would help establish a more generalized framework for analysis of dMRI data.

A significant proportion of the dMRI studies so far have made use of anisotropy information gained from using the tensor model. A limitation of the tensor model is its inability to resolve crossing fibres in complex white matter populations \(^{32}\), which has significant ramifications for the accuracy of tractography derived diffusivity measures. This presents a significant challenge in dMRI studies as it has been estimated that crossing fibres are present in at least two-thirds of brain voxels with FA > 0.2 \(^{91}\). Within these anatomical locations, the clinical interpretation of diffusivity measures is difficult, as voxels containing highly organised crossed white matter networks can exhibit paradoxical reduced anisotropy \(^{32}\). Higher-order models of diffusion have been developed to overcome this limitation and will provide improved insight into white matter injury and neural reorganization in CP.

Although dMRI studies play a useful role in clinical management of CP \(^{91}\), the full potential of this technology remains largely in the research domain. When sMRI is already indicated, adding dMRI sequences to the scan will add only a few minutes to the overall scan time. The major limitation is that for any quantitative analysis of the data, including tractography which is mandatory for any connectivity analysis, offline analysis needs to be performed. As highlighted by the studies
reviewed here, the preferred methodology for offline analysis is heterogeneous, and as such there are as yet no standards by which results can be reliably interpreted.

Conclusion

Diffusion imaging studies in CP are providing new insight into the specific injury and reorganization of white matter motor pathways. Small sample sizes and heterogeneous imaging acquisition and analysis strategies have impacted on the clinical interpretation of findings. Given this constraint, there is corroborating evidence showing that decreased FA and increased MD within descending corticomotor tracts, particularly the CST, are useful measures of white matter tract integrity, which correlate with measures of clinical severity of CP. There is also evidence to suggest that diffusion changes in ascending sensorimotor tracts, in particular the PTR, might provide novel information about corticomotor reorganization in CP. The link between these findings and motor function has been established, but less thoroughly investigated in larger clinical populations. Evidence for involvement of commissural and association fibres is limited and conflicting. There are no data on involvement of frontal, temporal, and occipital lobes. Although spasticity in varying motor distributions is well studied, children with athetosis are generally undersampled, and have been shown to have significantly different diffusion properties from those with spasticity in many brain regions. It may be of value for future studies to consider these types of CP separately.

2.3. Conclusion to Chapter 2

The literature review revealed sound evidence of corticospinal tract damage, which appears to bear good correlation to motor outcome. Damage to thalamic tracts was also evident and clinically important, however, studies these findings were relatively novel and less consistent amongst studies. Executive function domains were not yet researched using diffusion MRI. To try and identify particular areas of interest, a whole brain grey matter and white matter analysis was devised, with specific attention to the thalamus on grey matter analysis. Furthermore a tractography study was designed with specific attention to executive function networks.
Chapter 3. Automated Structural MRI Analysis

3.1. Introduction to Chapter 3

Cerebral palsy is a diagnosis made clinically, based on a set of symptoms and signs which represent a common endpoint for several discreet and heterogeneous pathological entities. This presents a significant barrier to the use of the clinical phenotype as a selection criterion for neuroimaging studies. Many studies recruit subjects based on their underlying pathology rather than their clinical picture. Therefore a disparity exists between many clinical studies and neuroimaging studies, making the link between structure and function difficult to interpret.

As outlined in Chapter 1, structural MRI has been well studied and summarised in CP, and diffusion MRI is a tool which is being increasingly utilised to explore the microstructural changes in these children. Prior to assessing microstructural white matter changes using diffusion MRI, first an understanding of gross morphology was sought, in particular of the cerebral cortex, thalamus and basal ganglia. While qualitative and semi-quantitative classification systems of structural MRI are widely used, there has been limited use of fully automated quantitative analysis.

This study aims to address these concerns using two independent fully automated quantitative approaches to assess both the cortex and basal ganglia in children with unilateral CP. The study group was selected based on clinical phenotype, and therefore the heterogeneous underlying pathological features allow identification of commonalities and differences between children with different underlying pathologies. The results of this study will be important to aid the methodology for subject selection and interpretation of results for future neuroimaging studies in unilateral CP.

3.2. Chapter 3 Publication


Cerebral palsy (CP) is a heterogeneous group of non-progressive brain injuries manifesting primarily as motor impairments. The diagnosis is made clinically, based on common motor
phenotypes: spasticity, diskinesia and ataxia \(^{42}\). Underlying the phenotype are heterogeneous pathological features, which relate to the timing and aetiology of the insult: brain malformations originating in the first or second trimester; white matter lesions, typically arising in early in the third trimester, most often in preterm children; and grey matter (GM) lesions typically arising late in the third trimester \(^{15}\).

Children with GM lesions have more severe motor impairment than other subtypes \(^{2}\), particularly when both the thalamus and basal ganglia are involved \(^{92}\). This may be due to reduced potential for plasticity within thalamocortical projections compared with corticomotor projections \(^{93}\). Thalamic changes are not limited to children with primary GM lesions; reduction in the thalamic volume without morphological or organisational change is also seen in children with periventricular white matter lesions \(^{94}, 95\).

Unilateral CP (UCP) is a clinical subgroup caused by either unilateral or bilateral lesions. Up to half of children with UCP have bilateral lesions (primarily children with PWM lesions), despite having unilateral clinical features \(^{16}, 92, 8\).

Quantitative assessment of GM using MRI can be carried out using several techniques. Application of such techniques to abnormal brain images poses a significant challenge. Voxel based morphometry (VBM) is one approach, involving registration of subject brains to a standard space allowing groupwise analysis of GM within each voxel \(^{26}\). VBM is most reliable where the grey-white interface is distinct, and signal to noise ratio is high, particularly the cortex. Cortical motor and sensory as well as thalamic and basal ganglia changes have been identified in children with spastic diplegia using this technique \(^{73}\). VBM is less reliable in deep GM, particularly the thalamus, where the grey-white interface is less distinct \(^{96}\). An alternative approach is segmentation of specific structures using probabilistic deformation of predefined structural templates \(^{50}, 96\). This technique relies on statistical mapping to predefined models, and therefore also becomes less reliable where brain morphology is abnormal. Whole brain surface-based parcellation techniques are often unable to adequately process images with abnormalities interfering with cortical continuity, such as middle cerebral artery infarcts or schizencephaly. One solution is to analyse only the subcortical structures \(^{96}\).

The aim of this study was to utilise these automated techniques to quantify the extent of GM change in children with UCP, and specifically to compare subcortical structure volumes and
morphology between children with secondary subcortical damage due to PWM lesions and children with direct subcortical damage from CDGM lesions. We hypothesise that subcortical structures, in particular the thalamus, will be reduced in volume similarly across all subtypes of UCP, despite heterogeneous aetiology. To assess GM changes in children with UCP, we employed two techniques. To analyse cortical and subcortical differences between groups we used VBM. For a more robust analysis of the deep GM structures we additionally employed a volumetric segmentation analysis.

Methods

Participants

Participants included 72 children with UCP (33 left, 39 right hemiparesis), all of whom were recruited for clinical studies requiring baseline MRI scans and clinical assessment prior to intervention. Inclusion criteria were children with a diagnosis of UCP, attending mainstream school and able to ambulate unaided. Children included were aged 5 years 1 month to 17 years 1 month (median 10 years 7 months), and included 31 females, 41 males. All children were assessed by an occupational therapist using the Gross Motor Function Classification Scale (GMFCS) \(^{97}\) (I=46, II=26) and Manual Ability Classification System (MACS) \(^{38}\) (I=29, II=43). Nineteen children with typically development, without brain pathology or indication for imaging were also recruited for the MRI protocol (Age range 7 years 8 months to 16 years 4 months; median 11 years 2 months; 11 females, 8 males). The Jebsen Taylor Hand Function Test (JTHFT) was used to assess both dominant and impaired hand function in 64 of the children with CP (30 left, 34 right hemiparesis). This is a unimanual measure of speed and dexterity in everyday tasks where lower scores represent faster performance in seconds \(^{98}\). The study was approved by the Ethics in Human Research Committees at the Royal Children’s Hospital, Brisbane and The University of Queensland, Brisbane (EHREC 41 and RCH 37). All parents/guardians gave written informed consent, and children gave verbal assent. Participants were given the opportunity to attend a mock scanner prior to imaging.

Image Acquisition

High-resolution structural images were acquired for each participant using a 0.9mm\(^3\) isotropic 3D T1-weighted magnetization-prepared gradient-echo (MPRAGE) sequence in a 3T MRI scanner.
(Siemens, Erlangen, Germany). The acquisition parameters were: FOV 24x25.6x17.6cm, TR/TE/TI 2300/2.26/900ms, flip angle 9°, with 0.9mm isotropic resolution.

**Image Classification**

Images were qualitatively assessed by a child neurologist (SF) according to a qualitative system as either cortical or deep grey matter lesions (CDGM), periventricular white matter lesions (PWM), brain malformations (BM), or miscellaneous. Each lobe and deep GM structure in each hemisphere was then systematically assessed for abnormalities, allowing a classification of each lesion as either unilateral (i.e. all abnormalities constrained to one hemisphere) or bilateral. For simplicity, we use the terminology “dominant” and “non-dominant” hemisphere, where the hemisphere contralateral to the side of impairment is considered non-dominant (functional dominance was not assessed in this study, and therefore what we refer to as the dominant hemisphere is not necessarily functionally dominant).

**Voxel Based Morphometry**

Structural images were analysed using FSL-VBM, contained within FMRIB’s Software Library (FSL: [http://www.fmrib.ox.ac.uk/fsl](http://www.fmrib.ox.ac.uk/fsl)). Brain extraction and GM segmentation was performed, followed by non-linear registration to a study-specific GM template in standard space. Templates were created using equal numbers of controls and subjects, with a specific template being created for each analysis. Registered GM images were then modulated using the Jacobian of the warp field, smoothed with an isotropic Gaussian kernel (σ=3mm). Output from each stage was visually assessed. A voxelwise general linear model was employed to account for age and gender. Voxels with significantly different GM concentration between the children with UCP and controls with typical development were identified using a voxelwise permutation based independent-samples t-test within the model, using the “randomise” function within FSL. A similar model was employed to identify voxels in which GM concentration was related to JTHFT scores. All analyses were adjusted for multiple comparisons across space using threshold-free cluster enhancement (TFCE), considered significant at corrected $p<0.01$. We use the terminology “GM concentration”, which refers to the likelihood of a voxel containing GM, not a physical property of the underlying GM.
Subcortical Parcellation and Analysis

Deep GM structure segmentation was performed using FMRIB’s Integrated Registration and Segmentation Tool (FIRST), contained within FSL. Two stage registration to standard space was performed: 12 degrees of freedom linear transformation followed by a second 12 degrees of freedom linear transformation using a subcortical mask. Segmentation was then carried out using training data included within FSL, derived from 336 subjects, including children and adults with both typical and pathological brains. Volumes of the thalamus, putamen, globus pallidus and caudate were reported. Segmentations were visually inspected in native subject space overlaid on T1 images. Where minor errors were noted, segmentations were manually corrected. Any hemisphere containing a major anatomical error was excluded. To control for brain size, total brain volume was calculated.

Statistical analysis of deep GM volumes was performed using Statsoft Statistica (version 12). General linear models were employed to account for age, gender and total brain volume. JTHFT performance and deep GM volumes were compared between unilateral and bilateral lesions, as well as between lesion types. One way ANOVA was used to identify structures with significantly different volumes between groups. Post-hoc analysis was then carried out on significant structures to elicit differences between groups and hemispheres, using Bonferroni correction. As multiple hypotheses were being tested in this study, results were considered significant at \( p<0.01 \). Relationships between dominant and impaired JTHFT scores and deep GM volumes were analysed using a single general linear model, built using forward stepwise analysis to include only statistically significant variables.

Results

Qualitative Description of Lesions

PWM lesions were observed in 53 children (73.6%). Lesions were unilateral in 32 children (60.4%), and bilateral in 21 (39.6%). CDGM lesions were observed in 17 children (23.6%). 15 of these subjects (88.2%) showed both cortical and subcortical damage, with the remaining two having isolated subcortical damage. Since this latter group was too small to allow for statistical comparisons, the subjects were grouped together in a single cohort. The majority (88.2%) of these CDGM lesions were unilateral, with two children having bilateral involvement (11.8%). Brain
malformations were observed in two children (2.8%), and excluded from further analyses due to insufficient numbers. Details are shown in Table 3.1, including abnormalities within the thalamus and lentiform nucleus (comprising the globus pallidus and putamen).
**Table 3.1. Participant characteristics.**

Number of participants with anatomical abnormalities within the thalamus and lentiform nucleus (putamen and globus pallidus) shown. BM – Brian malformations; CDGM – Cortical and/or deep grey matter lesions; PWM – Periventricular white matter lesions.

<table>
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</tr>
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**Hand Function**

JTHFT scores were not significantly different between children with unilateral (n=21) and bilateral lesions (n=43) ($p=0.017$; Bilateral lesions dominant hand mean: 47.1 [95% CI: 38.1-56.2], impaired hand mean: 187.2 [95% CI: 104.5-269.9]; Unilateral lesions dominant hand mean: 41.1 [95% CI: 36.6-45.6], impaired hand mean: 262.8 [95% CI: 193.0-332.7]). JTHFT scores were significantly different between children with PWM (n=47) and CDGM lesions (n=15) ($p=0.007$; CDGM dominant hand mean: 41.5 [95% CI: 32.3-50.7], impaired hand mean: 376.9 [95% CI: 234.0-519.8]; PWM dominant hand mean: 43.7 [95% CI: 38.7-48.7], impaired hand mean: 196.6 [95% CI: 142.0-251.2]). Post-hoc analysis showed children with CDGM lesions had significantly higher scores (higher impairment) in the impaired hand only ($p=0.010$).

**Voxel Based Morphometry**

The 70 included children with UCP were all satisfactorily registered to respective group specific templates: CDGM (not separated into unilateral and bilateral due to insufficient numbers), unilateral PWM and bilateral PWM. Children with CDGM lesions (n=17) showed widespread unilateral cortical and subcortical changes in both left and right hemiparesis groups ($p<0.01$ corrected). Cortical reduction in GM concentration in children with left hemiparesis was observed in all four lobes in the non-dominant hemisphere, and did not extend to the dominant hemisphere. Deep GM changes were also unilateral, involving the entire caudate as well as globus pallidus and medial thalamus. In children with right hemiparesis (n=10, including 2 bilateral lesions) changes were more pronounced, involving the majority of the caudate, putamen, globus pallidus and thalamus bilaterally, as well as the dorsal anterior cingulate and posterior cingulate bilaterally.

Children with bilateral PWM lesions (n=21) showed minor cortical involvement, with both left and right hemiparesis groups showing reduced GM concentration within the medial temporal cortex of the non-dominant hemisphere. Children with left hemiparesis (n=11) additionally showed reduced GM concentration within the postcentral gyrus and precuneus. In both children with left and right hemiparesis, deep GM changes involved the entire caudate in the non-dominant hemisphere as well as posterior and medial thalamus bilaterally, more pronounced in the non-dominant hemisphere.
GM changes in children with unilateral PWM lesions (n=32) only reached statistical significance in the right hemiparesis group (n=18), showing significant reduction in GM concentration in the caudate bilaterally, as well as unilateral changes in the posterior medial thalamus, putamen and extending into insular cortex.

There were no regions for any analysis where GM concentration was lower in controls than children with UCP. Results are shown visually in Figure 3.1.
Figure 3.1. Results of voxel-based morphometry.

Highlighted regions indicate significantly lower grey matter concentration within a voxel in participants compared with controls with typical development. For display purposes, results are shown on templates created by combining all participants within each group. Axial slices are taken at the levels shown.
No voxels were identified where GM concentration significantly correlated with JTHFT scores for either hand.

**Volumetric Analysis**

**Segmentation**

From 72 children with UCP, two scans (2.8%) were unable to be satisfactory segmented, due to poor registration (n=1) or inaccurate segmentation (n=1). Both excluded children had unilateral CDGM lesions and right hemiparesis. Of the included children, 11 (15.7%) contained unsatisfactory segmentations in the non-dominant hemisphere, and therefore volumes in this hemisphere were excluded (Left hemiparesis: unilateral CDGM n=6, bilateral PWM n=1; Right hemiparesis: unilateral CDGM n=3, bilateral PWM n=1). The caudate was slightly underestimated bilaterally in 7 children (10.0%) and unilaterally in 15 children (21.4%), 12 of which were on the non-dominant side, and was therefore excluded from further analysis. The thalamus was slightly overestimated bilaterally in 1 child (1.4%) and unilaterally in the non-dominant hemisphere in 2 children (2.9%). The putamen was slightly underestimated bilaterally in 1 child (1.4%) and unilaterally in 8 children (11.4%), all in the non-dominant hemisphere. In total, 35 scans (50%) were deemed to be entirely free from segmentation inaccuracies. All inaccurate thalamus and putamen segmentations were manually corrected (see Appendix for details and examples: Supplementary information to ). Examples of included, corrected and excluded segmentations are shown in Figure 3.2. All controls were segmented appropriately with the exception of one child (5.2%), for whom the caudate was slightly underestimated bilaterally.
Figure 3.2. Samples of deep grey volume segmentation in children with unilateral cerebral palsy.

(a) Samples of appropriate segmentation; (b) samples of minor errors, which were manually corrected (arrowheads indicate underestimation, arrow indicates overestimation); (c) samples of segmentation where only one hemisphere was able to be accurately segmented.

Raw volumes and 95% confidence intervals are given in the appendix (Supplementary information to ), as well as shown graphically (corrected for age, gender and total brain volume) in Figure 3.3.
Figure 3.3. Volumes of deep grey matter nuclei structures in children with typical development (CTD) and children with unilateral cerebral palsy.

Sorted by unilateral and bilateral lesions, and separately by lesion type (cortical or deep grey matter lesion [CDGM], periventricular white matter lesion [PWM] or brain malformation [BM]). Vertical bars show 95% confidence intervals, corrected for age, sex, and total brain volume.

Unilateral vs Bilateral Lesions

Dominant hemisphere deep GM volumes were significantly different between controls (n=19) and children with unilateral (n=45) and bilateral (n=25) lesions (p=0.008). Post-hoc analysis showed that compared with controls, children with unilateral lesions had significantly lower volume in the thalamus (p=0.004); children with bilateral lesions had significantly reduced volumes in the thalamus (p<0.001), and putamen (p<0.001). Children with bilateral lesions had significantly reduced volume in the thalamus (p<0.001) and globus pallidus (p=0.006) compared with children
with unilateral lesions (volumes with 95% confidence intervals shown in Figure 3.3). In a sub-
analys of children with unilateral lesions, there was no significant difference in deep GM
volumes between children with CDGM lesions and PWM lesions in either the dominant ($p=0.709$;
CDGM $n=13$, PWM $n=32$) or non-dominant ($p=0.068$; CDGM $n=4$, PWM $n=32$) hemispheres.

Non-dominant hemisphere deep GM volumes were also significantly different between controls
($n=19$) and children with unilateral ($n=36$) and bilateral ($n=23$) lesions ($p=0.001$). Post-hoc
analysis showed both children with bilateral and unilateral lesions had reduced volumes for all
structures compared with controls ($p<0.001$ for all structures and groups). Children with bilateral
lesions had significantly reduced volume in the thalamus ($p<0.001$) compared with children with
unilateral lesions. Volumes and 95% confidence intervals are shown in Figure 3.3.

**CDGM vs PWM Lesions**

Dominant hemisphere deep GM volumes were not significantly different between controls ($n=19$),
children with CDGM lesions ($n=15$) and children with PWM lesions ($n=53$) ($p=0.099$). Non-
dominant hemisphere deep GM volumes were significantly different between controls ($n=19$),
children with CDGM lesions ($n=6$) and children with PWM lesions ($n=51$) ($p=0.001$). Post-hoc
analysis showed that children with CDGM lesions had reduced volumes compared with controls
in the thalamus ($p=0.001$), putamen ($p<0.001$) and globus pallidus ($p<0.001$); children with PWM
lesions had reduced volumes compared with controls in the thalamus ($p<0.001$), putamen
($p<0.001$) and globus pallidus ($p<0.001$). Volumes were not significantly different between
children with PWM and CDGM lesions for any structure.

JTHFT scores did not significantly correlate with volumes for any structure.

**Discussion**

The present study utilised two independent automated analysis techniques to quantify GM changes
between subtypes of UCP. Results identified that cortical changes are minimal in children with
PWM lesions, whilst deep GM changes are not significantly different between CDGM and PWM
lesions. In addition, we have demonstrated that 39.6% of lesions are bilateral on MRI, similar to
previous studies (Cioni *et al* 16: 55% bilateral $[n=91]$; Holmefur *et al* 92: 37% bilateral $[n=27]$). Children with bilateral lesions had significantly reduced thalamic volumes bilaterally compared
with unilateral lesions. In children with unilateral lesions, despite the dominant hemisphere
appearing qualitatively normal, mean thalamic volume was reduced compared with controls. These results highlight the heterogeneity of GM changes that underlie UCP, and the similarities between primary and secondary deep GM nuclei changes.

The cohort for this study consisted of 73.6% PWM lesions, 23.6% CDGM lesions and 2.8% BM. This is a greater proportion of PWM lesions than previous studies (36% \(15\) - 45% \(16\)), likely due to inclusion of only children able to mobilise unaided (GMFCS I and II). Results may therefore differ from children with more severe hemiparesis. CDGM lesions were associated with significantly worse clinical outcomes than PWM lesions, consistent with previous studies \(^2\). Deep GM nuclei volumes were not significantly different between these two subtypes, despite the difference in aetiology. Diffusion MRI has identified reduced integrity of the thalamic radiations in children with CP, correlating with clinical function \(^{102}\). This relationship has been demonstrated in children with UCP and PWM lesions, with preservation of topographical organisation of fibres within the thalamus \(^{95}\). Lesions involving thalamic projections are the most likely cause of secondary reduction in thalamic volume. Similar changes have been observed in children with bilateral impairment due to PWM lesions \(^{94}\). The pathophysiology of these secondary changes to thalamic GM differs from that occurring in CDGM lesions, where the primary lesion directly impacts the GM. Children with bilateral PWM lesions also showed a reduction in GM in the medial temporal lobe, posterior cingulate and precuneus; all within the default mode network, the most highly structurally and functionally connected network within the brain \(^{103}\). Further investigation into the structural and functional connectivity of this network in these children is warranted.

The difference in hand function between children with unilateral and bilateral lesions did not meet statistical significance, similar to previous findings \(^92\).

The population for this study was sufficiently large to demonstrate several key findings, however, we could not comment on the significance of observed changes in the less common subtype of brain malformations. Adequate segmentation of data for volumes within the non-dominant hemisphere in children with CDGM lesions was limited (n=6). The inability to adequately segment highly abnormal brains is a technical limitation, which along with exclusion of the caudate, may cause selection bias towards more typical brains. The requirement for manual correction in a minority of subjects was undertaken to minimise this selection bias, at the cost of reduced
reproducibility. Thalamic volumes for controls were comparable with studies using both manual \cite{104} and alternate automated techniques (Freesurfer) \cite{105} in children of similar ages.

**Conclusion**

The aetiology of brain injury resulting in UCP is heterogeneous. Lesions may appear unilateral or bilateral qualitatively on MRI, however, even in unilateral lesions, deep GM nuclei may be impacted bilaterally. We did not identify a significant difference in function between bilateral and unilateral lesions. Lesions occurring primarily in the PWM appear to cause secondary changes to connected GM structures, particularly the thalamus and basal ganglia, but may also extend to cortical regions. These secondary changes result in similar volume reduction within the deep GM structures to those observed in children with primary CDGM lesions. Children with primary CDGM lesions, however, appear to have significantly worse clinical outcomes, which may be due to more extensive cortical involvement.

**3.3. Conclusion to Chapter 3**

This study highlights the heterogeneity inherent in the clinical diagnosis of unilateral CP. The study assessed primarily two discreet pathological entities: primary grey matter lesions and primary white matter lesions. Each of these groups is also heterogeneous, and could be further subcategorised either anatomically or by aetiology. One of the key findings in this study, however, is the common features across groups, particularly reduction in thalamic volume.

The selection of participants based on clinical phenotype allows clinical interpretation by relating structure to function, however, involves combining discreet pathological entities that exhibit separate anatomical and aetiological features. Studies selecting children based on these criteria should therefore take this into account and include sub-analyses of these groups, requiring larger subject numbers, or only include a specific pathological group.

An additional point highlighted by this study is the difficulty in applying fully automated techniques to abnormal brains. Automated techniques are developed based on subjects with typical development, usually adults, and therefore applying these techniques becomes increasingly difficult with morphological abnormalities, particularly in children. The consequence of this in studies of children with CP is that analysis of children with grey matter lesions are more likely to
be excluded. The study population for this thesis involves children with relatively mild physical impairment (both GMFCS and MACS scores of I or II), and therefore studies including those with more severe impairments would be expected to face increased difficulty for automated analyses.
Chapter 4. Whole Brain White Matter Analysis

4.1. Introduction to Chapter 4

Chapter 3 highlighted the differences in cortical and deep grey matter between clinical subtypes of children with unilateral CP. To elaborate on this, a similar fully automated quantitative analysis is applied in this study to elaborate differences in white matter. The application of fully automated whole brain analysis of diffusion MRI is significantly more complex than structural MRI, as the raw data within each voxel contains a vector of values for each direction (64 in this acquisition protocol), compared with a single scalar value for a T1 weighted structural image. The diffusion tensor model was then used to assign each voxel with a single tensor ellipsoid (i.e. three eigenvalues) which summarise the diffusion within the voxel. Data were then processed to produce a single scalar value, fractional anisotropy (FA), describing the uniformity of diffusion within the voxel. To perform a whole brain analysis we have used a methodology similar to the grey matter VBM analysis described in chapter 3, optimised for FA based white matter analysis. Adequate registration of children with cortical and deep grey matter lesions was unable to be achieved for this study, and therefore only children with periventricular white matter lesions were included.

The aim of this study is to build on the results of Chapter 3: characterising the extent of white matter change in these children; comparing the extent of white matter change between unilateral and bilateral lesions; and demonstrating a link between imaging features and clinical function.

4.2. Chapter 4 Publication


Unilateral cerebral palsy (UCP) is the result of a heterogeneous group of early brain injuries. Unilateral cerebral palsy (UCP) is the result of a heterogeneous group of early brain injuries. The clinical phenotype is primarily defined by motor dysfunction, fitting a pattern of either spasticity, dyskinesia or ataxia; and secondarily by impairments in executive functioning, cognitive reasoning and social interaction. Although the clinical phenotype is unilateral, approximately one third of these children show asymmetrical bilateral brain injury on MRI.
Several common patterns of brain injury have been observed. Each pattern is thought to bear an association with timing of insult. In particular, periventricular white matter lesions are thought to result from hypoxic-ischaemic insult early in the third trimester (which may be perinatal in preterm infants), when oligodendrocyte progenitors are most vulnerable. Such white matter lesions are a combination of both focal periventricular lesions, which may be unilateral, and diffuse components that tend to be more symmetrical. This may lead to secondary grey matter changes that can extend bilaterally even when brain lesions appear unilateral.

Autopsy studies originally identified the corticospinal tract as a key white matter tract impacted upon by periventricular white matter lesions. More recently, insight into the extent of these lesions has been gained from studies using models based on diffusion weighted MRI, such as diffusion tensor imaging (DTI). The Brownian motion of water molecules is restricted by axonal membranes, particularly when they are densely packed in neural tracts. DTI allows voxel derived metrics such as fractional anisotropy (FA - measuring the uniformity of diffusion), and mean diffusivity (measuring net amount of diffusion), which have been described as reflecting underlying neural fibre integrity. In children with UCP due to white matter lesions, these measures have been used to demonstrate multiple impacted regions and tracts, including the corticospinal tracts, thalamic radiations, frontal and parietal regions and corticopontocerebellar tracts. Similar findings have been demonstrated across other clinical and pathological subtypes of cerebral palsy. The clinical significance of these diffusion MRI changes is reinforced by the correlation between imaging derived measures and multiple measures of clinical function; including gross motor function, upper limb function and executive function.

To assess the extent of white matter injury in children with UCP due to white matter lesions, we use a well-documented and fully automated whole brain approach, allowing identification of the extent of impacted white matter in both unilateral and bilateral lesions. Furthermore we aim to identify regions where FA correlates with either impaired hand or dominant hand function. We hypothesise that decreased FA changes extend beyond the periventricular white matter, and may extend bilaterally even where lesions are unilateral. In contrast to a recent whole brain tractography approach in a largely overlapping cohort, the present methodology is aimed at defining specific anatomical regions where white matter is impacted (contrasted with defining specific tracts).
Material and methods

Subjects

A total of 80 children were identified through a population-based research database, recruited for one or more clinical studies requiring baseline MRI and clinical assessment (prior to any study specific intervention). Selection criteria included age 5-17 years, a confirmed diagnosis of UCP, attendance at a mainstream school, classification of either I or II in both the Gross Motor Function Classification System (GMFCS) and Manual Ability Classification System (MACS) as assessed by an occupational therapist or physiotherapist.

From these children, 57 were selected based on MRI brain image classification (periventricular white matter lesions only). T1 weighted images were reviewed by a child neurologist and classified according to the Krägeloh-Mann classification system as periventricular white matter lesions (the remainder included 20 with cortical or deep grey matter lesions, 2 with brain malformations and one child with a normal appearing brain). Of these, 46 children were able to be adequately processed by the software as outlined below. These children were aged 5-17 years, and included 23 with left hemiparesis and 23 with right hemiparesis. Images were assessed by a child neurologist using the sqMRI scale (developed for UCP) to systematically and reliably classify lesions as unilateral (n=30) or bilateral (n=16). The results of this classification as well as bilateral sqMRI severity scores are shown in Table 4.1. Children were assessed using the Jebsen Taylor Hand Function Test (JTHFT), measuring function of both the impaired and dominant hands.

Eighteen children with typical development (CTD) without any clinical indication for brain imaging, were also recruited as controls, aged 7-16.

The Institutional Review Board approved the study and written informed consent was obtained from the parent or guardian of each study participant as well as verbal assent from subjects. All participants were given the opportunity to attend a mock scanner prior to MRI to improve compliance; no sedation was used.
Table 4.1: Participant information.

* One child included as female had Turner’s syndrome

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Image Acquisition

MRI data were acquired using a 3T Siemens TIM Trio scanner (Siemens, Erlangen, Germany). Along with a number of other sequences, high angular resolution diffusion imaging (HARDI) scans were acquired using a commercial single shot echo planar multi-direction diffusion weighted sequence, employing a dual bipolar diffusion gradient and a double spin echo. The imaging parameters were: 60 axial slices; 2.5 mm slice thickness; field of view 30 x 30 cm; TR/TE 9500/116 ms; acquisition matrix 128 x 128, resulting in an in-plane resolution of 2.34 x 2.34 mm. Parallel imaging was employed with an acceleration factor of 2 to reduce susceptibility distortions. Sixty-four images were acquired at b=3000 s/mm<sup>2</sup>, in which the encoding gradients were distributed in space using the electrostatic approach<sup>117</sup> along with one minimally diffusion weighted image (b=0). A field map for diffusion data was acquired using two 2D gradient recalled echo images (TE1/TE2 4.92/7.38 ms) to assist in the correction for residual distortions due to susceptibility inhomogeneities.
Diffusion Preprocessing

An extensive preprocessing procedure was followed to detect and correct for image artefacts caused by head motion and image distortions. Image volumes impacted by within-volume movement were detected using the discontinuity index\textsuperscript{118} and excluded from further analysis. Image distortions caused by susceptibility inhomogeneities were reduced using the field map, using tools available with FMRIB’s Software Library (FSL: \url{http://www.fmrib.ox.ac.uk/fsl})\textsuperscript{99} and intensity inhomogeneities were removed using n3\textsuperscript{119}. Subsequently, signal intensity outlier voxels (caused by cardiovascular pulsation, bulk head motion, etc.) were detected and replaced using DROP-R\textsuperscript{120}. This includes between-volume registration to account for head movement during the scan time using FMAM\textsuperscript{121} including adjustment of the b-matrix\textsuperscript{122, 123}. DROP-R was modified from the originally proposed method to employ a higher order model for the detection and replacement of outliers suitable for high b-value diffusion data (HOMOR,\textsuperscript{124}) rather than the tensor model (RESTORE,\textsuperscript{125}).

Voxelwise Fractional Anisotropy Analysis

Whole brain groupwise analysis of FA images were carried out using a recently published pipeline\textsuperscript{126} based on tract based spatial statistics (TBSS)\textsuperscript{63} (included within FSL), shown to enhance specificity. Briefly, changes from the original pipeline include utilisation of alternative registration techniques and omission of the skeletonisation procedure. FA maps of all participants were non-linearly registered to a study specific FA template using ANTS-SyN (\url{http://picsl.upenn.edu/software/ants/})\textsuperscript{127}. Voxels were included in the analysis if they had an FA of more than 0.2 in more than 95% of subjects. Registration accuracy was assessed visually, and data with poor registration were excluded from further analysis (n=9). Permutation based testing was carried out using ‘randomise’ (included in FSL), which also corrected for multiple comparisons in space, using threshold free cluster enhancement with 5000 iterations\textsuperscript{100}. Using this software, a general linear model was employed including age and sex to perform a voxelwise analysis for each of the four groups (left and right hemiparesis; unilateral and bilateral lesions) against CTD, allowing identification of regions where FA is significantly lower in children with UCP (the relationship was not expected in the other direction and therefore not tested in order to minimise unnecessary comparisons). Initially maps were produced of regions where age and/or sex were significant factors. As sex was not determined to be a significant factor in these maps
(see Results), only age was included as a confounding factor in subsequent models. Separate general linear models were employed to identify voxels where FA correlated with JTHFT scores in the impaired or dominant hand in left and right hemiparesis groups. As a lower JTHFT score represents better performance relationships were only tested such that increased FA correlated with decreased JTHFT. To increase linearity of JTHFT results, the logarithm of each score was used. Results were considered significant at $p<0.05$ (corrected for multiple comparisons in space). Therefore all stated results account for age and are corrected for multiple comparisons.

Anatomical naming of white matter regions containing significant voxels was performed using the John Hopkins University white matter atlas included in FSL. For simplicity, we lateralise our results using the terminology “contralateral” and “ipsilateral” hemispheres, referring to the side of hemiparesis (i.e. the “contralateral” hemisphere contains the lesion in unilateral lesions).

**Results**

*Inclusion of Age and Sex in Models*

In the structural analysis (including all subjects and controls) age was found to significantly correlate with FA in almost all white matter (see bottom row of Figure 4.1) and was therefore included in all subsequent models. Effects of age on FA (as well as JTHFT) can therefore be assumed to be controlled for statistically. FA was not found to be significantly different between sexes in any voxels ($p>0.5$), and was therefore not included in subsequent models.
Figure 4.1: Regions where fractional anisotropy (FA) was significantly lower in children with unilateral cerebral palsy compared with children with typical development (CTD).

Age and sex accounted for statistically in the model; bottom row shows regions where FA correlated with age (across all groups). Blue lines or sagittal sections indicate level of axial sections. Results shown at $p<0.05$ corrected for multiple comparisons in space overlaid on group mean FA image.

**Differences between groups**

Children with unilateral lesions showed significantly reduced FA compared with CTD in voxels associated with the contralateral corticospinal tract in both left and right hemiparesis groups, extending superiorly from the superior corona radiata through the posterior limb of the internal capsule (with limited extension into the external capsule) through to the cerebral peduncle.
inferiorly. In the left hemiparesis group significant differences were restricted to this region and did not extend into the ipsilateral hemisphere; in the right hemiparesis group differences extended into voxels associated with the superior longitudinal fasciculus (visible on the third row in Figure 4.1) as well as across the midbody of the corpus callosum (coronal and sagittal sections on the third row of Figure 4.1). Ipsilateral differences did not extend beyond the corpus callosum.

Children with bilateral lesions showed widespread bilateral reduction in FA compared with CTD in both left and right hemiparesis groups. Voxels associated with the contralateral corticospinal tract were again impacted from the superior corona radiata through to the brainstem (see coronal sections in Figure 4.1); while the ipsilateral corticospinal tract did not demonstrate any significant differences. The corpus callosum was impacted along its entire sagittal axis in the midline. Bilateral differences were observed in voxels associated with the retrolenticular part of the internal capsule, superior longitudinal fasciculus (bilaterally in the right hemiparesis group only), inferior longitudinal fasciculus (more extensively in the contralateral hemisphere), cingulate along its entire length, and posterior thalamic radiations. Voxels associated with the external capsule were significant along its entire length in the contralateral hemisphere, with only the posterior aspect being impacted in the ipsilateral hemisphere. Differences within the cerebellar white matter were unilateral in the right hemiparesis group but bilateral in the left hemiparesis group. Findings are summarised in Table 4.2 and Figure 4.1.
Table 4.2. Anatomical regions with significantly lower fractional anisotropy (age accounted for) in children with unilateral cerebral palsy compared with children with typical development.

Note that these regions describe anatomical location, not necessarily specific tracts. C – Differences contralateral to side of hemiparesis; B – differences bilateral. Structures included where p<0.05. Anatomical regions identified using John Hopkins University White Matter Atlas 128.

<table>
<thead>
<tr>
<th>Hemiparetic side</th>
<th>Unilateral Lesions</th>
<th>Bilateral Lesions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Left</td>
<td>Right</td>
</tr>
<tr>
<td>Anterior Corona Radiata</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Superior Corona Radiata</td>
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<td>C</td>
</tr>
<tr>
<td>Posterior Corona Radiata</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anterior limb of internal capsule</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Posterior limb of internal capsule</td>
<td>C</td>
<td>C</td>
</tr>
<tr>
<td>Retrolenticular part of internal capsule</td>
<td></td>
<td></td>
</tr>
<tr>
<td>External capsule</td>
<td>C</td>
<td>C</td>
</tr>
<tr>
<td>Cerebral Peduncle</td>
<td>C</td>
<td>C</td>
</tr>
<tr>
<td>Superior longitudinal fasciculus</td>
<td>C</td>
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<tr>
<td>Inferior longitudinal fasciculus</td>
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<tr>
<td>Genu of corpus callosum</td>
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<td>Body of corpus callosum</td>
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<td>Splenium of corpus callosum</td>
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<tr>
<td>Cingulate</td>
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<tr>
<td>Posterior thalamic radiations</td>
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<tr>
<td>Cerebellum</td>
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</tbody>
</table>

**Correlation with hand function**

Impaired hand JTHFT scores correlated with FA in voxels associated with the contralateral corticospinal tract in the left hemiparesis group, extending from the corona radiata superiorly to the level of the posterior limb of the internal capsule inferiorly (see second row of Figure 4.2). No regions were statistically significant in the impaired hand of the right hemiparesis group.
Dominant hand JTHFT scores correlated with FA in the region associated with the posterior thalamic radiations bilaterally in both left and right hemiparesis groups; in both groups the significant region was larger in the left hemisphere. Additionally in children with left hemiparesis voxels within the brainstem, body of the corpus callosum and bilateral external capsules were significant (see top row of Figure 4.2). In children with right hemiparesis voxels associated with the inferior longitudinal fasciculi, the genu and splenium of the corpus callosum and ipsilateral posterior limb of the internal capsule were significant (see bottom row of Figure 4.2).

Figure 4.2. Regions where fractional anisotropy (FA) correlated with hand function as measured by the Jebsen Taylor Hand Function Test (age accounted for statistically).

Results shown at $p<0.05$ corrected for multiple comparisons in space. Right hemiparesis impaired hand not shown as no regions were statistically significant.

Discussion

Using a fully automated technique we have demonstrated that periventricular white matter lesions causing UCP may be largely unilateral and restricted to the periventricular white matter, or bilateral and extending into the white matter of all lobes of the cortex as well as into the brainstem and cerebellum. We have also demonstrated that impaired hand function in children with these lesions correlates with FA in the contralateral corticospinal tract (in the left hemiparesis group); and that dominant hand function correlates with white matter integrity across multiple brain regions, particularly the posterior thalamic radiations.
Although it is not possible to truly identify the pathophysiology of each white matter lesion from these images, the unilateral group are more likely to represent focal periventricular lesions with the bilateral group likely to include additional diffuse white matter change. In children with bilateral lesions the ipsilateral hemisphere was heterogeneously impacted (see sqMRI scores of in Table 4.1). Our results suggest that systematic classification of lesions on T1 weighted MRI images as unilateral or bilateral is reliable; in unilateral lesions white matter differences are restricted to the lesioned hemisphere, despite the corpus callosum being affected across the midline in the right hemiparesis group. These differences between right and left hemiparesis could be the result of differences in brain lesions (see sqMRI scores in Table 4.1), with slightly more severe lesions affecting in the contralateral hemisphere in the right hemiparesis group compared to the left hemiparesis group. In a study of deep grey matter in a largely overlapping cohort, bilateral thalamic grey matter differences were demonstrated even in children with unilateral lesions. In the group with bilateral lesions the extent of reduced FA is asymmetrical and extends well beyond the corticospinal tract. This is in keeping with previous studies showing altered diffusion parameters in the corpus callosum, superior longitudinal fasciculus, cingulate, posterior thalamic radiations and cerebellum. Further study on each of these regions is warranted, in particular a structure function relationship should be established where possible.

The corpus callosum showed reduced FA; in children with bilateral lesions this extended across its entire length in the midline. Functional MRI (fMRI) studies have provided insight into reorganisation and lateralisation of motor regions in children with UCP, with increased bilateral activation of sensorimotor cortex compared with unilateral activation in controls. Contralesional shifting of motor function is also a known phenomenon, however, this is uncommon. Our results suggest that FA differences in the corpus callosum are not just restricted to motor areas; commissural fibres across all lobes are impacted, and therefore in addition to motor function may impact cognitive, social and higher order functions. Furthermore our results suggest that the integrity of both the genu and splenium of the corpus callosum correlate with dominant hand function. Targeted tractography studies to identify connected structures of impacted transcallosal fibres and explore structure-function relationships may further define the significance of these differences.

Impaired hand function was correlated with FA in the ipsilateral corticospinal tract in the left hemiparesis group. A correlation between hand function and corticospinal tract integrity has been
demonstrated multiple times previously, and is likely due to direct focal damage to motor fibres leading to motor impairment. Differences in the site and severity of brain lesions may contribute to the difference in results between right and left hemiparesis groups in our cohort. According to lesion assessment on structural images, each group included similar numbers of unilateral or bilateral lesions, however, unilateral lesions were slightly more severe in the right hemiparesis group, possibly allowing for different mechanisms of brain plasticity determining motor hand control. Further studies on larger groups will allow for the understanding of the impact of unilateral or bilateral lesions on hand function in subjects with UCP.

In the region associated with the posterior thalamic radiations, FA was a significant predictor of dominant hand function. It has been suggested that damage to thalamic tracts may contribute more to motor impairment than corticospinal tract damage; several conflicting studies have compared the two, showing stronger motor function correlation with either thalamic sensory or motor tracts. The posterior thalamic radiations convey visual information to the occipital cortices as well as sensory information to regions of the parietal cortex involved in complex upper limb function and visuospatial tasks. It is possible that visuospatial and higher order functions are impacted on by damage in this region, explaining the observed impairment to function. Further study into these regions is warranted to define specific tracts and connected grey matter structures; consideration should also be given to the value of this tract as both a predictor of function and its response to successful rehabilitation therapy.

There are several limitations to the methodology applied in this study. Automated whole brain analyses have the advantage of removing the need for a priori hypotheses of impacted anatomical regions, therefore increasing the chance of finding unanticipated results. This may identify targets for future studies. The major disadvantage is the increased number of comparisons and therefore reduced statistical power, making subtle differences less likely to be identified. Additionally results are extensive and therefore more difficult to interpret and present meaningfully. Fully automated methodology typically requires either parcellation or registration of images, removing inter and intra observer reproducibility issues, however, subjects with more atypical brains are unable to be included, creating intrinsic bias towards less extensive lesions. In this study, registration techniques prohibited the inclusion of children with cortical or deep grey matter
lesions, as reliable registration could not be achieved in adequate numbers to include this group. It is important to note that the methodology here is based on the diffusion tensor model, and does not use tractography – therefore our results are region specific rather than tract specific (in contrast to a recent whole brain tractography based connectome study in a largely overlapping cohort). Reduced FA is classically interpreted as a reduction in underlying fibre integrity, however, since being first described its value has been known to be biologically most interpretable in anisotropic tissue (i.e. where fibres are running parallel). The major limitation of FA as a measure is that it may be paradoxically increased by damage to a sub-population of fibres that cross a larger tract within a voxel. This ‘crossing fibres’ problem may reduce sensitivity of some results from this study, particularly in regions such as the retrolenticular part of the internal capsule. Although our results highlight multiple regions with reduced FA, specific tracts cannot be explicitly implicated until studied further using tractography and ideally higher order models, particularly where results are in regions known to contain complex white matter architecture with multiple crossing fibres.

**Conclusion**

Children with UCP due to periventricular white matter lesions represent a heterogeneous pathological group which may be focal unilateral white matter insult or include additional widespread bilateral damage. Focal changes appear to affect impaired hand function while diffuse changes may play an important role in more global functions in these children. Several regions were identified which should be targets for future tractography studies including the corpus callosum, superior longitudinal fasciculus and in particular the posterior thalamic radiations where a relationship is demonstrated between dominant hand function and FA.

**4.3. Conclusions to Chapter 4**

This study has demonstrated a significant difference in the pattern of white matter change between bilateral and unilateral periventricular white matter lesions. As discussed above these findings are consistent with pathological studies describing two components of these of lesions: a focal periventricular component and a separate widespread and more symmetrical component. Interestingly, the study in chapter 3 demonstrated cortical grey matter changes in the bilateral periventricular white matter lesion group not evident in the unilateral group. In the unilateral group
findings were limited primarily to the thalamus and basal ganglia; all of which are connected to white matter tracts running through the periventricular white matter. It would be in keeping that the cortical changes seen in the bilateral lesion group appear to represent the default mode network, as this is the most highly structurally connected network in the brain, and therefore the most likely to be affected by widespread white matter damage.
Chapter 5. Anterior Cingulate Cortex Analysis

5.1. Introduction to Chapter 5

The two preceding chapters outline whole brain analyses without the requirement for specific brain regions to be selected prior to results. This type of analysis requires voxel based scalar measures, such as the FA. To perform more complex analyses, and fully utilise diffusion MRI with the use of higher order models, specific tracts must be selected for analysis, thereby reducing the number of multiple comparisons, and allowing multiple metrics to be compared for each tract. In the following study, a targeted analysis on executive function was performed; specifically targeted at the anterior cingulate.

Executive function is a major part of the clinical phenotype of cerebral palsy, and has become increasingly well studied over recent years. Unlike motor function, few studies have assessed the correlation between executive function measures and imaging parameters. Furthermore there is a paucity of studies in this population utilising structural and functional imaging of brain areas linked with executive function. The anterior cingulate was selected as the target region for the following study for its known role as a central hub of executive function.

In addition to the study of the anterior cingulate, a recently described analysis technique is introduced for this population; the apparent fibre density (AFD). This method exploits the fibre orientation distribution in each voxel to extract diffusion data in the direction of the tract being studied, significantly reducing bias introduced by crossing fibres (the major limitation of FA).

5.2. Chapter 5 Publication


Cerebral Palsy (CP) is a non-progressive disability caused by a heterogeneous group of brain pathologies. The clinical phenotype has been classically described in terms of motor impairment \(^42\), and consequently there has been a large focus in neurological research around motor regions of the brain in these children \(^2,15,42,102\). There has been increasing interest in exploring cognitive and
social functioning in children with CP ⁴⁻⁶, ⁸⁻¹¹, showing impairments across multiple executive function domains ⁵ with considerable impact on everyday life ⁴. Up to 50% of children with CP have an intellectual disability ¹² and 25-50% have attention deficit disorder or attention deficit hyperactivity disorder ⁸, ¹². Despite extensive research on the impact of cognitive and social impairment on the lives of people with CP, there is little understanding of the underlying neuropathology ⁶.

Lesions in CP typically involve periventricular white matter (56%) or either cortical or deep grey matter (18%), with global maldevelopments being less common (9%) (the remaining 17% are non-specific) ¹⁵. In the present study we assess children with periventricular white matter (WM) lesions. Early WM damage evident on structural MRI at term equivalent age is a significant predictor of executive function in very preterm children ¹¹, and diffusion properties of multiple WM regions have been shown to correlate with neuropsychological scores in children with spastic diplegia ¹¹⁵. In a whole brain connectome study in children with unilateral CP with WM lesions we have shown that the integrity of tracts connecting to the anterior cingulate cortex (ACC) may be compromised ¹¹⁰.

The ACC is a bilateral cortical structure in the medial wall of the brain, important for both cognitive and social functioning ¹³¹. Functional MRI studies have shown that the ACC plays a central role within the executive function brain network ¹³², being highly connected with the prefrontal cortex, premotor and supplementary motor areas and parietal cortex ¹³³, ¹³⁴.

To investigate WM integrity, we used two models derived from diffusion weighted MRI: the diffusion tensor model; and constrained spherical deconvolution. Multiple MRI measurements of Brownian motion of water molecules ²⁷ are acquired for each voxel, used to derive a mathematical model from which various properties can be extracted, relating to underlying tissue. The majority of diffusion MRI studies in children with CP to date have utilised the diffusion tensor model (reviewed in ¹⁰²) which characterises diffusion within each voxel using a single tensor ellipsoid ³⁰. From the tensor, quantitative measures such as fractional anisotropy (FA; often reported as a surrogate marker for white matter ‘integrity’) and mean diffusivity (MD) can be computed ²⁸. Tractography algorithms can also exploit tensor-derived fibre orientation information to estimate fibre bundle trajectories ³⁵. Surrogate markers for tract based ‘connectivity’ can then be computed by averaging quantitative measures within voxels traversed by tractography streamlines.
Where complex WM architecture exists, multiple fibres may cross within each voxel, and single ellipsoid tensor orientations can no longer be reliably used for tractography. FA results in these regions can be unintuitive and difficult to interpret. A higher order model of the diffusion signal is therefore more appropriate. The fibre orientation distribution (FOD), computed via constrained spherical deconvolution, is one such model. By resolving multiple fibres within each voxel, the FOD provides more accurate fibre directions for tractography in addition to tract specific quantification of white matter. The apparent fibre density (AFD), a recently developed fibre specific metric derived from the FOD lobe parallel to the direction of the streamline allows a result that reflects the individual tract being analysed, in contrast with the diffusion tensor model which provides a voxel-average FA or MD value. The AFD has been used to identify tracts with reduced fibre density in Motor Neurone Disease, Alzheimer’s disease, epilepsy, infants born preterm, adolescents born preterm, grey matter heterotopia, and Dravet Syndrome.

In this work we set out to analyse the connectivity of the ACC using both AFD and traditional tensor-derived measures (as this is the currently accepted approach in the majority of diffusion imaging studies in CP). We expect that due to anatomical location, WM tracts projecting from the ACC will pass through a high number of voxels with multiple fibre orientations and therefore AFD analysis will be more specific to the tracts being analysed than diffusion tensor-based analysis. We also aim to determine whether performance in an executive function task correlates with connectivity measures of these tracts.

**Methods**

**Participants**

A total of 71 children were identified through a population-based research database, recruited for one or more clinical studies requiring baseline MRI and clinical assessment (prior to any study specific intervention). Selection criteria included age 3-17 years, a confirmed diagnosis of unilateral cerebral palsy, attendance at a mainstream school, and a score of II (two) or below in both the Gross Motor Function Classification System (GMFCS) and Manual Ability Classification System (MACS) as assessed by an occupational therapist (scores shown in Table 5.1).
Of these children, 53 were selected based on MRI brain image classification. Images were reviewed by a child neurologist (SF) and classified according to the Krageloh-Mann classification system\textsuperscript{15} as periventricular white matter lesions (the remainder included 16 with cortical or deep grey matter lesions and 2 with brain malformations). Of these, 52 children were able to be adequately parcellated by the software as outlined below. These children were aged 5-17 years, and included 25 with left hemiparesis and 27 with right hemiparesis.

Seventeen children with typical development (CTD) without brain pathology were also included in all analyses as controls. The institutional review board approved the study and written informed consent was obtained from a parent or guardian, as well as verbal assent from each child.

\textit{Table 5.1. Participant information}

CTD - Children with typical development. GMFCS - Gross Motor Function Classification System. MACS - Manual Ability Classification Scale.

<table>
<thead>
<tr>
<th>Gender</th>
<th>GMFCS</th>
<th>MACS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
<td>n</td>
<td>Age</td>
</tr>
<tr>
<td>CTD</td>
<td>17</td>
<td>10.6 ± 0.5</td>
</tr>
<tr>
<td>Left Hemiparesis</td>
<td>25</td>
<td>9.9 ± 0.6</td>
</tr>
<tr>
<td>Right Hemiparesis</td>
<td>27</td>
<td>11.4 ± 0.6</td>
</tr>
</tbody>
</table>

\textit{Flanker Task}

A subset of children (7 left hemiparesis, 10 right hemiparesis, 14 CTD) participated in the Flanker task\textsuperscript{143}, which has previously been shown to be strongly linked to the ACC using functional MRI\textsuperscript{144}. This involved five symbols displayed in a horizontal line on a computer screen. The central symbol was either a left or right facing arrow. The remaining four symbols (the “Flanker” objects) were either congruent (e.g. ← ← ← ← ←); incongruent (e.g. → → ← → →); or neutral (e.g. – – ← – –). Subjects were given five seconds to press either the right or left button on a handheld control (using their preferred hand) to indicate the direction of the central arrow. This was repeated 120 times, with a 30 second break every after each set of 40 trials. To eliminate any group differences in motor performance, we used the mean time taken for a correct answer in the neutral condition as a baseline, and reported the mean additional time needed for a correct answer in the
incongruent condition as a measure of executive function (increased additional time representing poorer executive function). Results were statistically corrected for age (and gender where significant) using a general linear model (see statistical analysis below). We assessed the relationship between task performance and diffusion derived metrics (FA, MD and AFD) in both hemispheres, labelled as “contralateral” or “ipsilateral”, referring to the side of hemiparesis. For CTD ipsilateral was arbitrarily taken as the left hemisphere (as this is ipsilateral to the non-dominant hand in right handers, but still the non-dominant hemisphere in most left handers).

**Image Acquisition**

High resolution structural images were acquired for each participant using a 0.9 mm isotropic 3D T1-weighted magnetization-prepared gradient-echo (MPRAGE) sequence using a 3T MRI scanner (Siemens, Erlangen, Germany). The acquisition parameters were: FOV 24 x 25.6 x 17.6 cm, TR/TE/TI 2300/2.26/900 ms, flip angle 9 degrees. A high angular resolution diffusion imaging (HARDI) scan was performed using 60 axial slices, FOV 30 x 30 cm, TR/TE 9200/112 ms, 2.5 mm slice thickness, acquisition matrix 128 x 128 with a 2.3 mm in plane image resolution, an acceleration factor of 2 and a diffusion encoding gradient strength of b=3000 s mm-2. Sixty five diffusion weighted images were acquired at each location consisting of 1 low (b=0) and 64 high diffusion weighted images. A field map was acquired using two 2D gradient recalled echo images (TE1/TE2 4.76/7.22 ms) to assist the correction for distortion due to susceptibility inhomogeneity.

**Parcellation**

Cortical reconstruction and volumetric segmentation was performed on T1 images with the Freesurfer image analysis suite (http://surfer.nmr.mgh.harvard.edu/). This involves removal of non-brain tissue followed by automated segmentation of cortical and subcortical structures. Manual assessment was performed at each step and subjects were excluded if parcellation was either deemed to be inadequate or was unable to be processed. This resulted in exclusion of one subject (52 total included as well as 17 controls).

**Diffusion Preprocessing and Tractography**

Diffusion weighted images were corrected for subject motion by identifying volumes with head movement between subvolumes of the interleaved acquisition (i.e. within volumes) using the
discontinuity index \(^{118}\) and subsequently using the FMAM (Fit Model to All Measurements) method to correct movement between volumes \(^{121}\). Susceptibility distortions were corrected using the field map employing FUGUE \(^{146}\) and PRELUDE \(^{147}\) in raw image space (both contained within FMRIB’s Software Library (FSL) \(^{99}\)), with signal intensity correction \(^{148}\). Motion artefacts were identified and replaced using Detection and Replacement of Outliers Prior to Resampling (DROP-R) \(^{120}\), modified from the originally proposed method to incorporate an outlier detection technique suitable for high b-value diffusion data \(^{124}\). All images were normalised by dividing each diffusion weighted volume by the median value of all WM voxels in the b=0 image. Using the corrected data, the fibre orientation distribution (FOD) was estimated using the constrained spherical deconvolution method within the MRtrix package (http://www.mrtrix.org). The response function used to derive the FOD was representative of the group, created by taking the mean response function from all children with typical development and an equal number of children with CP (selected using a random number generator). Fibre tracking was also performed using MRtrix. Bidirectional streamlines were seeded in the anterior cingulate (n=100 000 each side), using a termination mask obtained from the structural image to ensure streamlines remained within brain tissue \(^{149}\).

**Connectivity Analysis**

The region of interest (ROI) used to drive the tractography analysis consisted of the Freesurfer parcellations of both caudal and rostral anterior cingulate (see Figure 4.1 for example parcellation). The number of streamlines passing through every other cortical and subcortical ROI was calculated. ROIs appropriate for further connectivity analysis were selected based on greatest number of ACC streamlines in the CTD group.

As our methodology is novel in this population, the corticospinal tract (CST) was also included as it has been well studied in this population using diffusion imaging \(^{102}\). The CST was derived using precentral gyrus, brainstem, thalamus and cerebellum parcellations. Bidirectional streamlines were seeded in the left and right precentral gyrus, again using the termination mask. Streamlines were selected if they reached the brainstem (a single parcellation, i.e. not divided into left and right) without passing through either the thalamus or cerebellum. Ten times more streamlines (i.e. n=1 000 000) were required in this ROI to achieve similar streamline counts as the ACC tracts, due to size of the ROI and spread of the seeded streamlines throughout the brain.
In this study we computed several measures as surrogate markers of tract connectivity. The first two measures were based on tensor-derived FA and MD. For each tract we computed the weighted mean of all FA and MD values across voxels traversed by tractography streamlines. A limitation of FA and MD is that they are voxel-average quantities, and are not tract-specific when unrelated tracts cross voxels traversed by the tract of interest. For this reason we also computed an alternative tract-specific connectivity measure based on AFD. At high diffusion gradient b-values (as used in this study) the AFD integral for a particular FOD ‘lobe’ is proportional to the intra-axonal volume of axons associated with that lobe. By summing the AFD integral for all FOD lobes associated with the tract streamlines, a measure can be computed relating to the total intra-axonal tract volume (note that streamlines are associated with FOD lobes if the streamline tangent is within 30 degrees of the FOD lobe peak orientation). However, since the total intra-axonal tract volume is a quantity that is tract length dependent, we divide by the mean streamline length to give a measure proportional to tract cross sectional area (something that should be more closely related to tract ‘connectivity’). These calculations were performed using the ‘afdconnectivity’ command in MRtrix3 (http://www.mrtrix.org).

To assess the extent of crossing fibres within these tracts (and hence whether AFD would be more appropriate than FA and MD) a previously described technique was used. This involved identifying the number of ‘peaks’ within each voxel, defined as FOD lobes with an amplitude greater than a threshold value (0.33). For each streamline the average number of peaks was calculated in each voxel traversed. We then assessed the percentage of voxels with more than one peak in each tract.

**Statistical Analysis**

Statistical analysis was performed using StatSoft Statistica version 12. To determine difference between groups for tractography parameters we employed a general linear model incorporating age and gender. ANOVA was then used to assess whether there was a significant difference between groups (CTD, children with left hemiparesis and children with right hemiparesis) across left and right hemispheres simultaneously. Where results were significantly different between groups, post-hoc analysis was performed using Dunnett’s test to determine in each hemisphere whether there was a significant difference between CTD and either children with left or right hemiparesis, correcting for multiple comparisons. Results were considered significant at p<0.05.
A forward stepwise general linear model was employed to investigate the relationship between Flanker performance and diffusion metrics in all participants. Ipsilateral and contralateral hemispheres were investigated separately. A separate model was used for FA, MD and AFD. Age was included in the model, with extra variables (gender and tract metrics) being added to the model in a stepwise fashion until no remaining variables met statistical significance (p<0.05). Where tracts were included into the model (i.e. met statistical significance) beta and p values were reported. This was all done in an automated fashion using Statsoft Statistica. To ensure outliers were not giving false positive results Flanker scores were excluded if they fell more than two standard deviations outside the mean.

Results

Selection of Tracts

The five regions reached by the largest number of streamlines generated in the ACC were, in order of highest to lowest, superior frontal gyrus, medial orbitofrontal cortex, isthmus cingulate, precuneus and rostral middle frontal gyrus (examples shown in Figure 5.1). This order was preserved in both hemispheres (intrahemispheric connections) for all groups. Streamline counts are shown in the supplementary material.
Figure 5.1. Anatomical regions selected for highest streamline count (streamlines seeded in Anterior Cingulate Cortex).

Samples are from a child with typical development. Data is overlaid on T1 MRI (top right) and 3D reconstruction (top left) as well as tractograms projected on T1 MRI (bottom).

**Tract Connectivity Results**

All FA, MD and AFD-based tract connectivity values with standard errors for selected tracts are shown graphically (corrected for age and gender) in Figure 5.2, with full raw data in the supplementary material (see Supplementary information to Chapter 5.2).

Streamlines connecting the ACC to the precuneus showed a significant difference in MD between groups (p<0.0001). Post-hoc analysis showed significantly increased MD in the right hemisphere of children with left hemiparesis compared with CTD (p=0.0143). AFD was also significantly
different between groups (p=0.0072), with no significant hemisphere specific differences between CTD and children with either left or right hemiparesis in post-hoc analysis.

Streamlines connecting the ACC to the superior frontal gyrus showed a significant difference between groups in both FA (p=0.0049) and MD (p=0.0031) but not AFD. In post-hoc analysis there were no significant hemisphere specific differences between CTD and children with either left or right hemiparesis.

Streamlines connecting the ACC to the isthmus cingulate (i.e. the cingulum bundle) showed a significant difference in MD between groups (p=0.0013). Post-hoc analysis did not show any significant differences between CTD and children with either left or right hemiparesis. Neither FA nor AFD were significantly different between groups.

Streamlines connecting the ACC to the medial orbitofrontal cortex were not significantly different between groups for FA, MD or AFD. The same was true for streamlines connecting the ACC to the rostral middle frontal gyrus.

The CST showed a significant difference between groups in FA (p<0.0001), MD (p<0.0001) and AFD (p<0.0001). Post-hoc analysis revealed children with left hemiparesis had reduced FA (p=0.0005), increased MD (p<0.0001) and reduced AFD (p=0.0018) compared with controls in the right hemisphere; and children with right hemiparesis had reduced FA (p<0.0001), increased MD (p=0.0008) and reduced AFD (p<0.0001) compared with CTD in the left hemisphere.
Figure 5.2. Fractional anisotropy (FA), mean diffusivity (MD) and apparent fibre density (AFD) of tracts of interest in children with typical development (Control) and children with left and right hemiparesis.

Vertical bars denote 95% confidence intervals.

**Correlation with Flanker Scores**

Children with hemiparesis took a significantly longer time (324±80ms; n=17) to get a correct answer with the incongruent condition (compared with neutral condition) compared with CTD (110±24ms; n=14) (p=0.0237). Two scores were excluded from the correlation analysis as they were greater than two standard deviations beyond the mean. Both excluded scores belonged to children with left hemiparesis.

Assessing all participants, FA scores did not correlate with Flanker performance for any tracts. MD of the ipsilateral CST significantly correlated with Flanker performance (p=0.0412, β=0.4020). AFD of the streamlines connecting the ACC to the superior frontal gyrus in the contralateral hemisphere correlated with Flanker performance (p=0.0045, β=−0.5856). These relationships are visualised in the supplementary data.
Crossing Fibre Analysis

More than 48% of voxels contained more than a single peak in all examined tracts. The CST had the highest percentage (56.8% ± 1.0%), followed by the tracts connecting the ACC to the superior frontal gyrus (53.8% ± 1.5%), medial orbitofrontal cortex (53.3% ± 1.6%), precuneus (52.0% ± 1.5%), rostral middle frontal gyrus (50.0% ± 1.5%) and the isthmus cingulate cortex (48.8% ± 1.5%).

Discussion

In the present study we assessed the structural connectivity of the anterior cingulate cortex in children with unilateral cerebral palsy as a result of periventricular white matter lesions. In particular we assessed connections to five cortical regions, selected for their high streamline count. We have examined these tracts using both the diffusion tensor model and the fibre orientation distribution, showing altered connectivity and a relationship to executive function using the Flanker task. Additionally we demonstrated a high number of crossing fibres to justify the use of a higher order model.

In addition to the ACC tracts, we analysed a previously well studied tract; the CST. Our results using the diffusion tensor model reflect previous studies (reviewed in 102), showing reduced FA and increased MD in the hemisphere contralateral to the hemiparetic side. The AFD was also significantly reduced in this hemisphere as expected. The MD of the ipsilateral CST correlated with Flanker performance. This result is surprising as we have controlled for motor performance in the executive function task, using response time in the neutral condition as a baseline. We speculate that MD in this region is a reflection of lesion severity. MD is a measure of overall diffusion within a voxel, and therefore in our population of children with periventricular white matter lesions, increased periventricular MD likely represents a more severe lesion, which we hypothesise correlates with impaired function across multiple domains including executive function. This is consistent with the relationship not being present for either FA or AFD, suggesting that the link with executive function does not specifically relate to the CST itself. Similar findings with MD in the corona radiata and posterior limb of the internal capsule (amongst
other regions) have been demonstrated previously in an ROI analysis of children with spastic
diplegia, showing a correlation with both IQ and neuropsychological scores 115.

The five ACC connections studied here are all well documented anatomical connections,
previously demonstrated in both primate studies and human imaging studies 152, 153. The changes
demonstrated within these connections were overall more subtle than those in the CST. The
connexions between the ACC and both the rostral middle frontal gyrus and the medial
orbitofrontal cortex did not show any significant differences between groups using either the
diffusion tensor or FOD model. This is not surprising, as these streamlines project posteriorly from
the ACC, away from the ventricles (although frontal involvement is not infrequent). The
connections between the superior frontal gyrus and ACC showed significant differences between
groups using diffusion tensor metrics, however, no difference using AFD. Such a result may
suggest that there are changes within the region not specific to this tract, or may reflect lack of
statistical power. We note that AFD was decreased in this tract bilaterally for all subjects, but not
to a statistically significant level. There was a significant correlation between Flanker performance
and AFD of this tract, highlighting the importance of the integrity if this tract. The superior frontal
gyrus is involved in higher order cognitive tasks including spatial cognition and working memory
154. Our results suggest that although this connection may not be significantly affected in children
with WM lesions as a combined group, there is heterogeneous integrity of this pathway, and
reduced integrity plays a role in impaired executive function. The selection criteria for this study
was based on extent of clinical impairment (GMFCS and MACS), rather than pathological extent
of brain lesions; therefore frontal lobe impairment amongst these children is intrinsically
heterogeneous. Those with reduced connectivity in this tract appear to have more severe executive
dysfunction. Early identification of pathology in this region may therefore be able to identify
children at risk of impaired executive function, where early intervention may induce plasticity 82,
155-157.

The connection between the ACC and the precuneus showed a significant difference between
groups in both AFD and MD, however, only MD was significant in post-hoc analysis. This
connection is involved with higher order cognitive functions and the integration of internal and
external information 153. This also forms part of the default mode network 158, the most highly
connected network in the brain, involved with internal thought. Pathology involving this
connection is likely to impact on higher order executive function, unable to be quantitatively
measured with simple tasks like the Flanker task. Of all the ACC connections examined, the connection with the precuneus runs in closest proximity to the ventricles, and is therefore more likely to be directly affected by the lesion.

The cingulum bundle itself was not significantly different between groups. This would suggest that the cingulum bundle remains relatively intact in children with white matter lesions. This is an interesting finding, as the cingulum bundle runs parallel to the ventricles. These results suggest that any damage to tracts connecting to the ACC occurs at a location beyond where fibres leave the cingulum bundle, with relative sparing of the cingulum bundle itself.

Our crossing fibre analysis showed that the cingulum bundle contained the fewest crossing fibres, with more than half of the voxels traversed by all tracts projecting out of the cingulate containing multiple fibre orientations. The CST showed the highest number of crossing fibres. The presence of many crossing fibres is reflected in the low mean FA values for all groups as shown in Figure 5.2. This suggests that FA group differences in these tracts are not reliable indicators of differences in connectivity. Group differences in the MD measure are more representative of underlying tissue than FA in crossing fibre regions \(^{32}\); however MD is not tract-specific. We therefore believe that the AFD results are the most biologically interpretable and robust analysis of the tracts of interest in this study. In addition, the AFD-based connectivity measure should theoretically be more related to actual tract connectivity since it combines information from both intra-voxel fibre density and tract cross sectional area (i.e. morphological information). In contrast, the mean FA and MD for a given tract are not necessarily affected by fibre bundle cross sectional area. For example two tracts with different cross sectional areas may have the same mean FA value, yet the thicker tract is likely to have more axons and therefore a higher ‘connectivity’. The tract-specificity and additional morphological information in the AFD-derived connectivity measure is the likely cause of discrepancies between the tensor and AFD derived results (see Figure 5.2). Most noticeable is the CST result (bottom row of Figure 5.2). The tensor FA and MD result suggests that the CST on the hemiparetic side is spared in CP. However the AFD connectivity measure demonstrates that while the contralateral CST is most altered, the CST on the ipsilateral side also has a reduced connectivity (not statistically significant), consistent with previous studies showing reduced grey matter bilaterally in children with unilateral CP \(^{106}\). This discrepancy is likely due to a reduction in the CST width in CP, which is not detectable using FA or MD as a surrogate marker for tract
connectivity. Application of this technique in children with grey matter lesions would be of particular interest, where the ipsilateral corticospinal tract undergoes hypertrophy.\(^{159}\)

The AFD-based connectivity measure is, however, not without limitations. The method used in this work identifies FOD lobes belonging to the tract of interest based on streamline traversal. The entire AFD integral for a given FOD lobe is assigned to the tract providing it is associated with at least one ROI streamline. It is possible, however, that a single FOD lobe may represent axons from multiple tracts (e.g. when different tracts merge into one near the cortex). This means that the AFD-based connectivity measure for a tract of interest may not be 100% tract specific if a tract merges with another at some point along its length. While this is a limitation of the current approach, it remains a significant improvement on the diffusion tensor derived metrics, as demonstrated above. We note that a more robust method for deriving a measure of tract connectivity based on tractography and AFD would be to use Spherical deconvolution Informed Filtering of Tractograms (SIFT)\(^{160}\). SIFT connectivity analysis requires robust DWI-T1 alignment to perform anatomically constrained tractography (ACT)\(^{161}\) and therefore ensure that all streamlines terminate at the grey white matter interface. While we did apply field map-based EPI distortion correction to our data in this study, we found that alignment was not sufficiently accurate on our 3T-acquired data (compared with superior methods that rely on additional b=0 images acquired with reverse phase encoding\(^{161}\)).

The analysis approach used in this work is entirely automated, which increases reproducibility, however, it prevents the inclusion of significantly atypical brains due to the limitations of automated parcellation. This restricted our study to children with WM lesions. We attempted parcellation of 16 subjects recruited using the same protocol with cortical or deep grey matter lesions; none were able to be parcellated adequately; these children were therefore unsuitable for this study protocol. We also note that the presence of gross morphological malformations in CP prevented us from performing a whole-brain AFD fixel-based analysis\(^{162}\) (due to imperfect image registration).

Finally, while our study had numbers comparable to other diffusion MRI studies in this population\(^{102}\), there were several trends seen which did not meet statistical significance, potentially limited by subject numbers and heterogeneity. Variation between scanning parameters and subsequent
preprocessing variability presents a barrier to pooling of data across studies and centres. To increase statistical power larger trials are required, which poses a significant resource challenge.

**Conclusion**

In children with unilateral cerebral palsy due to periventricular white matter lesions, connectivity to the anterior cingulate cortex appears to be altered, playing a role in impaired executive function. Anterior projections within the frontal cortex appear to be spared, as does the cingulum bundle itself. Connectivity between the superior frontal gyrus and the anterior cingulate appears to be heterogeneously impacted, with reduced connectivity relating to impaired executive function. Early identification of damage to this tract may therefore become important when targeting early intervention. Connectivity between the anterior cingulate and the precuneus is also compromised, which may play a role in higher order cognitive difficulties. In this study we measured connectivity using diffusion tensor and FOD derived measures. The discrepancy between the results from both models is likely caused by the large number of crossing fibres in the tracts, highlighting the advantage of employing higher order models in these regions.

**5.3. Conclusion to Chapter 5**

This study has outlined several important points, both in terms of methodology of diffusion MRI in these children, as well as the pathology underlying executive dysfunction. The use of higher order models is both validated and shown to be superior to DTI. The results demonstrate why FA and MD should be avoided in tract specific analyses, despite continuing to be the most commonly used measures. The AFD metric is one method of utilising a higher order model, however, multiple alternative methods exist, each with advantages and disadvantages, and at present there is no widely used standard. This will make comparison across multiple studies in the future difficult. This limitation will continue to improve as advanced diffusion MRI analysis techniques become more validated and readily available, and in particular as methods are developed to allow these techniques to be applied in morphologically abnormal brains.

Of interest, the path between the cingulate and the precuneus was shown to have altered diffusion; this pathway forms part of the default mode network. There are several suggestions from the results of these studies that this network may be impacted upon. It would be of interest to perform a resting state functional MRI study in the future in combination with tractography to assess this network,
and whether damage to it bears correlation to higher order function in these children, with potential use as both a predictor of function and a marker of response to therapy.
Chapter 6. Updated Systematic Review and Conclusion

6.1. Introduction to Chapter 6

The studies above have demonstrated several key findings relating to unilateral CP. Cortical and deep grey matter lesions were studied only with regards to grey matter changes; reduced grey matter was demonstrated in a widespread and bilateral distribution, with thalamic volume affected similarly to periventricular white matter lesions. Periventricular white matter lesions were shown to demonstrate either unilateral focal white matter change, in which changes appear to be localised lateral to the ventricle, or bilateral diffuse change, in which changes extend into all lobes of the brain bilaterally. These widespread white matter changes appear to cause secondary grey matter changes, most evident in the thalamus and basal ganglia, and cortical regions associated with the default mode network. Loss of integrity correlated with motor function in both the corticospinal tract and the posterior thalamic radiations, supporting previous studies. In the same population the anterior cingulate was specifically investigated, and shown to have heterogeneously altered connectivity, with integrity of the connection to the superior frontal gyrus significantly correlating with executive function.

At the conclusion of the above studies, there had been a significant number of new peer reviewed publications using diffusion MRI in CP. It was therefore fitting to conclude the thesis with an updated literature review, showcasing the utility of diffusion MRI in this population, and summarising current findings as well as making recommendations for future studies.

6.2. Chapter 6 Publication

Not published at time of thesis submission.

Cerebral palsy (CP) is the outcome of antenatal or perinatal brain insult resulting in long term clinical impairment.\textsuperscript{1,42} Both the underlying pathology and the clinical outcome are heterogeneous in nature and extent, and therefore patients with CP experience a vast spectrum of disability. Classically, CP is defined by motor dysfunction, described as spasticity, dyskinesia or ataxia; although more than one of these features may be present.\textsuperscript{1,42} Impairments in executive functioning, cognitive reasoning and social interaction are well described\textsuperscript{86} as are visual disorders
The underlying pathology comprises a heterogeneous group of early brain injuries, typically fitting one of several key patterns of injury \textsuperscript{15, 10}. These patterns are well described and give insight into presumed timing of the insult \textsuperscript{15}. Additionally, the site and extent of lesions, often assessed in the clinical setting qualitatively or quantitatively by structural MRI, contribute to the understanding of clinical outcome in CP \textsuperscript{2, 116, 164, 165}. Plasticity and microstructural changes beyond what can be appreciated on conventional structural MRI is known to further contribute to functional outcomes; for this reason advanced MRI techniques have been largely applied in this population.

Diffusion MRI (dMRI) exploits the interaction of diffusing water molecules and tissue microstructure to give information about biological tissue \textsuperscript{166, 167}. In particular, the microscopic diffusion of water is restricted by tissue microstructure, such as axonal membranes in neural tracts, and such restriction can be measured at a macroscopic scale using a diffusion weighted MRI sequence \textsuperscript{27}. Diffusion is measured along an arbitrary number of directions, equally distributed over a sphere (or hemisphere), for all voxels in the entire brain. This results in an array of 3-dimensional images for each acquisition. In the analysis of dMRI, mathematical models are employed to fit the acquired data within each voxel from which meaningful information can be extracted for interpretation by clinicians and researchers.

The most common dMRI model is the Diffusion Tensor (also known as diffusion tensor imaging [DTI]) whereby the diffusion within each voxel is described by a 3-dimensional Gaussian function \textsuperscript{30}. The diffusion tensor has been instrumental in enabling dMRI to become an important tool for both clinical and neuroscientific research, however, a major limitation is the diffusion tensor’s inability to model voxels with crossing fibres (i.e. voxels that contain two or more fibre pathways). Several alternative higher order models have therefore been proposed to more accurately characterise white matter in these regions (reviewed in \textsuperscript{32}). One such higher order model is the fibre orientation distribution (FOD), estimated via constrained spherical deconvolution \textsuperscript{33, 34}. FODs have been shown to provide more biologically interpretable quantitative measures \textsuperscript{135} and improve the accuracy of fibre tractography \textsuperscript{168}. An example of DTI and FOD voxels are shown in Figure 6.1.
Figure 6.1. Example of Diffusion Tensor Imaging and Fibre Orientation Distribution (computed via constrained spherical deconvolution). Models overlaid on T1-weighted image. Subject is a child with unilateral spastic CP (models computed using the methods outlined in Chapter 5).

The analysis of dMRI typically involves first extracting quantitative measures from the model used within each voxel. For example scalar values such as fractional anisotropy (FA), measuring the non-uniformity of diffusion, and mean diffusivity (MD), measuring net amount of diffusion are commonly estimated from the diffusion tensor $^{28}$ Quantitative measures can be computed across subjects at the voxel level (known as whole-brain voxel-based analysis) or by comparing averaged measures within a region of interest (ROI) (assuming a prior anatomical hypothesis). While ROIs can be determined manually, a common approach is to employ fibre tractography to delineate the location of the ROI (i.e. a tract of interest). Tractography is performed by repeatedly seeding in either a specified or random voxel and creating a streamline which propagates in the direction of the fibre inferred by the underlying model in each voxel traversed $^{35}$. Streamlines provide an estimate of fibre tract trajectories (they do not directly represent axons), which can be visualised via 3-dimensional rendering. Furthermore, the number of streamlines that represent a tract (or connect specific regions) is frequently reported as a quantitative measure of tract “integrity” (see Recommendations section below for a discussion on the validity of this).
In 2012 we published a systematic review of dMRI studies in CP\textsuperscript{102}. The review identified 22 studies, covering multiple aspects of CP neuropathology. The corticospinal tract was the most comprehensively studied tract, with studies repeatedly showing reduced FA and increased MD. Sensory tracts including the superior and posterior thalamic radiations showed similar findings. Clinical measures were frequently shown to correlate with both motor and sensory tract diffusion measures. Since 2012 there have been significant advances in diffusion MRI processing, as well as advances in the understanding of CP pathophysiology and rehabilitation. We therefore aim to provide an updated review of dMRI studies in CP assessing both clinical and technical aspects as well as giving specific recommendations to drive future studies.

**Method**

The search protocol from the previous review was utilised\textsuperscript{102}. This involved a literature search of relevant databases (Pubmed, Embase, Cinahl, Scopus and Psycinfo) ranging from 29 February 2012 to 12 December 2015 for the keywords: “cerebral palsy” and any of “tractography”, “diffusion imaging”, “diffusion magnetic resonance imaging”, “diffusion tensor imaging”, “high angular resolution diffusion imaging”, or “diffusion weighted imaging” (including associated acronyms). Only peer reviewed publications in English were considered. A full protocol is available as supplementary online material of the original review.

Studies were included if they met the following criteria: 1) Study type was cross sectional cohort study or case control study; 2) At least one subject group were clinically diagnosed as having CP; and 3) Diffusion MRI had been utilised (with or without tractography). Consequently, studies were excluded if they: 1) were studies of progressive, acute or traumatic brain injury; 2) were studies of animal models.

**Data Extraction**

Data was extracted independently, with reference to: imaging parameters used (magnet strength, \(b\) value, number of diffusion-weighted directions acquired, slice thickness [as this was reported more consistently than full image resolution]); methodology used (model, use of tractography, method of ROI placement, whole brain analysis); subjects (motor subtypes of CP, lesion description, age [all ages were included], number of subjects); clinical measures used; ROIs or WM tracts included; and finally diffusion metrics reported. Meta-analysis is not appropriate as
results are not directly comparable across different scanners, varying acquisition parameters and heterogeneous analysis methodologies. Of note we only include information pertaining to CP. For the same reasons we do not report statistical values as methodology is widely varied (we comment on validity of statistical methods, however, what is reported as statistically significant in the included studies is reported as significant in this review). Where studies include other groups, this data was not included in this review. Where studies use additional imaging modalities, results are discussed where directly relevant to diffusion MRI results.

The search on 17 December 2015 returned 361 results, of which 37 studies met inclusion criteria. Details of included studies are shown in Table 6.1.

**Terminology**

The terminology used varies significantly between studies. In this paper we adopt the SCPE terminology for clinical subtypes as spasticity, dyskinesia or ataxia. We use the Krägeloh-Mann classification of brain lesions as cortical or deep grey matter (CDGM), periventricular white matter (PWM), malformations or non-specific lesions.

For unilateral CP there is inconsistency around the terminology used to reference brain hemispheres. As lesions are often bilateral, the use of “lesioned/non-lesioned”, “affected/unaffected” or “ipsilesional/contralesional” hemisphere may be inappropriate. The terms “contralateral” and “ipsilateral” hemispheres have been used referring to the side of hemiparesis, however, unless used in full (e.g. “contralateral to the side of hemiparesis”) these terms are easily misinterpreted. The terms “dominant” and “non-dominant” hemisphere have also been used, however, hemispherical functional dominance has not been studied in this group, and therefore these terms may not reflect true functional dominance. In this study we use the terms “dominant” and “non-dominant”, as they are the simplest to interpret, accepting the caveat.
Table 6.1. Summary of included Studies

**n** – Number of subjects with CP (note some studies also included larger numbers of subjects without CP – these are not reported here)

**Age** – Age in years; range and mean are shown where reported; TEA – Term equivalent age

**Motor** - Motor subtype and distribution of CP; UCP – Unilateral CP; BCP – Bilateral CP; SCP – Spastic CP; USCP – Unilateral spastic CP; BSCP – Bilateral spastic CP; a Diplegia and Quadriplegia; b Diplegia

**Lesion**: PWM – Periventricular white matter lesions; CDGM – Cortical or deep grey matter lesions; a Periventricular leukomalacia; b Haemorrhagic parenchymal infarct; c Cystic periventricular leukomalacia

**dMRI Acquisition**: Mag – Magnet strength; Dir – Number of diffusion directions; B – b-value (s/mm²); ST – Slice thickness (mm); ? – not reported

**Processing**: Model – DTI – Diffusion tensor imaging; CSD – Constrained spherical deconvolution; Unspecified – Not specified whether probabilistic or deterministic tractography was used; TBSS – Tract based spatial statistics (alternative to tractography); Manual – ROIs were drawn manually on either diffusion or structural images; Automated – software algorithms were utilised to define ROIs; Whole brain – the entire brain was divided into ROIs; fMRI – functional MRI used to derive ROIs

**Other Imaging**: fMRI – Functional MRI; rs-fMRI – Resting state fMRI; sMRI – structural MRI; pMRI – perfusion MRI; TMS – Transcranial magnetic stimulation; PET – Positron emission tomography; MEG - Magnetoencephalography; MEP – Motor evoked potentials; USS – Ultrasound Scan

**Tracts / ROIs**: CST – Corticospinal tract; ATR – Anterior thalamic radiations; PTR – Posterior thalamic radiations; OR – Occipital radiations; IFOF – Inferior fronto-occipital fasciculus; ILF – Inferior longitudinal fasciculus; SLF – Superior longitudinal fasciculus; ACC – Anterior cingulate cortex; CC – Corpus callosum

**Recommendations**: Summary of recommendations (see text) which were met by each study
<table>
<thead>
<tr>
<th>Author (Year)</th>
<th>Subjects</th>
<th>dMRI Acquisition</th>
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<th>Other Imaging</th>
<th>Tracts / ROIs</th>
<th>Recommendations Met</th>
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<td>Abdelsalam (2014)</td>
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Notes:
- BSCP: Brain Simulated Canonical Pattern
- PWM: PWM Modelling
- USCP: Uniform Simulated Canonical Pattern
- CDGM: Confined DGM Pattern
- SCP: Scattered Canonical Pattern
- Q-ball: Q-ball tracking
- Multiple arbitrary ROIs: Manual placement of ROIs
- n/a: Not applicable
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<td>Age (years)</td>
<td>Motor</td>
<td>Lesion</td>
<td>Mag</td>
<td>Dir</td>
<td>B (s/mm²)</td>
</tr>
<tr>
<td>Park (2014)</td>
<td>23</td>
<td>16.0 - 50.0</td>
<td>Dyskinetic CP</td>
<td>CDGM</td>
<td>No lesion</td>
<td>3T</td>
</tr>
<tr>
<td>Park (2013)</td>
<td>6</td>
<td>7.0 - 20.0</td>
<td>USCP</td>
<td>CDGM</td>
<td>PWM</td>
<td>3T</td>
</tr>
<tr>
<td>Rai (2012)</td>
<td>22</td>
<td>mean 7.7</td>
<td>BSCP</td>
<td>Not reported</td>
<td>3T</td>
<td>30</td>
</tr>
<tr>
<td>Rickards (2014)</td>
<td>9</td>
<td>2.1 - 7.6</td>
<td>UCP</td>
<td>Not reported</td>
<td>1.5T</td>
<td>45</td>
</tr>
<tr>
<td>Roze (2015)</td>
<td>7</td>
<td>Birth (preterm)</td>
<td>USCP</td>
<td>PWM</td>
<td>1.5T</td>
<td>32</td>
</tr>
<tr>
<td>Scheck (2015)</td>
<td>52</td>
<td>5.0 - 17.0</td>
<td>USCP</td>
<td>PWM</td>
<td>3T</td>
<td>64</td>
</tr>
<tr>
<td>Schertz (2015)</td>
<td>20</td>
<td>7.8 - 13.6</td>
<td>USCP</td>
<td>Not reported</td>
<td>3T</td>
<td>19</td>
</tr>
<tr>
<td>Tsao (2013)</td>
<td>40</td>
<td>5.0 - 16.0</td>
<td>USCP</td>
<td>PWM</td>
<td>3T</td>
<td>64</td>
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<tr>
<td>Tsao (2015)</td>
<td>42</td>
<td>5.0 - 16.0</td>
<td>USCP</td>
<td>PWM</td>
<td>3T</td>
<td>64</td>
</tr>
<tr>
<td>Weinstein (2013)</td>
<td>14</td>
<td>7.2 - 14.3</td>
<td>USCP</td>
<td>Mixed</td>
<td>3T</td>
<td>19</td>
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<tr>
<td>Yoshida (2013)</td>
<td>18</td>
<td>2.0 - 15.0</td>
<td>SCP</td>
<td>Dyskinetic CP</td>
<td>1.5T</td>
<td>12</td>
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</table>
Specific Tracts

Corticospinal Tract

In the 2012 review, we identified 18 studies that assessed the corticospinal tract (CST) using dMRI, with the prominent finding being reduced FA and increased MD, and correlation between these measures and motor function. Here we have identified a further 22 studies assessing the CST (results of studies in the previous review are not discussed here). In unilateral CP ten studies showed significantly reduced FA in the non-dominant CST \(^{108-110, 112, 114, 169-173}\); eight studies also demonstrated increased MD \(^{108, 109, 112, 169-172, 174}\); five studies demonstrated reduced streamline count \(^{109, 112, 169, 171, 174}\); one study demonstrated reduced volume and cross sectional area \(^{175}\), and one study demonstrated a change in Apparent Fibre Density (a metric derived from constrained spherical deconvolution) \(^{112}\). One study demonstrated increased MD in the dominant CST in unilateral CP \(^{172}\). In bilateral CP, one study also demonstrated bilateral reduced CST streamline count \(^{176}\), and another study demonstrated that FA and MD changes are more pronounced on the “more affected” side \(^{171}\). One study looked at children with dystonia, and showed no difference in FA of the CST from controls \(^{177}\). FA and MD asymmetry were shown to be increased in unilateral CP compared with children with diplegia \(^{108}\). In bilateral CP, both FA and MD were shown to be more affected in children with quadriplegia compared with diplegia \(^{79}\).

Diffusion derived parameters of the CST have been shown to correlate with multiple clinical measures including the Melbourne Unilateral Upper Limb assessment \(^{109, 114}\), Box and Blocks test \(^{114}\), Assisting Hand Assessment \(^{109, 110, 174}\), Jebsen Taylor Test of Hand Function \(^{109}\), Paediatric Arm Function Test \(^{178}\) and mirror movements \(^{174}\). Additionally FA has been shown to be significantly different between children with different motor classifications according to the Gross Motor Function Classification Scale \(^{179, 180}\). One study demonstrated a relationship between hand function and FA in the dominant CST in children with unilateral CP \(^{181}\).

All of the above findings are similar to those evident at the 2012 review. There were, however, several novel findings relating to the CST. Diffusion MRI changes in the corticospinal tract were shown to be present at term equivalent age of preterm neonates who were later diagnosed with CP: asymmetry in unilateral CP \(^{111, 182}\); and reduced FA in children with cystic periventricular leukomalacia \(^{183}\). Furthermore, improvement of diffusion parameters (assumed to represent improved myelination) from MRI at the time of preterm birth to MRI at term equivalent age is a good prognostic feature \(^{111}\). Changes are less pronounced by one year after term equivalent age \(^{182}\), however, at age 18 years, preterm children
who developed CP have significantly reduced FA and MD compared with those with typical development.

**Sensory Tracts**

At the time of the 2012 review, there was increasing interest in the importance of damage to sensory tracts in CP. The earliest tractography study looking at these tracts was in 2002 whereby the posterior thalamic radiations were noted to be qualitatively different from controls in children with bilateral spastic CP. Subsequent studies showed that both the superior and posterior thalamic radiations exhibit altered FA and/or MD, and that these parameters correlate with motor function.

The superior thalamic radiations (the connection between thalamus and motor cortex) have been shown to exhibit reduced FA, increased MD as well as axial and radial diffusivity in both the non-dominant side of unilateral CP and bilateral CP (contralateral to “more affected” limb). One study also demonstrated increased MD in the dominant hemisphere in unilateral spastic CP. In bilateral CP there was also a reduced streamline count compared with controls. When assessed in further detail, the thalamus to postcentral gyrus tract showed conflicting results in UCP, one study showed significant reduction in FA, while two studies were unable to demonstrate a significant FA difference. The thalamus to precentral gyrus tract consistently showed reduced FA, which also correlated with Assisting Hand Assessment and sensorimotor function.

The posterior thalamic radiations were less frequently studied. One study showed reduced FA and increased MD in children with periventricular leukomalacia. Connections between the occipital lobe and multiple other regions were noted to have significantly reduced number of streamlines in children with bilateral CP and PWM lesions. In particular, FA in the optic radiations was shown to correlate with ophthalmological function in children with PVL. In children with CP who had normal appearance on structural MRI, no difference could be appreciated in FA or MD of the posterior thalamic radiations compared with controls.
Other Pathways

Extrapyramidal Motor Pathways

Two specific motor pathways other than the corticospinal tract were studied. The corticopontocerebellar tracts were shown to have an increased asymmetry (based on streamline count) in children with unilateral spastic CP, and this was shown to correlate with Assisting Hand Assessment scores \(^{113}\). The medial lemniscus was also studied, and shown to have reduced FA in children with unilateral CP who had normal structural MRI; this cohort was also not found to have any significant FA or MD changes in either the corticospinal tract or the posterior thalamic radiations \(^{185}\). Both of these findings highlight that motor impairment is not simply explained by corticospinal tract damage; and further assessment of other motor pathways is needed.

Language and Executive Function Pathways

Although research in executive function and language in CP has been rapidly progressing, there were relatively few studies using dMRI in these pathways. One study showed correlation between multiple neuropsychological scores and FA in multiple regions including the internal capsule, corona radiata, brainstem, corpus callosum in children with bilateral spastic CP \(^{115}\). Executive function as measured by flanker task performance was shown to correlate with Apparent Fibre Density of the connection between the anterior cingulate cortex and superior frontal gyrus in children with unilateral CP \(^{112}\). Of note, one study using multiple imaging modalities also found reduced functional connectivity using fMRI and reduced density of GABA-A receptors in the anterior cingulate using PET (diffusion findings were not specifically reported) \(^{186}\). A whole brain connectome based study in children with unilateral spastic CP showed reduced connectivity in a network comprising the anterior cingulate cortex, precuneus, medial frontal lobe \(^{110}\). This network likely represents the default mode network \(^{158}\). The connection between the precuneus and the posterior limb of the internal capsule was also shown to have reduced FA and increased MD compared with controls in unilateral CP, with both measures correlating with 2 point discrimination and stereognosis \(^{109}\). One study looked specifically at language tracts showing reduced volume but no change in FA in the right arcuate fasciculus in a mixed CP cohort \(^{187}\).

Long Range Fibres

In the 2012 review, we identified only one study that assessed the superior longitudinal fasciculus, showing decreased FA and increased MD in children with both dyskinetic and spastic CP \(^{78}\). One further
study has again demonstrated reduced FA in children with dyskinetic CP. It was also demonstrated that children with CP show specific vulnerability to long range fibres, and that increased severity of CP preferentially impacts long range fibres.

**Corpus Callosum**

In the 2012 review there were conflicting results surrounding the corpus callosum. Whole brain connectome studies have since demonstrated reduced FA in interhemispheric connections between the motor cortices (presumably representing primarily transcallosal motor fibres) in both children with unilateral and bilateral CP due to PWM lesions 110, 176. More specifically, children with unilateral CP were shown to have a reduced streamline count across the entire sagittal length of the corpus callosum, which correlated with Jebsen Taylor Test of Hand Function scores; reduced FA and increased MD were restricted to the midbody 174. This study also demonstrated a correlation between streamline counts of interhemispheric connections and intrahemispheric connections. In children with CP and partial dysgenesis of the corpus callosum, tractography was used to show qualitatively abnormal transcallosal fibres 175.

**Cortical Reorganisation**

Four studies utilised tractography to explore organisation of cortical fibres in children with CP. One study showed the utility of tractography in identifying ipsilateral control of the impaired hand in a child with unilateral spastic CP, which was corroborated by transcranial magnetic stimulation 156. In another child, the corticospinal tract was unable to be delineated prior to constraint induced movement therapy, but was able to be delineated after therapy, suggesting some level of plasticity in response to therapy (discussed in more detail below – see Rehabilitation) 155. One study demonstrated in children with PWM lesions that sensorimotor tracts show an atypical path, coursing in such a way to avoid the lesion 182. Topographical organisation of fibres by destination was explored in children with unilateral CP in both the thalamus and posterior limb of the internal capsule; in both cases the diffusion metrics within pathways were altered but the organisation was equivalent to that seen in controls 95, 109.

**Rehabilitation**

Ten studies have utilised diffusion MRI to assess response to therapy, in comparison with only one study in the 2012 review (showing children with bilateral spastic CP had improved FA in the corticospinal tract following botulinum toxin A therapy in conjunction with physiotherapy 82). In children with unilateral
spastic CP improvement in FA of the corticospinal tract was demonstrated following intensive occupational therapy \textsuperscript{169} or combined physiotherapy and occupational therapy \textsuperscript{173}. Improvement in FA and streamline count correlated with Functional Level of Hemiplegia Scale \textsuperscript{169}. In children with bilateral CP, FA increases were demonstrated in both motor and sensory tracts following six months of physiotherapy, correlating with improvement in the Gross Motor Function Measure \textsuperscript{188}. This study did not demonstrate any additional FA or functional improvement with the addition of Botulinum Toxin A. Another study from the same group demonstrated no FA or MD changes in multiple tracts including the corticospinal tract immediately following 6 months of physiotherapy and intramuscular Botulinum Toxin A, however, did demonstrate changes in perfusion \textsuperscript{189}. The authors conclude that perfusion changes immediately following therapy may precede changes appreciable on diffusion MRI. Bimanual therapy was shown to increase FA in the corticospinal tract in unilateral CP \textsuperscript{156, 181}. This response was found to be greatest in children with a lower baseline FA \textsuperscript{181}. Response to constraint induced movement therapy was assessed in unilateral CP by three studies: two showed improvement in FA of the CST \textsuperscript{155, 170} while one did not demonstrate any change \textsuperscript{178}. Children with higher baseline MD in the posterior limb of the internal capsule were shown to have higher functional improvement as measured by Jebsen Taylor Hand Function Test \textsuperscript{170}. Two studies also assessed response to experimental autologous cord blood therapy: one study showed increased FA in the posterior limb of the internal capsule as well as the spinothalamic tract \textsuperscript{24}, while the other showed improved total FA connectivity using a connectome approach, which correlated with the Gross Motor Function Measure \textsuperscript{157}. This study showed that children with higher baseline connectivity benefited most from cord blood transfusion. Of note there were also increased adverse outcomes with this therapy (the authors speculate this was likely due to the requirement for concurrent cyclosporine administration) \textsuperscript{24}.

**Methodology**

**Novel Methods and Innovations**

Seven studies have utilised whole-brain approaches to avoid the requirement for \textit{a priori} hypotheses about tracts or regions involved. Six of these studies used tractography-based connectome approaches \textsuperscript{110, 157, 165, 176, 179, 190} while two used whole brain voxel-based approaches \textsuperscript{179, 186} (one study utilised both \textsuperscript{179}). Whole-brain connectome approaches involve dividing the brain image into arbitrary segmentations, usually with a fully or semi-automated atlas-based registration \textsuperscript{191}. Tracts are then randomly seeded throughout the brain, with a “connectome” produced by identifying data (typically FA) from streamlines...
connecting each region to every other region. Fully automated statistical network-based analysis or graph analysis is then typically performed to correct for multiple comparisons\textsuperscript{192}. Voxel-based approaches, such as Tract Based Spatial Statistics\textsuperscript{63}, allow whole-brain voxel-wise analysis of measures such as FA between groups. The primary difference from the connectome approach is that this gives voxel specific results, allowing regional rather than tract-based localisation of results. These methods are advantageous in allowing a comprehensive analysis of the entire brain, at the cost of statistical correction for multiple comparisons causing reduced sensitivity to subtle changes. They share any caveats of the underlying model used; where the underlying model is DTI, results are less reliable in tracts or regions containing crossing fibres. This may explain some of the unexpected results such as increased FA in some connections\textsuperscript{110}. The extensiveness of the results (i.e. every region in both hemispheres to every other region in both hemispheres) can represent a significant challenge to present concisely, and therefore much of the data is typically not presented. Connectome studies were carried out in children with unilateral CP and PWM lesions\textsuperscript{110,165}, bilateral CP and PWM lesions\textsuperscript{157}, children with PWM lesions (motor type not specified)\textsuperscript{179}, a mixed cohort\textsuperscript{176} and one study comparing children with spasticity and dyskinesia, showing more widespread FA and MD changes in children with dyskinetic CP\textsuperscript{190}.

One study looked at reliability of FA analysis using manually drawn regions of interest in the posterior limb of the internal capsule; showing good reliability when inexperienced raters are excluded\textsuperscript{180}. Several novel diffusion MRI techniques were employed in children with CP including: use of tractography to determine topographical organisation of structures (the thalamus\textsuperscript{95} and posterior limb of the internal capsule\textsuperscript{109}); analysis of FA along tracts rather than using tract specific average – allowing regional identification of affected areas\textsuperscript{179}; and the use of a higher order model to avoid use of FA in crossing fibres (applied specifically to tracts connecting to the anterior cingulate)\textsuperscript{112}.

**Diffusion MRI used in conjunction with other imaging modalities**

Diffusion MRI has been increasingly used in conjunction with other imaging modalities to give information beyond structural connectivity. While diffusion MRI is almost always used in conjunction with structural MRI, one study used diffusion MRI to validate a semi-quantitative structural MRI classification system\textsuperscript{165}.

Functional MRI is a technique which measures the blood oxygenation level dependent (BOLD) signal while the subject is asked to perform a specific task, in order to identify active brain regions associated with the task\textsuperscript{193}. This method has been used to identify motor regions which can then be used as seed
points for tractography, increasing the probability of functionally important tracts (in contrast to anatomical seed points) \(^{182}\). Resting state functional MRI is a variation of functional MRI whereby the subject lays still with eyes closed and is instructed to avoid conscious thought, in order to identify brain regions active in the “resting state” \(^{194}\). This allows measurement of “functional connectivity”; identification of brain regions in which activation across time is correlated. This has been used in conjunction with structural connectivity measured by diffusion MRI \(^{114,182}\). This technique can be used to give an index of laterality \(^{170,171,174}\). Laterality indices were shown to correlate with FA in the posterior limb of the internal capsule \(^{174}\) as well as improve with constraint induced movement therapy (along with diffusion MRI measures) \(^{170}\). Resting state motor networks were shown to be altered in both unilateral and bilateral CP, with greater change in the non-dominant hemisphere in unilateral CP \(^{171}\). Perfusion MRI was also used to show that regions with diffusion MRI changes demonstrate reduced perfusion \(^{189}\).

Positron emission tomography (PET) was used to assess GABA-A receptor expression as a marker of brain injury in unilateral spastic CP, which also showed changes in the same regions identified by diffusion MRI \(^{186}\). Magnetoencephalography (MEG) was used to map somatotrophic organisation which correlated with tractography findings \(^{171}\). Transcranial magnetic stimulation (TMS) allows quantitative measurement of cortical excitability, which was shown to improve with bimanual intensive therapy along with diffusion MRI measures \(^{156}\). Additionally, the combination of these two techniques demonstrated ipsilateral control of the impaired hand in a child with unilateral CP \(^{156}\). This technique was also extended to give a measure of interhemispheric functional connectivity by measuring contralateral silent periods following stimulation, which correlated with Melbourne Unilateral Upper Limb scores alongside diffusion MRI measures \(^{114}\). Motor evoked potentials (MEP) were also used in conjunction with diffusion MRI to demonstrate the lack of abnormality within the corticospinal tract in children with dystonic CP \(^{177}\).

**Recommendations**

**Specific Recommendations and Rationale**

Based on the 18 studies included in the 2012 review and the additional 37 studies included in the present review we have postulated a series of recommendations for the use of diffusion MRI in children with CP.
Multiple studies have demonstrated diffusion differences between different clinical subtypes of CP, and there is indirect evidence to suggest different mechanisms of reorganisation according to pathological subtypes. We therefore recommend that studies should either include a single clinical or pathological subtype or ideally both (e.g. USCP with PWM lesions). Where more than one clinical or pathological subtype is included, details of clinical and pathological subtypes should be described and between-group analysis should be performed (which may require a larger sample size). Clinical subtypes should be allocated as per internationally accepted standards such as the Surveillance of Cerebral Palsy in Europe guidelines. Pathological subtypes should also be allocated as per internationally accepted standards, such as the Krägeloh-Mann classification system. This is the basis for recommendations (1) and (2).

1. **Motor subtypes and distribution of CP must be defined with different subgroups ideally analysed separately**
   
2. **Pathological subtypes of CP must be defined with different subgroups ideally analysed separately**

We found 24 (64%) studies met recommendation (1) and 26 (70%) studies met criteria (2).

Terminology is widely varied across the included studies. Reducing this heterogeneity is difficult due to author, regional and journal preferences. We strongly recommend the use of accepted standards, such as those outlined in the pathological and clinical classification systems mentioned above. We acknowledge that all terminology options relating to laterality of findings in children with unilateral CP has limitations; we cannot justify recommending any specific set of terms (“dominant/non-dominant”, “ipsilesional/contralesional”, “ipsilateral/contralateral to the side of hemiparesis”), but encourage uniformity with previous studies where possible. The terms “hemiparesis” and “hemiplegia” are used interchangeably, although classically hemiplegia refers to one sided paralysis with hemiparesis referring to one sided weakness. We therefore prefer use of the term “hemiparesis”, again acknowledging author, regional and journal preferences. We also recommend use of the term “unilateral cerebral palsy” rather than “congenital hemiplegia/hemiparesis”. With regards to DTI metrics, “mean diffusivity” is used in interchangeably across studies with “trace” or “mean apparent diffusion coefficient”. Mathematically MD is trace/3, therefore, between group analysis of trace or MD are equivalent. We encourage use of “mean diffusivity” as it is the most descriptive term and used in the two most commonly used software suites to process diffusion MRI in this cohort (FSL...

[http://www.fmrib.ox.ac.uk/fsl] and MRtrix [http://www.mrtrix.org/]). The term “apparent diffusion coefficient” should be avoided, as this is a direction specific measure (“mean apparent diffusion coefficient” is technically correct) 47. This is our basis for recommendation (3).

3. **Motor subtypes of CP must be defined with different subgroups ideally analysed separately**

We accept that these are loose recommendations subject to regional, author and journal preference and we therefore did not find any studies that used unacceptable terminology.

Preparing children for MRI with the use of a mock scanner has been shown to allow high quality MRI acquisition without anaesthesia or sedation 29. Our centre along with many others have successfully employed this strategy with multiple large cohorts of children with CP, typically developing children and other conditions. We therefore suggest that routine sedation or anaesthesia should be avoided for scanning children with CP, particularly if the scan is for research purposes only. Where scans are clinically warranted and adequate scans are not able to be achieved otherwise, sedation could be used (for example in neonates and infants). This is the basis for recommendation (4).

4. **Sedation should be avoided for MRI acquisition unless clinically indicated**

We found that 32 studies (86%) did not report unnecessary sedation. We acknowledge that the studies using routine sedation all report ethics committee approval, and therefore have justified their use of sedation or anaesthesia.

We acknowledge that acquiring high quality MRI images is a significant resource challenge, and there is a compromise between acquisition time and imaging protocol. We recommend greater than 45 directions for diffusion protocols, as lower than this has been shown to be detrimental in the corticospinal tract amongst other regions 197, 198. Where greater than 45 directions are used with a higher order model, the $b$ value should be as high as possible but not higher than 3000s/mm$^2$ (as no further benefit is gained beyond this 197) (note that $b$ values of 1000s/mm$^2$ are appropriate where DTI is used alone, which we recommend against – see below). We found that only 7 studies (19%) utilised scans with $>45$ directions.

Additionally, some studies have combined datasets acquired on different scanners with different acquisition protocols (shown on Table 6.1). This should be avoided as images are not necessarily comparable. Preprocessing of data is highly complex and variable between studies; standardisation of preprocessing protocols should be an aim for diffusion MRI studies in general but is beyond the scope
of this review. All preprocessing must be documented in detail and should follow previously published protocols where possible to reduce heterogeneity. As a minimum, preprocessing must include at least correction for head motion and visual quality assurance. This is our basis for recommendation (5). We acknowledge that this recommendation will likely become outdated quickly, with advances in diffusion MRI such as multi shell acquisition \(^{199,200}\) (yet to be applied in this population).

5. **Images must be acquired with acceptable acquisition parameters (>45 directions) with well documented and appropriate preprocessing**

Although the limitations of the diffusion tensor model are now well described \(^{32}\), and higher order models are being employed, DTI is still the standard, employed in all but one (97%) of the studies reviewed here (although often higher order models were used to drive probabilistic tractography). Importantly, DTI used to drive tractography (particularly where a deterministic approach is used) is inadequate for most brain regions \(^{32}\), and therefore tractography should be derived from higher order models. Furthermore, while the majority of studies use tractography to report FA and MD values from voxels traversed by streamlines as “tract specific”, this method is well documented as having limitations, as neither FA nor MD are tract specific measures (as they are influenced by all tracts within each voxel) \(^{31,201}\). MD is more appropriate than FA for this type of analysis as degeneration of a tract will cause only an increase in MD, whilst FA may be either increased or decreased depending on the number of crossing tracts within the voxel \(^{31,202}\). Streamline count as well as tract volume have been shown to be not biologically meaningful unless further mathematical modelling is applied \(^{203,204}\) (examples include Spherical deconvolution Informed Filtering of Tractograms (SIFT) \(^{160}\) and Convex Optimization Modelling for Microstructure Informed Tractography (COMMIT) \(^{205}\) amongst others). DTI results must be interpreted with caution, and studies must always include discussion around limitations of DTI and tractography methods utilised. The “mode” of diffusion is a novel metric derived from the diffusion tensor, and may assist in interpretation of FA analyses \(^{201,206}\). This is the basis for recommendation (6).

6. **Analysis should utilise up to date modelling techniques (DTI should not be used to drive tractography)**

We found 24 studies (65%) utilised up to date methodology.

Regions of interest are derived either by manual placement of ROIs, automated parcellation of anatomical regions, or more recently using functional MRI results. This leads to large variation between studies; for example the corticospinal tract may be a single manually defined region, or tracts crossing two regions
(e.g. the posterior limb of the internal capsule and premotor cortex), three regions, or other study specific protocols. Where manual placement is used, operators should be experienced and follow strict anatomical guidelines which must be documented clearly to ensure reproducibility. Where well studied tracts are included, such as the corticospinal tract, authors should endeavour to reproduce protocols of studies in similar cohorts to enhance pooling of information. Where guidelines are not previously published and shown to be reliable, a reliability study should be included. Automated parcellation poses a significant challenge in CP due to the inability of software to label highly atypical brains. Automated techniques should also be well documented and manually checked by a blinded operator in a clearly documented method, outlining any manual corrections or exclusion required. This is the basis for recommendation (7).

7. **Regions of interest must be derived either by an automated well documented method or manually defined by experienced operators with strict documentation (previously published where possible) and ideally with a reliability analysis**

We found 26 studies (70%) met this recommendation.

Finally, where relationships are sought between clinical measures and diffusion parameters, authors must specify specific hypotheses to reduce the number of multiple comparisons. Where multiple tracts are studied and/or multiple clinical scores are reported, if relationships are assessed between all possibilities, statistical correction (such as Bonferroni correction) should be carried out, which will significantly reduce sensitivity. (For example if 5 tracts are studied and 3 clinical measures are reported, searching all possibilities yields 15 (5*3) results; if overall statistical significance of $p<0.05$ is desired then Bonferroni correction would require results to be considered significant at $p<0.0033$ to avoid false positives). Of note, whole brain analysis software such as Network Based Statistics for connectome based studies and Threshold Free Cluster Enhancement for voxel based analyses use alternative and well documented statistical corrections. This is the basis for recommendations (8) and (9).

8. **Where clinical correlations are sought with imaging parameters, the number of clinical measures should be kept to a minimum, and should ideally be clinical measures with validity and reliability in the population of interest and widely used in similar studies**

9. **Where multiple statistical calculations are carried out (e.g. due to multiple tracts/ROIs, groups, and/or clinical measures) appropriate corrections must be made for multiple comparisons**
We found that 20 studies assessed clinical function, of these 13 (65%) minimised the use of multiple clinical measures with specific hypotheses. We identified 25 studies that used multiple comparisons, of which 14 studies (56%) applied appropriate statistical correction.
### Box 6.1: Specific Recommendations for the use of Diffusion MRI in CP

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<tr>
<td>1.</td>
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### Future Studies

The use of diffusion MRI is rapidly expanding and contributing significant knowledge to the understanding of the pathogenesis and response to therapy of children with CP. Changes in the corticospinal tracts as well as correlation between diffusion parameters and motor function is well
established; however, other motor pathways have been shown to be impacted, and further study into other motor tracts and regions are important. The correlation between specific sensory tracts and both motor and sensory function as well as higher order function is less well studied. Future studies identifying specific tracts (for example by using fMRI to identify specific motor regions) would be useful, as well as further elucidation into the anatomical pathways and importance of the posterior thalamic radiations. There has been some interest in language and executive function, however, this should be studied in further detail, and in different subgroups of CP. Although several tracts have been shown to correlate with executive function, no studies have yet assessed the response to therapy in these tracts. As yet there is no reliable way to identify the difference between primary changes seen due directly to the insult and secondary changes elsewhere in the brain, which may be due to a combination of plasticity and Wallerian degeneration; future studies are needed to explore this.

The predictive value of diffusion MRI has been demonstrated multiple times, predicting both the development of CP from neonatal images and the response to therapy in pre-therapy images. These principles should be explored in more detail with the goal of allowing clinical application.

The use of novel techniques to improve localisation of tractography results such as along-tract analysis and tractography derived parcellation\(^{95,109}\) is promising. Novel methods could improve understanding of damage to long-range fibres and organisation of fibres, for example in the corpus callosum. Investigators should aspire to understand the methodology and limitations of all dMRI software packages used for analysis, and utilise state of the art methods where possible; techniques such as multi shell acquisition should be applied in the near future\(^{199,200}\).

The statistical power of studies using diffusion MRI in CP is not well studied. Only one study has assessed reliability of FA in this cohort. Further studies into the reproducibility of diffusion MRI derived information in children with CP would be justified. As CP comprises a pathologically heterogeneous group, even within pathological subtypes, we hypothesise that subject number greatly affects the reliability of a study (particularly where more than one pathological or clinical subtype is included), however, quantification of this at present is purely speculative.

The use of other imaging modalities in conjunction with diffusion MRI is promising, in particular the use of functional MRI to define functional seed points to drive tractography. This technique overcomes the problems associated with automated atlas based parcellation in atypical brains as well as reliability bias in manual based ROIs. Perhaps more importantly, the use of functional ROIs is more appropriate
than anatomically derived ROIs as neuroplasticity is well documented in this population, and anatomy is atypical.

**Conclusion**

Diffusion MRI has been used extensively in CP research, giving widespread insight into underlying neuropathology behind clinical impairments. Multiple pathways have been studied in varying detail, and the organisation of multiple tracts has been investigated. Recent advances show promise for the predictive value of diffusion MRI, both in the neonatal period to predict outcome as well as prior to therapy to predict response. Response to therapy also causes changes in brain connectivity appreciable on diffusion MRI. Based on the studies reviewed, we postulate nine specific recommendations for future studies.

### 6.3. Thesis Limitations

There are several limitations within the studies included here. These have been discussed in detail in each study. Automated methods of either brain parcellation or registration have been utilised through the studies included. This is advantageous in avoiding inter-rater and intra-rater bias, and utilising well documented and accepted standards, however, introduces study limitations, particularly in this population where subjects can have atypical brains. Automated techniques rely on the software’s ability to recognise structures or features within the brain, therefore those subjects where these structures or features are atypical become unable to be processed. While manual placement of ROIs is avoided, manual quality assurance becomes necessary, therefore reintroducing some level of inter-rater and intra-rater bias. This limitation also prevented detailed white matter analysis of children with cortical and deep grey matter lesions, which make up approximately one quarter of the included cohort of children with unilateral CP.

Models used to process diffusion MRI data have been discussed in detail throughout the studies. DTI has well documented limitations, some of which were demonstrated in Chapter 5. The use of the Apparent Fibre Density measure again bears limitations; this method is also less well established and therefore less readily interpretable to the primary audience of the study.

The number of subjects requires is an unknown quantity in this population; no studies within this thesis, nor any of the studies included in either of the reviews, included a statistical power calculation. An
appropriate statistical power calculation may not be possible where the heterogeneity of the population is so wide, however, further research with recommendations for subject numbers would be beneficial.

6.4. Grand Discussion and Conclusion

As a final conclusion to the thesis, each specific hypothesis is discussed, with attention given to evidence supporting or refuting the hypothesis, unexpected or relevant incidental findings and barriers to investigation of the hypothesis.

1. Children with unilateral spastic cerebral palsy comprise a vastly heterogeneous pathological group; common pathological features may be present which contribute to the common phenotype

Due to restrictions of the automated methods utilised, children with periventricular white matter lesions were studied in the greatest detail. Comparison between this group and children with cortical and/or deep grey matter lesions was only able to be made with regards to grey matter using structural MRI (Chapter 3). Deep grey matter structures were shown to be similarly impacted between these two groups, in particular there was a significant reduction in thalamic volume compared with controls but not different between groups. Future study could further establish this hypothesis by using diffusion MRI to examine the thalamic radiations and compare between groups; successfully achieving this would require a robust method for identification of regions to drive tractography in children with cortical lesions; this would likely require a strict manual parcellation algorithm or fMRI based parcellation.

2. Children with lesions appearing unilateral on structural MRI may show subtle bilateral changes detectable using advanced structural MRI techniques and diffusion MRI

Several differences were demonstrated between children with unilateral and bilateral lesions in both grey matter and white matter analyses. Thalamic volume was shown to be reduced bilaterally compared with controls in children with unilateral lesions (children with bilateral lesions had again significantly reduced thalamic volume compared with children with unilateral lesions). In the white matter analysis, children with unilateral lesions did not demonstrate any bilateral changes in fractional anisotropy other than in the body of the corpus callosum. This study was limited by the use of fractional anisotropy as well as reduced sensitivity due to the whole brain nature of the analysis; further studies using higher order model tractography specifically targeting both the transcallosal fibres and connections with the subcortical grey
matter (particularly contralateral to lesioned hemisphere) could further elucidate the extent of subtle bilateral changes in children with unilateral lesions.

3. A significant correlation can be demonstrated between abnormal structural and diffusion MRI findings and motor function

Impaired hand function as measured by the Jebsen Taylor Hand Function Test correlated with fractional anisotropy in the contralateral corticospinal tract; dominant hand function correlated with bilateral fractional anisotropy in the posterior thalamic radiations. These findings are consistent with previous studies highlighting the importance of both of these connections. While the finding of corticospinal tract insult causing impaired hand dysfunction is more simple to interpret, insult to the posterior thalamic radiations causing dominant hand dysfunction is suggestive of impairment via higher order dysfunction. Bilateral thalamic insult was also demonstrated in the grey matter analysis, being consistent across different pathological subtypes (although no structure-function relationship was able to be demonstrated with grey matter volumes or density). Bilateral thalamic insult may impact the transmission of sensory information, however, involvement of the posterior thalamic radiations is suggestive that sensory connections to the higher order centres may be more important for preservation of dominant hand function than pure somatosensory connections.

4. Diffusion MRI may give insight into impact on executive function pathways; diffusion changes may bear correlation to clinical measures of executive function

Executive function as measured by the Flanker task correlated with the apparent fibre density of the connection between the anterior cingulate cortex and the superior frontal cortex. Diffusion MRI findings in motor pathways have been shown to bear useful prognostic value in neonates as well as measures of predictive and actual response to therapy; identification of important executive function pathways may allow future studies to apply these principals to therapy targeting executive function.

5. DTI is known to have many pitfalls yet remains widely used in studies of children with CP; superior methods are available and could be readily implemented in this population with a significant and measurable benefit

Constrained spherical deconvolution was used to drive tractography and derive a higher order metric (apparent fibre density) in this population; furthermore the advantages of this technique over diffusion tensor imaging was demonstrated as well as quantification of the high number of voxels with crossing
fibres. An updated systematic review showed that many studies in CP now utilise higher order models to drive tractography, however, the vast majority continue to report tensor derived metrics. Application of higher order models is not significantly more complicated or processing intensive than applying diffusion tensor imaging, however, there is at present less standardisation of techniques and therefore selection of appropriate novel methodology is less straightforward than with the well-established techniques using diffusion tensor imaging. The concluding review aims to familiarise authors with the limitations of the diffusion tensor model and encourage use of higher order models.
xi. Bibliography


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142. Raffelt D, Parker D, McMahon J, Scheffer I, Connelly A. Decreased Apparent Fibre Density in Dravet Syndrome. 2014;.


147. Jenkinson M. Improving the Registration of B0-distorted EPI Images using Calculated Cost Function Weights. 2004;.


Appendices

Appendix I. Ethics Approval

THE UNIVERSITY OF QUEENSLAND
Institutional Approval Form for Experiments on Humans
Including Behavioural Research

Chief Investigator: A/Prof Roslyn Boyd, A/Prof Stephen Rose
Project Title: Executive Function in Children with Congenital Hemiplegia - Phase 2: Neuroscience Outcomes
Supervisor: A/Prof Stephen Rose, A/Prof Roslyn Boyd
Co-Investigator(s): Lonnieke Welerink, Simon Sheck
Department(s): Qld Cerebral Palsy & Rehabilitation Research Centre; UQ Centre for Clinical Research
Project Number: 2011000393
Granting Agency/Degree:
Duration: 31st December 2011

Comments:
Expedited review on the basis of approvals from the Queensland Children’s Health Service (QCH) HREC, dated 24/05/2010 and 16/03/2011.

Name of responsible Committee:-
Medical Research Ethics Committee
This project complies with the provisions contained in the National Statement on Ethical Conduct in Human Research and complies with the regulations governing experimentation on humans.

Name of Ethics Committee representative:-
Professor Bill Vicenzino
Chairperson
Medical Research Ethics Committee

Date: 29.03.2011
Signature: 

Appendix II. Systematic Review Search Protocol (Chapter 2.2)

A literature search was conducted of five databases (Pubmed, Embase, Cinahl, Scopus and Psycinfo) on 29 February 2012 for the keywords: “cerebral palsy” and any of “tractography”, “diffusion imaging”, “diffusion magnetic resonance imaging”, “diffusion tensor imaging”, “high angular resolution diffusion imaging”, or “diffusion weighted imaging” (including associated acronyms). Search terms for each database are shown in Appendix II: Table 1.

Appendix II: Table 1. Search terms, dates and number of results returned. Times given are UTC+10:00 (Brisbane, Australia).

<table>
<thead>
<tr>
<th>Database</th>
<th>Search Term</th>
<th>Time of Search</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pubmed</td>
<td>((((((tractography)) OR (diffusion mri)) OR (hardi)) OR (high angular resolution diffusion imaging)) OR (DTI)) OR (diffusion tensor imaging)) OR (diffusion weighted imaging)) OR (diffusion imaging)) AND (cerebral palsy)</td>
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<td>103</td>
</tr>
<tr>
<td>EMBASE</td>
<td>('cerebral palsy'/exp OR 'cerebral palsy' AND ('tractography'/exp OR tractography OR ('diffusion'/exp OR diffusion AND ('imaging'/exp OR imaging)) OR 'diffusion mri'/exp OR 'diffusion mri' OR 'diffusion weighted imaging'/exp OR 'diffusion tensor imaging'/exp OR 'diffusion weighted imaging' OR 'diffusion tensor imaging') AND ('article'/it OR 'article in press'/it)</td>
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<td>64</td>
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<tr>
<td>PSYCinfo</td>
<td>(cerebral palsy) AND (tractography OR hardi OR (high angular resolution diffusion imaging) OR dti OR (diffusion tensor imaging) OR (diffusion mri) OR (diffusion weighted imaging) OR (diffusion imaging))</td>
<td>29/02/12 11:58</td>
<td>24</td>
</tr>
<tr>
<td>Database</td>
<td>Search Term</td>
<td>Time of Search</td>
<td>Results</td>
</tr>
<tr>
<td>----------</td>
<td>-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
<td>------------------------</td>
<td>---------</td>
</tr>
<tr>
<td>Scopus</td>
<td>((TITLE-ABS-KEY-AUTH(cerebral palsy)) AND ((TITLE-ABS-KEY(diffusion mri)) OR (TITLE-ABS-KEY-AUTH(diffusion imaging)) OR (TITLE-ABS-KEY-AUTH(tractography)) OR (TITLE-ABS-KEY-AUTH(diffusion tensor imaging)) OR (TITLE-ABS-KEY-AUTH(dti)) OR (TITLE-ABS-KEY-AUTH(high angular resolution diffusion imaging)) OR (TITLE-ABS-KEY-AUTH(hardi)) OR (TITLE-ABS-KEY-AUTH(diffusion weighted imaging))) AND (LIMIT-TO(DOCTYPE, &quot;ar&quot;) OR LIMIT-TO(DOCTYPE, &quot;ip&quot;))</td>
<td>29/02/12 12:01</td>
<td>132</td>
</tr>
<tr>
<td>Cinahl</td>
<td>(((cerebral palsy) or (MH &quot;Cerebral Palsy&quot;)] and ((diffusion imaging) or (diffusion mri) or (diffusion weighted imaging) or (diffusion tensor imaging) or (tractography)))</td>
<td>29/02/12 12:14</td>
<td>19</td>
</tr>
</tbody>
</table>

Process

The initial search returned 342 results, of which 180 were duplicates. Of the 162 unique results exclusions were made if all of the following criteria were not met:

- Study of human subjects only
- Study type either cross sectional cohort study or case control study
- At least one study group consisting entirely of subjects diagnosed clinically with cerebral palsy (this did not include progressive “cerebral palsy like” conditions)
- Use of diffusion MRI

After exclusions were made on the basis of these criteria, 20 articles remained for inclusion.
A further two peer reviewed studies known to the authors met the above criteria, but did not appear in any of the databases listed. These articles were also included. The entire search process is described visually in Appendix II: Figure 1.
Appendix II: Figure 1. Flowchart documenting the search process.
### Appendix III. Supplementary information to Chapter 3.2

*Appendix III Table 1. Volumes of deep grey matter nuclei in children with unilateral cerebral palsy, separated by lesion type. Values shown are mean and 95% confidence intervals.*

<table>
<thead>
<tr>
<th>Group</th>
<th>Thalamus (mm³)</th>
<th>Putamen (mm³)</th>
<th>Globus Pallidus (mm³)</th>
</tr>
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<td><strong>Dominant Hemisphere</strong></td>
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<td></td>
</tr>
<tr>
<td>Controls (n=19)</td>
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<td>1708</td>
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<td>1613</td>
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<td>n/a</td>
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Appendix III Table 2. Volumes of deep grey matter nuclei in children with unilateral cerebral palsy, separated by lesion type, before manual correction of thalamus and putamen segmentations. Values shown are mean and 95% confidence intervals

<table>
<thead>
<tr>
<th>Group</th>
<th>Thalamus</th>
<th>Putamen</th>
<th>Globus Pallidus</th>
</tr>
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<tbody>
<tr>
<td>Dominant Hemisphere</td>
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<tr>
<td>Controls (n=19)</td>
<td>8447</td>
<td>5323</td>
<td>1708</td>
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<td>Unilateral Lesions (n=45)</td>
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<td>5389-6570</td>
<td>3892-4819</td>
<td>1380-1611</td>
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</tbody>
</table>

| Brain Malformation (n=2)     | 7390     | 4822        | 1613            |
|                              | n/a      | n/a         | n/a             |
| Cortical or Deep Grey Matter | 7438     | 4785        | 1643            |
| Lesions (n=15)               | 6737-8139| 4310-5260   | 1520-1765       |
| Periventricular White Matter | 7376     | 4987        | 1642            |
| Lesions (n=15)               | 7029-7723| 4772-5201   | 1576-1708       |

| Brain Malformation (n=2)     | 6835     | 4580        | 1598            |
|                              | n/a      | n/a         | n/a             |
| Cortical or Deep Grey Matter | 6706     | 4076        | 1502            |
| Lesions (n=6)                | 4744-8667| 2410-5743   | 1130-1873       |
| Periventricular White Matter | 6538     | 4614        | 1568            |
| Lesions (n=51)               | 6170-6906| 4361-4867   | 1496-1639       |
Appendix III Table 3. Results of manual corrections to thalamus and putamen segmentation volumes. Values shown are mean ± standard deviation.

<table>
<thead>
<tr>
<th>Structure</th>
<th>Relative (%)</th>
<th>Absolute (mm³)</th>
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<tbody>
<tr>
<td>Thalamus (n=4)</td>
<td>-9.9 (±3.5)</td>
<td>-510 (±207)</td>
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<tr>
<td>Putamen (n=10)</td>
<td>11.8 (±4.5)</td>
<td>445 (±141)</td>
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Appendix III Figure 1. Samples of manually corrected segmentations of the thalamus (top two rows) and putamen (lower two rows).
**Appendix IV. Supplementary information to Chapter 5.2**

<table>
<thead>
<tr>
<th>n</th>
<th>Side</th>
<th>Cingulate Bundle</th>
<th>ACC - Medial Orbitofrontal Cortex</th>
<th>ACC - Precuneus</th>
<th>ACC - Rostral Middle Frontal Gyrus</th>
<th>ACC - Superior Frontal Gyrus</th>
<th>Corticospinal Tract</th>
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</thead>
<tbody>
<tr>
<td>CTD</td>
<td>L Streamlines</td>
<td>5.929 ± 598</td>
<td>8391 ± 765</td>
<td>3314 ± 441</td>
<td>1131 ± 186</td>
<td>20655 ± 2006</td>
<td>14017 ± 1254</td>
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<tr>
<td></td>
<td>FA</td>
<td>0.3576 ± 0.0077</td>
<td>0.2818 ± 0.0088</td>
<td>0.3530 ± 0.0077</td>
<td>0.3551 ± 0.0053</td>
<td>0.3933 ± 0.0072</td>
<td>0.4520 ± 0.0031</td>
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<tr>
<td></td>
<td>MD (*10^4)</td>
<td>6.0806 ± 0.0398</td>
<td>6.5841 ± 0.0476</td>
<td>6.0735 ± 0.04428</td>
<td>5.9518 ± 0.0327</td>
<td>6.1553 ± 0.0389</td>
<td>5.8912 ± 0.0417</td>
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<tr>
<td></td>
<td>AFD</td>
<td>18.58 ± 1.41</td>
<td>51.35 ± 2.89</td>
<td>17.80 ± 1.29</td>
<td>30.27 ± 2.54</td>
<td>116.60 ± 5.59</td>
<td>33.08 ± 1.32</td>
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<tr>
<td>R Streamlines</td>
<td>5.538 ± 534</td>
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<td>4127 ± 455</td>
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<td>14017 ± 1254</td>
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<td></td>
<td>FA</td>
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</tr>
<tr>
<td></td>
<td>AFD</td>
<td>13.30 ± 1.17</td>
<td>45.07 ± 3.04</td>
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<td>36.36 ± 2.95</td>
<td>122.99 ± 6.22</td>
<td>31.78 ± 1.20</td>
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<tr>
<td>Left Hemiplegia</td>
<td>L Streamlines</td>
<td>4.787 ± 466</td>
<td>8164 ± 1032</td>
<td>3699 ± 443</td>
<td>1467 ± 240</td>
<td>21412 ± 1454</td>
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<tr>
<td></td>
<td>FA</td>
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<td>0.3538 ± 0.0050</td>
<td>0.3429 ± 0.0064</td>
<td>0.4557 ± 0.0050</td>
</tr>
<tr>
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<td>6.1696 ± 0.0495</td>
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<td>101.57 ± 6.94</td>
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<td>R Streamlines</td>
<td>4.409 ± 584</td>
<td>6433 ± 831</td>
<td>2371 ± 467</td>
<td>2043 ± 251</td>
<td>23565 ± 1829</td>
<td>6237 ± 789</td>
</tr>
<tr>
<td></td>
<td>FA</td>
<td>0.3326 ± 0.0075</td>
<td>0.2792 ± 0.0053</td>
<td>0.3289 ± 0.0071</td>
<td>0.3491 ± 0.0080</td>
<td>0.3344 ± 0.0067</td>
<td>0.4174 ± 0.0066</td>
</tr>
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<td>MD (*10^4)</td>
<td>6.3184 ± 0.0539</td>
<td>6.6480 ± 0.0596</td>
<td>6.3232 ± 0.0530</td>
<td>6.1500 ± 0.0915</td>
<td>6.3044 ± 0.0648</td>
<td>6.3028 ± 0.0639</td>
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<td>AFD</td>
<td>12.24 ± 1.20</td>
<td>37.87 ± 2.36</td>
<td>11.67 ± 1.31</td>
<td>33.25 ± 2.32</td>
<td>102.69 ± 4.62</td>
<td>24.37 ± 1.78</td>
</tr>
<tr>
<td>Right Hemiplegia</td>
<td>L Streamlines</td>
<td>4.516 ± 403</td>
<td>8036 ± 891</td>
<td>2584 ± 370</td>
<td>1673 ± 416</td>
<td>21908 ± 1829</td>
<td>6237 ± 789</td>
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<td>FA</td>
<td>0.3678 ± 0.0073</td>
<td>0.2759 ± 0.0068</td>
<td>0.3566 ± 0.0076</td>
<td>0.3587 ± 0.0073</td>
<td>0.3388 ± 0.0084</td>
<td>0.4057 ± 0.0070</td>
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<td>MD (*10^4)</td>
<td>6.0663 ± 0.0684</td>
<td>6.5556 ± 0.0662</td>
<td>6.0800 ± 0.0692</td>
<td>5.9433 ± 0.0679</td>
<td>6.1752 ± 0.0747</td>
<td>6.3030 ± 0.1010</td>
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<td></td>
<td>AFD</td>
<td>17.11 ± 1.47</td>
<td>45.80 ± 4.97</td>
<td>16.32 ± 1.91</td>
<td>28.92 ± 3.08</td>
<td>104.64 ± 7.15</td>
<td>23.00 ± 1.75</td>
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<tr>
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<td>R Streamlines</td>
<td>4.263 ± 464</td>
<td>6567 ± 795</td>
<td>3440 ± 532</td>
<td>2802 ± 442</td>
<td>25063 ± 1587</td>
<td>17192 ± 1120</td>
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<tr>
<td></td>
<td>FA</td>
<td>0.3496 ± 0.0065</td>
<td>0.2878 ± 0.0060</td>
<td>0.3503 ± 0.0066</td>
<td>0.3619 ± 0.0067</td>
<td>0.3560 ± 0.0067</td>
<td>0.4582 ± 0.0041</td>
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<td>MD (*10^4)</td>
<td>6.0293 ± 0.0517</td>
<td>6.4678 ± 0.0635</td>
<td>5.9941 ± 0.0589</td>
<td>5.9752 ± 0.0728</td>
<td>6.0326 ± 0.0554</td>
<td>5.9267 ± 0.0503</td>
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<tr>
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<td>AFD</td>
<td>13.90 ± 1.37</td>
<td>44.55 ± 2.94</td>
<td>15.28 ± 1.64</td>
<td>39.66 ± 2.94</td>
<td>114.22 ± 8.41</td>
<td>28.54 ± 1.50</td>
</tr>
</tbody>
</table>

**Appendix IV Table 1: Connectivity Results**

CTD - Children with typical development. ACC – Anterior Cingulate Cortex. FA - Fractional anisotropy. MD - Mean diffusivity (mm^2/s). AFD - Apparent fibre density (Arbitrary Units). Values are group mean ± standard error. Tract values significantly different between groups (i.e. ANOVA comparing both hemispheres simultaneously) are shown in italics. Individual tract values significantly different from CTD in post-hoc analysis shown in bold.
Structural connectivity of the Anterior Cingulate in children with unilateral Cerebral Palsy due to white matter lesions: **Supplementary Figure**

Flanker performance plotted against diffusion properties

CTD - Children with typical development. MD - Mean diffusivity. AFD - Apparent fibre density.

ACC - Anterior cingulate cortex.