Abnormal deep grey matter development following preterm birth detected using deformation-based morphometry

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Preterm birth is a leading risk factor for neurodevelopmental and cognitive impairment in childhood and adolescence. The most common known cerebral abnormality among preterm infants at term equivalent age is a diffuse white matter abnormality seen on magnetic resonance (MR) images. It occurs with a similar prevalence to subsequent impairment, but its effect on developing neural systems is unknown. MR images were obtained at term equivalent age from 62 infants born at 24–33 completed weeks gestation and 12 term born controls. Tissue damage was quantified using diffusion-weighted imaging, and deformation-based morphometry was used to make a non-subjective survey of the whole brain to identify significant cerebral morphological alterations associated with preterm birth and with diffuse white matter injury. Preterm infants at term equivalent age had reduced thalamic volume without evidence of acute injury in these regions (t = 5.81, P < 0.05), and these alterations were more marked with increasing prematurity (t = 7.13, P < 0.05 for infants born at less than 28 weeks) and in infants with diffuse white matter injury (t = 6.43, P < 0.05). The identification of deep grey matter growth failure in association with diffuse white matter injury suggests that white matter injury is not an isolated phenomenon, but rather, it is associated with the maldevelopment of remote structures. This could be mediated by a disturbance to corticothalamic connectivity during a critical period in cerebral development. Deformation-based morphometry is a powerful tool for modelling the developing brain in health and disease, and can be used to test putative aetiological factors for injury.

Introduction

Preterm birth is associated with long-term neurodevelopmental impairment including cognitive and behavioral problems (Marlow et al., 1993; McCormick et al., 1996), which are more severe with prolonged exposure to the extra uterine environment (Bhutta et al., 2002; Marlow et al., 2005). The most common known cerebral abnormality in surviving preterm infants at term equivalent age is diffuse white matter injury (Figs. 1a and b), which is seen on conventional imaging in one half to two thirds of survivors and is quantifiable as increased apparent diffusion coefficient (ADC) values on diffusion-weighted imaging (DWI) (Maalouf et al., 1999; Counsell et al., 2003). However, it is not known how diffuse white matter injury affects neural development or function, and this significantly hinders the development of strategies to reduce the problems suffered by preterm infants.

Cystic periventricular leucomalacia (PVL) is associated with loss of thalamic volume in late infancy (Lin et al., 2001), which draws attention to the role of white matter injury in thalamic development. An important role for the deep grey matter in preterm brain injury is suggested by its volume reduction in a dose-dependent manner with degree of prematurity at birth (Inder et al., 2005), which mirrors the prevalence of subsequent neurocognitive impairment among survivors (Bhutta et al., 2002; Marlow et al., 2005).

Cystic PVL is uncommon and cannot account for the high prevalence of neural dysfunction seen in preterm infants. There have been no reports of the effect of the common but poorly understood diffuse white matter lesion on other brain regions, at least in part because of the technological difficulties of making such observations.

To investigate the effect of prematurity and diffuse white matter injury on brain development in the neonatal period, we have combined conventional imaging with DWI and deformation-based morphometry (DBM), which uses image registration and statistical analysis to quantify structural differences between groups. We have...
applied this quantitative morphometric method to make a non-hypothesis-based survey of the whole brain, to identify structural alterations apparent in preterm infants at term equivalent age, and to examine the effects of diffuse white matter injury on brain structure in early development.

Materials and methods

Subjects

Ethical permission for MR studies was granted by the Hammersmith Hospital Research Ethics Committee, and informed parental consent was obtained. Preterm infants were recruited from the Neonatal Intensive Care Unit (NICU) at Queen Charlotte’s and Chelsea Hospital (QCCH) between February 2001 and December 2002. Exclusion criteria were infants with congenital central nervous system infection; exposure to postnatal steroids; cystic PVL; hemorrhagic parenchymal infarction, post-hemorrhagic ventricular dilatation and porencephalic cysts. The MR images of 62 preterm infants (median GA 29.71 weeks, range 24–33 completed weeks) were analyzed at term equivalent (median GA 40.43 weeks, range 37–44.57) with 12 term born controls recruited from the postnatal wards at QCCH (median 39.57 weeks, range 36–41.86; imaged on postnatal day 4, median) (Table 1). All of the infants in the preterm group had serial cranial ultrasound scans as part of standard clinical care during their stay on the NICU: 13 had evidence of germinal matrix hemorrhage—intraventricular hemorrhage (grade 1 to 2) on early cranial ultrasound examination. There was no significant difference between the weights of the two groups at the time of image acquisition ($P = 0.21$). Preterm infants were sedated with chloral hydrate, and control infants were examined in natural sleep. We use chloral hydrate in the preterm group because it increases the likelihood of successful acquisition, and we are unaware of data suggesting that it alters the magnetic resonance or diffusion properties of cerebral tissue. Ear protection was used (Natus MiniMuffs, Natus Medical Inc, San Carlos, CA).

MR image acquisition

For volumetric studies, a 1.5 T MR Eclipse system (Philips Medical Systems) was used to acquire high-resolution RF spoiled T1-weighted (TR = 30 ms, TE = 4.5 ms, flip angle = 30°) volume datasets with a voxel size of 1.0 × 1.0 × 1.6 mm. For DWI we used the following parameters: TR 6000 ms; TE 100 ms; 100 × 100 matrix, FOV 24 cm, slice thickness 5 mm. A reference image was obtained with a $b$ value of 0 (nominal value), and DWIs were obtained with a $b$ value of 1000 s/mm$^2$ in the read, phase, and slice directions.

Table 1

<table>
<thead>
<tr>
<th>Summary of patient characteristics</th>
<th>Preterm infants at term equivalent age $n = 62$</th>
<th>Term born controls $n = 12$</th>
</tr>
</thead>
<tbody>
<tr>
<td>PMA at birth/weeks (median and range)</td>
<td>29.71 (24–34)</td>
<td>39.57 (36–41.86)</td>
</tr>
<tr>
<td>Gender/M:F</td>
<td>27:35</td>
<td>7:5</td>
</tr>
<tr>
<td>Birth weight/gram (median and range)</td>
<td>1270 (610–2178)</td>
<td>3220 (2700–3654)</td>
</tr>
<tr>
<td>Corrected PMA at scan/weeks (median and range)*</td>
<td>40.43 (37–44.57)</td>
<td>40.43 (36.57–43.14)</td>
</tr>
<tr>
<td>Weight at time of image acquisition/gram (mean and range)*</td>
<td>3079 (1899–4380)</td>
<td>3220 (2700–3654)</td>
</tr>
<tr>
<td>Diffuse white matter injury (ADC value &gt;2 SD above mean of controls in one or more white matter region, but with ADC values within 1sd of the mean of the controls in deep grey matter and the posterior limb of the internal capsule)**</td>
<td>28/60</td>
<td>–</td>
</tr>
<tr>
<td>Intrauterine growth restriction</td>
<td>17</td>
<td>–</td>
</tr>
<tr>
<td>Perinatal sepsis</td>
<td>19</td>
<td>–</td>
</tr>
<tr>
<td>Chronic lung disease (persistent oxygen requirement at 28 days of postnatal life)</td>
<td>16</td>
<td>–</td>
</tr>
</tbody>
</table>

* No significant difference between groups.
** DWI was available for 60/62 preterm infants at term equivalent age.
Deformation-based morphometry

To map the anatomy of each subject into the anatomy of a common template (the image of a healthy term control infant), we used a non-rigid registration algorithm (Rueckert et al., 1999). This algorithm combines an affine transformation which adjusts for differences in scale and position between subjects and the template, with a local transformation to achieve precise spatial correspondence of structures between each subject and the template. The local transformation is modeled using a free-form deformation (FFD)-based on B-splines, which allows detailed modelling of 3D deformable objects. In essence, FFDs deform an object by manipulating an underlying mesh of control points. The resulting deformation controls the shape of the 3D object and can be written as the 3D tensor product of the 1D cubic B-splines, where c denotes the control points that parameterize the transformation.

\[ T_{local}(x) = \sum_{i} \sum_{j} \sum_{k} \sum_{m} \sum_{n} B_i(u)B_j(v)B_k(w)c_{i+j+k+m+n}, \]

where \( B_i \) is the ith B-spline basis function, \( c \) denotes a \( mx \times my \times mz \) lattice of control points which parameterize the FFD, \( i, j, k \), denote the indexes of the control points, and \( u, v, w \) correspond to the relative positions of \( x \) in lattice coordinates.

The optimal transformation is found by minimizing a cost function associated with the global and local transformation parameters. The cost function comprises two competing goals: the first term represents the cost associated with the voxel-based similarity measure, in this case normalized mutual information (Studholme et al., 1998), while the second term is based on the squared sum of the second derivatives of the deformation field (Rueckert et al., 1999). This term corresponds to a regularization term which constrains the transformation to be smooth. The resulting transformation \( T \) maps each point in the anatomy of the reference subject to the corresponding point in the anatomy of the subjects. The algorithm has been previously optimized and evaluated in a neuroimaging study (Crum et al., 2004).

An MR image taken from a term born control infant was selected as the template space to which the images from all subjects in the study group were brought into alignment using a sequential multi-resolution scheme of control point spacings of 20 mm, 10 mm, 5 mm, and 2.5 mm. The resulting 2.5–mm control point mesh defines a continuous and analytic representation of the deformation field which describes the point-wise 3D displacement vectors required to warp each dataset to the template. Cases where registration achieved qualitative alignment of anatomical structures were analyzed (Fig. 2). Seven additional images could not be brought into spatial alignment with the template due to motion artefact in the source image, and they are not included in the analysis.

Apparent diffusion coefficient values

Circular regions of interest were positioned bilaterally in frontal, central, and posterior white matter at the level of the centrum semiovale on the transverse slice above the level of the lateral ventricles where the central sulcus was at its maximum depth, on the reference image (\( b = 0 \)) and on the phase, read, and slice DWIs (Fig. 1c). Care was taken to position regions of interest to avoid partial volume averaging from cerebrospinal fluid and cortical grey matter. Regions of interest were positioned in the posterior limb of the internal capsule, the thalamus and the lentiform nuclei bilaterally on the slice in which each structure was largest in the transverse plane (Fig. 1d). The ADC values for each ROI were calculated using previously described methods (Counsell et al., 2003).

The mean area (standard deviation) for each ROI in the native diffusion space was anterior white matter = 24.8 ± 2.3 mm² (4.31 voxels, SD 0.4); central white matter = 23.2 ± 2.2 mm² (4.03 voxels, SD 0.38); posterior white matter = 25.1 ± 2.4 mm² (4.36 voxels, SD 0.42); posterior limb of the internal capsule = 16.9 ± 2.0 mm² (2.93 voxels, SD 0.35); thalamus = 17.51 ± 2.4 mm² (3.04 voxels, SD 0.42); and lentiform nucleus = 21.79 ± 2.7 mm² (3.78 voxels, SD 0.47).

Data analysis

Volume change comparisons

The determinant of the Jacobian operator of the deformation field was used to quantify local volume differences between registered images and the template. This metric provides an estimate of voxel-wise volume change for all transformed images with respect to the template. The volume change maps for subjects in each group were analyzed with a two-sample \( t \) test implemented in SPM99 to identify areas of statistically significant differences in tissue volume between groups (http://www.fil.ion.ucl.ac.uk/spm). Corrections for multiple comparisons were made using the family wise error rate controlled by random field theory, and significance thresholds were set at \( P = 0.05 \). Only voxel clusters greater than 10 were considered in the statistical model. Statistical parametric maps are displayed in the template anatomy in consecutive 1–2 mm slices to include all areas of volume difference for any given map.

Fig. 2. An example of a deformation field. Panel a is an example sagittal section of the reference anatomy. Panel b is a midline slice taken from a preterm infant at term equivalent age, which differs in size, shape, position, and local anatomy. Following non-rigid registration the preterm brain occupies the spatial coordinates of the reference space, and the information required to achieve spatial correspondence of structures is retained within the deformation field (c). The deformation field displayed in this illustration is a magnified two-dimensional representation of the 3D field.
Characterizing white matter injury using DWI

Regions of interest where ADC values exceeded the mean ADC value of the control group by more than 2 standard deviations were defined as abnormal: infants with one or more abnormal region were classified as having white matter injury. Infants with ADC values within 1 standard deviation of the mean of the control group in all white matter regions were classified as having normal white matter.

Robustness analysis and interobserver variability

We have previously shown that this image registration algorithm performs consistently when different neonatal brain template images are used (Boardman et al., 2003), but here, we used methods described by Bland and Altman (1986) to compare the degree of agreement obtained from the transformations to two different templates with that between two manual observers. The observers performed manual measurements of lateral and third ventricular volume on individual subjects, and these were compared to the volumes obtained from the automatic process for those same subjects. The mean difference in ventricular volume using the DBM approach was $-0.11 \text{ cm}^3$ (95% limits of agreement $-0.99$ to $0.77 \text{ cm}^3$), and that measured interactively by two observers was $-0.77 \text{ cm}^3$ (95% limits of agreement $-2.65$ to $1.11 \text{ cm}^3$). This analysis provides further evidence that DBM results are repeatable using different templates, and that agreement between volume measurements using DBM is as good or better than that obtained using interactive methods.

Results

Structural brain changes after preterm birth

The DBM analysis showed an expected volumetric increase in the posterior horns of the lateral ventricles ($t = 5.81$, $P < 0.05$), which has been described in previous studies (Peterson et al., 2003). However, the most striking difference between preterm and term infants was an unexpected reduction in the volume within deep grey matter, predominantly the thalami and lentiform nuclei ($t = 5.81$, $P < 0.05$) (Fig. 3), which was not apparent on conventional analysis of the T1- and T2-weighted images.

To establish whether the structural changes detected by DBM could be due to random variation, the subjects were divided into two random groups and compared. There were no significant morphological differences in deep grey matter or the ventricular system between these groups. We next considered the effect of gender on brain morphology as sexual dimorphism has been reported in childhood (Giedd et al., 1997; Reiss et al., 2004), and early gender differences in the cognitive functioning of preterm infants have been suggested (Hindmarsh et al., 2000). However, there were no significant morphological differences robust to the correction for multiple comparisons.

The gestational age in the preterm group varied between 24 and 33 completed weeks, so to investigate whether prematurity had a dosage effect on deep grey matter abnormalities, we compared those born at less than 28 completed weeks of gestation ($n = 16$) and infants born between 32 and 33 completed weeks ($n = 17$) to the control group. Volume reduction was identified in deep grey matter in the more immature group ($t = 7.13$, $P < 0.05$), and no significant clusters were identified in the more mature infants. A further analysis dichotomizing the group by birth weight showed a similar pattern of deep grey matter volume reduction in infants with birth weight <1000g ($n = 11$) and among infants weighing 1000–2000g at birth ($n = 33$) with respect to term born controls.

We examined a series of clinical variables which might account for changes in brain development: intraterine growth restriction ($n = 17$); chronic lung disease indicated by a persistent oxygen requirement at 28 days of postnatal life ($n = 16$); and confirmed perinatal sepsis (positive blood cultures or viral isolation in the infant, or a clinical diagnosis of maternal infection together with raised maternal C-reactive protein and/or white blood cell count) ($n = 19$). None were associated with significant additional morphometric effects.

Regional tissue characterization by DWI

ADC values characterize tissue injury in the perinatal period (Neil et al., 1998; Huppi et al., 1998; Counsell et al., 2003; Rutherford et al., 2004), and we used them to investigate the nature of changes in the deep grey matter in preterm infants at term equivalent age. There were no significant differences in the ADC values of lentiform, thalamus or the posterior limb of the internal capsule between preterm infants at term and the controls. One preterm infant at term equivalent age had an ADC value $>2$ standard deviations from the mean of the control group in the thalamus, none had abnormal values in the lentiform nuclei, and 14 had high ADC values in the posterior limb of the internal capsule. Visual examination of conventional MR images also showed no evidence of tissue damage or destruction in the deep grey matter structures.

Although ADC values in deep grey matter did not differ between the two groups, values in the white matter were significantly increased in preterm infants at term equivalent age (Fig. 4), which is consistent with diffuse white matter injury (Counsell et al., 2003). Twenty-eight subjects had abnormally high ADC values in one or more white matter region (with normal ADC values in deep grey matter and the posterior limb of the internal capsule), and 11 infants had normal ADC values in all regions (Table 2). The mean gestational age at birth of these groups was not significantly different (29.14 weeks and 30.29 weeks, respectively, $P = 0.20$).

White matter injury and structural abnormality

We explored whether the morphological abnormalities seen in the deep grey nuclei, and not apparently the result of acute injury, may be related to diffuse white matter injury. Infants with white matter damage showed volumetric reduction within deep grey matter nuclei but the group with normal ADC values in white matter did not have significantly altered deep grey matter structures compared with term born controls (Fig. 5). These data show that diffuse white matter injury is associated with significant abnormalities in deep grey matter structure.

Discussion

This is the first use of DBM to perform a non-subjective survey of the whole brain in the neonatal period. We chose a DBM
approach rather than the widely used technique of voxel-based morphometry (VBM) for two reasons related to unique physical properties of the brain in the neonatal period. The white matter is predominantly unmyelinated, and tissue classification is problematic, so there are currently no published VBM protocols to classify neonatal cerebral tissue, which is a requisite step in VBM (Ashburner and Friston, 2000). In addition, the neonatal brain is more variable in size and shape than the adult brain, so the high-dimensional registration procedure used in DBM may be more appropriate for spatial normalization than an affine or coarse deformation-based spatial normalization currently used in voxel-based approaches. An advantage of the DBM approach is that it requires neither prior hypotheses nor tissue segmentation; rather it surveys the brains of groups of infants for morphometric changes within and between anatomical structures, providing a powerful tool to explore brain development, particularly when combined with the quantitative definition of tissue characteristics provided by DWI and other advanced imaging techniques.

DBM identified volume reduction in the thalamus and lentiform nuclei in association with preterm birth, particularly in infants with diffuse non-cystic white matter injury. These changes could not be detected by conventional analysis of the T1- and T2-weighted images. Previous studies have suggested that deep grey matter volume might be decreased in preterm infants with PVL, but tissue classification difficulties and the use of small patient groups leave room for doubt about the significance of these findings as a neurological substrate for the impairments seen in the majority of preterm infants (Lin et al., 2001). A different study that used an automatic tissue segmentation algorithm to classify and quantify cerebral tissue types observed that preterm infants at term equivalent age had reduced cortical and subcortical grey matter volumes, and although young gestational age at birth was associated with subcortical grey matter volume reduction, white matter abnormality, assessed qualitatively, was not (Inder et al., 2005). This might be explained by methodological differences between the work of Inder et al. and this study. First, the patient...
group presented in this study did not contain infants with major parenchymal lesions such as cystic PVL or IVH. Second, we used DWI to define diffuse matter injury quantitatively thereby reducing the potential for observer bias or imaging artefact. Third, DBM does not rely on tissue classification prior to volumetric analysis; a significant advantage as this is particularly difficult in the predominantly unmeylinated brain.

These data show that infants thought to be suffering from specific white matter abnormalities in fact have more widespread neuroanatomical abnormalities. Recent research has successfully focused upon white matter injury and in particular on the role of the preoligodendrocyte, which is vulnerable to free radical and inflammatory injury during the preterm period. It has been suggested that preferential loss of preoligodendrocytes (Haynes et al., 2003) results in a failure of normal white matter tract development and myelination (for review, see Volpe, 2003). In addition, protein expression studies suggest that white matter damage may represent a primary axonopathy as well as preoligodendrocyte damage (Meng et al., 1997; Arai et al., 1995; Dammann et al., 2001).

However, it now becomes essential to understand the role of deep grey matter abnormality in the syndrome of preterm brain injury, and specifically, its association with diffuse white matter injury. Deep grey matter abnormalities had neither conventional MRI, nor DWI features of acute tissue injury, and so were not apparently due to acute tissue damage, nor were they specifically linked to adverse clinical events such as perinatal infection or chronic lung disease (oxygen dependency at 28 days of postnatal life). Severe bronchopulmonary dysplasia (BPD) has been associated with subsequent extra-pyramidal movement disorders (Perlman and Volpe, 1989; Majnemer et al., 2000), which are presumed to originate in the deep grey matter structures. As only three of the infants in the study group had severe bronchopulmonary dysplasia (requiring oxygen at term equivalent age/home oxygen), this study cannot assess whether the abnormalities seen in the deep grey matter are more apparent in this small subgroup. We have previously shown that focal brain lesions in preterm infants are strongly associated with antenatal infection and inflammation (Duggan et al., 2001), but neither this nor intrauterine growth retardation was associated with additional morphometric abnormalities to those seen in the deep grey matter.

It is possible that growth failure might be caused by disconnection of the thalamus from the developing cortex due to impaired white matter tract development, which is supported by DWI and DTI studies of preterm brain injury. Increased ADC values in the white matter of the centrum semiovale reflect an increase in water content and/or a decrease in the restriction of water motion which arises due to loss of the normal barriers to diffusion such as axonal membranes, myelin neurofilaments, and macromolecules (Beaulieu, 2002). We have previously shown that the ADC value provides a useful objective measure of the diffuse white matter lesion, and that the diffusion properties of the diffuse lesion are similar to those in tissue affected by cystic PVL, indicating that the lesion represents significant tissue damage (Counsell et al., 2003). Furthermore, relative anisotropy, a measure of the directionality of water diffusion, is reduced in the white matter of preterm infants at term equivalent age compared with term born infants, which may be due to a reduction in fibre number or a delay in myelination of fiber tracts (Huppi et al., 1998). Either event would be expected to result in reduced connectivity between the cortex and basal ganglia/thalami. The interpretation of ADC values in the deep grey matter is less well understood, but the similarity in values between the controls and the preterm infants at term equivalent age does suggest that acute tissue injury was not responsible for the structural changes seen in the preterm group. Although we found no significant difference in the ADC values of the PLIC between the two groups, this could reflect partial volume averaging from neighboring voxels, given the resolution of DWI.
Injury to the preoligodendrocyte or axon could result in impairment to thalamocortical tracts within white matter causing decoupling between cortex and thalamus, and aberrant neuronal differentiation and organization at these sites (Marin-Padilla, 1999). Damage to the transient cortical subplate might also mediate corticothalamic decoupling. This neuronal population are among the first postmitotic cortical neurons (Allendoerfer and Shatz, 1994), are present in superficial white matter, and are required to guide and sustain early thalamocortical tract development and organization (for review, see Volpe, 1996). Disconnection of the thalamus from the cortex might also help explain some abnormalities in cortical morphology found in older children with specific neurocognitive impairments (Peterson et al., 2000; Isaacs et al., 2001; Isaacs et al., 2003; Isaacs et al., 2004; Reiss et al., 2004; Woodward et al., 2005). However, this literature is limited as the effects of preterm birth may be modified by later events. The opportunity to consider pathological changes and development at the time of postulated vulnerability are advantages of the present study.

Although parcellation and classification approaches have reported reductions in tissue classified as cortical grey matter volume among preterm infants at term equivalent age (Peterson et al., 2003; Inder et al., 2005), this was not detected using DBM. This may be because DBM is highly sensitive to local change, and summated changes detected by global tissue classification obscures considerable local diversity to which DBM is sensitive. The inconsistency of regional change then means that the numbers of infants with change in a particular region is too small to create statistically significant differences in our group while maintaining our highly stringent approach to the correction for multiple comparisons used in the statistical analyses of the deformation fields. We predict that larger numbers or a group of infants with similar cortical abnormality may be required to identify regional cortical volume change.

These results draw attention to changes in deep grey matter in preterm infants and to the development of normal connectivity.
between thalamus and cortex. This has implications both for theories of causation and potential therapies for these infants and suggests that new imaging technologies may be valuable tools in further understanding the alterations to brain development that underlie these complex developmental problems.

Acknowledgments

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