MS CLAIRE KELLY (Orcid ID : 0000-0002-6564-0872)

DR JOY ELIZABETH OLSEN (Orcid ID : 0000-0002-8006-0139)

DR ALICIA JANE SPITTLE (Orcid ID : 0000-0002-6535-661X)

[OA; 3 Figs; 1 Table; 8 supplementary items]

Brain structure and neurological and behavioural functioning in infants born preterm

CLAIRE E KELLY[1]

DEANNE K THOMPSON[1,2,3]

JEANIE LY CHEONG[1,4,5]

JIAN CHEN[1,6]

JOY E OLSEN[1,4]

ABBEY L EELES[1]

JENNIFER M WALSH[1,4,5,7]

MARC L SEAL[1,2]

PETER J ANDERSON[1,8]

LEX W DOYLE[1,2,4,5]

ALICIA J SPITTLE[1,4,9]

1 Murdoch Children's Research Institute, Melbourne, Victoria; **2** Department of Paediatrics, The University of Melbourne, Melbourne, Victoria; **3** Florey Institute of Neuroscience and Mental Health, Melbourne, Victoria; **4** Newborn Research, Royal Women's Hospital, Melbourne, Victoria; **5** Department of Obstetrics and Gynaecology, The University of Melbourne, Melbourne, Victoria; **6** Department of Medicine, Monash Medical Centre, Monash University, Melbourne, Victoria; **7** Pediatric, Infant, Perinatal Emergency Retrieval

(PIPER), Royal Children's Hospital, Melbourne, Victoria; **8** Monash Institute of Cognitive and Clinical Neurosciences, Monash University, Melbourne, Victoria; **9** Department of Physiotherapy, The University of Melbourne, Melbourne, Victoria, Australia.

Correspondence to Claire E Kelly, Victorian Infant Brain Studies (VIBeS), Murdoch Children's Research Institute, The Royal Children's Hospital, 50 Flemington Road, Parkville, Melbourne, Victoria, Australia, 3052. E-mail: claire.kelly@mcri.edu.au

ABBREVATIONS

HNNE	Hammersmith Neonatal Neurological Examination
NNNS	Neonatal Intensive Care Unit Network Neurobehavioral Scale
TEA	Term-equivalent age

AIM To examine: (1) relationships between brain structure, and concurrently assessed neurological and behavioural functioning, in infants born preterm at term-equivalent age (TEA; approximately 38–44wks); (2) whether brain structure–function relationships differ between infants born very (24–29wks) and moderate-late (32–36wks) preterm.

METHOD A total of 257 infants (91 very preterm, 166 moderate-late preterm; 120 males, 137 females) had structural magnetic resonance imaging (MRI) and neurological and behavioural assessments (Prechtl's general movements assessment, Neonatal Intensive Care Unit Network Neurobehavioral Scale [NNNS] and Hammersmith Neonatal Neurological Examination [HNNE]). Two hundred and sixty-three infants (90 very preterm, 173 moderate-late preterm; 131 males, 132 females) had diffusion MRI and assessments. Associations were investigated between assessment scores and global brain volumes using linear regressions, regional brain volumes using Voxel-Based Morphometry, and white matter microstructure using Tract-Based Spatial Statistics.

RESULTS Suboptimal scores on some assessments were associated with lower fractional anisotropy and/or higher axial, radial, and mean diffusivities in some tracts: NNNS attention and reflexes, and HNNE total score and tone, were associated with the corpus callosum and

optic radiation; NNNS quality of movement with the corona radiata; HNNE abnormal signs with several major tracts. Brain structure-function associations generally did not differ between the very and moderate-late preterm groups.

INTERPRETATION White matter microstructural alterations may be associated with suboptimal neurological and behavioural performance in some domains at TEA in infants born preterm. Brain structure–behaviour relationships are similar for infants born very preterm and moderate-late preterm.

[Boxed content to appear on page 2]

What this paper adds

- Brain volume is not related to neurological/behavioural function in infants born preterm at term.
- White matter microstructure is related to some neurological/behavioural domains at term.
- Brain–behaviour relationships are generally similar for infants born very preterm and moderate-late preterm.

[First page footer]

[Left page footer]

[Right page footer]

[Main text]

Assessments have been developed to evaluate an infant's neurological integrity and behavioural functioning at around term-equivalent age (TEA), from 38 weeks to 44 weeks' gestational age. Neurological assessments can include evaluation of an infant's muscle tone, movement quality, and reflexes, while behavioural assessments can include evaluation of an

infant's ability to regulate their level of arousal and states, and to attend, orient, and habituate.[1] Infants born preterm (<37wks gestational age) often have atypical performance on neurological and behavioural assessments at TEA compared with infants born at term (≥37wks).[2]

More atypical performance on neurological and behavioural assessments has previously been related to brain injury and impaired brain growth in both the white and grey matter, as assessed by qualitative scoring of magnetic resonance imaging (MRI).[3–8] This previous research established that atypical neurological and behavioural performance at TEA has neural correlates, suggesting the assessments provide insight into the integrity of the developing brain. MRI can provide additional information beyond qualitative brain abnormality scores, such as information on brain volumes and white matter microstructure. At TEA, infants born preterm have smaller global and regional brain volumes,[9] and altered microstructure in many major white matter fibre tracts,[10] compared with infants born at term. However, the relationships between brain volumes and microstructure at TEA, and concurrent neurological and behavioural outcomes, are not well defined. Only one study has reported associations between white matter microstructure and neurological and behavioural outcomes in infants born preterm (<34 wks) at TEA.[11] Additional studies in this area might help to improve knowledge of the neural correlates of neurological and behavioural outcomes in infants born preterm at TEA.

Furthermore, most previous research has focused on infants born very preterm (<32wks' gestational age), and there has been much less research on infants born moderate-late preterm (32–36wks' gestational age). Rates of atypical neurological and behavioural performance at TEA are highest in infants born very preterm, but are still higher in infants born moderate-late preterm compared with infants born at term.[8] Brain development is rapid during the late preterm period, and infants born moderate-late preterm have less mature brains than infants born at term.[12] On MRI, infants born moderate-late preterm have smaller brains[13] and altered white matter microstructure[14] compared with infants born at term at TEA. Brain MRI has been related to neurological and behavioural performance at TEA in infants born very preterm.[4–6,8,11] However, it is unclear whether brain MRI relates to neurological and behavioural performance at TEA in infants born moderate-late preterm, in the same way that it does in infants born very preterm, or whether brain–behaviour relationships differ between infants born very preterm and infants born moderate-late preterm. We have recruited and obtained early MRI and neurological and behavioural data for cohorts of infants born

very preterm and moderate-late preterm,[1,13] enabling us to address this research question. Our previous study was the first to relate qualitative MRI scores to neurological and behavioural outcomes in both infants born very preterm and moderate-late preterm,[8] but this is yet to be done using quantitative MRI measures.

The aims of the current study were (1) to explore the relationships between brain structure, including global and regional brain volumes and white matter microstructure, and neurological and behavioural performance at TEA in infants born preterm; and (2) to investigate whether brain structure–behaviour relationships differ between infants born very preterm and moderate-late preterm.

METHOD

Participants

Participants were derived from two prospective, longitudinal cohort studies recruited from the Royal Women's Hospital, Melbourne. The first cohort of 201 infants was recruited between November 2009 and November 2012, and infants were included if they were born moderate-late preterm (32–36wks' gestational age).[13] The second cohort of 149 infants was recruited between January 2011 and December 2013, and infants were included if they were born very preterm (<30wks' gestational age).[1] For both cohorts, infants were excluded if they had congenital abnormalities known to affect development, or if they had non-English speaking parents because the larger study involved many English-based questionnaires. Ethical approval for both studies was obtained from the Human Research Ethics Committees of the Royal Women's Hospital and the Royal Children's Hospital, Melbourne, and written informed consent was obtained from parents. Of the recruited infants, 104 were born very preterm and 198 were born moderate-late preterm and had MRI between 38–44 weeks' gestational age. Ninety-one infants born very preterm and 167 infants born moderate-late preterm had structural images of sufficient quality for global brain volume analysis (i.e. 13 infants born very preterm and 31 infants born moderate-late preterm were excluded because of structural image artefacts). Ninety-one infants born very preterm and 166 infants born moderate-late preterm had structural images of sufficient quality for voxel-based morphometry (i.e. one additional infant born moderate-late preterm was excluded because of poor registration during Voxel-Based Morphometry), and 90 infants born very preterm and 174 infants born moderate-late preterm had diffusion images of sufficient quality for Tract-Based Spatial Statistics (i.e. 14 infants born very preterm and 24 infants born moderate-late preterm were excluded because of incomplete diffusion acquisitions and/or diffusion image

artefacts). Of those infants who had usable global brain volume data, 91 born very preterm and 166 born moderate-late preterm also had at least one neurological or behavioural assessment between 38 weeks and 44 weeks' gestational age (numbers differed slightly for the various assessments). Of those infants who had usable Voxel-Based Morphometry data, 91 born very preterm and 165 born moderate-late preterm also had at least one assessment between 38 weeks and 44 weeks' gestational age. Of those infants who had usable Tract-Based Spatial Statistics data, 90 born very preterm and 173 born moderate-late preterm also had at least one assessment between 38 weeks and 44 weeks' gestational age. The numbers of participants included in the current study are summarized in Figure S1 (online supporting information).

MRI

Infants had brain MRI at TEA at the Royal Children's Hospital, Melbourne on a 3T Siemens Magnetom Trio, Tim system (Siemens Healthcare GmbH, Erlangen, Germany).

Infants were fed, swaddled, placed in a MedVac bag (CFI Medical Solutions Inc, Fenton, MI, USA), and scanned during natural sleep without sedation. T_2-weighted images were acquired with turbo spin echo sequences (repetition time 8910ms; echo time 152ms; flip angle 120°; field of view 192mm x 192mm; matrix 192 x 192; 1mm^3 isotropic voxels]. Diffusion-weighted images were acquired with an echo planar imaging sequence, which was the same for all infants (repetition time 20400ms; echo time 120ms; field of view 173mm x 173mm; matrix 144 x 144; 1.2mm^3 isotropic voxels; 45 non-collinear gradient directions with multiple b-values ranging from 100s/mm^2 to 1200s/mm^2; three b=0s/mm^2 volumes).[14]

Structural images were bias corrected,[15] skull-stripped,[16] and segmented into tissue types.[17] Tissue segmentations in each infant's native space were used to examine global brain volumes (intracranial volume and total brain tissue, cortical grey matter, white matter, cerebrospinal fluid, subcortical grey matter, cerebellum and brainstem volumes). Tissue segmentations registered non-linearly to the standard space of a neonatal template[18] were analysed using Voxel-Based Morphometry.

Diffusion images were processed using the Functional MRI of the Brain Software Library. This included (1) motion correction (with b-vector reorientation); (2) echo planar imaging distortion correction using an average gradient echo field map (based on n=10; an average map was used because field maps were not acquired for each infant) and the

Functional MRI of the Brain's Utility for Geometrically Unwarping Echo Planar Images tool; and (3) diffusion tensor fitting using the weighted linear least squares method to generate fractional anisotropy and axial, radial, and mean diffusivity images. Diffusion tensor images were analysed using Tract-Based Spatial Statistics,[19] as previously described.[14]

Neurological and behavioural assessments

Infants had three assessments at TEA. All assessments were administered according to their standardized procedures by trained assessors who were unaware of the infants' clinical histories, as previously detailed.[1,8]

Assessment 1

Prechtl's assessment of general movements[20] is an observational assessment from video-recordings of the global quality of an infant's spontaneous whole body movements. General movements were categorized as normal (fluent, variable in speed and amplitude, and involving complexity of movement patterns) or abnormal (poor repertoire, cramped-synchronized, or chaotic).

Assessment 2

The Neonatal Intensive Care Unit Network Neurobehavioral Scale (NNNS)[21] assesses infant neurological integrity, behavioural function, and responses to stress using 45 items. Thirteen summary scores were calculated using local norms[22] and categorized as suboptimal for scores less than the 10th centile on the attention, quality of movement, and regulation summary scores, or greater than the 90th centile for the handling, non-optimal reflexes, asymmetrical reflexes, hypotonicity, hypertonicity, excitability, lethargy, arousal, and stress/abstinence scores. Habituation summary scores were not included as most infants were not in the appropriate behavioural state (asleep) to administer these items.

Assessment 3

The Hammersmith Neonatal Neurological Examination (HNNE)[23] primarily evaluates neurological function. It includes 34 items, with six subtotal scores (tone, tone patterns, reflexes, spontaneous movements, abnormal signs, and behaviour), which are summed to a total score. Subtotal and total scores were categorized as suboptimal for scores less than the 10th centile using local norms.[22]

Statistical analysis

Statistical analysis of global brain volumes was performed using Stata version 14 (Statcorp, College Station, TX, USA). For the primary aim, volumes were compared between infants with optimal and suboptimal scores for each neurological or behavioural assessment using linear regressions adjusted for gestational age at MRI. Linear regressions were modelled using generalized estimating equations to account for correlations between data from multiple births. For the second aim, interaction terms between assessment scores (suboptimal or optimal) and preterm subgroup (very preterm or moderate-late preterm) were added to all the models to investigate whether the brain structure–behaviour associations varied between infants born very preterm and moderate-late preterm. Each analysis was multiple-comparison corrected for the number of brain volumes (eight) using the false discovery rate method. Thus, in the results section, the results are described in terms of whether there were significant associations that had a *p*-value less than 0.05, false discovery rate-corrected.

Voxel-wise statistical analysis of the Voxel-Based Morphometry and Tract-Based Spatial Statistics data was performed using the Functional MRI of the Brain Software Library's 'Randomise' tool (version 2.9), a non-parametric, permutation-based method.[24] For the primary aim, voxel-wise cortical grey matter and white matter volumes and white matter diffusion values were compared between infants with optimal and suboptimal scores for each assessment, adjusted for gestational age at MRI. Voxel-wise volume analyses were also performed with and without adjusting for intracranial volume. For the second aim, models were created to investigate whether there were interactions between assessment scores and preterm subgroup for the voxel-wise cortical grey and white matter volumes and diffusion values; that is, to investigate whether the voxel-wise brain structure–behaviour associations varied between infants born very preterm and moderate-late preterm. All analyses were performed with 5000 permutations, threshold-free cluster enhancement and voxel-wise multiple comparison correction, using the family-wise error rate method. Thus, in the results section, the results are described in terms of whether there were significant associations that had a *p*-value less than 0.05, family-wise error rate-corrected. Clusters were localized to anatomical brain regions and tracts with the aid of a neonatal atlas.[25]

RESULTS

Participants

Participant characteristics for the very preterm and moderate-late preterm groups are shown in Table I. The number of infants who had major brain injuries was low in both the very preterm and moderate-late preterm groups.

Neurological and behavioural assessments were generally performed on the same day as each other, but sometimes the general movements assessment was on a different day to the NNNS and HNNE. Additionally, all three assessments were generally performed on the same day as the MRI, but were occasionally performed on a different day (Table SI, online supporting information).

Neurological and behavioural outcomes for the larger cohort have been published previously.[8] For the current cohort with volumetric and diffusion data, the percentage of infants with abnormal scores on the general movements assessment was slightly higher in the very preterm group compared with the moderate-late preterm group. The percentage of infants with suboptimal scores on the NNNS subscales was generally similar between very preterm and moderate-late preterm groups, although for some subscales the percentage was higher in the very preterm group than the moderate-late preterm group, particularly the non-optimal reflexes, hypotonicity, and stress subscales. The percentage of infants with suboptimal HNNE subscale scores was generally higher in the very preterm group compared with the moderate-late preterm group (Tables SII and SIII, online supporting information).

Perinatal characteristics were generally similar between the participants born moderate-late preterm who were included in the analyses and the participants born moderate-late preterm who could not be included. However, the included participants born very preterm had a slightly older gestational age at birth, larger birthweight, and a lower rate of postnatal infection compared with the participants born very preterm, who could not be included in the current study (Tables SIV and SV, online supporting information).

General movements and brain structure

There was no significant association between abnormal general movements and global brain volumes (Fig. 1; Table SVI, online supporting information). There was an association between abnormal general movements and smaller volume in the right external capsule in 12 voxels only (Fig. 2a); this association was not significant after adjusting for intracranial volume. Additionally, abnormal general movements were associated with small clusters of increased axial diffusivity and mean diffusivity in the right internal capsule and corona radiata (and external capsule for axial diffusivity only; Figs. 2b and 2c).

NNNS and brain structure

There was no significant association between NNNS scores and global brain volumes (Fig. 1; Table SVI).

Suboptimal lethargy scores were associated with smaller anterior insular cortex volume (Fig. 2d); however, this association was not significant after adjusting for intracranial volume.

Suboptimal attention scores were associated with lower fractional anisotropy in the corpus callosum, sagittal stratum, optic radiation, inferior fronto-occipital fasciculus, external capsule, and cingulum (Fig. 2e), higher radial diffusivity in the corpus callosum, optic radiation and left cingulum (Fig. 2f), and higher mean diffusivity in the left optic radiation (Fig. 2g).

Suboptimal scores for non-optimal reflexes were associated with lower fractional anisotropy in the corpus callosum, sagittal stratum, optic radiation, inferior fronto-occipital fasciculus, and cingulum (Fig. 2h).

Suboptimal hypotonicity was associated with lower axial diffusivity and mean diffusivity in the left corona radiata (Figs. 2i and 2j).

Suboptimal quality of movement was associated with higher axial, radial, and mean diffusivity in the right internal capsule, corona radiata, and superior longitudinal fasciculus (Figs 2k–2m).

Suboptimal regulation was associated with higher axial diffusivity in the left optic radiation (Fig. 2n).

HNNE and brain structure

There was no significant association between HNNE scores and global brain volumes (Fig. 1; Table SVI).

Suboptimal HNNE total scores were associated with larger lingual gyrus (Fig. 2o), and right optic radiation (Fig. 2p) volumes. However, after adjusting for intracranial volume, suboptimal scores were only associated with larger volume in 6 voxels in the lingual gyrus and no voxels in the optic radiation.

Suboptimal HNNE total scores were also associated with lower fractional anisotropy, and higher radial and mean diffusivity in the corpus callosum, optic radiation, and cingulum (Figs 2q–2s).

Suboptimal tone was associated with lower fractional anisotropy in the corpus callosum (Fig. 2t).

Suboptimal scores for abnormal signs were associated with lower fractional anisotropy and higher axial, radial, and mean diffusivity in many tracts (Figs 2u–2x). These tracts included the internal capsule, corona radiata, and superior longitudinal fasciculus, as well as the sagittal stratum (for all parameters except axial diffusivity), corpus callosum and cingulum (for all parameters except fractional anisotropy), the right external capsule for axial diffusivity, and the cerebral peduncle for radial and mean diffusivity.

Very preterm compared with moderate-late preterm

There were generally no significant interactions between assessment scores and preterm subgroup for global brain volumes. The one exception was the NNNS asymmetrical reflexes and preterm subgroup interaction for brainstem volume, which had *p*-value equal to 0.048. This was because of a stronger relationship in the very preterm group (*p*=0.003) compared with the moderate-late preterm group (*p*=0.4).

There were generally no significant interactions between assessment scores and preterm subgroup for voxel-wise volumes. There were, however, significant interactions between NNNS asymmetrical reflexes score and preterm subgroup for cortical grey matter and white matter volumes, predominantly in the occipital and parietal lobes (Fig. 3a shows the clusters that had significant interactions; Fig. 3b shows these cluster volumes split by preterm subgroup). Given this interaction, we ran the voxel-wise analyses for the preterm subgroups separately, and for grey matter, suboptimal NNNS asymmetrical reflexes were significantly associated with smaller volume in the very preterm group (Fig. 3c), but were not significantly associated with volume in the moderate-late preterm group. For white matter, associations between volume and NNNS asymmetrical reflexes were not significant in voxel-wise analyses of the groups separately (despite there being trends for suboptimal asymmetrical reflexes being associated with smaller volume in the very preterm group and larger volume in the moderate-late preterm group, as seen in Fig. 3b).

There were generally no significant interactions between assessment scores and preterm subgroup for voxel-wise diffusion values (only 6 voxels and 1 voxel had a significant interaction between NNNS handling and preterm subgroup for axial and mean diffusivity respectively; data not shown).

DISCUSSION

We found little evidence that global and regional brain volumes were related to neurological and behavioural performance in infants born preterm at TEA. However, there was some

evidence that alterations in white matter microstructure of various tracts at TEA were related to atypical performance on some neurological and behavioural domains of the NNNS and HNNE in infants born preterm at TEA. Additionally, relationships between brain structure and neurological and behavioural performance were overall relatively similar between infants born very preterm and moderate-late preterm.

Our associations between neurological and behavioural scores and white matter diffusion values were generally in the direction of suboptimal scores being associated with lower fractional anisotropy and/or higher axial, radial, and mean diffusivity (except for suboptimal NNNS hypotonicity scores, which were associated with lower axial and mean diffusivity). Fractional anisotropy and axial, radial, and mean diffusivity are thought to provide insight into white matter microstructural development. White matter development during the last trimester of gestation involves many processes, including axonal overproduction and pruning, premyelination (proliferation of oligodendrocyte precursor cells), and myelination (ensheathment of oligodendrocytes around axons and maturation of the myelin sheath).[26] At TEA (the time our cohort had MRI), only the cerebellar white matter and posterior limb of the internal capsule are myelinated; myelination of other tracts occurs predominantly during the first postnatal year and continues into adolescence for some tracts.[26] These processes in white matter development are accompanied by many microstructural changes, including increases in axon density, increases in macromolecular concentration, decreases in water content, decreases in the distance between axons, decreases in the permeability of cell membranes, and increases in axon diameter.[26] There is growing literature showing that diffusion tensor values appear to be sensitive to these white matter developmental changes in children. Many different studies have found that fractional anisotropy increases and axial, radial, and mean diffusivity decrease in the white matter between the preterm period and TEA.[27–29] Additionally, infants born preterm at TEA have lower fractional anisotropy and higher axial, radial, and mean diffusivity in many white matter tracts compared with infants born at term,[10,14,27,30–33] which suggests that infants born preterm have atypical white matter development. Despite this sensitivity of diffusion tensor values, they are not specific to any single aspect of white matter microstructure.[34] This means our associations between lower fractional anisotropy and/or higher axial, radial, and mean diffusivity and suboptimal assessment scores could suggest that these suboptimal scores are associated with atypical white matter development, but it is not possible for us to conclude which specific changes to white matter microstructure suboptimal scores are related to (e.g. whether suboptimal scores are more related to axonal injury or impairments to the

premyelination/myelination processes). It is also possible that suboptimal scores are related to a combination of white matter microstructural changes, since many processes are occurring concomitantly during white matter development.[26] Interpretation of diffusion tensor values is particularly problematic in regions of crossing white matter tracts,[34] where myelination of one tract during development could be expected to increase fractional anisotropy and decrease diffusivities, but myelination of the second crossing tract could be expected to decrease fractional anisotropy and increase diffusivities,[26] in which case decreased fractional anisotropy and increased diffusivities could reflect more mature white matter development. This could explain unexpected findings in some tracts, such as higher fractional anisotropy and/or lower diffusivities previously reported in infants born preterm compared with infants born at term in corticospinal projection tracts,[33] or potentially, the lower axial and radial diffusivity in the corona radiata in association with suboptimal hypotonicity in the current study.

The neuroanatomical locations of associations in the white matter also differed between some neurological and behavioural domains. Generally, suboptimal scores for the attention, non-optimal reflexes and regulation scales on the NNNS, and tone and total scales on the HNNE, were associated with altered microstructure particularly in the corpus callosum and optic radiation. Suboptimal scores for quality of movement and hypotonicity on the NNNS were more strongly associated with altered microstructure in the internal capsule and/or corona radiata. The only other study we are aware of that has reported associations between white matter microstructure and neurological and behavioural performance in infants born preterm at TEA found that altered microstructure (higher axial and radial diffusivity) particularly in the corpus callosum, optic radiation, and corona radiata were related to neurological outcomes (abnormal tonic regulation).[11] Thus, it is possible these tracts are particularly important for early neurological and behavioural outcomes. Neonatal corpus callosum microstructure has previously been related to longer-term cognitive and motor outcomes in children born very preterm,[35] while optic radiation microstructure has previously been related to visual outcomes,[36] and corona radiata microstructure has been related to motor outcomes.[37] Our results might suggest that performance on some early neurological and behavioural assessments relies on the development of tracts involved in these functions.

In our study, brain volumes did not appear to be associated with neurological and behavioural scores at TEA. In contrast, previous studies have reported associations between reduced brain size and volume, particularly of the cerebellum, and general movements

assessment scores and HNNE scores after term (3mo–2y) in children born very preterm.[3,38] General movements also did not appear to be associated with brain volume and microstructure in our study. In contrast, another study found that aberrant general movements after term (at 10–15wks) in 47 infants born very preterm were associated with lower volume of the corpus callosum and right central frontal white matter, and lower fractional anisotropy in widespread white matter tracts.[39] Unlike our study of neurological and behavioural performance at TEA, these previous studies examined neurological and behavioural performance post-TEA, so it is possible that relationships between brain structure and neurological and behavioural performance strengthen later in childhood. This is supported by a previous study that found that relationships between white matter microstructure at TEA and neurological and behavioural scores were stronger when assessments were performed at 44 weeks, compared with 40 weeks.[11]

We also found that relationships between MRI measures and neurological and behavioural assessment scores were generally similar between infants born very preterm and moderate-late preterm, except that NNNS asymmetrical reflex scores were more strongly related to brain volumes in the very preterm group compared with the moderate-late preterm group. Very little research has been carried out on the brain basis of functional outcomes in the moderate-late preterm population. The importance of the current result is that it suggests that, in general, brain development is associated with neurological and behavioural performance at TEA in infants born moderate-late preterm in the same way that it is associated with neurological and behavioural performance at TEA in infants born very preterm. Speculatively, any insults in the perinatal period may affect brain development in both infants born very preterm and moderate-late preterm, leading to functional impairments in both populations.[12] Our finding is similar to our previous study, in which we found that relationships between qualitative brain abnormality scores and suboptimal neurological and behavioural scores generally did not differ between infants born very preterm and moderate-late preterm, except abnormal general movements were more strongly related to global brain and cortical grey matter abnormality scores in infants born very preterm compared with infants born moderate-late preterm.[8]

Strengths of our study include the large sample of infants born preterm across the gestational age spectrum, the broad range of early neurological and behavioural outcomes, and early imaging at TEA. Both brain MRI[40,41] and neurological and behavioural assessments[42] at TEA have been related to longer-term developmental outcomes in children born preterm. As such, our work is important because both early brain MRI and neurological

and behavioural assessments could help to identify infants who require interventions to improve their long-term developmental outcomes. Because brain–behaviour relationships were generally similar between infants born very preterm and moderate-late preterm in the current study, interventions to improve early brain structure and behavioural performance might be beneficial in both the very preterm and moderate-late preterm populations. In future, it will be necessary to determine the importance of our findings for long-term outcomes.

Limitations of our study include that we completed many different statistical tests and have relatively few relationships where evidence for associations between brain structure and neurological and behavioural outcomes are strong, raising the possibility of type-1 errors in some of our findings. We regard our analyses as exploratory and hypothesis generating. Additionally, even though brain–behaviour associations were generally similar between the very preterm and moderate-late preterm groups, it is also important to note that statistical tests for interactions inherently have low power.[43] There are factors influencing the generalizability of our results to other cohorts. These include that our sample was from a single tertiary centre, and that the participants born very preterm included in our study had lower rates of medical complications than the participants born very preterm who could not be included in our study, so our participants may not be fully representative of the larger population. Inherent limitations with the MRI analysis techniques reduce our ability to localize findings to specific neuroanatomical regions and cellular properties.[34] Some, but not all, of the neurological and behavioural assessment subscales correlated with each other (data not shown), and given this, the separate associations between the white matter microstructure measures and the various subscales should be interpreted with some caution.

In conclusion, our study suggests brain volumes are not strongly related to neurological and behavioural performance at TEA in infants born preterm. However, white matter microstructural alterations may be associated with suboptimal performance on some neurological and behavioural assessments at TEA in infants born preterm. Additionally, relationships between brain volumes and microstructure and neurological and behavioural performance at TEA are generally similar between infants born very preterm and moderate-late preterm.

SUPPORTING INFORMATION

The following additional material may be found online:

Table SI: Days between neurological and behavioural assessments and magnetic resonance imaging

Table SII: Rates of suboptimal neurological and behavioural outcomes for the participants with volumetric data

Table SIII: Rates of suboptimal neurological and behavioural outcomes for the participants with diffusion data

Table SIV: Perinatal characteristics contrasted between participants who could and could not be included in the volumetric analysis

Table SV: Perinatal characteristics contrasted between participants who could and could not be included in the diffusion analysis

Table SVI: *P*-values for associations between global brain volumes and assessment scores after false discovery rate correction

Figure S1: Graphical summary of the number of participants included in the current study

REFERENCES

1 Spittle AJ, Thompson DK, Brown NC, et al. Neurobehaviour between birth and 40 weeks' gestation in infants born <30 weeks' gestation and parental psychological wellbeing: predictors of brain development and child outcomes. *BMC Pediatr* 2014; **14**: 111.

2   Brown NC, Doyle LW, Bear MJ, Inder TE. Alterations in neurobehavior at term reflect differing perinatal exposures in very preterm infants. *Pediatrics* 2006; **118**: 2461–71.

3   Spittle AJ, Doyle LW, Anderson PJ, et al. Reduced cerebellar diameter in very preterm infants with abnormal general movements. *Early Hum Dev* 2010; **86**: 1–5.

4   Pineda RG, Tjoeng TH, Vavasseur C, Kidokoro H, Neil JJ, Inder T. Patterns of altered neurobehavior in preterm infants within the neonatal intensive care unit. *J Pediatr* 2013; **162**: 470–6.

5   Olsen JE, Brown NC, Eeles AL, et al. Early general movements and brain magnetic resonance imaging at term-equivalent age in infants born <30weeks' gestation. *Early Hum Dev* 2016; **101**: 63–8.

6   Brown NC, Inder TE, Bear MJ, Hunt RW, Anderson PJ, Doyle LW. Neurobehavior at term and white and gray matter abnormalities in very preterm infants. *J Pediatr* 2009; **155**: 32–8.

7   Spittle AJ, Brown NC, Doyle LW, et al. Quality of general movements is related to white matter pathology in very preterm infants. *Pediatrics* 2008; **121**: e1184–9.

8   Eeles AL, Walsh JM, Olsen JE, et al. Continuum of neurobehaviour and its associations with brain MRI in infants born preterm. *BMJ Paediatr Open* 2017; **1**: e000136.

9   Inder TE, Warfield SK, Wang H, Huppi PS, Volpe JJ. Abnormal cerebral structure is present at term in premature infants. *Pediatrics* 2005; **115**: 286–94.

10   Thompson DK, Lee KJ, Egan GF, et al. Regional white matter microstructure in very preterm infants: predictors and 7 year outcomes. *Cortex* 2014; **52**: 60–74.

11   Weinstein M, Marom R, Berger I, et al. Neonatal neuropsychology: emerging relations of neonatal sensory-motor responses to white matter integrity. *Neuropsychologia* 2014; **62**: 209–19.

12   Kinney HC. The near-term (late preterm) human brain and risk for periventricular leukomalacia: a review. *Semin Perinatol* 2006; **30**: 81–8.

13 Walsh JM, Doyle LW, Anderson PJ, Lee KJ, Cheong JL. Moderate and late preterm birth: effect on brain size and maturation at term-equivalent age. *Radiology* 2014; **273**: 232–40.

14   Kelly CE, Cheong JL, Gabra Fam L, et al. Moderate and late preterm infants exhibit widespread brain white matter microstructure alterations at term-equivalent age relative to term-born controls. *Brain Imaging Behav* 2016; **10**: 41–9.

15   Tustison NJ, Avants BB, Cook PA, et al. N4ITK: improved N3 bias correction. *IEEE Trans Med Imaging* 2010; **29**: 1310–20.

16   Smith SM. Fast robust automated brain extraction. *Hum Brain Mapp* 2002; **17**: 143–55.

17   Beare RJ, Chen J, Kelly CE, et al. Neonatal brain tissue classification with morphological adaptation and unified segmentation. *Front Neuroinform* 2016; **10**: 12.

18   Kuklisova-Murgasova M, Aljabar P, Srinivasan L, et al. A dynamic 4D probabilistic atlas of the developing brain. *Neuroimage* 2011; **54**: 2750–63.

19   Smith SM, Jenkinson M, Johansen-Berg H, et al. Tract-based spatial statistics: voxelwise analysis of multi-subject diffusion data. *Neuroimage* 2006; **31**: 1487–505.

20   Einspieler C, Prechtl HF, Bos AF, Ferrari F, Cioni G. Prechtl's method on the qualitative assessment of general movements in preterm, term and young infants. London: Mac Keith Press, 2004.

21   Lester BM, Tronick EZ, Brazelton TB. The Neonatal Intensive Care Unit Network Neurobehavioral Scale procedures. *Pediatrics* 2004; **113**: 641–67.

22   Spittle AJ, Walsh J, Olsen JE, et al. Neurobehaviour and neurological development in the first month after birth for infants born between 32-42 weeks' gestation. *Early Hum Dev* 2016; **96**: 7–14.

23   Dubowitz L, Mercuri E, Dubowitz V. An optimality score for the neurologic examination of the term newborn. *J Pediatr* 1998; **133**: 406–16.

24   Winkler AM, Ridgway GR, Webster MA, Smith SM, Nichols TE. Permutation inference for the general linear model. *Neuroimage* 2014; **92**: 381–97.

25   Oishi K, Mori S, Donohue PK, et al. Multi-contrast human neonatal brain atlas: application to normal neonate development analysis. *Neuroimage* 2011; **56**: 8–20.

26   Dubois J, Dehaene-Lambertz G, Kulikova S, Poupon C, Hüppi PS, Hertz-Pannier L. The early development of brain white matter: a review of imaging studies in fetuses, newborns and infants. *Neuroscience* 2014; **276**: 48–71.

27   Hüppi PS, Maier SE, Peled S, et al. Microstructural development of human newborn cerebral white matter assessed in vivo by diffusion tensor magnetic resonance imaging. *Pediatr Res* 1998; **44**: 584–90.

28   Kersbergen KJ, Leemans A, Groenendaal F, et al. Microstructural brain development between 30 and 40 weeks corrected age in a longitudinal cohort of extremely preterm infants. *Neuroimage* 2014; **103**: 214–24.

29   Rose J, Vassar R, Cahill-Rowley K, Guzman XS, Stevenson DK, Barnea-Goraly N. Brain microstructural development at near-term age in very-low-birth-weight preterm infants: an atlas-based diffusion imaging study. *Neuroimage* 2014; **86**: 244–56.

30   Alexandrou G, Mårtensson G, Skiöld B, Blennow M, Adén U, Vollmer B. White matter microstructure is influenced by extremely preterm birth and neonatal respiratory factors. *Acta Paediatr* 2014; **103**: 48–56.

31   Anjari M, Srinivasan L, Allsop JM, et al. Diffusion tensor imaging with tract-based spatial statistics reveals local white matter abnormalities in preterm infants. *Neuroimage* 2007; **35**: 1021–7.

32   Pogribna U, Yu X, Burson K, et al. Perinatal clinical antecedents of white matter microstructural abnormalities on diffusion tensor imaging in extremely preterm infants. *PLoS One* 2013; **8**: e72974.

33   Rose SE, Hatzigeorgiou X, Strudwick MW, Durbridge G, Davies PS, Colditz PB. Altered white matter diffusion anisotropy in normal and preterm infants at term-equivalent age. *Magn Reson Med* 2008; **60**: 761–7.

34   Jones DK, Knösche TR, Turner R. White matter integrity, fiber count, and other fallacies: the do's and don'ts of diffusion MRI. *Neuroimage* 2013; **73**: 239–54.

35   Thompson DK, Inder TE, Faggian N, et al. Corpus callosum alterations in very preterm infants: perinatal correlates and 2 year neurodevelopmental outcomes. *Neuroimage* 2012; **59**: 3571–81.

36   Bassi L, Ricci D, Volzone A, et al. Probabilistic diffusion tractography of the optic radiations and visual function in preterm infants at term equivalent age. *Brain* 2008; **131**: 573–82.

37   Kaukola T, Perhomaa M, Vainionpaa L, et al. Apparent diffusion coefficient on magnetic resonance imaging in pons and in corona radiata and relation with the neurophysiologic measurement and the outcome in very preterm infants. *Neonatology* 2010; **97**: 15–21.

38   Lind A, Parkkola R, Lehtonen L, et al. Associations between regional brain volumes at term-equivalent age and development at 2 years of age in preterm children. *Pediatr Radiol* 2011; **41**: 953–61.

39   Peyton C, Yang E, Msall ME, et al. White matter injury and general movements in high-risk preterm infants. *AJNR Am J Neuroradiol* 2017; **38**: 162–9.

40   Anderson PJ, Cheong JL, Thompson DK. The predictive validity of neonatal MRI for neurodevelopmental outcome in very preterm children. *Semin Perinatol* 2015; **39**: 147–58.

41   Cheong JL, Thompson DK, Spittle AJ, et al. Brain volumes at term-equivalent age are associated with 2-year neurodevelopment in moderate and late preterm children. *J Pediatr* 2016; **174**: 91–7.

42   Spittle AJ, Walsh JM, Potter C, et al. Neurobehaviour at term-equivalent age and neurodevelopmental outcomes at 2 years in infants born moderate-to-late preterm. *Dev Med Child Neurol* 2017; **59**: 207–15.

43   Altman DG, Bland JM. Interaction revisited: the difference between two estimates. *BMJ* 2003; **326**: 219.

44   Papile LA, Burstein J, Burstein R, Koffler H. Incidence and evolution of subependymal and intraventricular hemorrhage: a study of infants with birth weights less than 1,500 gm. *J Pediatr* 1978; **92**: 529–34.

Table I: Perinatal medical characteristics of the participants included in the volumetric and diffusion analyses, contrasted between the very preterm and moderate-late preterm groups

	Volumetric cohort[a]		Tract-Based Spatial Statistics cohort	
	Very preterm, *n*=91	Moderate-late preterm, *n*=166	Very preterm, *n*=90	Moderate-late preterm, *n*=173
Gestational age at birth in weeks, mean (SD, min–max)	27.9 (1.4, 24.6–29.9)	34.3 (1.2, 32.0–36.9)	27.9 (1.3, 24.6–29.9)	34.4 (1.2, 32.0–36.9)
Weight at birth in grams, mean (SD, min–max)	1081 (254, 528–1638)	2138 (448, 1000–4522)	1061 (241, 528–1630)	2172 (455, 1000–4522)
Weight SD score at birth, mean (SD, min–max)	–0.3 (1.0, –3.6, 2.0)	–0.4 (1.1, 3.1–4.2	-0.4 (1.0, –3.6 to 2.0)	–0.3 (1.1, –3.1 to 4.2)
Males, *n* (%)	48 (53)	72 (43)	47 (52)	84 (49)
Cystic periventricular leukomalacia,[b] *n* (%)	1 (1)	0 (0)[c]	1 (1)	0 (0)[c]
Intraventricular haemorrhage grade 3/4, *n* (%)	3 (3)	0 (0)	3 (3)	0 (0)
White matter signal abnormalities, *n* (%)	8 (9)	9 (5)	9 (10)	9 (5)
Cerebellar haemorrhage, *n* (%)	4 (4)	7 (4)	3 (3)	8 (5)
Corrected age at MRI in weeks, mean (SD, min–max)	42.4 (1.5, 39.0–44.9)	41.4 (1.2, 38.4–44.1)	42.4 (1.4, 39.7–44.9)	41.4 (1.1, 38.4–44.1)
Corrected age at general movements assessment in weeks, mean (SD, min–max)	42.6 (1.5, 39.0–44.9)[d]	41.4 (1.1, 38.4–44.1)[e]	42.5 (1.4, 39.9–44.9)[f]	41.4 (1.0, 38.4–44.1)[g]
Corrected age at NNNS assessment in weeks, mean (SD, min–max)	42.4 (1.5, 39.0–44.9)[c]	41.5 (1.0, 38.9–44.1)[h]	42.4 (1.4, 39.4–44.9)[i]	41.5 (1.0, 38.9–44.1)[j]
Corrected age at HNNE assessment in weeks, mean (SD, min–max)	42.4 (1.5, 39.0–44.9)[i]	41.4 (1.2, 38.4–44.1)[k]	42.3 (1.4, 39.4–44.9)[l]	41.4 (1.1, 38.4–44.1)[m]

[a]Data are based on the participants who contributed global volume data; the participants who contributed Voxel-Based Morphometry data are the same minus one moderate-late preterm participant who was excluded because of having poor registration during Voxel-Based Morphometry. [b]Major brain injuries including cystic periventricular leukomalacia and intraventricular haemorrhage were diagnosed from cranial ultrasounds before term. Intraventricular haemorrhage was graded according to Papile et al.[44] [c]*n*=90; [d]*n*=75; [e]*n*=129; [f]*n*=78; [g]*n*=135; [h]*n*=154; [i]*n*=89; [j]*n*=163; [k]*n*=162; [l]*n*=88; [m]*n*=170. SD, standard deviation; MRI, magnetic resonance imaging; NNNS, Neonatal Intensive Care Unit Network Neurobehavioral Scale; HNNE, Hammersmith Neonatal Neurological Examination.

[Figure legends]

Figure 1: Global brain volumes and neurological and behavioural outcomes.

Differences in global brain volumes between infants with optimal and suboptimal scores for each neurological and behavioural assessment. In the graphs, points represent mean volume differences (cm^3) adjusted for gestational age at magnetic resonance imaging, and error bars represent 95% confidence intervals (CI) for the differences. Points to the left of the zero line indicate volumes for infants with suboptimal scores are lower than volumes for infants with optimal scores; points to the right of the zero line indicate volumes for infants with suboptimal scores are higher than volumes for infants with optimal scores. GMs, general movements; HNNE, Hammersmith Neonatal Neurological Examination; NNNS, Neonatal Intensive Care Unit (NICU) Network Neurobehavioral Scale.

Figure 2: Regional brain volumes and white matter microstructure and neurological and behavioural outcomes.

Representative brain slices showing regions, in red-yellow, where cortical grey matter volume, white matter volume, or white matter microstructure differed at $p<0.05$, family-wise error rate (FWE)-corrected, between infants with abnormal and normal general movements (a–c), suboptimal and optimal Neonatal Intensive Care Unit (NICU) Network Neurobehavioral Scale (NNNS) subscale scores (d–n) and suboptimal and optimal Hammersmith Neonatal Neurological Examination (HNNE) total and subscale scores (o–x). The number of voxels that differed at $p<0.05$, FWE-corrected, are also provided in the text above each image. The percentage refers to the percentage of the total number of voxels in the grey matter, white matter or mean fractional anisotropy (FA) skeleton. AD, axial diffusivity; MD, mean diffusivity; RD, radial diffusivity; TFCE, threshold-free cluster enhancement.
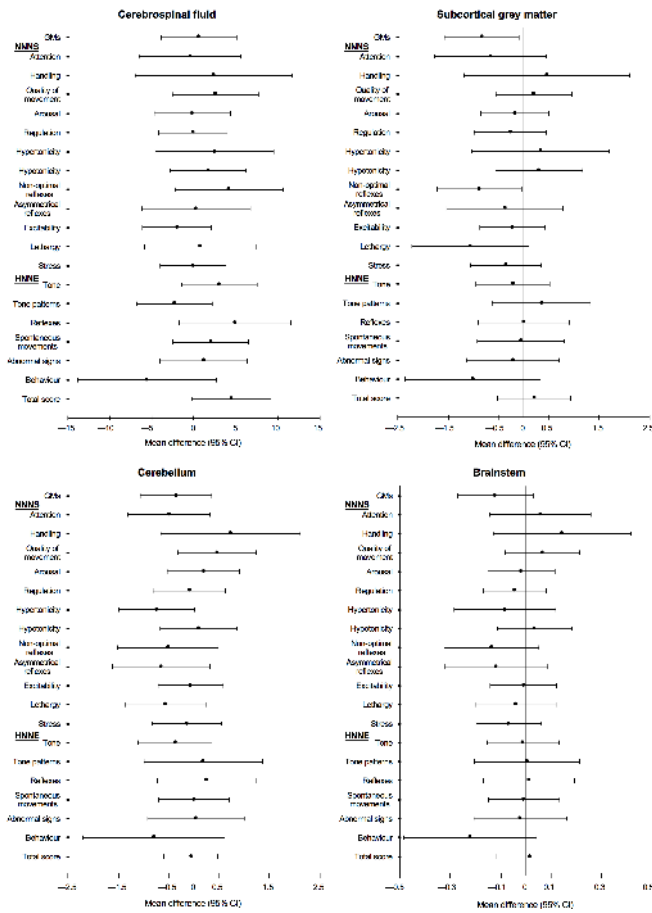
Figure 3: Results of the interaction analyses between preterm subgroup and assessment scores.

(a) Representative brain slices showing the regions, in red-yellow, where relationships between cortical grey matter or white matter volumes, and Neonatal Intensive Care Unit (NICU) Network Neurobehavioral Scale (NNNS) asymmetrical reflexes scores, differed between infants born very preterm (VPT) and moderate-late preterm (MLPT) at $p<0.05$, family-wise error rate (FWE)-corrected.

(b) The entire cortical grey matter and white matter cluster volumes from (a), plotted by preterm subgroup and assessment score.

(c) Given the significant interactions in (a), associations between voxel-wise cortical grey matter and white matter volumes, and NNNS asymmetrical reflex scores, were analysed separately for the very preterm and moderate-late preterm groups. The regions where lower cortical grey matter volume was associated with suboptimal NNNS asymmetrical reflexes in the very preterm group are shown (in the moderate-late preterm group there were no associations at $p < 0.05$, FWE-corrected, hence no images are shown). There was also no association between white matter volume and NNNS asymmetrical reflexes at $p < 0.05$, FWE-corrected, in either the very preterm or moderate-late preterm group, hence no images are shown.

dmcn_14084_f1a.tiff
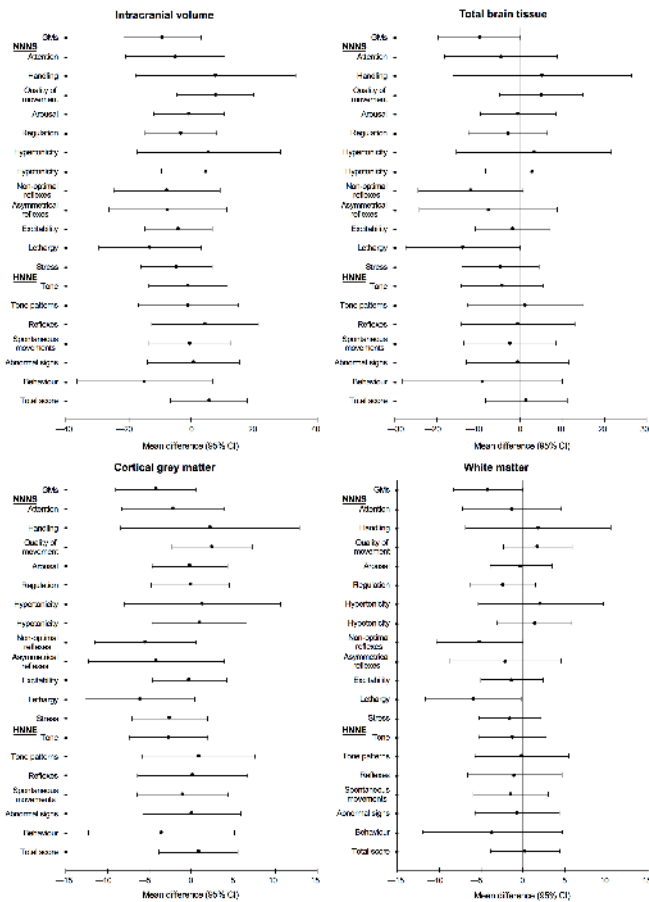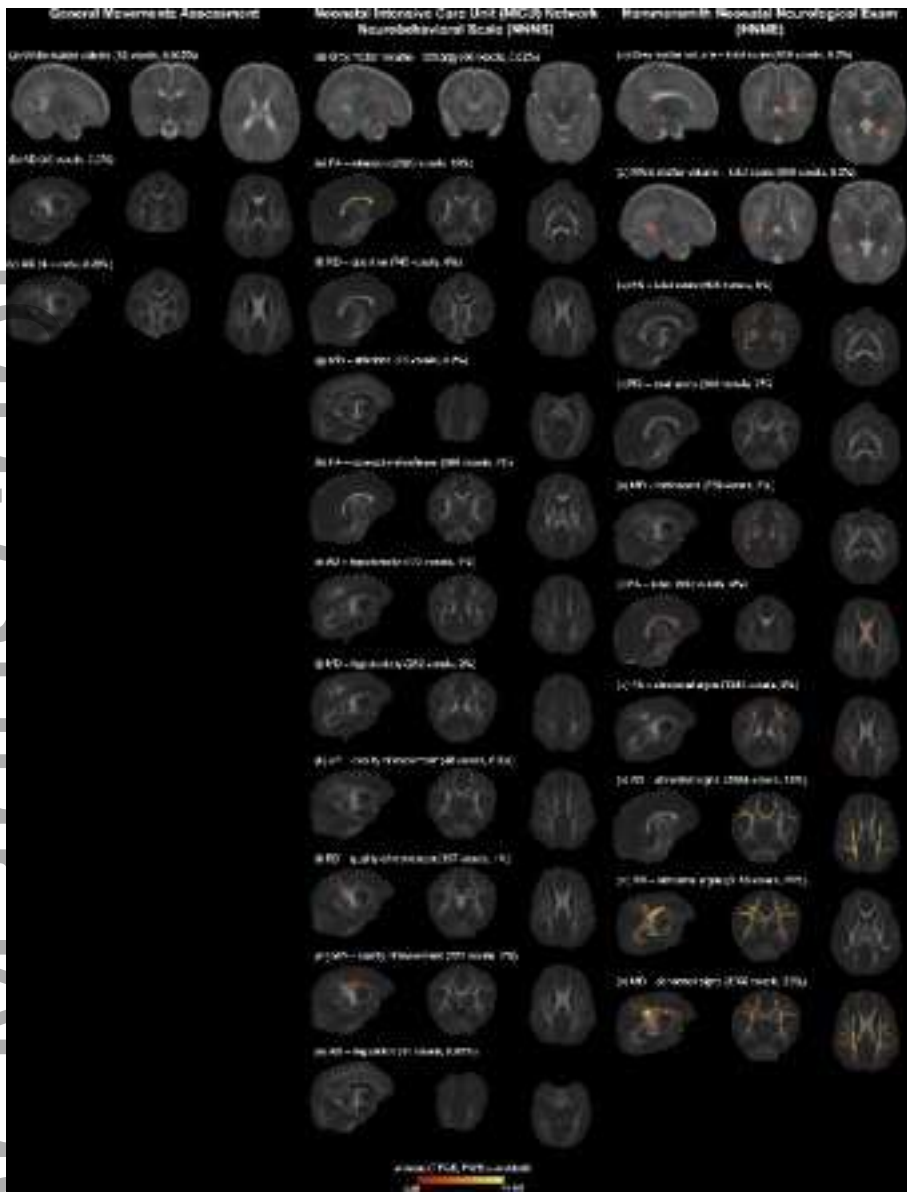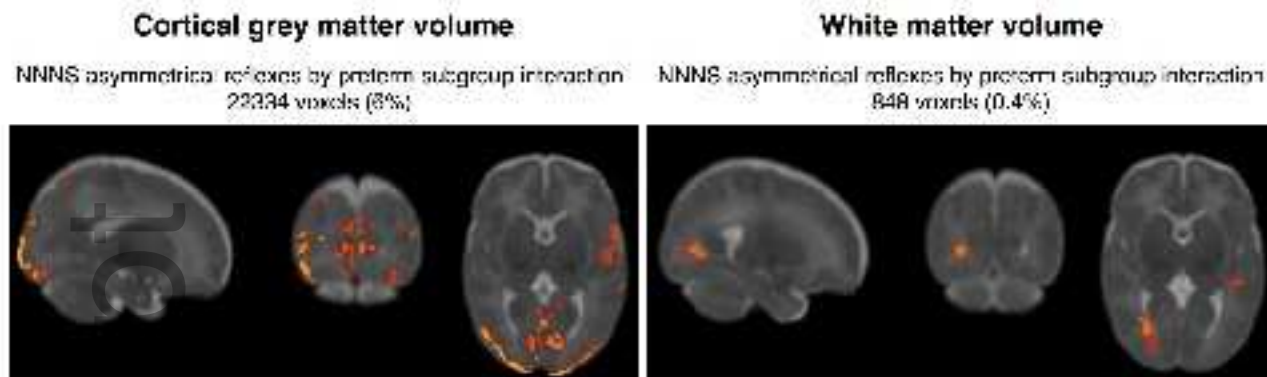
dmcn_14084_f1b.tiff

dmcn_14084_f2.tiff

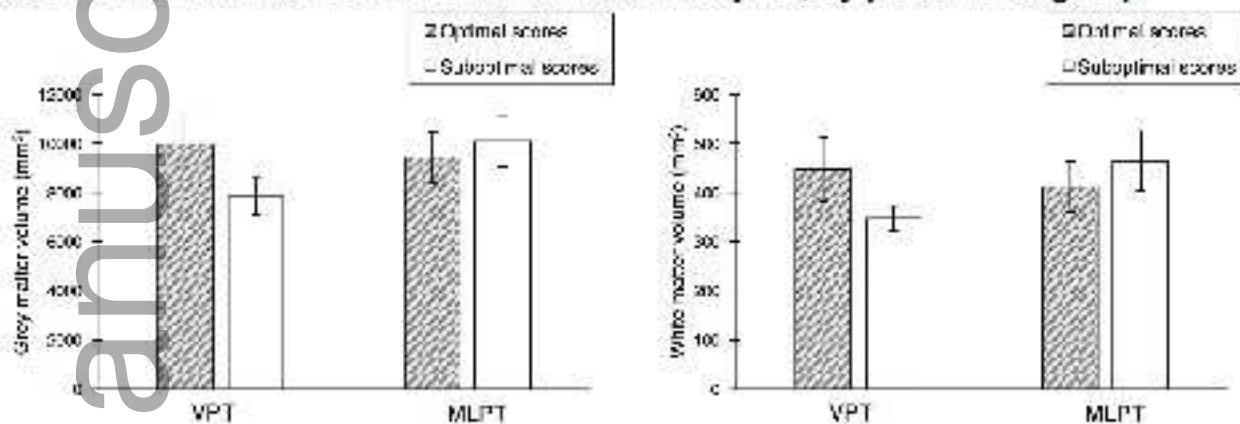## (a) Results of voxel-wise interaction analyses

Cortical grey matter volume

NNNS asymmetrical reflexes by preterm subgroup interaction
22334 voxels (6%)

White matter volume

NNNS asymmetrical reflexes by preterm subgroup interaction
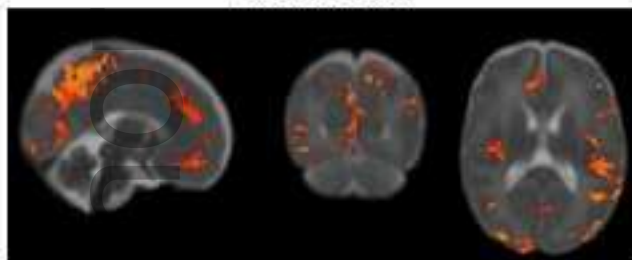848 voxels (0.4%)

(b) Cluster volumes from the voxel-wise interaction analyses, by preterm subgroup

(c) Results of voxel-wise analyses for the VPT group

Cortical grey matter volume - NNNS asymmetrical reflexes in VPT group
27903 voxels (8%)

dmcn_14084_f3.tiff

Author/s:
Kelly, CE; Thompson, DK; Cheong, JLY; Chen, J; Olsen, JE; Eeles, AL; Walsh, JM; Seal, ML; Anderson, PJ; Doyle, LW; Spittle, AJ

Title:
Brain structure and neurological and behavioural functioning in infants born preterm

Date:
2019-07-01

Citation:
Kelly, C. E., Thompson, D. K., Cheong, J. L. Y., Chen, J., Olsen, J. E., Eeles, A. L., Walsh, J. M., Seal, M. L., Anderson, P. J., Doyle, L. W. & Spittle, A. J. (2019). Brain structure and neurological and behavioural functioning in infants born preterm. DEVELOPMENTAL MEDICINE AND CHILD NEUROLOGY, 61 (7), pp.820-+. https://doi.org/10.1111/dmcn.14084.

Persistent Link:
http://hdl.handle.net/11343/284872

File Description:
Accepted version