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Alterations in functional connectivity for language in prematurely born adolescents

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Recent data suggest recovery of language systems but persistent structural abnormalities in the prematurely born. We tested the hypothesis that subjects who were born prematurely develop alternative networks for processing language. Subjects who were born prematurely ($n = 22$; 600–1250 g birth weight), without neonatal brain injury on neonatal cranial ultrasound, and 26 term control subjects were examined with a functional magnetic resonance imaging (fMRI) semantic association task, the Wechsler Intelligence Scale for Children-III (WISC-III) and the Clinical Evaluation of Language Fundamentals (CELF). In-magnet task accuracy and response times were calculated, and fMRI data were evaluated for the effect of group on blood oxygen level dependent (BOLD) activation, the correlation between task accuracy and activation and the functional connectivity between regions activating to task. Although there were differences in verbal IQ and CELF scores between the preterm (PT) and term control groups, there were no significant differences for either accuracy or response time for the in-magnet task. Both groups activated classic semantic processing areas including the left superior and middle temporal gyri and inferior frontal gyrus, and there was no significant difference in activation patterns between groups. Clear differences between the groups were observed in the correlation between task accuracy and activation to task at $P < 0.01$, corrected for multiple comparisons. Left inferior frontal gyrus correlated with accuracy only for term controls and left sensory motor areas correlated with accuracy only for PT subjects. Left middle temporal gyri correlated with task accuracy for both groups. Connectivity analyses at $P < 0.001$ revealed the importance of a circuit between left middle temporal gyri and inferior frontal gyrus for both groups. In addition, the PT subjects evidenced greater connectivity between traditional language areas and sensory motor areas but significantly fewer correlated areas within the frontal lobes when compared to term controls. We conclude that at 12 years of age, children born prematurely and children born at term had no difference in performance on a simple lexical semantic processing task and

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activated similar areas. Connectivity analyses, however, suggested that PT subjects rely upon different neural pathways for lexical semantic processing when compared to term controls. Plasticity in network connections may provide the substrate for improving language skills in the prematurely born.

Keywords: connectivity; fMRI; volumetric; preterm; language

Abbreviations: BA = Brodman's area; BOLD = blood oxygen level dependent; CCCT = correlation coefficient comparison test; fMRI = functional magnetic resonance imaging; H = hippocampus; IFG = inferior frontal gyrus; MTG = middle temporal gyrus; pTG = posterior middle and inferior temporal gyrus; PT = preterm; ROI = region(s) of interest; STG = superior temporal gyrus; SMA = sensorimotor association cortex

Introduction

The cognitive deficits associated with preterm (PT) birth have been described, and include diminished attention, memory and reasoning skills relative to full-term peers (Saigal and Doyle, 2008). Recent studies have, however, documented functional recovery over a range of cognitive skills on the part of the prematurely born, especially by late childhood and early adolescence, and an increasing number of children born before week 33 of gestation and weighing <1500 g at birth are successfully maturing into adulthood (Rushe *et al.*, 2001; Allin and Nosarti, 2005; Saigal *et al.*, 2006; Ment and Constable, 2007; Saigal and Doyle, 2008).

Cognitive behaviour of prematurely born children that is indistinguishable from that of children born full term suggests non-differential localization between the two groups in neuro-cognitive studies employing fMRI brain imaging. At the same time, evidence of such non-differential localization in PT children is unanticipated because structural abnormalities associated with PT birth persist through early development (Peterson *et al.*, 2000*b*) and are still observed during adolescence (Nosarti *et al.*, 2008). These abnormalities occur even in the absence of severe disability and take the form of smaller cortical and sub-cortical volumes, larger ventricular volumes and decreases in callosal projections and in fibre tract organization in general (Santhouse *et al.*, 2002; Kesler *et al.*, 2004; Gimenez *et al.*, 2006; Constable *et al.*, 2008; Mullen *et al.*, 2008). The default assumption is that such structural differences would result in a difference in the localization of cognitive function.

An alternative view is that localization could be the same for the two groups when behaviour is equivalent, but in such cases the network underlying the areas of activation would differ. This account of functional recovery in a structurally altered brain would permit the possibility for activations to task in PT and term born children to be largely overlapping, though the connectivity between regions may differ. Connectivity refers to the coordination of response across spatially distinct brain areas (Raichle and Snyder, 2007). Work on resting state connectivity, examining brain activity during sleep states and in anaesthetized animals, indicates that connectivity is at least partially anatomically determined, dependent upon white and grey matter fibres (Greicius *et al.*, 2003; Hampson *et al.*, 2006; Vincent *et al.*, 2007). Thus we expect modifications in connectivity in the context of altered anatomy.

This work explores the neurobiological underpinnings of functional recovery in a set of 12-year-old PT children. When tested earlier, at age eight, they evidenced the cognitive deficits frequently documented in PT children including impaired performance on basic semantic tasks and a failure to make use of familiar semantic pathways when assessed by functional magnetic resonance imaging (fMRI) (Peterson *et al.*, 2002). Yet in light of previous work indicating recovery of basic semantic processing during adolescence (Saavalainen *et al.*, 2006), we adopted the null hypothesis for their performance on the simple semantic association task presented to them and to a term born control group during a 12-year-old follow-up fMRI scan. The blood oxygen level-dependent (BOLD) response was used to localize any group differences in activation during task performance. In addition, to verify the persistence of structural differences, we examined data from volumetric scans available for a majority of the subjects from the two groups. Finally, the network underlying the areas of activation was identified *post hoc* by analysing the connectivity between regions responding to task in the PT and the control group. These analyses constitute the first examination of functional connectivity in the developing PT brain.

Methods

This study was performed at Yale University School of Medicine, New Haven, CT, Warren Alpert Brown Medical School, Providence, RI, and Stanford University, Palo Alto, CA. The protocols were reviewed and approved by institutional review boards at each location. All scans were obtained and analysed at Yale University with the exception of voxel-based and volumetric morphometric analyses which were performed at the Stanford Center for Interdisciplinary Brain Sciences Research.

Subjects

Seventy-four children provided written assent, and parents provided written consent for the study. As has been our custom for all imaging studies, children were reimbursed $100 to participate in each scanning session. PT children included in this study were screened for intraventricular haemorrhage, periventricular leukomalacia and low pressure ventriculomegaly, and showed no evidence of neonatal brain injury. Twenty (9 PT) were excluded from the analyses due to incomplete scanning sessions, response recording errors or excessive motion; six additional PT children with a history of intraventricular haemorrhage and/or periventricular leukomalacia were also excluded from the analysis. FMRI data from the remaining 48 subjects were analysed.

The 22 PT subjects (10 males) participated as part of a 12 year follow up to the Multicenter Randomized Indomethacin Intraventricular

Haemorrhage Prevention Trial (Ment *et al.*, 1994). The PT subjects were recruited from the original cohort based on geographical proximity to New Haven, Connecticut and are representative of the original cohort in sex, handedness, full-scale intelligence quotient scores, race/ethnicity and maternal education. Twenty six children born at term were recruited from the local community. They were group-matched with the PT children for age, sex and minority status. The assessments of neonatal health status and neurodevelopmental outcome have been previously described. Blinded assessment of intelligence was performed in a separate session from the fMRI scan using the Wechsler Intelligence Scale for Children-III (WISC-III) (Wechsler, 1991). Children also underwent measures of language function, including portions of the Clinical Evaluation of Language Fundamentals (CELF) Third Edition (Semel *et al.*, 1995), the Peabody Picture Vocabulary Test-Revised (PPVT-R) (Peabody, 1981) and the Gray Silent Reading Test (Wiederholt and Blalock, 2000). The PPVT-R provides a receptive vocabulary score, while the CELF and the Gray Silent Reading Test measure the ability to understand spoken and written language.

Volumetric MRI studies

High-resolution volumetric magnetic resonance imaging scans were collected on 37 of the 48 subjects (15 PT). Volumetric images were acquired with a three-dimensional volumetric radiofrequency spoiled gradient echo pulse sequence 124 contiguous 1.2 mm sagittal slices (256×192 matrix, TE = 5 ms, $\alpha = 45$, TR = 24 ms, NEX = 1, field of view = 30 cm).

fMRI task paradigm

The fMRI paradigm consisted of two block-design tasks, semantic association and non-word rhyme, each presented across four runs consisting of six task blocks and seven baseline blocks. Because of difficulties with the non-word rhyme stimuli, this task had to be eliminated from the study. All blocks were 18 s in length and contain four 4.5 s fixed time trials, for a total of 24 task trials and 28 baseline trials per run, 96 task trials and 112 baseline trials across the four runs. Stimuli were presented using Psyscope (Macwhinney *et al.*, 1997) and timed via a Macintosh PowerPC running the programme. Responses were registered using a fiber optic button box. Dependent behavioural measures are response time and response accuracy.

Task stimuli consisted of 56 word pairs presented on a single line on a screen. Twenty items were repeated once in identical order and 20 items were repeated once in inverse order to yield 96 trials. Subjects were required to decide whether the two words were associated; in 50 of the 96 trials, the items did so, e.g. *dry wet* or *bronze chrome*. We refer to these as the matched trials; they include instances of near synonyms, of antonyms and of items that share a category. Accurate response in these trials demand lexical access and semantic association. In non-matched trials words were not readily related, e.g. *bride cloud* or *pin rag*. The baseline involved the visual presentation of a pair of symbol strings consisting of forward and back-slashes. Subjects were required to decide if the pair matched or not through visual inspection with respect to the single parameter, direction of slant. Of the 112 baseline trials, 54 were matches and 48 were non-matches.

fMRI imaging

Images were collected from all 48 subjects on a 1.5T Siemans Sonata scanner with a standard bird-cage head coil. Functional images were acquired with a gradient-echo, echo-planner imaging sequence of 24 axial-oblique slices set parallel to the ac–pc line (6 mm thick, 0 skip) with gradient echo planar imaging (64×64 matrix, TE = 45 ms, $\alpha = 80$, TR = 2 s, 2112 kHz bandwidth). BOLD acquisitions were obtained at 122 images per slice per run. The 24 6 mm slices permitted full brain coverage for every subject. The scanning series included both T1- and T2-weighted axial-oblique 2D anatomic scans and a 3D Sagittal SPGR anatomic series. Stimuli presentation was timed to start on a signal from the scanner at the beginning of each run; there was a programmed delay for discarded acquisitions in order to allow magnetization to reach steady-state. This sequence required a minimum total time in the scanner of 1 h, 10 min.

Data analysis

Behavioural data analysis

Task accuracy and response time data were input to a 2×2 ANOVA in SPSS (trial type by diagnostic group) in which subject age at time of scan was factored in as a covariate. Because semantic association occurred only in matched trials, if a difference were to be found in the 2×2 ANOVA, group contrast in accuracy and response time would be examined separately in these trials. For completeness, accuracy and response time to the baseline task was examined by univariate ANOVA. In addition, to verify that the well-established connection between PT structural irregularities and cognitive performance (Peterson *et al.*, 2000*a*) was instantiated in our data, accuracy scores were correlated with a representative measure of structural divergence, left ventricle volume.

Volumetric analysis

Volumetric images were transferred digitally to Stanford University. Raters blinded to group membership visually inspected the images to exclude those evidencing excessive motion. Scans were imported into BrainImage version 5.x (http://spnl.stanford.edu/tools/brainimage.htm) for semiautomated whole brain segmentation and quantification in the sagittal plane using previously described methods (Kesler *et al.*, 2004). The process yields results for whole brain grey matter, white matter and cerebrospinal fluid, including subcortical grey matter. Measurements for each group were compared using general linear modelling adjusting for total brain volume and age at scan.

Imaging data analysis

Functional images were motion corrected using SPM99 (Friston *et al.*, 1995), and registered to a three-dimensional reference brain as implemented in the Yale BioImage Suite software package (http://www.bioimagesuite.org). Individual subject data were analysed within BioImage Suite (Papademetris *et al.*, 2006) using a General Linear Model with the semantic task as a regressor on each voxel in the entire brain volume. The data were normalized to a signal measure of 100 and were spatially smoothed with an 8.0 mm Gaussian kernel over a 3 voxel radius to account for variations in the location of activation across participants. The output maps are normalized beta-maps that are in the acquired space ($3.44 \times 3.44 \times 6$ mm).

To take these data into a common reference space, three registrations were calculated within the BioImage Suite software package using the intensity-only component of the method reported in (Papademetris *et al.*, 2004). The first registration performs a linear registration between the individual subject raw functional image stripped of the skull and that subject's 2D anatomical image. The 2D anatomical image is then linearly registered to the individual's 3D anatomical image. The 3D differs from the 2D in that it has

a $1 \times 1 \times 1$ mm resolution whereas the 2D *z*-dimension is set by slice-thickness and its *x*–*y* dimensions are set by voxel size. Finally, a non-linear registration is computed between the individual 3D anatomical image and a reference 3D image. The reference brain used was a selected normal subject brain from an age-matched child who did not participate in the study. All three registrations were applied sequentially to the individual normalized beta-maps to bring all data into the common reference space.

Data were converted to AFNI format [(Cox, 1996) http://afni.nimh.nih.gov] for group analysis. Because males show a different trajectory of recovery than female PT children, we applied a 2×2 ANOVA (gender type by diagnostic group) in which subject was treated as a random factor using the GroupAna programme from the AFNI Matlab library (http://afni.nimh.nih.gov/afni/matlab/). In addition to group analysis, we examined activation associated with semantic processing via a regression analysis performed using 3DRegAna (http://afni.nimh.nih.gov/pub/dist/doc/program_help/3dRegAna.html) on whole brain activation and accuracy scores on the matched trials, those in which semantic association occurred. In both these analyses individual voxel thresholds were set at a $P < 0.01$. Results were masked and converted back into ANALYZE format for viewing in BioImage Suite. Data were corrected for multiple comparisons by spatial extent of contiguous suprathresholded individual voxels at an experiment-wise $P < 0.01$. In a Monte Carlo simulation within the AFNI software package and using a smoothing kernel of 8 mm and a connection radius of 7.2 mm on $3.44 \times 3.44 \times 6$ mm voxels, it was determined that an activation volume of 102 original voxels (2754 microliters) satisfied the $P < 0.01$ threshold.

ROI connectivity analysis

The synchrony of response between regions responding highly to task was examined *post hoc*. Functional connectivity in each group was substantiated using cross-subject correlations between mean activations within regions isolated via the imaging data analysis. A cross-subject correlation is the correlation between the mean activation for each subject in each region being compared (Horwitz *et al.*, 1998, 2000; Pugh *et al.*, 2000). In the absence of time course information from resting state runs, the correlation across-subjects of two or more regional mean activations during task offers a good approximation of temporal connectivity, the correlation between the time course of BOLD responses in two or more regions or across voxels in the brain.

Taking this approach, it was necessary to identify a set of ROI that manifested a robust response to semantic processing. We isolated ROI via the regression analysis mentioned above correlating whole brain activation and accuracy scores on matched trials. Individual voxel thresholds were set at a $P < 0.025$. At this threshold a set of distinct regions was exposed consisting of well-established frontal and temporal language areas plus a less expected region in sensorimotor association cortex (SMA). The language areas include three regions in IFG Brodman's area (BA) 44, 45 and 47 and four temporal semantic processing areas, the hippocampus in medial temporal lobe, the anterior inferior temporal gyrus BA 21, MTG at BA 21/22, and an area within the posterior temporal gyrus (pTG) extending superiorly from middle temporal gyrus in BA 39, through posterior BA 22 into inferior temporal gyrus in BA 37. We further expanded the set to include five homologues that were not already present at this threshold, so that each area and its homologue would be included in the analysis. The anatomical connections between homologues are well-established, and their activity strongly correlates in healthy brains. Including them in our larger probe of the network connections supplied a baseline threshold by which to compare other connections. Talairach coordinates are shown for these ROI in Table 1.

Based on the differential BOLD results discussed fully in the next section, we formed two hypotheses concerning functional connectivity between ROI. First, the similar intensity of response across groups within the left IFG and the left MTG that we observed in the composite maps of activation to task suggested a functional connection between these regions in both groups. Second, we hypothesized group differences in connectivity would pattern with the two group differences observed in the BOLD activation-task accuracy correlation results, where SMA was significant only in the PT group and left IFG was significant only in the term control group.

ROI analysis was completed using BioImage Suite. Output was a mean beta weight per subject per ROI describing the activation across runs in that ROI for the subject. These data were input to SAS 9.1.3 and cross-subject correlations were calculated between the ROI betas for each subject within each group. A cross-subject correlation at $P = 0.001$ two-tailed was treated as a significant connection between two ROI since the mean significance of the correlations between homologues in the term control data was 0.002.

In order to identify when *R*-values derived from the corresponding cross-subject correlations for PT and term control subjects were significantly different, these independent correlation coefficients were statistically compared by performing a Fisher R to z transform (Hinkle *et al.*, 1988) using the interface available at www.fon.hum.uva.nl/Service/Statistics/Two_Correlations.html. This computation takes into account the size of each subject group when determining whether an apparent difference in correlation coefficients is significant. Henceforth we refer to this procedure as the 'correlation coefficient comparison test', and *P*-values resulting from this test reported below are labeled CCCT. 128 correlations were required per subject group to examine the correlations among the complete set of ROI. Because of the multiple comparisons, we applied a more stringent threshold for significance, $P \leqslant 0.001$. For regions that were hypothesized to be connected in both groups, we expected equivalently significant *R*-value results from cross-subject correlations within both groups and no significant difference between these *R*-values in the CCCT. For regions that were hypothesized to be connected in only one group, we expected a significant *R*-value result from cross-subject correlations in the relevant group and a significant difference in the pair of corresponding *R*-values when submitted to the CCCT.

Table 1 Talairach Coordinates for the ROI

Region	Brodmann's area	Hemisphere	Talairach coordinates		
Anterior/ventral IFG	BA 47	Left	−42,	20,	4
		Right	38,	19,	4
Posterior/dorsal IFG	BA 45	Left	−34,	32,	13
		Right	32,	32,	23
Posterior/dorsal IFG	BA 44	Left	−40,	5,	29
		Right	38,	5,	27
MTG	BA 20/21	Left	−60,	−14,	−11
		Right	58,	−14,	−11
STG	BA 22	Left	−48,	−39,	19
		Right	46,	−38,	17
AG	BA 39	Left	−41,	−48,	12
		Right	42,	−48,	10
Hippocampus		Left	−32,	−29,	−1
		Right	32,	−29,	−3
Sensorimotor cortex		Left	−29,	−30,	62
		Right	32,	−26,	61

Results

Subject assessment

As shown in Table 2 the PT subjects had a mean gestation age of 28.6 ± 2 weeks and a mean birth weight of 1013.2 ± 139.2 g. Children in the two groups were well matched in gender and race. Mothers of term control subjects had significantly more years of education ($P = 0.033$) and were more likely to have graduated from high school ($P = 0.04$). As shown in Table 3, there were no significant differences in the numbers of right-handed subjects or subject height at scan. PT subjects were on average 6 months older than term controls ($P = 0.048$). Data in Table 3 also demonstrate that verbal IQ, verbal comprehension IQ, performance IQ and full-scale IQ scores were all significantly lower for the 22 PT subjects when compared to the 26 term control children ($P = 0.007$, 0.012, 0.001 and 0.003, respectively). Similarly, CELF receptive language and total scores and PPVT-R scores were also significantly lower for PT subjects ($P = 0.002$, 0.008 and 0.016, respectively). None of the subjects had a VIQ score < 70.

In-magnet behavioural results

The PT and term groups performed equally well on the semantic association task. As reported in Table 3, no significant difference for trial type or group was observed on either dependent variable, accuracy or response time. Likewise no significant difference between the two groups on the baseline task was observed for either accuracy or response time. As expected, there was a significant inverse correlation between accuracy and left ventricle volume only for PT subjects ($R_{Preterm} = -0.744$, $p_{Preterm} = 0.001$, $R_{Term} = -0.282$, $p_{Term} = 0.203$).

Brain volumes for the study subjects

Brain volume data for a subset of the study children are shown in Table 4 and demonstrate significant differences between the PT and term study children for the deep gray regions bilaterally ($P < 0.001$ for left and 0.002 for right), for the left frontal, temporal and parietal white regions ($P = 0.04$, 0.05 and 0.05, respectively), for the right temporal and deep white matter ($P = 0.02$ and 0.04, respectively) and for right and left lateral ventricular cerebrospinal fluid volumes ($P = 0.036$ and 0.002, respectively).

Table 2 Neonatal Data

	Preterm	Term	*P*-value
Number	22	26	
Number of males	10 (45%)	11 (42%)	1.0
Birth weight (grams)	1013.2 ± 139.2		
Gestational age (weeks)	28.6 ± 1.9		
Non-white	10 (45%)	7 (27%)	0.23
Chorioamnionitis	5 (23%)		
Randomization to indomethacin	11 (50%)		
Bronchopulmonary dysplasia	6 (27%)		
Maternal education (years)	12.9 ± 2.1	15.0 ± 2.8	0.033
Maternal education < high school	4 (18%)	0	0.038

Functional imaging results

There was no significant difference at $P < 0.01$, corrected, in activation patterns between the two study groups. This null result was not a threshold effect: no significant differences were observed if we lowered our significance threshold to $P < 0.05$, corrected. Composite group activation maps at $P < 0.001$ corrected for the 22 PT and 26 term control subjects are shown in Fig. 1 and reveal that both the PT subjects and those born full term present the greatest activation to task in the left IFG and in the left STG/MTG.

Maps of the correlation between accuracy on semantically associated items with whole brain activation to task for both study groups are shown in Fig. 2A. For both study groups, task accuracy significantly correlates with activation in the left MTG, in BA 22 for the PT subjects and in BA 39 for the term controls ($P < 0.01$, corrected). Activation in the left SMA of PT subjects also significantly correlated with accuracy, as did that in the left IFG of the term controls ($P < 0.01$, corrected). For illustrative purposes, a plot of the correlation between activation within the left IFG and accuracy on semantic items is shown in Fig. 2B. The plot demonstrates a high degree of correlation for term control subjects ($R = 0.632$, $P < 0.001$, R-square = 0.40) but not for prematurely born study subjects ($R = 0.001$, $P = 0.996$, R-square = 0.00) despite the fact that we know from the composite maps in Fig. 1 that both groups robustly activate left IFG.

Table 3 Developmental data including cognitive test and task results

	Preterm	Term	*P*-value
Age at scan (years)	12.8 ± 2.1	12.2 ± 0.4	0.048
Height at scan (m)	153.3 ± 11.5	152.3 ± 7.9	NS
# right-handed	18 (86%)	24 (92%)	0.64
WISC III IQ scores			
VIQ	97.5 ± 14.4	107.7 ± 14.1	0.007
VCIQ	98.4 ± 14.1	108.0 ± 13.9	0.012
PIQ	90.2 ± 13.8	104.9 ± 15.6	0.001
FSIQ	93.3 ± 12.9	106.9 ± 14.8	0.003
CELF			
Receptive language	94.3 ± 12.9	108.0 ± 16.2	0.002
Expressive language	96.2 ± 14.5	102.2 ± 13.4	0.22
Total score	94.0 ± 12.9	104.8 ± 14.7	0.008
Gray Silent Reading quotient	95.4 ± 16.7	107.2 ± 23.4	0.07
PPVT - R	94.7 ± 18.4	106.9 ± 19.5	0.016
In magnet task accuracy			
Matched trials	77.09% ± 13.6	73.77% ± 15.0	0.568
Unmatched trials	80.0% ± 24.2	75.77 ± 20.0	
In magnet task resp. time			
Matched trials	1383.03 ms ± 406.19	1316.13 ms ± 399.83	0.568
Unmatched trials	1562.19 ms ± 491.11	1479.87 ± 460.66	
In magnet task baseline			
Accuracy	75.77% ± 14.6	74.73% ± 13.0	0.639
Response time	1291.93 ms ± 350.0	1221.35 ± 323.17	0.129

Table 4 Volumetric data (least square means, $mm^3 \pm SEM$)

Region	Preterm $n=15$	Term $n=22$	*P*-value
Gray matter			
L Frontal gray	114.1 ± 1.86	110.7 ± 1.49	0.17
R Frontal gray	114.4 ± 1.76	112.7 ± 1.41	0.47
L Temporal gray	62.1 ± 1.85	59.4 ± 1.48	0.28
R Temporal gray	60.6 ± 1.80	58.0 ± 1.44	0.28
L Parietal gray	69.3 ± 1.72	66.4 ± 1.38	0.21
R Parietal gray	69.3 ± 1.65	65.8 ± 1.32	0.11
L Occipital gray	37.9 ± 0.93	35.7 ± 0.75	0.09
R Occipital gray	36.6 ± 1.06	36.3 ± 0.85	0.81
L Deep gray	17.7 ± 0.40	19.7 ± 0.31	<0.001
R Deep gray	17.3 ± 0.40	19.1 ± 0.31	0.002
White matter			
L Frontal white	82.3 ± 1.94	87.7 ± 1.56	0.04
R Frontal white	86.9 ± 1.81	90.5 ± 1.45	0.14
L Temporal white	45.4 ± 1.68	49.8 ± 1.35	0.05
R Temporal white	44.4 ± 1.93	50.4 ± 1.55	0.02
L Parietal white	72.6 ± 1.45	76.4 ± 1.16	0.05
R Parietal white	71.0 ± 1.59	73.9 ± 1.27	0.16
L Occipital white	29.3 ± 0.98	28.8 ± 0.79	0.70
R Occipital White	27.5 ± 1.26	27.2 ± 1.01	0.87
L Deep white	22.7 ± 0.38	23.0 ± 0.31	0.48
R Deep white	22.5 ± 0.39	23.6 ± 0.31	0.04
CSF			
L Ventricle CSF	7.10 ± 4.72	4.16 ± 1.33	0.0038
R Ventricle CSF	6.02 ± 3.10	4.10 ± 1.37	0.0425

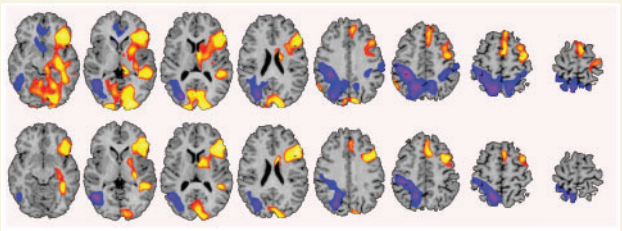

Figure 1 fMRI group composite maps. Activation to task in each group, $P<0.001$, corrected for multiple comparisons, $n=26$ Term born controls (top row) and $n=22$ Prematurely born children (bottom row). Images are radiologic: left is on the right. Initial slice at approximately Talairach $z=9, -8,$ 8 mm increments. There is no significant difference between these two sets of maps.

ROI connectivity results

R-values and *P*-values from cross-subject correlations are listed in Table 5 together with the results of the CCCT. Included are data from all regions whose cross-subject correlation met the threshold of $P \leqslant 0.001$ for at least one group. All significant correlations in activity between non-homologous regions are depicted in Fig. 3. Significant connections shared by both groups are drawn in blue; those unique to one group are drawn in red. Results can also be viewed on rotating 3D brain images at http://research.yale.edu/bioimagesuite/MovieGallery/PTconnectivitymovie.html.

As shown in Table 5 and Fig. 3, the correlation in activity between the left pTG and left anterior/ventral inferior frontal gyrus, BA 47, was highly robust in both groups ($R=0.687$, $P=0.0004$ and $R=0.686$, $P<0.0001$ for PT and term control subjects, respectively). No difference was found between these two *R*-values ($p_{ccct}=0.995$). The correlations between these two ROI were among the most robust connections in our data set. Other non-homologous regions whose *R*-values were significant for both groups were found within both frontal and temporal regions. Homologous regions evidencing significant correlations in both groups are listed on Table 5.

Differences between the two groups were identified in three types of connections: fronto-temporal, cross-hemispheric frontal, and those involving the SMA. Among fronto-temporal cross-subject correlations, two pairs of *R*-values were found to significantly differ between groups. Interestingly these connections crossed hemispheres: the correlation between right BA 22 and left BA 44 was found to be significant only for the term control subjects ($R=0.618$, $P=0.001$, $p_{ccct}=0.0042$); the correlation between left pTG and right BA 47 was found to be significant only for the PT subjects ($R=0.698$, $P=0.0003$, $p_{ccct}=0.049$). Two pairs of significantly correlating frontal regions occurred only in the term control data where subjects evidenced significant cross-subject correlations between right BA 47 and left BA 45 and between right BA 47 and right BA 45 ($R=0.716$, $P<0.0001$, $p_{ccct}=0.064$ for the former and $R=0.906$, $P<0.0001$, $p_{ccct}=0.00066$ for the latter).

Finally, significant cross-subject correlations involving the SMA occurred only in PT data. The *R*-values for the PT and term control groups significantly differed between right SMA and right hippocampus ($R_{R\ H}=0.67$, $p_{R\ H}=0.0006$, $p_{ccct}=0.013$), and between left SMA and three regions: the right hippocampus, and right and left BA 22 ($R_{R\ H}=0.718$, $p_{R\ H}=0.0002$, $p_{ccct}=0.048$; $R_{L22}=0.701$, $p_{L22}=0.0003$, $p_{ccct}=0.034$; $R_{R22}=0.662$, $p_{R22}=0.001$, $p_{ccct}=0.044$).

All connections exclusive to one group are diagrammed in red in Fig. 3. This figure clearly illustrates a difference in connections with left IFG between the two groups. Significant direct connections from right IFG and right MTG to the left IFG are observed in the term control data. The SMA and right temporal regions participate more prominently in the network isolated in PT children.

Discussion

Using fMRI to examine lexical semantic processing, we have demonstrated that children born prematurely and term control subjects engage neural systems for language differently at age 12 years. The basis of this conclusion is a set of key findings. These include two similarities between the groups: the PT and term control subjects performed equally well on a visual semantic association task, and their overall composite group activations to task were non-distinct. Three critical differences between the groups were also isolated. First was a structural difference. Similar to examinations of volumetric and microstructural changes in other cohorts reported during late childhood and early adolescence (Lodygensky *et al.*, 2005;

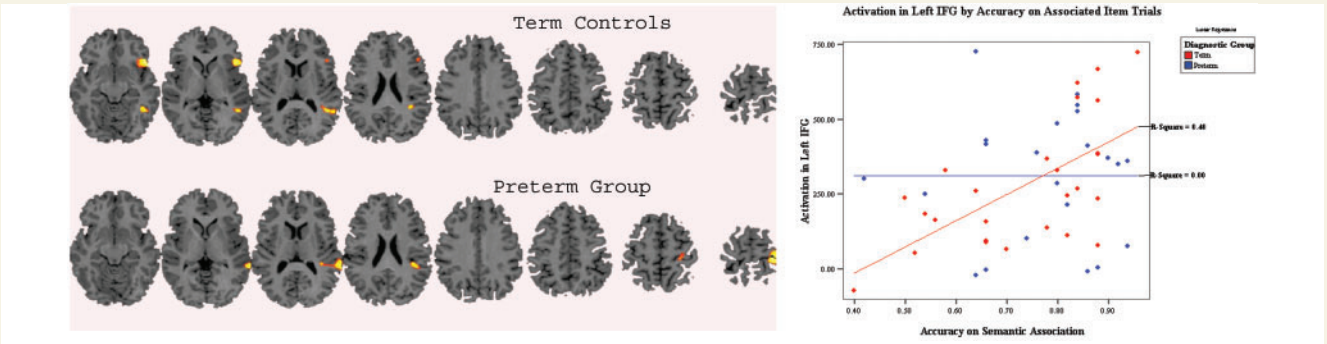

Figure 2 Correlation between accuracy and activation to task. (**A**) Maps of the correlation between accuracy on semantically associated items with whole brain activation to task for Term born controls (top row) and Prematurely born children (bottom row). Images are radiologic: left is on the right. Initial slice at approximately Talairach $z = 9$, −8 mm, 8 mm increments. Maps are displayed at $P < 0.01$, corrected for multiple comparisons: $n = 26$ Term controls, $R = 0.49$; $n = 22$ Prematurely born subjects, $R = 0.53$. (**B**) Plot of correlation between accuracy and activation within the IFG ROI for Term Controls ($R = 0.632$, $P = 0.001$, R-square = 0.40) and PT subjects ($R = 0.001$, $P = 0.996$, R-square = 0.00).

Table 5 Cross–subject correlations per group

Region 1	Region 2	Term ($n = 26$)		PreTerm ($n = 22$)		Coefficient comparison	Significant in group
		R-value	*P*-value	*R*-value	*P*-value		
Frontal connections							
L 47	R 47	0.566	0.001	0.499	0.018	0.763	
	L 45	0.592	0.001	0.485	0.022	0.626	
	L 44	0.662	0.0002	0.683	0.0005	0.902	both
R 47	L 45	0.716	<0.0001	0.315	0.153	0.0644	term
	R 45	0.906	<0.0001	0.421	0.051	0.00066	term
	L 44	0.603	0.001	0.343	0.118	0.272	
	R 44	0.782	<0.0001	0.52	0.013	0.126	
L 45	R 45	0.673	0.0002	0.478	0.024	0.34	
	L 44	0.62	0.001	0.726	0.0001	0.529	both
R 45	R 44	0.844	<0.0001	0.628	0.002	0.109	
FrontoTemporal connections							
L 47	L pTG	0.686	<0.0001	0.687	0.0004	0.995	both
R 47	L pTG	0.247	0.224	0.698	0.0003	0.0487	preterm
L 44	R 22	0.618	0.001	−0.164	0.465	0.00421	term
R 44	R pTG	0.53	0.005	0.668	0.001	0.484	
Temporal connections							
L 21	R 21	0.63	0.001	0.718	0.0002	0.601	both
	L H	0.637	0.0005	0.635	0.002	0.991	both
	L 22	0.642	0.0004	0.612	0.002	0.873	both
R 21	R H	0.461	0.018	0.742	<0.0001	0.141	
	L 22	0.356	0.074	0.654	0.001	0.186	
L H	R H	0.604	0.001	0.705	0.0002	0.566	both
	L 22	0.506	0.008	0.735	<0.0001	0.218	
	R 22	0.424	0.031	0.662	0.001	0.267	
R H	L 22	0.49	0.011	0.739	<0.0001	0.184	
L 22	R 22	0.787	<0.0001	0.802	<0.0001	0.896	both
R 22	L pTG	0.69	<0.0001	0.456	0.033	0.251	
Sensory motor association cortex connections							
L SMA	R 21	0.301	0.135	0.641	0.001	0.147	
	R H	0.282	0.162	0.718	0.0002	0.0478	preterm
	L 22	0.209	0.306	0.701	0.0003	0.034	preterm
	R 22	0.171	0.403	0.662	0.001	0.0442	preterm
	R SMA	0.619	0.001	0.629	0.002	0.958	both
R SMA	R H	0.042	0.838	0.67	0.0006	0.0132	preterm

Table includes all correlations with R values meeting threshold $P = 0.001$. Results from the CCCT are provided in column five for each pair of correlations. Significant connections are noted in the final column. Non–homologous significant connections are included in Figure 3.

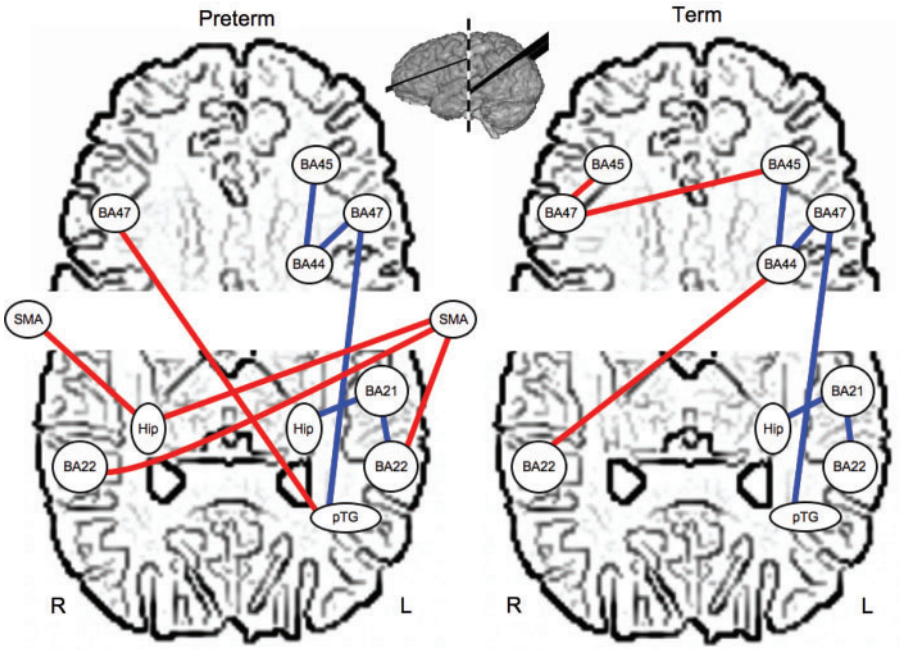

Figure 3 Functional connectivity in each group. Cross-subject correlations between non-homologous regions and satisfying the threshold, $P = 0.001$. *R*-values and *P*-values are reported in Table 5. Blue lines join regions significantly correlating in both groups. Red lines join regions significantly correlating in a unique group.

Gimenez *et al.*, 2006; Nosarti *et al.*, 2008), the PT children in our study were found to have significant decreases in left frontal and bilateral temporal white matter volumes when compared to term controls. Second, despite the fact that both groups activated cortical regions well-known to be associated with semantic processing, the left IFG and MTG, correlations between activations and accurate performance revealed group differences, including a correlation only found for the PT children between task accuracy and activation in the left SMA. Lastly, we observed differences in the connectivity between the regions that were engaged by children in both groups when accurately performing the task. Particularly, only in PT children was activation in the temporal areas functionally connected with activation in the SMA, and only in term-born children was activation in the left IFG functionally connected with activation in right IFG and right MTG.

The cross-subject correlation method employed here constitutes an extension of previous fMRI studies of prematurely born subjects that have mainly focussed on the comparison of activation patterns between PT and term control groups. Using a measure of network connectivity, we speculate functional recovery through network innovation. Recovery in cognitive performance is often associated with some form of neuro-anatomical compensation, such as the recruitment of adjacent regions or homologues (Perani *et al.*, 2003; Saur *et al.*, 2006) or completely distinct localizations (Peterson *et al.*, 2002). The present work revealed a case in which the PT children and the term control group did not significantly differ in either task performance or in the group composite localized BOLD response to task, but did so in the functional connectivity of the regions of significant BOLD response.

The examination of functional connectivity confirmed our hypothesis that connectivity would reflect the left hemisphere co-activation to task in left IFG and MTG observed in both groups. Both groups evidenced functional connectivity between left IFG in BA47 and the left pTG ROI that contains substantial portions of left MTG. We conclude that the connection between these two regions predominantly subserved the semantic task in both groups consistent with the role of these areas across studies of lexical semantics in adults (Friederici *et al.*, 2000; Martin, 2003; Noppeney *et al.*, 2004). Yet clear differences in the network feeding into and enhancing the activation in this circuit were observed and these underlie the findings in Fig. 2. Specifically, in the term control data, ROI significantly correlating with the left IFG–pTG circuit are in right IFG and right MTG and are connected directly with activation in left IFG; by comparison, in prematurely born subjects, ROI significantly correlating with the left IFG–pTG circuit include bilateral SMA which is directly connected not with left IFG, but with the left temporal lobe. Thus our hypothesis concerning SMA connectivity in the PT group was confirmed without exception. SMA appears to mediate connections between language areas in the PT brain. We also identified a subset of frontal connections available only to the term control group, consistent with our expectations.

Since the groups did not differ in task performance or composite BOLD activations to task, we interpret the differences in functional connectivity in the language network recruited by the two groups for task processing as a response to the structural distinctions in cerebral white matter volumes that we report. Persistent structural anomalies in the PT sample, evidenced in these volumetric differences, may prevent the formation of the parsimonious network

found in the term control children, so alternative pathways may be recruited by PT children. This process of reorganization of functional connectivity may explain the developmental delays observed in the acquisition of language skills in the prematurely born (Ment *et al.*, 2003; Hack *et al.*, 2005).

In addition to the distinctions in brain volumes, the two groups of children differed in maternal education. While behavioural measures are known to correlate with maternal education, physiological measures, such as measures of BOLD activation, are not. Our analyses also demonstrated that there was no significant correlation across subjects between BOLD activation and maternal education. Thus it cannot be the case that maternal education is a significant factor in the group differences in recruitment of SMA or connections with left IFG during task performance.

The relationship suggested by our results between brain volume differences, differences in functional connectivity in the language network and language function in PT children supplements previous volumetric imaging studies that evaluated the cerebral structural sequelae of PT birth. This work has consistently reported alterations in those regions subserving language, the left temporal and frontal regions. FMRI studies of auditory and phonologic processing in older PT subjects with thinning of the corpus callosum have demonstrated transfer of function to the right STG and right superior frontal gyrus when the PT groups were compared with term controls (Santhouse *et al.*, 2002; Rushe *et al.*, 2004). Our previous fMRI study of semantic processing in PT subjects at age 12 years suggested that PT and term control children demonstrated positive BOLD signals for the same regions during an auditory semantic task but that the PT group showed lower BOLD signals for each region when compared to term controls (Ment *et al.*, 2006). This finding would be consistent either with a PT network consisting of fewer correlating regions than the term control network or with indirectly or less efficiently connected regions, such as the network we observe in which correlations to areas showing the greatest activation to task are mediated through SMA.

The limitations of this study include the moderate sample size and the limited numbers of tests administered that assess semantic processing. Direct assessment of temporal connectivity using temporal BOLD correlations within subjects has shown sensitivity to reading performance in normal adults with varied reading abilities (Hampson *et al.*, 2006). In future studies we plan to assess connectivity in PT individuals using this emerging methodology. Functional connectivity can also be related to assessments of the underlying white matter axonal integrity through diffusion tensor imaging and such studies are underway in our laboratory. These studies will allow us to continue to examine the relationship between microstructural connectivity and those cerebral structural abnormalities associated with PT birth (Kesler *et al.*, 2008).

Although the relation between observed connectivity and later neurologic outcome has not been investigated, BOLD signal changes in PT children in response to language, memory and executive function are just beginning to be explored. This work establishes that functional connectivity studies may reveal differences in the networks engaged in processing and how the nodes within these networks interact, and suggests that the dynamic nature of neural connectivity can intercede for recovery in the PT brain.

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