January 2016

Language At Rest: A Longitudinal Study Of Intrinsic Functional Connectivity In Preterm Children

Megan Alysse Rowlands
Yale University, megan.a.rowlands@gmail.com

Language at Rest: A Longitudinal Study of Intrinsic Functional Connectivity in Preterm Children

A Thesis Submitted to the

Yale University School of Medicine

in Partial Fulfillment of the Requirements for the

Degree of Doctor of Medicine

by

Megan Alysse Rowlands, MPH

2016

LANGUAGE AT REST: A LONGITUDINAL STUDY OF INTRINSIC FUNCTIONAL CONNECTIVITY IN PRETERM CHILDREN

Megan A. Rowlands, Dustin Scheinost, Cheryl Lacadie, Betty Vohr, Fangyong Li, Karen C. Schneider, R. Todd Constable, Laura R. Ment. Department of Pediatrics, Yale University, School of Medicine, New Haven, CT.

Preterm (PT) children show early cognitive and language deficits and display altered cortical connectivity for language compared to term (T) children. Developmentally, functional connectivity networks become more segregated and integrated through the weakening of short-range and strengthening of long-range connections. The specific aims of this study are: (1) To use residual fMRI data to investigate intrinsic connectivity development from ages 8 to 16 years in PT vs. T controls; and (2) To correlate intrinsic connectivity to cognitive and language scores. Longitudinal intrinsic connectivity distribution (ICD) values were assessed in PT (n = 13) compared to T children (n = 12) at ages 8 vs. 16 years using a Linear Mixed Effects model. Connectivity values in regions generated by the group x age interaction analysis were correlated with scores on full IQ (FSIQ), verbal IQ (VIQ), verbal comprehension IQ (VCIQ), performance IQ (PIQ), Peabody Picture Vocabulary Test-Revised (PPVT-R), and Rapid Naming Composite (RDRL_cmp). The group x age analysis revealed significant ICD differences in the following regions: bilateral Brodmann area (BA) 47-BA11-BA10-L BA45 ($p=0.0002$) and L fusiform-BA18-BA19 ($p=0.008$). The larger frontal region (bilateral BA47-BA11-BA10-L BA45) was separated into subregions for further analysis, which showed the following significant ICD group x age differences: L and R BA47 ($p=0.03$ and $p=0.0006$, respectively), bilateral BA11 ($p=0.0008$), L and R BA10 ($p=0.0005$ and 0.005, respectively), and L BA46 ($p=0.03$). Over time, PT ICD increased in: bilateral BA47-BA11-BA10-L BA45 ($p<0.0001$), L and R BA47 ($p=0.02$ and <0.0001, respectively), bilateral BA11 ($p<0.0001$), L and R BA10 ($p<0.0001$ for both), and L BA46 ($p=0.002$). In addition, PT showed decreased ICD in L fusiform-BA18-BA19 ($p=0.002$). In contrast, the T subjects had no significant changes in ICD values over time. At age 16, PT had greater ICD than T in: bilateral BA47-BA11-BA10-L BA45 ($p=0.0002$), L & R BA47 ($p=0.03$ & $p=0.0007$), bilateral BA11 ($p=0.0009$), L & R BA10 ($p=0.0006$ & $p=0.005$), and L BA46 ($p=0.03$). PT had less ICD than T in L fusiform-BA18-BA19 ($p=0.04$). L fusiform-BA18-BA19 ICD positively correlated to scores on VIQ ($p=0.021$), PIQ ($p=0.041$), and FSIQ ($p=0.015$). None of the other regions correlated to scores on the cognitive tasks. The L fusiform-BA18-BA19 region includes the visual word form area, which has long been associated with reading performance and complex visual processing. These data demonstrate for the first time that, over the course of adolescence, prematurely-born children undergo widespread developmental changes in intrinsic connectivity that differ from term-born children. The development of resting state connectivity in prematurely-born children does not reflect compensatory alterations but rather appears to underscore and perpetuate impairment in language and cognitive processing.

Acknowledgements: I would like to personally thank Dr. John Forrest, Donna Carranzo, and Mae Geter from the Yale Office of Student Research for their support. I would also sincerely like to thank Dustin Scheinost, Cheryl Lacadie, R. Todd Constable, Fangyong Li, Karen C. Schneider, and especially Laura R. Ment, without whom this project would not have been possible. This work was supported by stipends from the Yale Office of Student Research, NIH R01 NS 27116 and DHHS Grant # 2T35HL00764926. This work has been accepted for publication in NeuroImage: Clinical (doi:10.1016/j.nicl.2016.01.016).

Table of Contents

1. Introduction

1.1. Cognitive impairment is prevalent in preterm-born children

Preterm (PT) birth is a major global health burden, with up to 11% of all live born infants worldwide being born at less than 37 weeks gestation (1,2), and as many as one-third of prematurely born infants suffering from significant cognitive impairments during early childhood (3-6). While Saigal et al. (7) demonstrated that, by adulthood, preterms are comparable to their term (T) peers in educational attainment and functional independence, several studies have shown that preterms persistently display global impairment in cognition, language, and motor function (8-10).

Even in early childhood, language deficits are evident. At age 2.5 years, preterms had lower scores on tasks of cognition, receptive and expressive communication, with over 10% of preterm children showing moderate-severe delay in these areas (11). Similarly, at age 6 years, preterms had significantly poorer reading, vocabulary, and comprehension than terms (12).

Promisingly, there is some evidence to suggest that these early language deficits in preterms may improve with age. Luu et al. found that from ages 3 to 12, preterms had poorer receptive vocabulary compared to controls, but they improved over time and nearly approached the normative values by age 12 (13). These "catch-up gains" in receptive vocabulary were also seen at age 16, although preterms continued to show impairment in phonology (14).

1.2 Structural and functional organization of neural networks

Cognitive functions are mediated by neuronal networks of varying complexity. Neurons communicate by receiving synaptic inputs along their dendritic trees and then transmitting

information via axon outputs to other neurons. These connections form larger networks, which are critical for communicating, integrating, and processing information in the brain. Morphologically, the brain contains visibly distinct tissues, termed gray and white matter. Neural gray matter includes neurons, dendrites, synapses, and local axons, while white matter refers to the long-distance, myelinated axon tracts between cortical areas. The vast majority of axonal connections exist between local neurons, while the likelihood of two distant neurons being connected is only one in a million. Thus, most neuronal communication occurs within local networks, and only a small proportion of these signals are communicated distantly by the relatively few neurons with long-range connections (15).

The development of this structural connectivity is substantially restricted by material, energy, and physical constraints. To economize resources, the brain has adapted to reconfigure axonal connections between neurons over time. Synapses and axons are remodeled, redundant connections are eliminated, and the sensitivities of these connections are continually modified. While this may succeed in minimizing structural costs, the challenge lies in preserving the functional integrity and efficiency of neuronal communication and processing (15).

The functional properties of neural networks are not strictly defined by structural connections but rather are influenced by the interplay of the activity and connections across the entire network. While functional connectivity is mediated by structural connections, not all structural connections between neurons confer an efficient functional relationship. As such, as the brain evolves over the course of a person's life, unnecessary or redundant connections may be eliminated while others are created in order to maximize functional efficiency (16). Evidence of this remodeling has been observed in a number of structural neuroimaging studies. White matter tends to increase linearly, peaking at age 50, for example, while gray matter density

decreases in a non-linear fashion up to age 40 (17). Optimally, these changes serve to maximize functional efficiency while conserving precious structural resources.

1.3. Altered neural structures in PTs

Preterm birth substantially alters the neurodevelopment of both gray and white matter. In early childhood, the brains of preterms are 5-6% smaller and display diffuse white matter abnormalities compared to matched term controls (18-20). During childhood and adolescence, preterm brains fail to undergo white matter expansion and gray matter pruning in temporal and frontal lobes that characterize normal development in term controls (21), resulting in significant decreases in left frontal and bilateral temporal white matter volumes (22). At young adulthood, preterm subjects continue to display alterations in both regional volumes and microstructural connectivity, particularly in language areas including the left frontal language regions, temporal and parietal cortices, and both cerebellar hemispheres (18,23-26). These observations suggest that the effects of prematurity on brain development are both widespread and long-lasting.

Nosarti et al. observed that preterms had diffuse decreases in both white and gray matter volumes compared to terms, which correlated with greater cognitive impairment (27). Parker et al. also showed that preterms had reduced total cerebellar volumes compared to terms. Although initial cognitive measures revealed a positive correlation with cerebellar volume, this association did not persist after controlling for white matter volume (28).

Notably, Schafer et al. found that, despite preterms having significant differences in functional connectivity to language areas as well as a reduction in left frontal and bilateral temporal white matter, preterm subjects performed comparably on semantic language tasks to normal term controls (22). Lubsen et al. echoed these findings, demonstrating that despite

preterms having lower fractional anisotropy values, which are a marker of microstructural connectivity, in several language regions, they performed comparably to terms (29).

1.4. Development of resting state functional connectivity networks

Recently, resting state functional connectivity MRI has come into focus as a method for identifying functional neural networks. It is based on the finding that distinct neural regions that display temporally related spontaneous blood oxygen level dependent (BOLD) fluctuations at rest reflect a functionally connected network (30).

Few studies have investigated resting state connectivity (RSC) in children or across development. Of these studies, it has been reported that the functional organization of the brain in children significantly differs from that of adults. Children display more local, short-range connections between adjacent brain regions, which eventually shift to more long-range, distributed connections in adults (31). This developmental trend encompasses "segregation" of neural networks via the weakening of short-range connections and concurrent "integration" of distant regions into functional networks via the strengthening of long-range connections (32).

The evolving functional architecture over development appears to correspond to maturing behavioral and cognitive abilities. Task-based fMRI studies show that more mature brains exhibit both greater task-activation and greater anti-task-deactivation in corresponding regions with age (33). Resting state studies have similarly demonstrated that this synchrony of stronger activation within networks and enhanced deactivation of antagonistic networks correlates with more mature performance on tasks of higher order executive function, such as attention, working memory, and regulatory control (34,35).

While this trend of "segregation and integration" seems to characterize maturation in a number of neural networks, little is known about the development and refinement of language network connectivity or how this development is affected by preterm birth. At adolescence, preterms display globally stronger intra- and inter-hemispheric connectivity to the superior temporal lobes than terms, but functional connectivity between these language regions and overall network efficiency are reduced in preterms (36). Preterms continue to demonstrate greater connectivity at age 20 in hypothesized language processing areas, including left temporal-parietal areas, left and right inferior temporal lobes, and the medial frontal lobes (37). Although previous studies have demonstrated significant connectivity differences between preterms and terms (36,37), it is unclear whether these connections are present at birth and are not pruned through the course of development or if they develop over time.

Traditionally, the use of resting state functional MRI data has had several limitations. For one, it relies on pre-selected regions of interest (ROI) to be investigated. ROI refers to a cluster of neural voxels in fMRI analysis that corresponds to an anatomical area of the brain that an investigator chooses to study. For example, if an investigator were interested in studying connectivity in visual networks, then an area of visual cortex would likely be selected as an ROI or "seed". Connectivity to and from this region would then be analyzed, while other neural regions are disregarded (38). Although this method may help to answer questions about specific regions, the use of pre-selected ROIs may overlook non-selected areas that display notable connectivity. Also, the use of resting state functional connectivity relies on arbitrarily defined correlation thresholds to describe functional connectivity differences.

To overcome these limitations, Scheinost et al. developed the intrinsic connectivity distribution (ICD) metric. ICD allows for the characterization of all connections via a voxel-by-

voxel whole-brain survey without requiring *a priori* defined ROIs or connectivity thresholds (37). It measures the connectivity of each voxel to all other neural voxels and allows for elaboration of a specific voxel's degree of connectivity throughout the brain without being limited to connectivity within a pre-defined network (39).

2. Statement of Purpose

The purpose of this longitudinal study is to investigate how intrinsic functional connectivity is altered from childhood through adolescence in preterms compared to terms, as well as how these changes in connectivity relate to cognitive, semantic, and phonologic testing scores. I hypothesize that, when compared to term controls, preterm subjects will display altered connectivity trajectories between childhood and adolescence. Functional connectivity will be correlated with performance on language tasks.

3. Materials and Methods

This study was performed at the Yale University School of Medicine, New Haven, CT and Brown Medical School, Providence, RI. The protocols were reviewed and approved by institutional review boards at each location. Children provided written assent; parent(s) provided written consent for the study. All scans were obtained at Yale University and were analyzed at Yale University.

3.1. Subjects

The preterm cohort consisted of children who were enrolled in the follow-up MRI component of the Multicenter Randomized Indomethacin Intraventricular Hemorrhage Prevention Trial (40). Newborns were identified as preterm if they weighed between 600 and 1250g at birth, and they were recruited for the IVH Prevention Trial within 6 hours of birth. Only those preterm children without evidence of intraventricular hemorrhage, periventricular leukomalacia and/or low-pressure ventriculomegaly and who lived within 200 miles of the Yale study center were included in the IVH trial. Term control children were recruited from the local communities of the study children. They were group-matched to the preterm children for age, sex, and minority status. Minority status was defined as being of non-Caucasian race and was reported by parents at the time of the assessment. Only preterm subjects and term controls with data collected at both 8 years and 16 years of age were included.

3.2. Neurodevelopmental assessments

Serial standardized neuropsychological assessments were performed by testers blinded to the randomization status of the subjects in the IVH prevention study. Intellectual ability was measured using the Weschler Intelligence Scale for Children, Third Edition (WISC-III), from which the verbal IQ (VIQ), performance IQ (PIQ), verbal comprehension IQ (VCIQ) and full-scale IQs (FSIQ) were obtained. Specific language skills were assessed with the Peabody Picture Vocabulary Test—Revised (PPVT-R) and the Rapid Naming Composite (RDRL_Cmp). The PPVT tests receptive vocabulary, while the RDRL_Cmp measures the efficiency of retrieving names of digits and letters, which is suggested to be a component of phonologic coding (41).

3.3. Residual data between task paradigms during fMRI scanning

For the 16-year-old subjects, each subject performed an event-related cue-target identity task that required a match/mismatch judgment between pictures and words that were presented acoustically and/or in printed form on each trial. Responses were made via a button press. Between 8 and 10 runs were completed per subject. This task is described in detail in Frost et al. (42). For the 8-year-old subjects, each subject passively listened to the Ugly Duckling story presented either normally, with words scrambled, or with a low pass filter applied. This task is described in detail in Ment et al. (43). For both tasks, the task-based data were analyzed using a general linear model described in each of the respective papers. Then the model fit was subtracted from the raw data to create a residual data set, which was used as the input to the connectivity analysis.

The use of residuals has been described previously by Finn et al. (44). The effect of task was regressed out to leave residual fluctuations that we believe are more closely representative of intrinsic, spontaneous neural activity. By avoiding task-based data, we prevent our results from being dominated by activation coupled to the onset and processing of each stimulus and instead are able to examine spontaneous fluctuations. Furthermore, the use of purely continuous resting-state scan data may introduce confounding "tasks", such as mind-wandering, which are likely inhibited in task-based studies. Thus, we believe that the use of residual data during a task-based study may more accurately reflect spontaneous neural changes and better enhance underlying functional organization between the groups.

3.4. Preprocessing

All data were converted from Digital Imaging and Communication in Medicine (DICOM) format to Analyze format using XMedCon (http://xmedcon.sourcefroge.net/). During

the conversion process, the first four images at the beginning of each of the ten functional series were discarded to enable the signal to achieve steady state, leaving 209 measurements for analysis. Images were first slice time corrected using sinc interpolation and then motion corrected using SPM5 (http://www.fil.ion.ucl.ac.uk/spm/software/spm5/). Runs with linear motion in excess of 1.5 mm or rotation greater than 2° were discarded. All voxels with signal less than 5% of the maximum were set to zero, and drift removal (up to 3rd order) and temporal Gaussian smoothing (standard deviation = 1) were then performed on the time-course of each voxel. Finally, the global time-course was regressed out. As group differences in motion have been shown to confound functional connectivity results as seen in Van Dijk et al. (45), average frame-to-frame displacement was calculated for each run and compared between the four groups: 8-year preterm, 8-year term, 16-year preterm and 16-year term. To level the mean displacement across groups to 0.054 mm, runs with displacement greater than 0.09 mm for the 8-year preterm (58 of 150 runs), 0.08mm for the 8-year term (29 of 62 runs), and 0.12mm for the 16-year preterm (55 of 288 runs) were additionally discarded. No runs were additionally discarded from the 16-year term subjects (349 runs).

3.5. Residual functional connectivity maps

All remaining residual data runs for each subject were concatenated, and the functional connectivity of each voxel, as measured by ICD, was calculated as described in Scheinost et al. (37). Similar to most voxel-based functional connectivity measures, ICD involves correlating the time course for any given gray-matter voxel with the time course of every other gray-matter voxel in the brain, and then summarizing these correlations with a network theory metric. Specifically, ICD models the entire distribution of the network measure of degree, therefore

eliminating the need to specify a connection threshold. A histogram of these positive correlations was constructed to estimate the distribution of connections to the voxel in question. This distribution of connections was converted to a survival function and the survival function was fitted with a stretched exponential with unknown variance, α. As alpha controls the spread of the distribution of connections, a larger alpha indicates a greater number of high correlation connections. Finally, this process was repeated for all voxels in the gray matter resulting in a parametric image of the alpha parameter for each subject, which was used in all between group and correlational analyses. Each subject's ICD map was then normalized by subtracting that subject's mean and dividing by the standard deviation across all voxels. This normalization process removed the large global connectivity differences to better investigate the more subtle relative connectivity differences (46). Finally, a 6mm Gaussian filter was applied to each normalized ICD map.

3.6. Registration to a common reference space

To take individual subject data into a common reference space, three registrations were calculated within the Yale BioImage Suite software package (http://www.bioimagesuite.org) (47) and then concatenated and applied as one registration. The first was a linear registration between the individual subject's raw functional data and the subject's T1 anatomical image collected at the same slice locations. The second linear registration was between the individual's T1 anatomical image and the individual's 1 mm isotropic MP-Rage anatomical image. Finally, a non-linear registration was computed between the individuals' MP-Rage anatomical image and the Colin27 Brain (48) in order to transform data into the standardized space defined by the

Montreal Neurological Institute (MNI). The inverse transformation from the MNI space to the individual functional space was also computed.

3.7 Group comparison Linear Mixed Effects model

To compare the longitudinal changes of ICD across the groups, a Linear Mixed Effects model (LME) using age (8 year vs. 16 year) and group (preterm vs. term) as factors was computed using AFNI. The age by group interaction was investigated using a threshold significance of $p<0.05$ with a conjoint cluster of 184 voxels corresponding to a $p<0.05$ family-wise error (FWE) correction as determined by AFNI's AlphaSim program.

3.8. Other statistical analyses

Demographic data were analyzed using Fisher's exact test for categorical variables and t test for continuous variables. Mixed model repeated measures analysis was performed to compare the longitudinal changes in both resting state functional connectivity and neurocognitive scores between preterms and terms, with covariate adjustment for gender, race and maternal education status and inclusion of time by group interaction. Linear contrasts were performed to examine the changes in both connectivity and cognitive scores from age 8 to 16 for each group, compare groups at each age, and compare these connectivity and cognitive score changes between groups. Pearson correlation analysis was performed to examine the correlations between all ROIs and cognitive scores at age 16. The correlation analysis was also stratified by preterms and terms. The significance level was $p < 0.05$, two-sided.

3.9 My contribution

After participating in a similar research project with Dr. Ment during my first year summer, I became interested in further characterizing connectivity in preterm subjects versus term controls. I performed an extensive background literature review, which revealed a knowledge gap in longitudinal studies of connectivity in preterm children and adolescents. Thus, when I proposed to revisit the neuroimaging data with Dr. Ment, I suggested that we focus on longitudinal data. From Dr. Ment's cohort of study patients, I identified the subjects for whom we had longitudinal data at both 8 and 16 years. I then coordinated with the neuroimaging data analysts and described my interest in identifying regions of the brain that underwent significant connectivity changes between ages 8 and 16 in preterms versus terms. They recommended that we utilize a new method of connectivity analysis, the intrinsic connectivity distribution, for quantifying connectivity from residual fMRI data. The neuroimaging analysts provided me with the raw values for relative connectivity from the group x age interaction analyses. I discussed the project with statisticians at the Yale Center for Analytic Studies, emphasizing that I wanted to explore the correlations between the changes in these connectivity values over time and scores on various neurocognitive assessments. I provided them with the ICD data and neurocognitive scores, and they sent me the results of their analyses. I independently interpreted the results of these analyses, recognized the striking decrease in left fusiform-BA18-BA19 connectivity in preterm subjects, and described the implications for its positive correlation to scores on VIQ, PIQ, and FSIQ. I performed an extensive literature review to establish context for these results, which led me to conclude that reductions in connectivity in this region are contributing to the pervasive and persistent neurocognitive deficits that we observed in the prematurely-born children.

4. Results

4.1. Subjects

Thirteen preterm children and twelve term children were included. Demographic data for all subjects are reported in Table 1. Perinatal data for the preterm subjects are also shown in Table 1. There were no significant differences between the two groups in the number of males, handedness, race, or years of maternal education. Both groups were comparable at age of scan, although preterms were slightly older than terms at age 16, which trended toward significance (16.31 vs. 16.12 years, $p = 0.059$).

Table 1: Demographic data for the study children (mean ± SD)

	Preterm	Term	*p*
Number	13	12	-
Males	9 (69%)	6 (50%)	0.428
Right-handed	13 (100%)	12 (100%)	-
Minority Status	5 (38%)	4 (33%)	1.000
Age at 8yo Scan	8.89 ± 0.49	8.80 ± 0.49	0.654
Age at 16yo scan	16.31 ± 0.29	16.12 ± 0.18	0.059
Mat ed < HS	0	0	-
Birthweight (grams)	948.46 ± 188.05	-	-
Gestational Age (weeks)	27.31 ± 2.43	-	-

As shown in Table 2, there were no significant differences between preterms and terms in cognitive scores at age 8, but PIQ and FSIQ trended towards significance ($p = 0.06$ and $p = 0.07$,

respectively), with preterms having poorer performance. Similarly at age 16, preterms scored lower than terms in VIQ, PIQ, and FSIQ ($p = 0.04$, $p = 0.006$ and $p = 0.006$, respectively).

Comparing changes in testing scores from age 8 to 16 by group, preterm children's scores worsened with increasing age, while scores for the term controls improved, with PIQ and FSIQ reaching significance ($p = 0.03$ and $p = 0.03$, respectively). Preterms also performed significantly worse on FSIQ at age 16 than at age 8 ($p = 0.03$).

Table 2: Cognitive data adjusted for gender, race, and maternal education. Presented as least squares means (95% confidence interval) and p values.

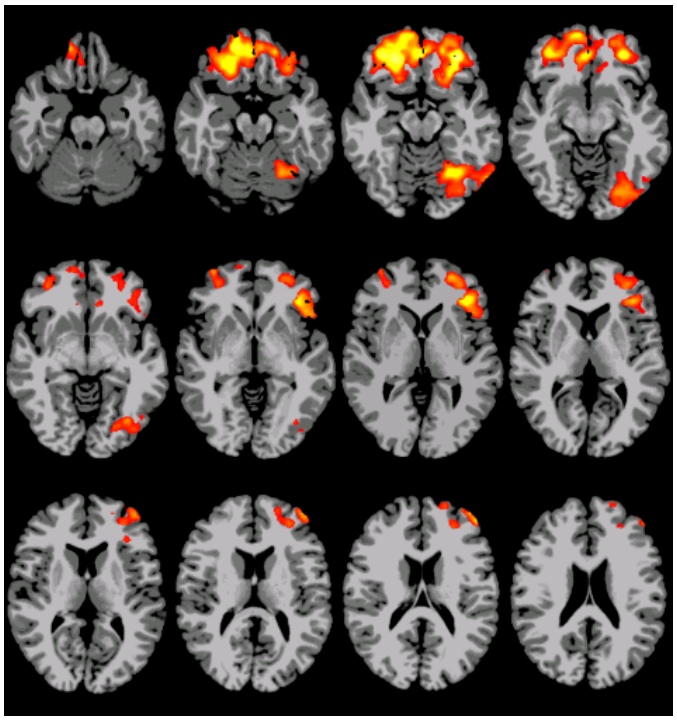
	Outcomes	Pre-Term	Term	Group difference	Interaction (slope difference)
VIQ	8 years	104.1 (96.7, 111.5)	109.8 (102.3, 117.4)	-5.7 (-16.3, 4.9) P=0.27	
	16 years	98.3 (90.8, 105.8)	109.6 (101.6, 117.6)	-11.4 (-22.4, -0.3) **P=0.04**	
	Changes from 8 to 16years	-5.9 (-12.6, 0.9) P=0.09	-0.2 (-7.6, 7.2) P=0.95		-5.6 (-15.8, 4.5) P=0.26
PIQ	8 years	97.2 (87.8, 106.7)	110.0 (100.5, 119.5)	-12.8 (-26.2, 0.7) P=0.06	
	16 years	94.0 (84.1, 103.9)	114.9 (104.7, 125.1)	-20.9 (-35.1, -6.6) **P=0.006**	
	Changes from 8 to 16 years	-3.2 (-8.0, 1.6) P=0.18	4.9 (-0.3, 10.1) P=0.06		-8.1 (-15.2, -1.0) **P=0.03**
FSIQ	8 years	100.6 (92.2, 108.9)	111.3 (102.9, 119.7)	-10.7 (-22.6, 1.2) P=0.07	
	16 years	95.8 (87.5, 104.1)	113.5 (104.9, 122.0)	-17.7 (-29.6, -5.7) **P=0.006**	
	Changes from 8 to 16 years	-4.8 (-9.0, -0.5) **P=0.03**	2.2 (-2.5, 6.8) P=0.34		-6.9 (-13.2, -0.7) **P=0.03**

VCOMPIQ	8 years	106.5 (98.7, 114.2)	109.2 (101.3, 117.1)	-2.8 (-13.9, 8.4) P=0.61	
	16 years	100.5 (93.1, 108.0)	108.2 (100.2, 116.1)	-7.6 (-18.6, 3.3) P=0.16	
	Changes from 8 to 16 years	-5.9 (-13.6, 1.7) P=0.12	-1.0 (-9.3, 7.2) P=0.79		-4.9 (-16.1, 6.3) P=0.38
PPVT	8 years	101.0 (90.3, 111.6)	113.6 (102.8, 124.4)	-12.7 (-27.9, 2.6) P=0.10	
	16 years	104.0 (93.5, 114.5)	117.5(106.8, 128.1)	-13.5 (-28.5, 1.6) P=0.08	
	Changes from 8 to 16 years	3.0 (-4.1, 10.1) P=0.39	3.8 (-3.6, 11.3) P=0.30		-0.8 (-11.1, 9.5) P=0.87
RDRL_Cmp	8 years	-	-	-	
	16 years	98.8 (82.1, 115.5)	100 (88.3, 111.7)	-1.2 (-13.2, 10.9) P=0.84	
	Changes from 8 to 16 years	-	-		-

4.2 Generated regions

The group x age interaction analysis revealed several regions with significant differences in longitudinal changes in connectivity, including left fusiform-BA18-BA19 (occipito-temporal cortex), bilateral BA47-BA11-BA10-left BA45 (inferior frontal gyri, orbitofrontal and anterior prefrontal cortices) for preterm compared to term controls (Fig. 1). This included some areas that had been both previously published in the literature and identified in our previous studies (22,49,50).

Figure 1: Interaction result from Linear Mixed Effects model using age (8 year vs. 16 year) and group (preterm vs. term), with p<0.05 threshold significance. These areas were mapped to: left fusiform-BA18-BA19 (occipito-temporal cortex) and bilateral BA47-BA11-BA10-left BA45 (inferior frontal gyri, orbitofrontal and anterior prefrontal cortices). Regions were selected from this analysis.



Additional normalized ICD maps were generated comparing groups at ages 8 and 16 years. Several connectivity differences were observed between groups at age 8, including L BA18, L BA19, L BA21, L and R BA37, and the cerebellum (Fig. 2). However, these areas did not undergo significant longitudinal changes in connectivity and thus did not persist in the group x age interaction map. The interaction map at age 16 showed significant connectivity group differences, including bilateral BA9, bilateral BA10, bilateral BA11, L BA13, L BA24, L BA32, R BA44, bilateral BA45, bilateral BA46, bilateral BA47 (Fig. 3). Several of these regions overlapped with the connectivity differences generated by the group x age interaction map.

Figure 2: Interaction result showing group differences at age 8, with p < 0.05 threshold significance. These areas included: L BA18, L BA19, L BA21, L and R BA37, and the cerebellum. They did not undergo significant longitudinal connectivity changes, however.

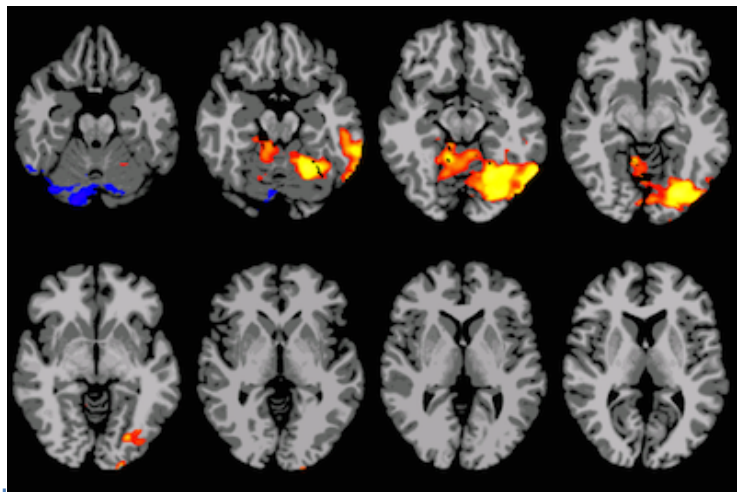
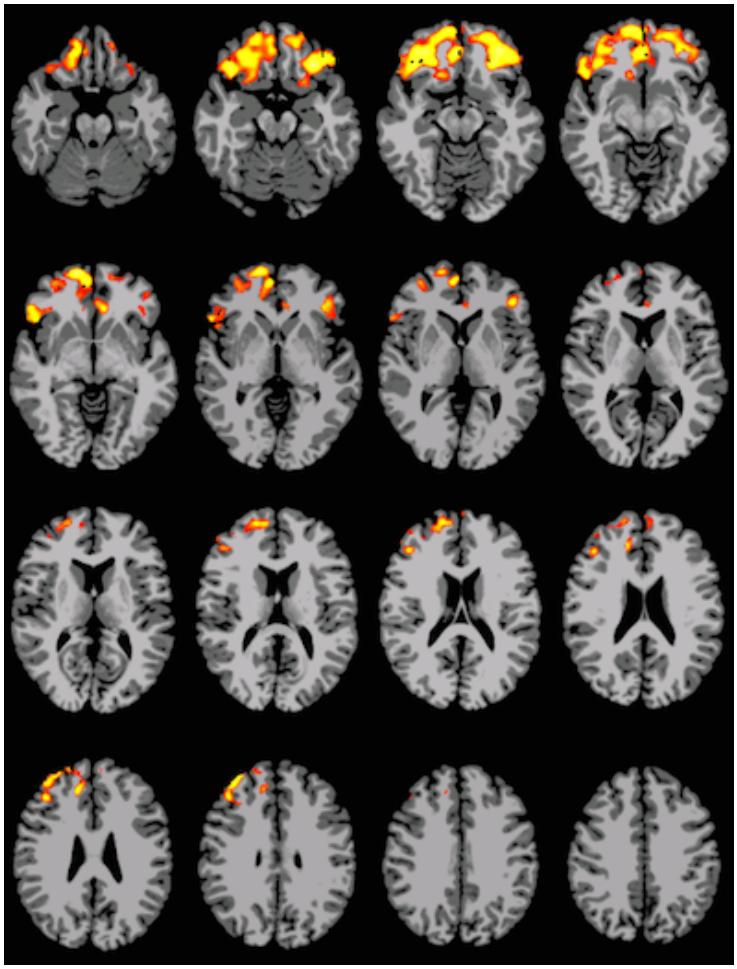


Figure 3: Interaction result showing group differences at age 16, with p < 0.05 threshold significance. These areas included: bilateral BA9, bilateral BA10, bilateral BA11, L BA13, L BA24, L BA32, R BA44, bilateral BA45, bilateral BA46, bilateral BA47.



The larger frontal ROI was separated into seven individual ROIs based on Brodmann areas for further investigation (Fig. 4). All regions were defined in reference space, with the center of mass MNI coordinates listed in Table 3, as defined in the Yale Bioimagesuite Software (Bioimagesuite.org). For all regions, the inverse transformation from reference space was used to warp each region back to individual functional space.

Figure 4: Regions generated from group by age interaction analysis.

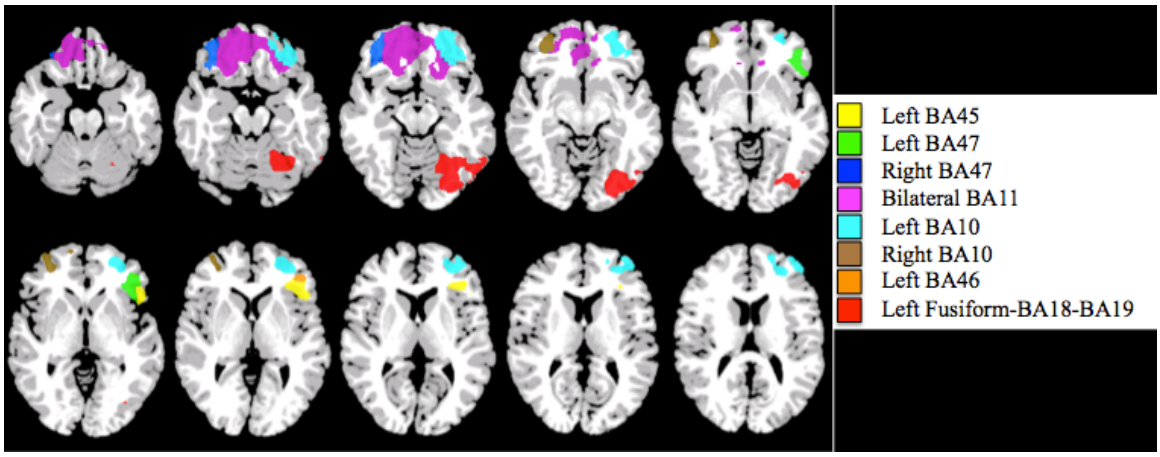


Table 3: Montreal Neurological Institute (MNI) coordinates of generated regions.

Brodmann's areas (BA)	Center of Mass MNI (x, y, z)
Bilateral BA47-BA11-BA10-Left BA45	-6, 43, -9
Left BA 45	-43, 28, 2
Left BA 47	-43, 31, -4
Right BA 47	36, 41, -15
Bilateral BA 11	8, 43, -16
Left BA 10	-30, 48, -3
Right BA 10	33, 52, -4
Left BA 46	-44, 40, 1
Left Fusiform-BA18-BA19	-33, -71, -13

4.3. Intrinsic connectivity over time

Least squares means for the resting state ICD data for each ROI are shown in Figure 5 and Table 4; these were adjusted for sex, race, and maternal education. Preterm subjects demonstrated significant increases in connectivity from age 8 to age 16 years in bilateral BA47-BA11-BA10-Left BA45 (LSM 0.61, $p < 0.0001$), left and right BA 47 (0.27, $p = 0.02$ and 0.89, $p < .0001$, respectively), bilateral BA 11 (0.74, $p < 0.0001$), left and right BA 10 (0.48, $p < 0.0001$ and 0.74, $p < 0.0001$), and left BA 46 (0.49, $p = 0.002$). Preterms also displayed significant decreases in connectivity in left fusiform-BA18-BA19 over time (-0.20, $p = 0.002$). Terms did not undergo significant alterations in connectivity over time in any ROIs, although the increase observed in right BA 10 trended towards significance (0.23, $p = 0.06$).

At age 8, none of the regions showed different connectivity between two groups. At age 16, the majority of the interrogated regions displayed significantly different connectivity in preterms compared to term controls. Preterms had greater connectivity than terms at age 16 in the following regions: bilateral BA47-BA11-BA10-Left BA45 (0.53, $p = 0.0002$), left and right BA 47 (0.37, $p = 0.03$ and 0.68, $p = 0.0007$), bilateral BA11 (0.60, $p = 0.0009$), left and right BA 10 (0.46, $p = 0.0006$ and 0.51, $p = 0.005$), and left BA 46 (0.46, $p = 0.03$). Conversely, terms had greater connectivity than preterms in left fusiform-BA18-BA19 (-0.21, $p = 0.04$).

Finally, in comparing the changes in connectivity over time in preterms versus terms, significant differences were again seen in bilateral BA47-BA11-BA10-Left BA45 (0.53, $p = 0.0002$), left fusiform-BA18-BA19 (-0.24, $p = 0.008$), left and right BA 47 (0.38, $p = 0.03$ and 0.68, $p = 0.0006$), bilateral BA 11 (0.60, $p = 0.0008$), left and right BA 10 (0.46, $p = 0.0005$ and 0.51, $p = 0.005$), and left BA 46 (0.46, $p = 0.03$).

Figure 5: ICD plots and p values for group by age interaction. At age 8, PTs and Ts displayed similar connectivity in regions. PT but not T underwent significant increases in connectivity by age 16 in all areas except left fusiform-BA18-BA19 & left BA45.

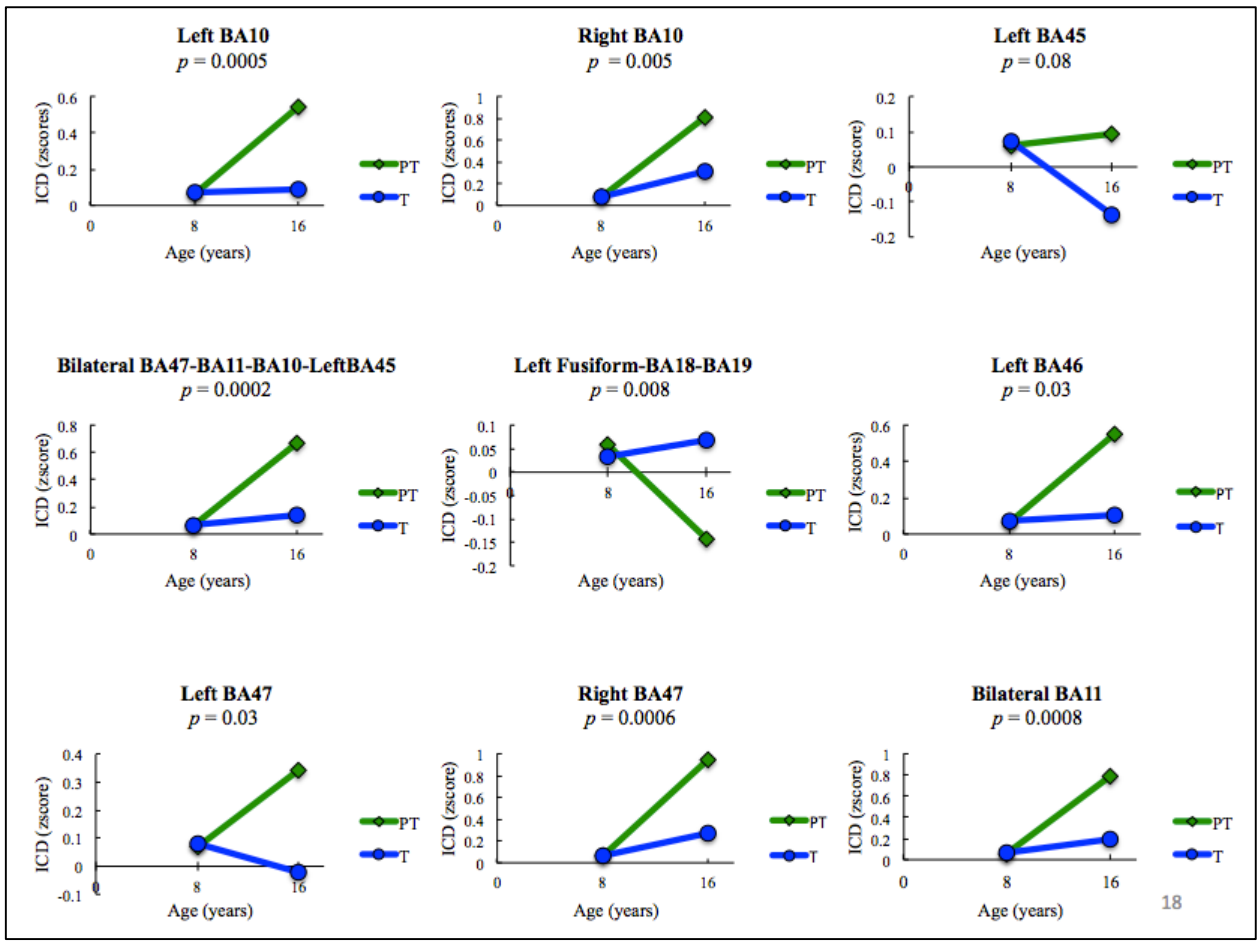


Table 4: Generated region outcomes adjusted for gender, race, and maternal education. Presented as LS means (95% confidence interval) and p values.

	Outcomes	Pre-Term	Term	Group difference	Interaction (slope difference)
Bilateral BA47_BA11_BA10_LeftBA45	8 years	0.06 (0.05, 0.07)	0.06 (0.06, 0.07)	-0.00 P=0.58	
	16 years	0.67 (0.50, 0.84)	0.14 (-0.03, 0.32)	0.53 **P=0.0002**	
	Changes from 8 to 16 years	0.61 (0.44, 0.77) **P <.0001**	0.08 (-0.1, 0.25) P=0.37		0.53 (0.29, 0.77) **P=0.0002**

Left Fusiform_BA18_BA19	8 years	0.06 (0.04, 0.08)	0.04 (0.02, 0.05)	0.02 (-0.00, 0.05) P=0.08	
	16 years	-0.14 (-0.26, -0.03)	0.07 (-0.05, 0.19)	-0.21 (-0.38, -0.05) **P=0.04**	
	Changes from 8 to 16 years	-0.20 (-0.32, -0.08) **P=0.002**	0.04 (-0.08, 0.16) P=0.53		-0.24 (-0.41, -0.07) **P=0.008**
Left BA45	8 years	0.06 (0.06, 0.07)	0.07 (0.06, 0.08)	-0.01 (-0.02, 0.00) P=0.13	
	16 years	0.10 (-0.09, 0.28)	-0.14 (-0.33, 0.06)	0.24 (-0.03, 0.52) P=0.08	
	Changes from 8 to 16 years	0.03 (-0.15, 0.22) P=0.71	0.21 (-0.41, -0.01) P=0.33		0.24 (-0.03, 0.52) P=0.08
Left BA47	8 years	0.07 (0.06, 0.08)	0.08 (0.07, 0.09)	-0.01 (-0.02, 0.01) P=0.40	
	16 years	0.34 (0.12, 0.57)	-0.03 (-0.26, 0.21)	0.37 (0.04, 0.69) **P=0.03**	
	Changes from 8 to 16 years	0.27 (0.04, 0.50) **P=0.02**	-0.10 (-0.34, 0.13) P=0.37		0.38 (0.05, 0.71) **P=0.03**
Right BA47	8 years	0.06 (0.05, 0.07)	0.06 (0.05, 0.07)	-0.01 (-0.02, 0.02) P=0.96	
	16 years	0.95 (0.71, 1.19)	0.27 (0.02, 0.52)	0.68 (0.33, 1.03) **P=0.0007**	
	Changes from 8 to 16 years	0.89 (0.65, 1.1) **P<.0001**	0.21 (-0.04, 0.46) P=0.10		0.68 (0.33, 1.03) **P=0.0006**
Bilateral BA 11	8 years	0.06 (0.05, 0.07)	0.06 (0.05, 0.07)	-0.00 (-0.02, 0.01) P=0.70	
	16 years	0.79 (0.57, 1.01)	0.19 (-0.04, 0.43)	0.60 (0.28, 0.92) **P=0.0009**	

	Changes from 8 to 16 years	0.74 (0.52, 0.95) **P <.0001**	0.13 (-0.09, 0.36) P = 0.23		0.60 (0.28, 0.92) **P=0.0008**
Left BA10	8 years	0.06 (0.06, 0.07)	0.07 (0.06, 0.07)	-0.00 (-0.01, 0.00) P=0.50	
	16 years	0.55 (0.38, 0.7)	0.08 (-0.08, 0.25)	0.46 (0.23, 0.70) **P=0.0006**	
	Changes from 8 to 16 years	0.48 (0.32, 0.64) **P <.0001**	0.02 (-0.15, 0.19) P=0.83		0.46 (0.23, 0.70) **P=0.0005**
Right BA10	8 years	0.07 (0.07, 0.08)	0.08 (0.07, 0.09)	-0.00 (-0.01, 0.01) P=0.94	
	16 years	0.81 (0.58, 1.05)	0.31 (0.07, 0.55)	0.51 (0.17, 0.85) **P=0.005**	
	Changes from 8 to 16 years	0.74 (0.51, 0.97) **P< .0001**	0.23 (-0.01, 0.47) P = 0.06		0.51 (0.17, 0.85) **P=0.005**
Left BA46	8 years	0.07 (0.06, 0.08)	0.07 (0.06, 0.08)	0.00 (-0.01, 0.01) P=0.99	
	16 years	0.56 (0.28, 0.84)	0.10(-0.18, 0.39)	0.46 (0.06, 0.86) **P=0.03**	
	Changes from 8 to 16 years	0.49 (0.21, 0.77) **P=0.002**	0.03 (-0.26, 0.33) P=0.81		0.46 (0.05, 0.86) **P=0.03**

4.4. Intrinsic connectivity and language scores

Exploratory analysis using Pearson correlations were performed to correlate ICD in ROIs with cognitive measures of all study subjects (i.e. PT and T) at age 16 years; results are listed in Table 5. In these analyses, significant correlations were only seen between connectivity of the left fusiform-BA18-BA19 and VIQ ($r = 0.467$, $p = 0.021$), PIQ ($r = 0.419$, $p = 0.041$), and FSIQ ($r = 0.491$, $p = 0.015$). Scores on the other cognitive tasks were not significantly associated with

connectivity in the other regions. Furthermore, in comparing preterm and term control subjects, there were no statistically significant differences between these groups in the correlations of ROI connectivity to cognitive scores at age 16.

When interrogating the relationship between changes in ICD from ages 8 to 16 years and overall cognitive outcome measures (ages 8 and 16), again only the left fusiform-BA18-BA19 region was significantly associated with scores on VIQ ($r = 0.466$, $p = 0.022$), PIQ ($r = 0.431$, $p = 0.036$), and FSIQ ($r = 0.498$, $p = 0.013$). Comparisons of the association of connectivity changes from ages 8 to 16 and cognitive scores at age 16 revealed no significant group differences between preterms and terms. Furthermore, no significant group differences between preterms and terms were seen when comparing connectivity at age 16 to changes in cognitive scores from ages 8 to 16.

Table 5: Unadjusted Pearson correlations between generated regions' connectivity and score at age 16.

		VERBIQ	VCIQ	PERFIQ	FULLIQ	PPVT	RDRL_cmp
Bilateral BA47_BA11_BA10_LeftBA45	Pearson Correlation Coefficients	-0.0750	-0.05056	-0.27232	-0.20537	-0.15723	0.23503
	p	0.7266	0.8145	0.1980	0.3357	0.4529	0.2581
Left Fusiform_BA18_BA19	Pearson Correlation Coefficients	0.46707	0.36750	0.41928	0.49088	0.24065	0.10950
	p	**0.0214**	0.0773	**0.0414**	**0.0149**	0.2466	0.6023
Left BA45	Pearson Correlation Coefficients	-0.07473	-0.05031	-0.10132	-0.09214	0.04644	0.17545
	p	0.7286	0.8154	0.6376	0.6685	0.8255	0.4015

Left BA47	Pearson Correlation Coefficients	-0.17325	-0.14823	-0.18649	-0.19468	-0.00563	0.11965
	p	0.4182	0.4894	0.3829	0.3620	0.9787	0.5689
Right BA47	Pearson Correlation Coefficients	-0.03099	-0.06313	-0.26783	-0.18179	-0.15021	0.31215
	p	0.8857	0.7695	0.2058	0.3952	0.4736	0.1287
Bilateral BA11	Pearson Correlation Coefficients	-0.01667	0.02428	-0.23584	-0.15659	-0.16158	0.18251
	p	0.9384	0.9103	0.2672	0.4649	0.4403	0.3826
Left BA10	Pearson Correlation Coefficients	-0.10211	-0.12229	-0.30733	-0.23721	-0.12313	0.31724
	p	0.6350	0.5692	0.1441	0.2644	0.5576	0.1223
Right BA10	Pearson Correlation Coefficients	-0.30007	-0.18210	-0.13887	-0.23281	-0.28134	-0.01274
	p	0.1543	0.3944	0.5175	0.2736	0.1731	0.9518
Left BA46	Pearson Correlation Coefficients	-0.24017	-0.24532	-0.26274	-0.27499	-0.06541	-0.07850
	p	0.2583	0.2479	0.2148	0.1934	0.7561	0.7092

5. Discussion

Developmental changes in connectivity differ in prematurely-born subjects compared to healthy term controls during that critical period of late childhood through adolescence. To the

best of our knowledge, this is the first report of longitudinal changes in intrinsic connectivity in preterm subjects and term controls from 8 to 16 years. At age 8, preterms and terms displayed grossly equivalent connectivity in the interrogated regions but then underwent significant alterations through age 16 years. Preterm but not term children demonstrated significant increases in connectivity in the regions of interest over time, especially in left and right BA47, bilateral BA11, left and right BA10, and left BA46. Ultimately, preterms showed greater connectivity than terms at age 16 in all areas of interest except the left fusiform-BA18-BA19, in which preterms underwent a significant decrease in connectivity in this region. Finally, in both preterms and terms, left fusiform-BA18-BA19 connectivity was significantly and positively associated with scores on the Full Scale IQ, Performance IQ, and Verbal IQ.

5.1. Altered development of resting state connectivity

Several studies have investigated the development of preterm resting state connectivity in infancy and early childhood. Seed-based correlation analysis has shown that preterms, when studied at term in the neonatal period, demonstrate resting state networks that closely topographically resemble those of term controls (51). However, network studies show that preterm resting state networks have weaker connectivity and complexity, especially in those networks subserving higher-order functions, e.g. language, frontoparietal control, and default mode networks (52). By age 4, network analysis studies demonstrate that the strong, predominantly local resting state connections at birth eventually shift to increased inter-hemispheric connectivity (53).

Although our study did not attempt to map out the geography of these connections, we found that at age 8, both preterms and terms displayed quantitatively similar connectivity in the

interrogated regions. When solely comparing relative connectivity between preterms and terms at age 8, we did observe significant group differences. This is in keeping with previous findings of connectivity and volumetric differences at age 8 (21,49). However, these areas of contrast in our 8-year-old interaction map did not undergo significant longitudinal changes, and thus were not generated by our group x age interaction map, which was the variable of interest in this study.

ROI studies show that at several time points in childhood and adolescence, functional connectivity to various language regions differs significantly between preterms and terms (37,49,50). At age 8, for example, preterms displayed greater connectivity from Wernicke's region (L BA22) to L BA40, R BA40, and R BA44/45 compared to terms; they also displayed increased connectivity to and involvement of right-sided neural circuits than their term counterparts (49). At age 16, functional connectivity in preterms was increased between Wernicke's area (L BA22) and R BA40 relative to terms, further demonstrating altered connectivity and increased right hemisphere involvement for language (50). Finally, at age 20, significant functional connectivity differences between preterms and terms were observed in the left temporal-parietal area (L BA39, L BA40, L BA7, L BA19, L BA21, and L BA 22), the right inferior temporal lobe (R BA21 and R fusiform gyrus), the left inferior temporal lobe (L fusiform gyrus, L BA19, and L BA21), the left cerebellum (biventer, inferior semilunar, superior semilunar, and quadrangular lobules), and bilateral medial frontal lobes (R BA6, bilateral BA8, bilateral BA9, and L BA32) (37).

These findings are similarly confirmed by microstructural analyses and appear to confer cognitive, functional implications. In preterm adolescents, for example, the microstructural integrity of white matter tracts within ventral and dorsal language pathways, which were significantly altered compared to terms, positively correlated with performance on semantic and

phonological tasks, respectively (54). Our present findings are of particular importance because they suggest that differences in preterm and term neural connectivity are not simply reflections of early and preserved perturbations, but rather are the result of the preterm subjects more rapidly altering the number of connections to these areas over the course of adolescence.

5.2. Maturation of the Visual Word Form Area

Interestingly, we found only a few regions that underwent reductions in resting state connectivity from childhood through adolescence, with only left fusiform-BA18-BA19 reaching significance in the preterm group. This area is primarily involved in visual processing. The fusiform gyrus is a region of the occipital temporal cortex, which is composed of several subregions that subserve visual functions of varying complexity. Higher-order functional regions tend to be located in the more anterior portion of the fusiform gyrus and include areas that process faces (fusiform face area)(55), bodies (fusiform body area)(56), or visual word forms (visual word form area)(57). These higher-order regions also show greater lateralization, with visual face processing being predominately right-lateralized, and visual word form processing being predominately left-lateralized (58). The posterior fusiform gyrus has been associated with processing of object-related visual cues and visual language perception (58).

Both structural and functional connectivity analyses have revealed extensive networks that link the fusiform gyrus to the occipital, temporal, parietal, frontal, insular, and subcortical regions (58). The posterior fusiform gyrus, for example, has been found to be connected to the amygdala, hippocampus, dorso-lateral occipital cortex, and posterior lingual cortex through the inferior longitudinal fasciculus (59). It is also connected to the dorsolateral and inferolateral frontal cortices via the inferior frontal-occipital fasciculus (60). More anteriorly, the anterior

ventral occipito-temporal cortex is connected to parietal, temporal, and frontal cortical regions via the long and short fiber bundles that comprise the superior longitudinal fasciculus (58,60).

The visual word form area (VWFA) is an area encompassed by the left occipito-temporal cortex (61); given our particular interest in language for preterm children, the VWFA will be the focus of discussion from here forward. As early as the first few months of life, infants already display left hemisphere lateralization for language, long before they are capable of producing speech themselves (62). By age 7, the VWFA has become strongly left-lateralized and demonstrates prominent activation to word forms (63). Although the predominance is for left lateralization, the right homologous region has been shown to assume visual word form processing function in the case of early disruption of left VWFA. For example, a child reported in the literature underwent a left occipital lobe resection involving the VWFA and temporal white matter at age 5. By age 10, she had learned to read normally. Neuroimaging studies revealed that word forms elicited a strong activation in her right VWFA homologue, suggesting that this area had compensated for normal left VWFA function (64). This neuroplasticity appears to be limited to a certain age window, however. Another subject underwent a complete left hemispherectomy for epilepsy at age 15. Prior to surgery, she had developed normally with speech and reading abilities similar to those of typically-developing adolescents. Postoperative, she was consistently impaired in tasks of letter naming, word recognition, word reading, and phonology. These findings suggest that once the cortical language areas have become lateralized, the contralateral hemisphere is no longer able to compensate (65).

A longitudinal fMRI study of the left occipital-temporal sulcus found that the size of the VWFA region activation to visual word stimuli increased from ages 8 to 12, then decreased from ages 13 to 15 years, and remained largely stable into adulthood (66). Resting state connectivity

studies have shown that the involvement of VWFA in different resting state networks also changes across development. In children, for example, VWFA participates in the visual resting state network and then transitions to the fronto-parietal network at adolescence (67).

Studies of anatomic connections from the VWFA show predominate extension to hypothesized language areas, including left superior temporal gyrus, posterior medial temporal gyrus, Broca's area, and within the left occipito-temporal sulcus (61,68). These areas are interconnected by the arcuate fasciculus, inferior fronto-occipital fasciculus, and inferior longitudinal fasciculus (69). The reduction in VWFA functional connectivity observed in our preterm adolescents may represent either a global decrease in these connections to VWFA or a loss of connections along specific white matter tracts. Furthermore, the observed connectivity loss may be the result of a steady decrease in resting state connectivity or an initial expansion and later pruning, as observed in Ben-Shachar et al. (66).

5.3. Association between VWFA connectivity and cognition

In our study cohort, preterms scored lower on all cognitive tasks compared to terms and scored lower on all tasks at age 16 than at age 8 except for the PPVT-R. However, this decline in score over time only reached significance for the FSIQ. Although our sample size was particularly small, the larger cohort from which these preterms were selected, which included 373 8-year-olds and 326 16-year-olds, also demonstrated decreases in the FSIQ over time (mean scores of 91 and 87 at ages 8 and 16, respectively) (14).

Notably, despite such remarkable and widespread connectivity changes over time, only left VWFA connectivity correlated to scores on Full Scale IQ, Performance IQ, and Verbal IQ before correction for multiple comparisons. The Full Scale IQ is a composite of both the Verbal

IQ and Performance IQ, taking into account scores on both. The Verbal IQ measures verbal comprehension and working memory, while the Performance IQ measures perceptual organization and processing speed.

Interestingly, left VWFA connectivity did not correlate with the other language tasks, such as the Peabody Picture Vocabulary Test or Rapid Digit and Letter Naming Tasks, suggesting that it does not participate in semantic or word retrieval processing. This conflicts with other studies, however, which have demonstrated VWFA involvement in various naming and object recognition tasks (70,71). Further, the other interrogated regions did not correlate to performance on any of the cognitive tasks. This is interesting given that several of these regions have been associated with cognitive functioning in other studies. BA 45 and BA 47, for example have been implicated in semantic and lexical processing (72-74). BA 11 is involved in creativity and reward-based behavior (75,76). BA 10 shows activation during tasks of prospective memory and "mentalizing" (77,78), and BA 46 is involved in episodic memory retrieval (79).

The involvement of the VWFA in reading has long been established. Early lesion studies demonstrating pure alexia (80) have since been corroborated by task-based fMRI studies showing VWFA activation during reading tasks (66,81,82). Children with greater left occipito-temporal activation display more advanced reading skills, while disruptions in parieto-temporal and occipito-temporal activation are associated with reading impairment, including dyslexia (83). Additionally, DTI studies have shown that VWFA receives white matter input from visual cortex and projects fiber tracts to cortical language areas, further speaking to its role in visual language processing (61,68).

Cohen and Dehaene (84) argue that VWFA represents a "specialized" region for responding to visual words, which they propose is necessary and apparent given how seamlessly

we can recognize words despite the complexities of orthography, e.g. changes in font, case, size, and location. To support their hypothesis, they provide evidence that VWFA exhibits three forms of specialization: functional specialization, reproducible localization, and regional selectivity, albeit partial regional selectivity (84). Functional specialization theorizes that different cortical areas are specialized for different functions, i.e. VWFA for word recognition. In order to demonstrate this, VWFA must show specific activation to written words that are distinct from generic visual processing. Several studies have supported this hypothesis, showing that the VWFA displays stronger activation to letters than to pseudo-characters or digits (85-87) and is equally responsive to words regardless of case (88). Interestingly, the VWFA is also preferentially activated by strings of letters and words that conform to orthographic rules as opposed to random strings of consonants (85,89-92).

Reproducible localization refers to activation of a reproducible area during tasks. This has been observed for the VWFA, as several studies have shown that a portion of the left fusiform gyrus, approximated at MNI coordinates (-46, -53, -20), is reproducibly activated during reading (57,93,94). Thirdly, regional selectivity postulates that regions of cortex are exclusively dedicated to performing a specific function, such as letter or word recognition, and do not respond to other non-letter or non-word stimuli. Cohen and Dehaene argue that the VWFA exhibits partial regional selectivity. This region of occipito-temporal cortex is likely more generally involved in complex visual processing in early development, and then becomes "re-purposed" and increasingly but not exclusively specialized for reading as an individual continues to learn and refine these skills (84,95).

Although its name implies specific involvement in visual word processing, recent studies have argued against this specificity. For one, although resting state connectivity correlations have

been shown between purported “reading regions” (96), resting state whole brain analyses have not demonstrated a designated “reading network” (67,97,98). Furthermore, resting state studies demonstrate that the VWFA is only weakly correlated with hypothesized reading regions and is most strongly correlated with the dorsal attention network (99). This suggests that, while the VWFA plays a pivotal role in visual word processing, it is not exclusively dedicated to this function. Rather, it is likely more generally involved in the processing of complex visual stimuli (100,101).

In further support of this, the VWFA has been shown to be active during tasks of object or picture naming, despite these tasks not explicitly requiring the processing of visual word forms (70,71). For example, positron emission tomography studies show greater VWFA activation when subjects silently named a picture of an object than when they silently read the name of the object (70). This has been similarly reproduced, where stronger activation was demonstrated to naming objects than reading the written names of the same objects (102). If the VWFA were solely involved in word form recognition, it would be expected to be most active during “explicit” reading of the written word as opposed to “implicit” naming of objects, which was not observed (103). Moreover, even unfamiliar non-objects without names elicited VWFA activation relative to visual noise (104). Thus, the role of the VWFA in word form recognition or naming cannot be consistently ascribed, as it appears to be involved in a number of visual processes. Curiously, despite these other studies showing involvement of the VWFA in naming, we did not observe a correlation between rapid letter or digit naming and VWFA connectivity.

Interestingly, the VWFA does not seem to be exclusively limited to visual stimuli either. VWFA became activated during auditory word tasks (105) and has even been activated by congenitally blind subjects tactilely reading Braille (90). Although more strongly activated by

visual stimuli, the ability for auditory and tactile stimuli to also activate this region further casts doubt on its dedicated role in visual word form processing (103).

For these reasons, Price and Devlin counter that naming this region the VWFA is both misleading and inaccurate (103). While involved in visual word form processing, this function is nonspecific. They posit that multimodal activation in this region suggests that: (1) this region may contain multiple neuronal populations subserving different functions; (2) a single cognitive function may be underlying these responses; or (3) these neurons may be multifunctional as a result of converging communication with other cortical areas (103).

While Cohen and Dehaene (84) strongly disagree with several of the methodologies and conclusions drawn by Price and Devlin (103), they agree that since reading is a relatively new advent and that the skill itself develops and improves through learning over time, one should not expect a predetermined region of cortex to have evolved to be exclusively devoted to visual word form processing. They also agree that this area is likely composed of neurons that preferentially respond to letter and word stimuli but also likely contains neurons that are involved in other visual processes, such as face and object recognition (106). Thus, they conclude that it is not surprising that functional neuroimaging studies demonstrate activation of the VWFA to word and non-word stimuli. Furthermore, they posit that even if a portion of VWFA were to demonstrate regional selectivity and cortical delineation from neighboring regions, the limitations in spatial resolution of current neuroimaging modalities would prevent us from accurately differentiating between distinct cortical areas.

The topography of VWFA connectivity appears to divergently influence reading performance in children compared to adults. Koyama et al. (107), for example, found that reading performance in children was inversely related to RSC between VWFA and the left

inferior frontal gyrus and left inferior parietal lobule. Reading competence was positively associated with connectivity between VWFA and both the precuneus/posterior cingulate cortex and ventromedial prefrontal cortex, which are part of the default network. The opposite was true for adults, whose reading competence was positively associated with connectivity between the VWFA and phonology-related regions, i.e. Broca's area and left inferior parietal lobule, and negatively associated with connectivity between VWFA and default network regions. This suggests that enhanced RSC between VWFA, Broca's area, and the left inferior parietal lobule mediates improved reading performance in the mature adult brain but not in children. Furthermore, the negative relationship seen in adults between reading competence and the VWFA-default network connectivity reflects that "segregation" of these regions contributes to maturation and optimization of the adult brain.

Thiebaut de Schotten et al. studied the effect of literacy on white matter microstructure and found that reading performance positively correlated with an increased fractional anisotropy (FA) in the posterior left arcuate fasciculus. The posterior left arcuate fasciculus connects the posterior temporal lobe, which includes the VWFA, with the inferior parietal lobule and posterior superior temporal regions. Fractional anisotropy values in the posterior arcuate were consistently increased by literacy, regardless of whether literacy was acquired in childhood or adulthood. FA was also positively correlated with the level of VWFA activation to written letter strings as well as the level of planum temporale activation to spoken sentences. The authors conclude that the act of learning to read induces functional changes in VWFA, planum temporale, and the white matter connections between them (69).

In light of the positive correlation we found between left VWFA connectivity and cognitive and verbal performance, the decrease in connectivity for preterms is paradoxical. It

indicates that they are not effectively or adaptively recruiting connections in these regions. Furthermore, this active loss of connections does not reflect an enhanced, "mature" brain (32,108), but rather is associated with inferior cognitive performance. Preterms' worsening scores on Verbal IQ, Performance IQ, and Full Scale IQ from ages 8 to 16 can be attributed in part to this decrease in connectivity. Interestingly, we did not observe a correlation between VWFA connectivity and rapid naming scores (RDRL_Cmp). This differs from expected given that several studies have found an association between letter and word naming and VWFA (66,81,82). As we did not adjust for multiple comparisons, the significance of these correlations in this exploratory analysis should be interpreted with caution (109).

Additionally, although terms in our study did not display any significant quantitative changes in connectivity in the left VWFA over time, this does not indicate that the topography of these connections was similarly unaltered. It may be that terms similarly undergo "segregation" and "integration" of these connections, but that the overall degree of connectivity remains the same. Likewise, it may be that preterms' loss of connections from VWFA to specific neural regions is what is underlying the observed cognitive deficits and is not due to the reduction in connectivity overall.

5.4 Educational interventions to enhance VWFA connectivity

Our results suggest that if one were able to increase connectivity in left fusiform-BA18-BA19, this may help to mediate some of the cognitive deficits we observed in the preterms. This may be addressed by educational neuroscience, which has emerged as a focus aimed at designing and implementing educational exercises to evoke neural plasticity and enhance functional specialization in cognitive circuits (110).

In a longitudinal DTI study by Keller and Just, 8- to 10-year-old poor readers underwent 100 hours of remedial reading instruction, and resulting changes in their cortical fractional anisotropy (FA) values were assessed (111). FA is a marker of the microstructural integrity of white matter and is influenced by a number of axonal features, including myelination, axonal packing density, axonal diameter, or orientation of axons (112). At baseline, compared to the good readers, these poor readers exhibited significantly lower FA values. After the 100-hour remediation program, the poor readers displayed significant increases in both reading ability and FA. Radial diffusivity significantly decreased after remediation, while axial diffusivity was unchanged. This indicates that the increase in FA was reflective of increased myelination. These remediation effects are promising, as increasing myelination may enhance the speed and efficiency of neural transmission, in turn augmenting functional connectivity. These observations differed from those of a group of good readers and to a control group of untreated poor readers, who did not display significant changes in FA. Similarly, the findings by Thiebaut de Schotten et al. of literacy's effects on increasing left posterior arcuate FA further supports an intervention aimed at improving reading ability and, ultimately, VWFA microstructural connectivity (69).

Brem et al. studied the effect of grapheme-phoneme training on sensitization of the visual word form area in kindergarten-aged, non-reading children (113). Their study children alternately practiced a computerized grapheme-phoneme game and nonlinguistic control game for 8 weeks and were subsequently imaged with fMRI and event related potential at various time points during the training period. At baseline, the children were similarly familiar with letters but were rudimentary in reading ability. Prior to training, fMRI revealed bilateral ventral posterior occipito-temporal activation when presented with word or pseudoword stimuli. Over the 8 week course of grapheme-phoneme training but not the control training, the children demonstrated

increased activation and sensitivity to print words primarily in the posterior visual word form system, including the left and right posterior occipito-temporal lobes (fusiform gyrus and inferior temporal gyrus) and cuneus. On further investigation of regions of interest in the occipito-temporal lobes, only left R4 (MNI coordinates [x,y,z] ±46, −78, −12) showed significant training effects with increased responses to word stimuli. Event related potential analyses confirmed the fMRI findings of increased response to word stimuli as opposed to pseudoword stimuli after grapheme-phoneme training, specifically in the left occipito-temporal cortex (fusiform gyrus and lingual gyrus), right cuneus, and posterior cingulate. This study provides striking evidence that educational experiences can induce specific alterations in the neural activity of children. Notably, however, the increase in printed word sensitivity was only temporary and declined after the subjects discontinued their grapheme-phoneme training.

Functional cortical specialization has also been shown to depend on the modality of learning and training. James et al. investigated the effect of training on functional specialization of the VWFA (114). Twelve healthy pre-school aged, pre-literate children were trained for letter recognition through either manual sensori-motor training by writing out a given letter or through visual training by verbally identifying a presented letter. Prior to training, fMRI showed that at baseline, the left VWFA demonstrated greater activation during letter perception than during perception of shapes or pseudo-letters. The right fusiform gyrus, in contrast, showed similar degrees of activation to all stimuli. These hemispheric differences support that even prior to literacy, functional specialization for printed letters is beginning to emerge. The authors note that all subjects had some familiarity with letters, i.e. singing the alphabet song, writing their names, which may account for this early left hemisphere localization. After completion of sensorimotor training, the amplitudes of the BOLD signals in both the left VWFA and right anterior fusiform

gyrus dramatically increased during letter perception; these phenomena were not observed in the visual training group. This heightened activation of the VWFA in reading after sensorimotor training highlights its role in synthesizing multiple sensory modalities as opposed to being functionally limited to visual processing alone. On behavioral tasks of letter recognition, the sensorimotor trained group showed greater improvement in performance than the visual-only trained group, although these differences did not reach significance. This suggests that functional neural alterations can be induced by sensorimotor training and may be early precursors to behavioral changes.

Given the potentially enormous benefits in neuroimaging-based educational interventions, several agencies have organized to share these innovations with the public. The Organization for Economic Cooperation and Development (OECD), for example, has launched a website (www.OECD.org) with several forums, one of which serves as a platform where neuroimaging and educational researchers can share their interventional educational materials. The goal is that eventually these websites will include multilingual educational exercises, descriptions of efficacy for specific target groups, and information for parents, children, teachers, and researchers to reference (115).

5.5. Limitations

One limitation of this study is the use of interleaved residual fMRI data to approximate resting state connectivity. Although Fair et al. (116) have described that interleaved residual data are both quantitatively and qualitatively similar to continuous resting data and may be an adequate alternative for RSC analyses, it is possible that these approximations are not an accurate measure of true RSC. Other limitations include the relatively small sample size of only

13 preterm and 12 term children, unadjusted Pearson correlations, and analyzing longitudinal data from only two time points. Additionally, we cannot interpret the changes in connectivity as being attributed solely to time or age differences due to the possibility of confounding from magnet effects. Regardless, this does not minimize the observation that preterm connectivity changes are significantly different than term connectivity changes.

To the best of our knowledge, this is the first longitudinal study evaluating the development of intrinsic functional connectivity and language in preterm children through adolescence. The children who participated in this study are part of a well-studied cohort with neuroimaging available from early in the neonatal period and extending through young adulthood. Future longitudinal studies should assess connectivity at several time points over the course of childhood through adolescence, ideally with larger numbers of preterm and term children. It may be pertinent to correlate changes in cortical thickness to RSC as a metric for brain maturation.

5.6. Conclusions

Prematurely-born children undergo significant expansion of resting state connections over time, which differs markedly from term children. Most notably, preterm subjects showed paradoxical decreases in resting state connectivity in the left occipito-temporal cortex, which includes the VWFA, that was significantly correlated with verbal and IQ measures. These data suggest that the development of resting state connectivity in prematurely-born children does not reflect compensatory alterations in connectivity but rather may underscore and perpetuate impairment in language and cognitive processing. Promisingly, the advent of educational neuroscience in the future may serve to increase connectivity in this region and mediate the cognitive deficits that were observed.

References

1. Blencowe H, Cousens S, Oestergaard MZ, et al. National, regional, and worldwide estimates of preterm birth rates in the year 2010 with time trends since 1990 for selected countries: a systematic analysis and implications. *The Lancet.* 2012;379(9832):2162-2172.
2. Beck S, Wojdyla D, Say L, et al. The worldwide incidence of preterm birth: a systematic review of maternal mortality and morbidity. *Bulletin of the World Health Organization.* 2010;88(1):31-38.
3. Blencowe H, Lee AC, Cousens S, et al. Preterm birth-associated neurodevelopmental impairment estimates at regional and global levels for 2010. *Pediatric research.* 2013;74(S1):17-34.
4. Neubauer V, Griesmaier E, Pehbock-Walser N, Pupp-Peglow U, Kiechl-Kohlendorfer U. Poor postnatal head growth in very preterm infants is associated with impaired neurodevelopment outcome. *Acta paediatrica.* 2013;102(9):883-888.
5. Robertson CM, Watt MJ, Dinu IA. Outcomes for the extremely premature infant: what is new? And where are we going? *Pediatric neurology.* 2009;40(3):189-196.
6. Saigal S, Doyle LW. An overview of mortality and sequelae of preterm birth from infancy to adulthood. *The Lancet.* 2008;371(9608):261-269.
7. Saigal S, Stoskopf, B., Streiner, D., Boyle, M., Pinelli, J., Paneth, N., Goddeeris, J. Transition of Extremely Low-Birth-Weight Infants from Adolescence to Young Adulthood. *JAMA.* 2006;295(6):667-675.
8. Botting N, Powls A, Cooke RWI, Marlow N. Cognitive and educational outcome of very-low-birthweight children in early adolescence. *Developmental Medicine & Child Neurology.* 1998;40(10):652-660.
9. Bowen JR, Gibson FL, Hand PJ. Educational outcome at 8 years for children who were born extremely prematurely: A controlled study. *Journal of Paediatrics and Child Health.* 2002;38(5):438-444.
10. Hack M. Adult Outcomes of Preterm Children. *Journal of Developmental & Behavioral Pediatrics.* 2009;30(5):460-470.
11. Mansson J, Stjernqvist K. Children born extremely preterm show significant lower cognitive, language and motor function levels compared with children born at term, as measured by the Bayley-III at 2.5 years. *Acta paediatrica.* 2014;103(5):504-511.
12. Pritchard VE, Clark CA, Liberty K, Champion PR, Wilson K, Woodward LJ. Early school-based learning difficulties in children born very preterm. *Early human development.* 2009;85(4):215-224.
13. Luu TM, Vohr BR, Schneider KC, et al. Trajectories of receptive language development from 3 to 12 years of age for very preterm children. *Pediatrics.* 2009;124(1):333-341.
14. Luu TM, Vohr BR, Allan W, Schneider KC, Ment LR. Evidence for catch-up in cognition and receptive vocabulary among adolescents born very preterm. *Pediatrics.* 2011;128(2):313-322.
15. Laughlin SB, Sejnowski TJ. Communication in neuronal networks. *Science.* 2003;301(5641):1870-1874.

16. Bullmore E, Sporns O. Complex brain networks: graph theoretical analysis of structural and functional systems. *Nature Reviews Neuroscience.* 2009;10(3):186-198.
17. Toga AW, Thompson PM, Sowell ER. Mapping brain maturation. *Focus.* 2006.
18. Boardman JP, Craven C, Valappil S, et al. A common neonatal image phenotype predicts adverse neurodevelopmental outcome in children born preterm. *NeuroImage.* 2010;52(2):409-414.
19. Nosarti C, Al-Asady, M.H.S., Frangou, S., Stewart, A.L., Rifkin, L., Murray, R.M. Adolescents who were born very preterm have decreased brain volumes. *Brain : a journal of neurology.* 2002;125(7):1616-1623.
20. Peterson BS, Vohr, B., Staib, L.H., Cannistraci, C.J., Dolberg, A., Schneider, K.C., Katz, K.H., Westerveld, M., Sparrow, S., Anderson, A.W., Duncan, C.C., Makuch, R.W., Gore, J.C., Ment, L.R. . Regional brain volume abnormalities and long-term cognitive outcome in preterm infants. *JAMA.* 2000;284(15):1939-1947.
21. Ment LR, Kesler S, Vohr B, et al. Longitudinal brain volume changes in preterm and term control subjects during late childhood and adolescence. *Pediatrics.* 2009;123(2):503-511.
22. Schafer RJ, Lacadie C, Vohr B, et al. Alterations in functional connectivity for language in prematurely born adolescents. *Brain : a journal of neurology.* 2009;132(Pt 3):661-670.
23. Haldipur P, Bharti U, Alberti C, et al. Preterm delivery disrupts the developmental program of the cerebellum. *PloS one.* 2011;6(8):e23449.
24. 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. *Pediatric radiology.* 2011;41(8):953-961.
25. Nosarti C, Mechelli A, Herrera A, et al. Structural covariance in the cortex of very preterm adolescents: a voxel-based morphometry study. *Human brain mapping.* 2011;32(10):1615-1625.
26. Tam EW, Ferriero, D.M., Xu, D., Berman, J.I., Vigneron, D.B., Barkovich, A.J., Miller, S.P. . Cerebellar development in the preterm neonate: effect of supratentorial brain injury. *Pediatric research.* 2009;66(1):102-106.
27. Nosarti C, Giouroukou E, Healy E, et al. Grey and white matter distribution in very preterm adolescents mediates neurodevelopmental outcome. *Brain : a journal of neurology.* 2008;131(Pt 1):205-217.
28. Parker J, Mitchell A, Kalpakidou A, et al. Cerebellar growth and behavioural & neuropsychological outcome in preterm adolescents. *Brain : a journal of neurology.* 2008;131(Pt 5):1344-1351.
29. Lubsen J, Vohr B, Myers E, et al. Microstructural and functional connectivity in the developing preterm brain. *Seminars in perinatology.* 2011;35(1):34-43.
30. Cohen AL, Fair DA, Dosenbach NU, et al. Defining functional areas in individual human brains using resting functional connectivity MRI. *NeuroImage.* 2008;41(1):45-57.
31. Dosenbach NU, Nardos B, Cohen AL, et al. Prediction of individual brain maturity using fMRI. *Science.* 2010;329(5997):1358-1361.
32. Fair DA, Cohen AL, Power JD, et al. Functional brain networks develop from a "local to distributed" organization. *PLoS computational biology.* 2009;5(5):e1000381.
33. Marsh R, Zhu H, Schultz RT, et al. A developmental fMRI study of self-regulatory control. *Human brain mapping.* 2006;27(11):848-863.

34. Barber AD, Caffo BS, Pekar JJ, Mostofsky SH. Developmental changes in within- and between-network connectivity between late childhood and adulthood. *Neuropsychologia.* 2013;51(1):156-167.
35. Rubia K. Functional brain imaging across development. *European child & adolescent psychiatry.* 2013;22(12):719-731.
36. Wilke M, Hauser TK, Krägeloh‐Mann I, Lidzba K. Specific impairment of functional connectivity between language regions in former early preterms. *Human brain mapping.* 2014;35(7):3372-3384.
37. Scheinost D, Benjamin J, Lacadie CM, et al. The intrinsic connectivity distribution: a novel contrast measure reflecting voxel level functional connectivity. *NeuroImage.* 2012;62(3):1510-1519.
38. Etzel JA, Gazzola V, Keysers C. An introduction to anatomical ROI-based fMRI classification analysis. *Brain Research.* 2009;1282:114-125.
39. Scheinost D, Finn ES, Tokoglu F, et al. Sex differences in normal age trajectories of functional brain networks. *Human brain mapping.* 2015;36(4):1524-1535.
40. Ment LR, Oh W, Ehrenkranz RA, et al. Low-dose indomethacin therapy and extension of intraventricular hemorrhage: a multicenter randomized trial. *The Journal of pediatrics.* 1994;124(6):951-955.
41. Wagner R, Torgesen J, Rashotte C. Comprehensive test of phonological processing (CTOPP). *Pro-Ed, Austin, Texas.* 1999.
42. Frost SJ, Landi N, Mencl WE, et al. Phonological awareness predicts activation patterns for print and speech. *Annals of dyslexia.* 2009;59(1):78-97.
43. Ment LR, Peterson BS, Meltzer JA, et al. A functional magnetic resonance imaging study of the long-term influences of early indomethacin exposure on language processing in the brains of prematurely born children. *Pediatrics.* 2006;118(3):961-970.
44. Finn ES, Shen X, Holahan JM, et al. Disruption of functional networks in dyslexia: a whole-brain, data-driven analysis of connectivity. *Biological psychiatry.* 2014;76(5):397-404.
45. Van Dijk KR, Sabuncu MR, Buckner RL. The influence of head motion on intrinsic functional connectivity MRI. *NeuroImage.* 2012;59(1):431-438.
46. Mitchell MR, Balodis IM, DeVito EE, et al. A preliminary investigation of Stroop-related intrinsic connectivity in cocaine dependence: associations with treatment outcomes. *The American journal of drug and alcohol abuse.* 2013;39(6):392-402.
47. Duncan JS, Papademetris X, Yang J, Jackowski M, Zeng X, Staib LH. Geometric strategies for neuroanatomic analysis from MRI. *NeuroImage.* 2004;23 Suppl 1:S34-45.
48. Holmes CJ, Hoge R, Collins L, Woods R, Toga AW, Evans AC. Enhancement of MR images using registration for signal averaging. *Journal of computer assisted tomography.* 1998;22(2):324-333.
49. Gozzo Y, Vohr B, Lacadie C, et al. Alterations in neural connectivity in preterm children at school age. *NeuroImage.* 2009;48(2):458-463.
50. Myers EH, Hampson M, Vohr B, et al. Functional connectivity to a right hemisphere language center in prematurely born adolescents. *NeuroImage.* 2010;51(4):1445-1452.
51. Doria V, Beckmann CF, Arichi T, et al. Emergence of resting state networks in the preterm human brain. *Proceedings of the National Academy of Sciences of the United States of America.* 2010;107(46):20015-20020.

52. Smyser CD, Inder TE, Shimony JS, et al. Longitudinal analysis of neural network development in preterm infants. *Cerebral cortex.* 2010;20(12):2852-2862.
53. Lee W, Morgan BR, Shroff MM, Sled JG, Taylor MJ. The development of regional functional connectivity in preterm infants into early childhood. *Neuroradiology.* 2013;55 Suppl 2:105-111.
54. Mullen KM, Vohr BR, Katz KH, et al. Preterm birth results in alterations in neural connectivity at age 16 years. *NeuroImage.* 2011;54(4):2563-2570.
55. Kanwisher N, Yovel G. The fusiform face area: a cortical region specialized for the perception of faces. *Philosophical Transactions of the Royal Society B: Biological Sciences.* 2006;361(1476):2109-2128.
56. Peelen MV, Downing PE. Selectivity for the human body in the fusiform gyrus. *Journal of neurophysiology.* 2005;93(1):603-608.
57. Cohen L, Dehaene S, Naccache L, et al. The visual word form area. *Brain : a journal of neurology.* 2000;123(2):291-307.
58. Caspers J, Zilles K, Amunts K, Laird AR, Fox PT, Eickhoff SB. Functional characterization and differential coactivation patterns of two cytoarchitectonic visual areas on the human posterior fusiform gyrus. *Human brain mapping.* 2014;35(6):2754-2767.
59. Catani M, Jones DK, Donato R. Occipito‐temporal connections in the human brain. *Brain : a journal of neurology.* 2003;126(9):2093-2107.
60. Catani M, Howard RJ, Pajevic S, Jones DK. Virtual in vivo interactive dissection of white matter fasciculi in the human brain. *NeuroImage.* 2002;17(1):77-94.
61. Yeatman JD, Rauschecker AM, Wandell BA. Anatomy of the visual word form area: adjacent cortical circuits and long-range white matter connections. *Brain and language.* 2013;125(2):146-155.
62. Dehaene-Lambertz G, Dehaene S, Hertz-Pannier L. Functional neuroimaging of speech perception in infants. *Science.* 2002;298(5600):2013-2015.
63. Gaillard W, Balsamo L, Ibrahim Z, Sachs B, Xu B. fMRI identifies regional specialization of neural networks for reading in young children. *Neurology.* 2003;60(1):94-100.
64. Cohen L, Lehéricy S, Henry C, et al. Learning to read without a left occipital lobe: Right-hemispheric shift of visual word form area. *Annals of Neurology.* 2004;56(6):890-894.
65. PATTERSON K, VARGHA-KHADEM F, POLKEY CE. Reading with one hemisphere. *Brain : a journal of neurology.* 1989;112(1):39-63.
66. Ben-Shachar M, Dougherty RF, Deutsch GK, Wandell BA. The development of cortical sensitivity to visual word forms. *Journal of cognitive neuroscience.* 2011;23(9):2387-2399.
67. Vogel AC, Church JA, Power JD, Miezin FM, Petersen SE, Schlaggar BL. Functional network architecture of reading-related regions across development. *Brain and language.* 2013;125(2):231-243.
68. Bouhali F, de Schotten MT, Pinel P, et al. Anatomical connections of the visual word form area. *The Journal of Neuroscience.* 2014;34(46):15402-15414.
69. Thiebaut de Schotten M, Cohen L, Amemiya E, Braga LW, Dehaene S. Learning to read improves the structure of the arcuate fasciculus. *Cerebral cortex.* 2014;24(4):989-995.

70. Bookheimer SY, Zeffiro TA, Blaxton T, Gaillard W, Theodore W. Regional cerebral blood flow during object naming and word reading. *Human brain mapping.* 1995;3(2):93-106.
71. Murtha S, Chertkow H, Beauregard M, Evans A. The neural substrate of picture naming. *Journal of cognitive neuroscience.* 1999;11(4):399-423.
72. Heim S, Alter K, Ischebeck AK, et al. The role of the left Brodmann's areas 44 and 45 in reading words and pseudowords. *Cognitive Brain Research.* 2005;25(3):982-993.
73. Heim S, Eickhoff SB, Amunts K. Specialisation in Broca's region for semantic, phonological, and syntactic fluency? *NeuroImage.* 2008;40(3):1362-1368.
74. Hagoort P. On Broca, brain, and binding: a new framework. *Trends in Cognitive Sciences.* 2005;9(9):416-423.
75. Chavez-Eakle RA, Graff-Guerrero A, Garcia-Reyna JC, Vaugier V, Cruz-Fuentes C. Cerebral blood flow associated with creative performance: a comparative study. *NeuroImage.* 2007;38(3):519-528.
76. Pochon JB, Levy R, Fossati P, et al. The neural system that bridges reward and cognition in humans: an fMRI study. *Proceedings of the National Academy of Sciences of the United States of America.* 2002;99(8):5669-5674.
77. Burgess PW, Gonen-Yaacovi G, Volle E. Functional neuroimaging studies of prospective memory: what have we learnt so far? *Neuropsychologia.* 2011;49(8):2246-2257.
78. Gilbert SJ, Gonen-Yaacovi G, Benoit RG, Volle E, Burgess PW. Distinct functional connectivity associated with lateral versus medial rostral prefrontal cortex: a meta-analysis. *NeuroImage.* 2010;53(4):1359-1367.
79. Fletcher PC, Shallice T, Frith CD, Frackowiak RS, Dolan RJ. The functional roles of prefrontal cortex in episodic memory. II. Retrieval. *Brain : a journal of neurology.* 1998;121(7):1249-1256.
80. Greenblatt SH. Alexia without agraphia or hemianopsia. *Brain : a journal of neurology.* 1973;96(2):307-316.
81. Wandell BA. The neurobiological basis of seeing words. *Annals of the New York Academy of Sciences.* 2011;1224:63-80.
82. Wandell BA, Rauschecker AM, Yeatman JD. Learning to see words. *Annual review of psychology.* 2012;63:31-53.
83. Shaywitz BA, Shaywitz SE, Pugh KR, et al. Disruption of posterior brain systems for reading in children with developmental dyslexia. *Biological psychiatry.* 2002;52(2):101-110.
84. Cohen L, Dehaene S. Specialization within the ventral stream: the case for the visual word form area. *NeuroImage.* 2004;22(1):466-476.
85. Price CJ, Wise RJ, Frackowiak RS. Demonstrating the implicit processing of visually presented words and pseudowords. *Cerebral cortex.* 1996;6(1):62-70.
86. Allison T, McCarthy G, Nobre A, Puce A, Belger A. Human extrastriate visual cortex and the perception of faces, words, numbers, and colors. *Cerebral cortex.* 1994;4(5):544-554.
87. Polk T, Stallcup M, Aguirre GK, et al. Neural specialization for letter recognition. *Cognitive Neuroscience, Journal of.* 2002;14(2):145-159.
88. Polk TA, Farah MJ. Functional MRI evidence for an abstract, not perceptual, word-form area. *Journal of Experimental Psychology: General.* 2002;131(1):65.

89. Beauregard M, Chertkow H, Bub D, Murtha S, Dixon R, Evans A. The neural substrate for concrete, abstract, and emotional word lexica a positron emission tomography study. *Cognitive Neuroscience, Journal of.* 1997;9(4):441-461.
90. Büchel C, Price C, Friston K. A multimodal language region in the ventral visual pathway. *Nature.* 1998;394(6690):274-277.
91. Cohen L, Martinaud O, Lemer C, et al. Visual word recognition in the left and right hemispheres: anatomical and functional correlates of peripheral alexias. *Cerebral cortex.* 2003;13(12):1313-1333.
92. Xu B, Grafman J, Gaillard WD, et al. Conjoint and extended neural networks for the computation of speech codes: the neural basis of selective impairment in reading words and pseudowords. *Cerebral cortex.* 2001;11(3):267-277.
93. Cohen L, Lehéricy S, Chochon F, Lemer C, Rivaud S, Dehaene S. Language‐specific tuning of visual cortex? Functional properties of the Visual Word Form Area. *Brain : a journal of neurology.* 2002;125(5):1054-1069.
94. Dehaene S, Le Clec'H G, Poline J-B, Le Bihan D, Cohen L. The visual word form area: a prelexical representation of visual words in the fusiform gyrus. *Neuroreport.* 2002;13(3):321-325.
95. Dehaene S. Pre-emption of human cortical circuits by numbers and language: The "neuronal recycling" hypothesis. *From monkey brain to human brain. Vol in press. Cambridge, Massachusetts.* 2004.
96. Koyama MS, Kelly C, Shehzad Z, Penesetti D, Castellanos FX, Milham MP. Reading networks at rest. *Cerebral cortex.* 2010;20(11):2549-2559.
97. Power JD, Cohen AL, Nelson SM, et al. Functional network organization of the human brain. *Neuron.* 2011;72(4):665-678.
98. Yeo BT, Krienen FM, Sepulcre J, et al. The organization of the human cerebral cortex estimated by intrinsic functional connectivity. *Journal of neurophysiology.* 2011;106(3):1125-1165.
99. Vogel AC, Miezin FM, Petersen SE, Schlaggar BL. The putative visual word form area is functionally connected to the dorsal attention network. *Cerebral cortex.* 2011:bhr100.
100. Vogel AC, Petersen SE, Schlaggar BL. The left occipitotemporal cortex does not show preferential activity for words. *Cerebral cortex.* 2012:bhr295.
101. Vogel AC, Petersen SE, Schlaggar BL. The VWFA: it's not just for words anymore. *Frontiers in human neuroscience.* 2014;8.
102. Moore CJ, Price CJ. Three distinct ventral occipitotemporal regions for reading and object naming. *NeuroImage.* 1999;10(2):181-192.
103. Price CJ, Devlin JT. The myth of the visual word form area. *NeuroImage.* 2003;19(3):473-481.
104. van Turennout M, Ellmore T, Martin A. Long-lasting cortical plasticity in the object naming system. *Nature neuroscience.* 2000;3(12):1329-1334.
105. Price C, Winterburn D, Giraud A, Moore C, Noppeney U. Cortical localisation of the visual and auditory word form areas: a reconsideration of the evidence. *Brain and language.* 2003;86(2):272-286.
106. Puce A, Allison T, McCarthy G. Electrophysiological studies of human face perception. III: Effects of top-down processing on face-specific potentials. *Cerebral cortex.* 1999;9(5):445-458.

107. Koyama MS, Di Martino A, Zuo XN, et al. Resting-state functional connectivity indexes reading competence in children and adults. *The Journal of neuroscience : the official journal of the Society for Neuroscience.* 2011;31(23):8617-8624.
108. Stevens MC, Pearlson GD, Calhoun VD. Changes in the interaction of resting-state neural networks from adolescence to adulthood. *Human brain mapping.* 2009;30(8):2356-2366.
109. Bender R, Lange S. Adjusting for multiple testing—when and how? *Journal of clinical epidemiology.* 2001;54(4):343-349.
110. McCandliss BD. Educational neuroscience: The early years. *Proceedings of the National Academy of Sciences.* 2010;107(18):8049-8050.
111. Keller TA, Just MA. Altering cortical connectivity: remediation-induced changes in the white matter of poor readers. *Neuron.* 2009;64(5):624-631.
112. Beaulieu C. The basis of anisotropic water diffusion in the nervous system–a technical review. *NMR in Biomedicine.* 2002;15(7‐8):435-455.
113. Brem S, Bach S, Kucian K, et al. Brain sensitivity to print emerges when children learn letter-speech sound correspondences. *Proceedings of the National Academy of Sciences of the United States of America.* 2010;107(17):7939-7944.
114. James KH. Sensori‐motor experience leads to changes in visual processing in the developing brain. *Developmental science.* 2010;13(2):279-288.
115. Posner MI, Rothbart MK. Influencing brain networks: implications for education. *Trends in Cognitive Sciences.* 2005;9(3):99-103.
116. Fair DA, Schlaggar BL, Cohen AL, et al. A method for using blocked and event-related fMRI data to study "resting state" functional connectivity. *NeuroImage.* 2007;35(1):396-405.