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Research report

The motor and visual networks in preterm infants: An fMRI and DTI study

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ABSTRACT

Knowledge regarding the association between functional connectivity and white-matter (WM) maturation of motor and visual networks in preterm infants at term equivalent age (TEA) and their association with behavioral outcome is currently limited. Thirty-two preterm infants born <34 weeks gestational-age without major brain abnormalities were included in this study, underwent resting-state fMRI at TEA. Thirteen infants also underwent diffusion tensor imaging (DTI). Neurobehavioral assessments were performed at one and two years corrected age using the Griffiths Mental Developmental Scales. Functional connectivity between homolog motor and visual regions were detected, which may reflect that a level of organization in these domains is present already at TEA. DTI parameters of WM tracts at TEA demonstrated spatial-temporal variability, with the splenium of the corpus-callosum (CC) found to be the most mature fiber bundle. Correlations between DTI parameters, functional connectivity and behavioral outcome were detected, yet did not show the same pattern of diffusivity changes in the different networks. Visual functional connectivity was negatively correlated with radial-diffusivity (RD) in the optic radiation, while motor functional connectivity was positively correlated with RD in the splenium. In addition, axial-diffusivity (AD) and RD in the genu and midbody of the CC were positively correlated with neurobehavioral outcome at one and 2 years of age. This study highlights the importance of understanding the spatial-temporal changes occurring during this sensitive period of development and the potential effect of extrauterine exposure on the microstructural changes as measured by DTI; their correlation with functional connectivity; and their long term relationship with neuro-behavioral development.

1. Introduction

Brain development in the third trimester and early neonatal period is a dynamic and complex process (Dubois et al., 2014). During brain development, several mechanisms take place including the maturation and functional specialization of gray matter (GM) regions along with the formation and myelination of white matter (WM) connections between the different neural regions (Dubois et al., 2014, Vasung et al., 2013). Some processes are time dependent with critical times for maturation. Factors such as epigenetic, intrauterine and extrauterine environmental also have major impact on development. Extrauterine exposure to stress, pain, visual input and nutrition may affect brain development (Anjari et al., 2007; Bhutta and Anand, 2002; Keunen et al., 2015; Tsuneishi and Casaer, 2000; Valeri et al., 2015).

Magnetic resonance imaging (MRI) enables noninvasive mapping of both structural and functional networks. This methodology may aid in understanding the complex interplay between anatomical and functional brain development (Sui et al., 2014), which is

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particularly interesting in the premature brain since it provides an opportunity to look at a very sensitive time window.

Resting state functional connectivity MRI (fcMRI) examines temporal correlations in low-frequency (<0.1 Hz) spontaneous fluctuations in blood oxygen level-dependent (BOLD) signal. This method has revealed a number of networks, such as the somatosensory network, which were consistently reported and represent specific patterns of synchronous BOLD fluctuations, even in the absence of a task (Biswal et al., 1995; Fox and Raichle, 2007). Bilateral functional connectivity of several networks including visual, sensorimotor and auditory was shown to be present already in the neonatal and preterm brain (Fransson et al., 2007, 2009; Hoff et al., 2013; Lin et al., 2008). Additionally, default mode and executive control resting state networks have been reported in very preterm to late preterm infants (Doria et al., 2010). The development of networks is spatially-temporally dependent, with some networks such as the visual and auditory networks appearing to be more developed at the neonatal stage than others, such as language related networks, (Doria et al., 2010; Hoff et al., 2013; Lin et al., 2008).

Diffusion tensor imaging (DTI) provides indirect quantitative measures of microstructural properties of the tissue, with the various diffusivity parameters related to WM integrity. Tractography analyses method can reconstruct in vivo the trajectories of WM fasciculi connecting different brain areas, providing information regarding structural networks. During development, an overall decrease in mean diffusivity (MD) and an increase in fractional anisotropy (FA) are detected as age increases (Barnea-Goraly et al., 2005; Cascio et al., 2007; Dubois et al., 2014; Mukherjee et al., 2001). A decrease in the radial diffusivity (RD), more pronounced than the decrease in axial diffusivity (AD), was reported in newborns and infants from birth to childhood resulting in an actual increase in FA (Huppi et al., 1998; Mukherjee et al., 2001; Neil et al., 1998; Song et al., 2003; Verhoeven et al., 2010). However, recent studies have shown that this typical pattern does not always occur at the pre-natal stage or in preterm infants. Moreover, spatio-temporal variation with a non-linear relationship between diffusivity parameters and development were reported in preterm infants (Aeby et al., 2012; Nossin-Manor et al., 2015). Therefore, interpretation of the level of maturation based on diffusivity parameters is not straightforward.

In addition to normal maturation with age, extrauterine exposure has an effect on the development of both functional and structural networks. DTI studies in preterm infants have shown reduced FA in specific WM tracts such as the corpus callosum (Anjari et al., 2007; Jo et al., 2012; Rose et al., 2008) while in other WM tracts such as the sagittal stratum, higher FA was found compared to term born controls (Gimenez et al., 2008). Functional connectivity studies demonstrated lower correlations and more limited distribution, including decreased long-range functional connectivity in preterm infants scanned at term equivalent age (TEA) compared to term born controls especially in thalamocortical connections (Smyser et al., 2010).

Structural and functional networks measured at TEA were associated with both neonatal and future neurodevelopment. Higher AD and RD in the CC and optic radiation (OR) were detected in preterm infants with atypical sensory reactivity and tonic regulation at TEA compared with preterm infants with no abnormalities (Weinstein et al., 2014b). Significantly lower FA values in the posterior limb of the internal capsule and higher ADC/lower FA values of the splenium at TEA were reported in preterm infants with psychomotor delay detected around two years of age (De Bruine et al., 2013; Rose et al., 2009). In addition, higher FA in the optic radiations was correlated with better visual function in premature infants at TEA (Bassi et al., 2008; Berman et al., 2009; Groppo et al., 2014). Recently, a serial fMRI-DTI study on auditory development demonstrated different thalamic activation patterns between preterm infants and term controls associated with maturation of subcortical WM bundles and correlation between activation patterns and with outcome (Baldoli et al., 2015). A recent review concluded that the approach of combining DTI and fMRI data is very valuable yet stressed that it is important to take into account that essential relationships between structural brain architecture and the underlying dynamic functions is not yet understood (Zhu et al., 2014).

Relatively few studies have focused on the relation of structural and functional connectivity with neurobehavioral outcome. In the current study we set to explore the motor and visual domains in preterm infants, using resting state functional connectivity to characterize the functional networks and DTI tractography to study WM maturation, and to assess the association between functional connectivity and WM maturation in relation to neurobehavioral outcome at one and two years of age.

2. Results

2.1. Conventional MRI findings

A total of 32 preterm infants born at <34 weeks' gestational age without major brain abnormalities (see inclusion and exclusion criteria in Section 4.1), detected on cranial ultrasound underwent MRI at TEA and neurobehavioral assessments at one and two years corrected age. Detailed description of the demographic and clinical characteristics of the infants is given in Table 1. Diffuse excessive high signal intensity (DEHSI) was present in the cerebrum of 60% of infants, cerebellar punctuate lesions in 25% and grade I haemorrhage in 22%.

2.2. Functional networks in preterm infants

Motor and visual related networks were identified in all preterm infants, using seed correlation analysis demonstrating temporally correlated BOLD signal on resting state fcMRI data (see Fig. 1 for examples of the functional connectivity maps). The Fisher z-transformed correlation coefficients of the motor network were $Z(r)=0.97\pm0.18$ and of the visual network $Z(r)=0.94\pm0.29$.

Gestational age (GA) at birth, postnatal age (GA at MRI) and the time interval from birth to the TEA MRI scan were not correlated with functional connectivity in either network. No differences in functional connectivity were detected in any of the networks when comparing preterm infants with DEHSI to those without.

Table 1
Infants' characteristics.

Variable	Mean ± SD	Frequency
Gestational Age at birth (w)	29.0 ± 2.7	
Gestational Age at MRI test (w)	37.6 ± 1.6	
Birth weight (g)	1258 ± 459	
Male	17	53%
Twin	23	72%
Intra-uterine growth restriction (IUGR)	0	0
Arterial pressure at entrance to NICU	40.3 ± 7.4	
Respiratory distress syndrome	23	72%
Hypotension	1	3%
Bronchopulmonary dysplasia	8	25%
Antenatal steroids	32	100%
Postnatal sepsis	6	19%

N=32.

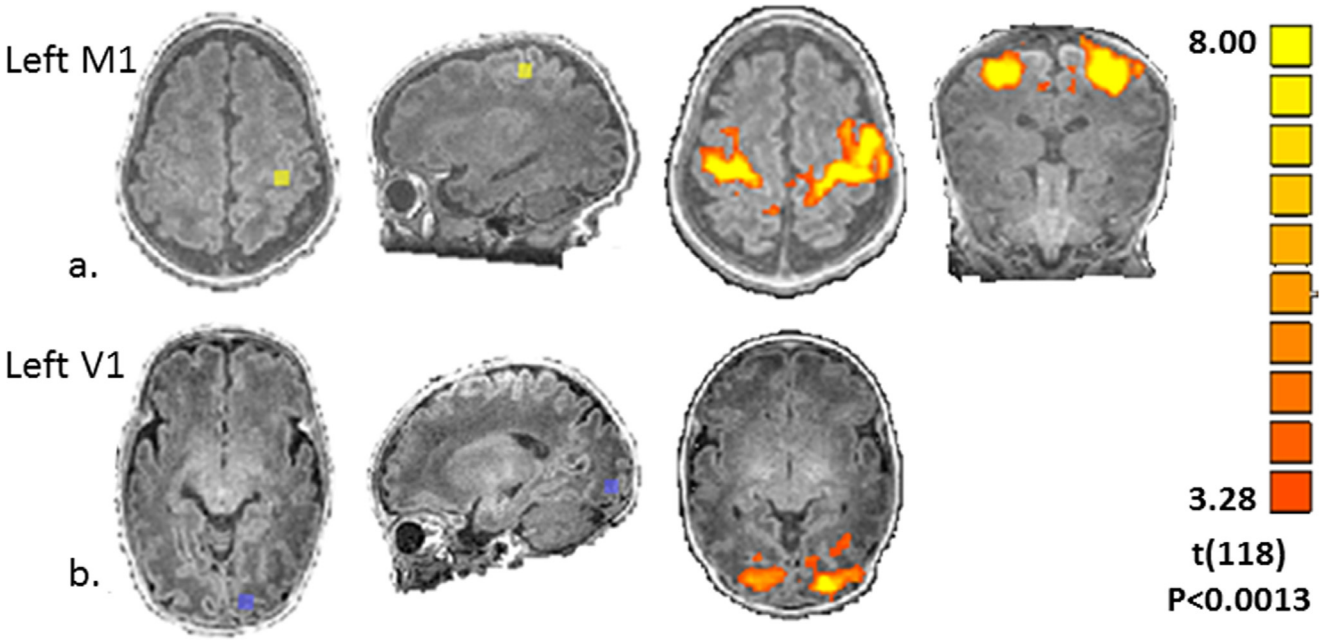

Fig. 1. Location of seed region of interest (ROIs) and resulting functional connectivity maps: a. Left motor cortex (M1), b. Left visual cortex (V1).

2.3. *Structural networks in preterm infants: level of maturation of WM tracts*

DTI stream-line tractography analyses of the left and right cortico-spinal tracts (CST), corpus callosum (CC) and optic radiations (OR) were performed in preterm infants who had DTI data (n=13). Segmentation of the CC was further performed based on Witelson Segmentation into genu, midbody and splenium. Fig. 2 shows examples of the fiber tracts reconstructed using tractography analysis.

Mean DTI parameters were measured within each WM tract for each infant, and mean values of all preterm infants are given in Table 2.

For comparison between diffusivity parameters of the different WM tracts, a repeated measures ANOVA was used. Mauchly's test (Mauchly, 1940) indicated a violation of the assumption of sphericity for the FA and AD values, therefore for these parameters the Greenhouse-Geisser correction was used. Significant differences in the DTI parameters of different WM tracts were found. Mean FA, AD and RD values differed significantly between WM tracts, FA: ($F_{(2.55,\ 30.55)}=15.12$, $p<0.0001$, AD: ($F_{(2.48,\ 29.8)}=56.07$, $p<0.0001$), RD: $F_{(5,\ 60)}=42.27$, $p<0.0001$). Post hoc tests using the Bonferroni correction revealed that FA of the CC splenium was significantly higher than FA in the CC genu, CC midbody and OR but not significantly different from the FA in the left and right CST.

Table 2
DTI parameters values in the WM tracts.

Tracts	AD ($\times 10^{-3}$ mm^2/s)	RD ($\times 10^{-3}$ mm^2/s)	FA
CC-genu	1.577 ± 0.089	0.801 ± 0.088	0.418 ± 0.040
CC-midbody	1.589 ± 0.176	0.900 ± 0.128	0.373 ± 0.045
CC-splenium	1.540 ± 0.123	0.691 ± 0.092	0.485 ± 0.043
L-CST	1.173 ± 0.079	0.575 ± 0.082	0.446 ± 0.066
R-CST	1.123 ± 0.083	0.542 ± 0.093	0.453 ± 0.079
OR	1.360 ± 0.081	0.760 ± 0.081	0.368 ± 0.055

N=13, AD=axial diffusivity, RD=radial diffusivity, FA= fractional anisotropy, CC=corpus callosum, L=left, R=right, CST=cortico spinal tract, OR=optic radiations.

AD in the splenium of the CC was also significantly lower than AD in the OR, while RD was not significantly different. AD and RD in the left and right CST were significantly lower compared to all other tracts.

GA was negatively correlated with FA in the OR (r=−0.736, $p=0.007$). The time interval from birth until the MRI scan was negatively correlated with RD in the genu of the CC (r=−0.595, $p=0.032$) and with AD in the midbody of the CC (r=−0.611, $p=0.026$). Post-conceptual age (PCA) was not correlated to any of the DTI parameters in the various WM tracts.

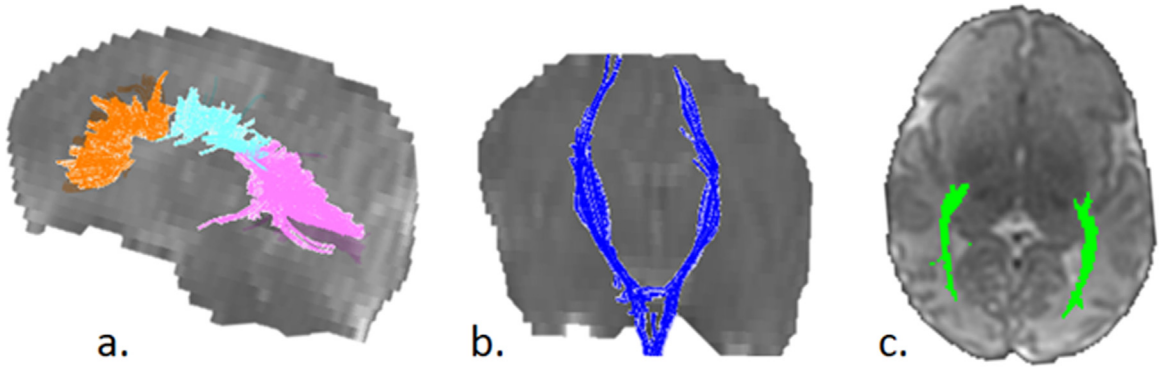

Fig. 2. Data obtained from a male preterm infant born at 33 weeks' GA and scanned at 36 weeks GA. Reconstructed fiber tracts are shown on b0 image: A. CC segmented to genu (orange), midbody (light blue) and splenium (pink) – sagittal view, B. Cortico spinal tracts (blue) – coronal view, C. Optic radiations – (green) axial view.

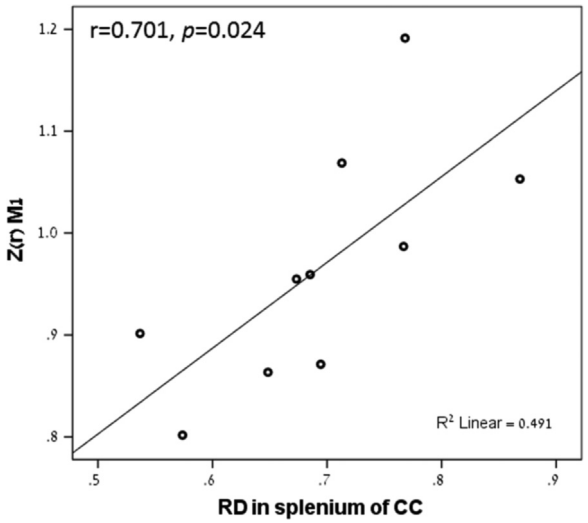

Fig. 3. Scatter-plot demonstrating the correlation between Dr in the splenium of the CC and functional connectivity between the homolog primary motor areas.

2.4. Correlation between functional and structural networks

Significant correlation was detected between functional connectivity of the homolog primary motor areas and RD in the splenium of the CC (n=10, r=0.701, p=0.024) (Fig. 3). No significant correlations were found between functional connectivity of the homolog primary motor areas and WM integrity of the cortico-spinal tract.

A trend towards significance was found between functional connectivity of the primary visual areas and FA and RD of the OR (n=10, FA: r=0.602, p=0.065; RD: r=−0.576, p=0.081; Fig. 4).

2.5. Neurobehavioral outcomes at 1 and 2 years corrected age

Outcome at one and two years (ages corrected for prematurity) was assessed by the Griffiths Mental Development Scales (Huntley, 1996), results are given in Table 3. Mean total GMDS score for all subjects was within the normal range; at one year 92 ± 5.4; and at two years 95 ± 7.6 with some discrepancy among scales. Mean scores were within one standard deviation of the norm (GMDS norms: 100 ± 15) in all subscales except for the performance subscale at one year corrected age where subjects scored slightly lower than the norm (see Table 3). Significantly higher scores were found in the language (t(25)=−3.212, p=0.004) and performance (t(25)=−3.93, p=0.001) subscales and developmental quotient (t(25)=−2.14, p=0.042) at two years corrected age compared to one year corrected age. The motor subscale was lower at two years corrected age compared to one year corrected age (t(25)=2.07, p=0.048).

Table 3
GMDS subscale scores at 1 and 2 years corrected age.

GMDS outcome scores	1 year		2 years	
	Mean	Std. Deviation	Mean	Std. Deviation
Motor	95	9.7	91	8.1
Social	107	9.0	109	14.9
Language	90	11.0	98	14.2
Hand-eye coordination	88	9.1	85	8.3
Performance	82	8.2	90	9.0
Total DQ	92	5.4	95	7.6

DQ=Developmental quotient; 1 year: n=31, 2 years: n=26.

2.6. Correlations between imaging parameters and neurobehavioral outcome

No significant correlations were detected between functional connectivity of the motor and visual homolog areas and GMDS scores on all scales at both one and two years.

No significant correlations were detected between diffusivity parameters in the OR and CST with any of the GMDS subscales at one and two years corrected age. Yet, significant correlations between diffusivity parameters mainly within the genu of the CC and neurobehavioral outcome at both one and two years corrected age were detected. More specifically, higher AD in the genu of the CC was positively correlated with hand-eye coordination and performance GMDS subscales at 1 year of age (r=0.619, p=0.024; r=0.575, p=0.040, respectively). Higher AD in the midbody of the CC was positively correlated with the personal-social subscale scores (r=0.716, p=0.006). See Fig. 5 for scatter-graphs demonstrating the correlations between the diffusivity parameters in the CC and neurobehavioral outcome at one year corrected age.

Lower FA and higher RD in the genu of the CC were correlated with hand-eye coordination at two years (FA: r=−0.668, p=0.035, RD: r=0.688, p=0.028), as well as with performance

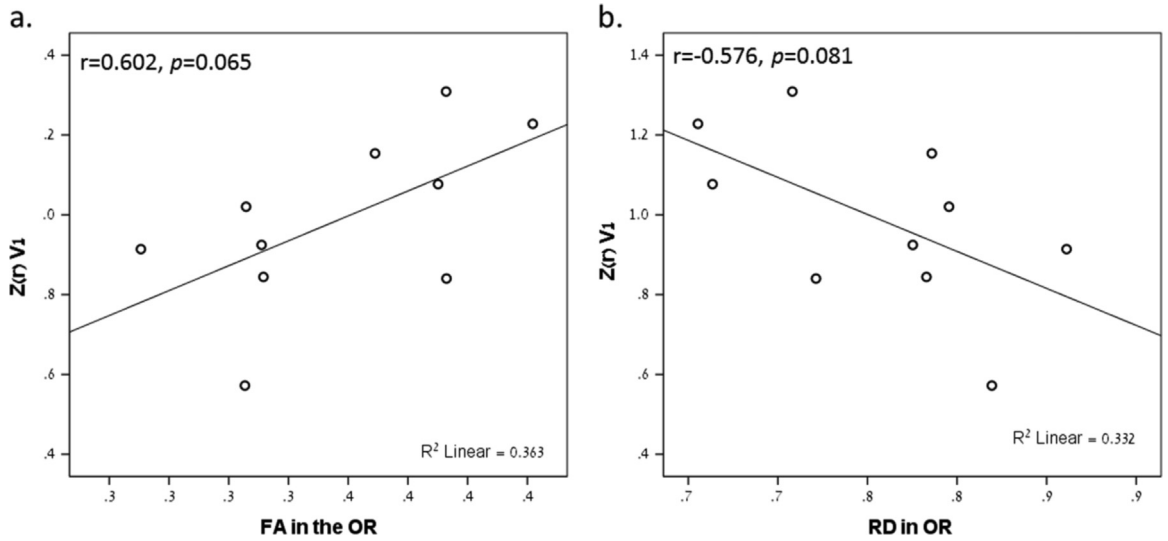

Fig. 4. Scatter-plots demonstrating the correlation between FA and Dr in the OR and functional connectivity between the homolog primary visual areas.

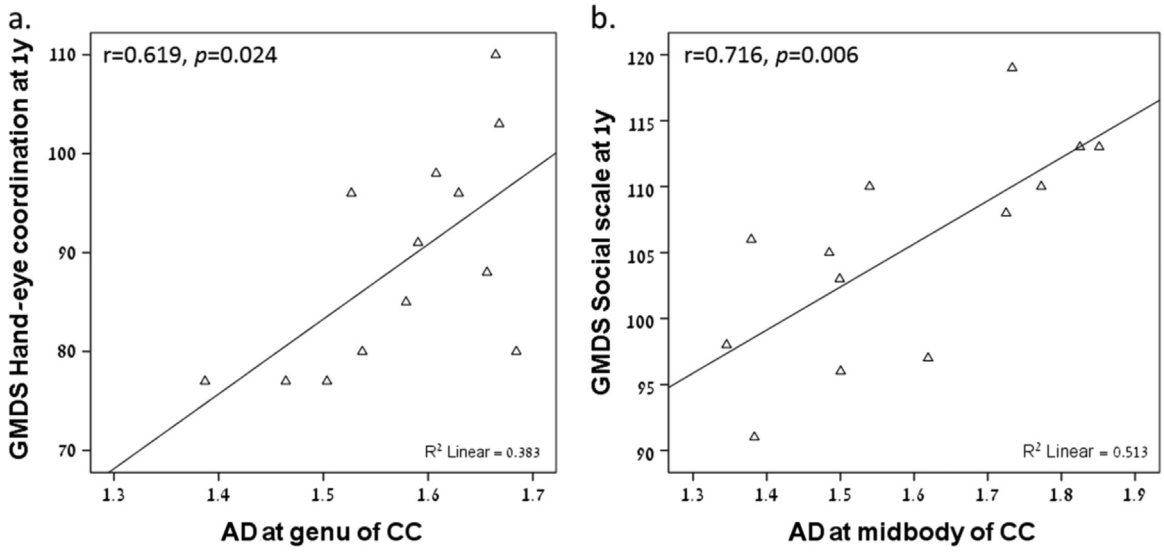

Fig. 5. Scatter-plots demonstrating the correlation between: a. Da at the genu of the CC and hand eye coordination scale at one year corrected age, b. Da at midbody of the CC and social scale at one year corrected age.

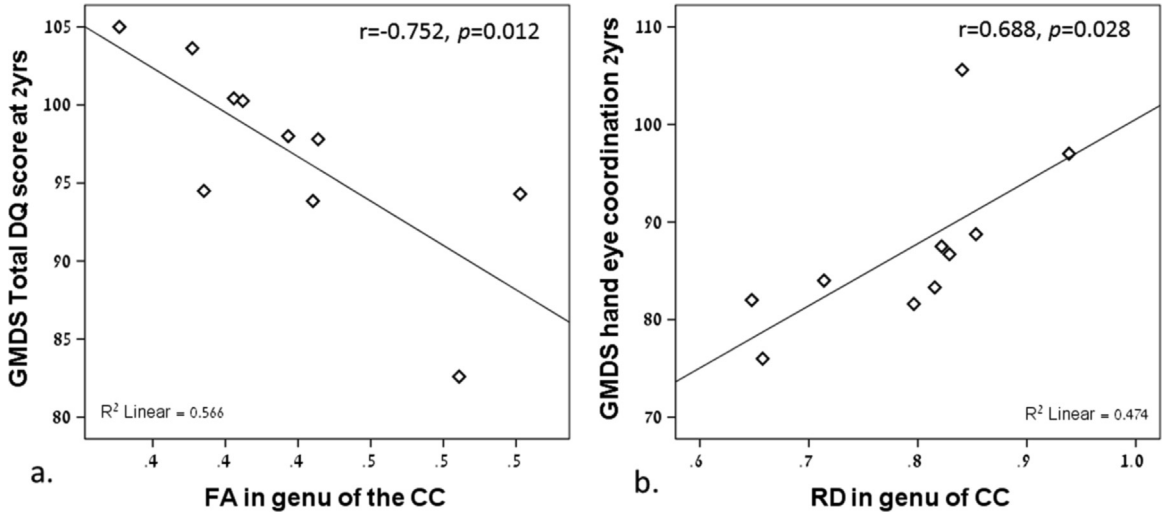

Fig. 6. Scatter-graphs demonstrating the correlation between: a. FA at the genu of the CC and total DQ score at two years corrected age, b. Dr at genu of the CC and eye hand coordination scale at two years corrected age.

(RD: r=0.639, p=0.047), personal-social scores (FA: r=−0.710, p=0.021) and with total developmental quotient (RD: r=0.642, p=0.045; FA: r=−0.752, p=0.012) at 2 years corrected age. See Fig. 6 for scatter-graphs demonstrating the correlations between the DTI parameters in the CC and neurobehavioral outcome at two years corrected age.

3. Discussion

In this study we demonstrated a relationship between functional networks and WM maturation in motor and visual domains, and significant associations between WM maturation and neurobehavioral outcome at one and two years of age. High functional connectivity between homolog primary motor and visual regions was already present in premature infants at TEA. When looking at the maturation level of the WM tracts based on DTI, spatial-temporal variability was detected. The correlations between structural and functional connectivity in the OR (which demonstrated a trend towards significance) could be explained in line with the typical pattern of changes of the DTI parameters during development, characterized by an increase in FA and a decrease in AD and RD with development. However, the correlations between structural and functional connectivity in the CC and with future neuro-behavior presented an opposite trend. These findings may be explained by a different maturation pattern demonstrated in the premature brain, or by the effect of the extrauterine exposure.

In the current study we demonstrated strong interhemispheric functional connectivity of the motor and visual networks in preterm infants at TEA. Fransson et al. (2007) were the first to demonstrate that resting-state networks driven by spontaneous signal fluctuations are already present in the preterm infant's brain at TEA. Smyser et al. (2010) further showed decreased long-range functional connectivity in preterm infants compared to term born controls. These networks demonstrated a region-dependent age-specific pattern of development, with more mature forms consisting of localized interhemispheric connections between homotopic counterparts.

Severe WM injury was found to be related to pronounced resting state fcMRI abnormalities such as reduced interhemispheric connectivity (Smyser et al., 2013). Our preterm infants did not have major brain abnormalities, yet a large proportion (60%)

displayed mild to moderate diffuse-excessive high signal intensity (DEHSI) at TEA MRI. We have previously demonstrated in this cohort that infants with DEHSI present with higher AD and RD values in regions with DEHSI (Weinstein et al., 2014a) compared to infants with no DEHSI and that the longer the exposure to the extra-uterine environment the less DEHSI was detected (Leitner et al., 2014). In the current study, we did not detect any effect of DEHSI on the motor and visual functional networks, which may explain the lack of association between DEHSI and neurobehavioral outcome at one year of age (Leitner et al., 2014).

The splenium was found in this study to be the most mature part of the CC compared to the genu and midbody, reflected by high FA and low RD values. This finding is in line with the general pattern of WM maturation, from central to peripheral and from posterior to anterior direction (Oishi et al., 2011), and with previous studies in preterm infants (Hasegawa et al., 2011; Rose et al., 2014; Thompson et al., 2011). Changes in DTI parameters in these WM tracts seem to follow the typical developmental trajectory, with FA increasing and MD, AD, and RD decreasing with age, reflecting increased WM maturation likely due to increased fiber organization, axonal coherence, and myelination (Dubois et al., 2008; Dubois et al., 2014; Mukherjee et al., 2002; Partridge et al., 2004).

No relationship between the maturation level of the CC segments and PCA or GA were detected, however there were correlations with the time interval from birth to the time of the MRI scan. These findings imply that longer exposure to the extrauterine environment may be associated with increased maturation. This is in line with prior studies that noted higher values of FA at TEA in preterm infants when compared with term controls in areas corresponding to fiber tracts of the neuro-sensory pathways of vision and hearing (Gimenez et al., 2008) and revealed an effect of the period of premature extra-uterine life beyond the degree of prematurity (Groppo et al., 2014; Leitner et al., 2014).

Correlations between functional networks and WM integrity were detected both in the motor and visual domains. In the visual domain, the higher the FA and the lower the RD in the OR, the higher the interhemispheric connectivity detected between the primary visual areas. In the CC a seemingly opposite relationship between structural and functional connectivity emerged, demonstrating a positive correlation between RD in the splenium and functional connectivity between the homolog primary motor areas. A similar pattern was also shown between the DTI parameters at TEA and neurobehavioral outcome at one and 2 years of age; with higher AD and RD and lower FA in the genu of the CC at TEA associated with better neurobehavioral outcome, mainly with hand-eye coordination. Previous studies also reported an inconsistent pattern of correlations between diffusivity parameters and neurobehavioral outcome. Duerden et al. (2015) reported a significant positive association between diffusivity parameters within the CC in neonates scanned between 30 and 33 weeks of age and motor skills at 18 months of age. However, these correlations were not found in preterm neonates scanned between 37 and 41 weeks, and the only positive correlation was detected between FA (within the genu of the CC) and motor outcome in that age group (Duerden et al., 2015).

In general, maturational trajectories in preterm infants may not reflect the typical maturational trajectory of term born infants. Indeed, recent studies focusing on the perinatal period of development demonstrated spatio-temporal variation with a non-linear relationship between diffusivity parameters and maturation (Aeby et al., 2012; Nossin-Manor et al., 2015). For example, Aeby et al. demonstrated that there are fluctuations in FA changes with age in occipital and temporal regions, decreasing between 34 and 39 weeks followed by an increase from 40 to 43 weeks. fluctuations in FA changes were also demonstrated in the intermediate zone which showed a decrease in FA and an increase in MD with age, while the subplate region showed no change in FA (and an increase in MD) (Nossin-Manor et al., 2015). When studying premature infants, additional factors, such as perinatal neural injury and premature exposure to the extra-uterine environment, should be taken into account.

In children with major neurobehavioral impairments, correlations between DTI parameters and outcome do seem to follow the typical pattern, with poorer outcome associated with reduced FA and increased AD, RD and MD. Previous studies reported reduced FA in the CC and the posterior limb of the internal capsule at TEA, associated with cerebral palsy and psychomotor delay (De Bruine et al., 2013; Rose et al., 2009). This study demonstrated that in preterm infants with no major structural abnormalities, this relationship is not always present.

There may be several possible explanations of WM maturational trajectories at different stages of development reported in preterm infants, including the distinct relation between diffusivity parameters and maturation; the different microstructural changes that occur both during normal and abnormal development; and the exposure to environmental stimuli at very early stage of development. Our study along with other studies demonstrate the complexity of the associations between DTI parameters, WM maturation and outcome showing large variability. Additional studies are needed to better understand and define these spatial-temporal changes and their effect on neurobehavioral outcome.

A limitation of this study is the relatively small number of subjects that had both DTI data enabling tractography analysis and resting state fMRI data. However the demonstration of significant relationships in this small number of subjects suggests the potential of this approach. A control group of term born infants would have added valuable information but was not possible due to restrictions of the ethical committee which approved perinatal MRI only for infants with prominent clinical indications. Six fraternal twin pairs were included in the study, this should be taken into consideration due to genetic factors that may impact neurodevelopment. Finally, in our study overall neurobehavior was examined and we did not focus on visual function, therefore limiting the sensitivity to detect correlations between maturation of the OR and visual function, which have been previously reported in premature infants at term age (Bassi et al., 2008; Berman et al., 2009; Groppo et al., 2014).

In summary, this study shows correlation between structural and functional connectivity in the visual and motor domains in preterm infants without major brain abnormalities, at TEA. Furthermore, associations between DTI parameters and neurobehavioral outcome at one and two years of age were demonstrated. This study highlights the importance of understanding the spatial-temporal changes occurring during this sensitive period of development, reflecting microstructural neural changes as measured by DTI already present at term age and their long-term relationship with neurobehavioral development.

4. Experimental procedure

The Ministry of Health and the local Institutional Review Board approved this study, and informed consent was obtained from all parents.

4.1. Participants

This study is part of a longitudinal study of premature infants born at <34 weeks' gestational age. Infants in this study underwent an MRI examination between 2009 and 2012 and continued neurobehavioral follow-ups until two years corrected age (Leitner

et al., 2014; Weinstein et al., 2014a). Inclusion criteria: minimal to moderate echogenicity as identified on routine cranial US performed within a week from birth in the neonatal intensive care unit (NICU). Exclusion criteria were as follows: infants with intraventricular haemorrhage grade II–IV, major congenital malformations, cortical or corpus-callosal and cerebellar malformations as identified on cUS or MRI, genetic syndromes, congenital infections (e.g. cytomegalovirus, rubella), central nervous system infection major system (CNS or extra-CNS) anomalies, intra uterine growth restriction (IUGR) unstable medical condition or contra-indication to MRI. A total of 32 preterm infants were included in the current study, 31 had rs-fMRI data, 13 had DTI-tractography (due to different protocol), and 10 had both rs-fcMRI and DTI data.

4.2. MRI set-up and data acquisition

MR setup was performed without sedation according to the guidelines by Mathur et al. (2008). Infants were transported to the MRI scanner, accompanied by a registered nurse or physician. During the MRI procedure, a thermo-neutral environment was maintained with warming blankets. Neonatal ear muffs were used to block out MRI noise. During MRI examination, the infants were continuously monitored by a pulse oximeter and electrocardiogram and closely observed by a neonatologist. Imaging was done immediately after the infant was fed to induce drowsiness and reduce head motion. All scans were performed during natural sleep.

MRI scans were performed using a 3.0 T MRI scanner (GE Signa EXCITE) with eight-channel head coil. MRI protocol included: 3D T1 weighted spoiled gradient echo sequence, matrix size / field of view (FOV)=256 × 256/180 mm, slice thickness/gap =1/0 mm; fMRI images acquired using gradient-echo EPI images, matrix/FOV=128 × 18/200 mm; TR/TE=3000/35 ms, 30–32 axial slices with thickness/gap=3/0 mm, flip angle=90°. DTI images were acquired along 16 non-collinear gradient directions with b values of 700 s/mm^2, and one that served as a reference with no applied diffusion gradient, TR/TE =8000/89 ms, matrix size/field of view (FOV)=256 × 256/200 mm and slice thickness/gap=3/0 mm axial slices were prescribed to cover the entire brain.

4.3. fMRI preprocessing

Functional MRI data were analyzed with the BrainVoyager analysis package (version 2.1; Brain Innovation). The first six volumes were discarded to allow for T1 equilibrium. Preprocessing of functional scans included 3D motion correction to account for head motion within and across runs, slice scan time correction, linear trend removal, and low-pass filtering data (Gausian FWHM bandpass filtered between 0.01 and 0.08 Hz). The functional images were then superimposed on 3D anatomical images and incorporated into the 3D datasets through trilinear/sinc interpolation. Several sources of spurious variance were removed from the signal time-course of each voxel through linear regression (Cole et al., 2010): 1) the average signal from each subject's ventricles, 2) the average signal from each subject's WM voxels, and 3) the motion correction predictors generated during FMR preprocessing.

4.4. Seed correlation analysis

To analyze the dynamics of the functional connectivity, we first manually defined seed regions of interest (ROIs) in the motor (left motor hand region in the primary motor cortex) and visual (left primary visual cortex) domains. This methodology was previously used to study rs-fcMRI of the motor network in preterm infants (Doria et al., 2010; Smyser et al., 2010). Seed regions were 5 mm boxes centered on regions of interest. The average BOLD time series for the seed regions chosen was cross-correlated with all other voxels in the brain, generating correlation maps identifying regions with functional connection to the ROI. We used the false discovery rate (FDR) procedures for the selection of thresholds, which was found to be an effective technique, selecting thresholds automatically and adaptively across subjects (Benjamini et al., 2001; Genovese et al., 2002). The FDR (q value) chosen in the present study was 0.05. The resulting contralateral homolog region was saved as a ROI and the correlation between the two homologous ROIs was measured. Correlation coefficients were then converted to a normal distribution by Fisher's z transformation.

4.5. DTI analysis

DTI analysis was performed using DTIStudio software (Johns Hopkins University, Baltimore, MD, USA). First, the diffusion tensor was estimated on a voxel-by-voxel basis and AD, RD and FA maps were calculated. The main CC, OR and left and right CST were reconstructed using streamline fiber tracking method with Fiber Assignment by Continuous Tracking (FACT) algorithm (Mori et al., 1999). Fiber tracking was terminated when it reached a pixel with an FA value lower than 0.2, or when the turning angle was $>70°$. The CC was extracted using a single ROI defined on a color coded mid-sagittal FA image (Mori et al., 1999; Mori and van Zijl, 2007; Wakana et al., 2004). Further segmentation of the CC into three segments was performed based on Witelson parcellation scheme (Witelson, 1989): genu- comprising the anterior third, midbody-comprising the anterior and posterior midbody and the isthmus, and splenium comprising the posterior fifth. The CST tracts were extracted using a multiple ROI approach, defining fibers that pass through the unilateral pons, posterior limb of the internal capsule, and motor and premotor cortex (Ben Bashat et al., 2011). The OR were reconstructed using a single ROI placed in the in the WM adjacent to the lateral geniculate nuclei according to the method in (Bassi et al., 2008). Mean values of AD, RD and FA, were calculated for each fiber.

4.6. Neurobehavioral assessment

Outcome at one and two years (ages corrected for prematurity) was assessed by the Griffiths Mental Development Scales (GMDS) (Huntley, 1996). The GMDS provides an overall developmental quotient with subscales assessing six key skill areas (locomotor, personal-social, hearing and speech, hand-eye coordination, performance). In addition, a neurological examination was performed by pediatric neurologists. GMDS normative score is 100 ± 15.

4.7. Statistical analysis

The time interval from GA at birth until the GA at MRI scan was calculated. Descriptive statistics were calculated for the rs-fcMRI networks and for the various fiber tracts. One way analysis of variance (ANOVA) was performed to test differences in functional connectivity between infants with DEHSI compared to those without DEHSI. To study maturational differences between the different WM tracts, the AD, RD and FA values of the different tracts were analyzed using a repeated measures analysis of variance (ANOVA). Mauchly's sphericity test was used to test the assumption of sphericity. This procedure was followed by Bonferroni-corrected post-hoc tests. Correlations were measured between maturational factors (GA, and time interval from GA at birth until the GA at MRI) and DTI and rs-fMRI parameters. Correlations were measured between the diffusivity parameters of the CST and CC with the rs-fcMRI motor z(r); and the diffusivity parameters of the OR with the rs-fcMRI visual z(r). Correlation analysis was

performed between DTI and rs-connectivity parameters and GMDS subscales at one and two years of age.

Conflict of interest

There is no conflict of interest for any of the authors.

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