Developmental Science

Developmental Science 11:2 (2008), pp 237–252 DOI: 10.1111/j.1467-7687.2008.00674.x

PAPER

Functional neuroimaging of speech perception during a pivotal period in language acquisition

Elizabeth Redcay,[1] Frank Haist[2] and Eric Courchesne[3]

1. Department of Psychology, University of California, San Diego, USA
2. Department of Psychiatry, University of California, San Diego, USA
3. Department of Neurosciences, University of California, San Diego, Rady Children's Hospital, and UCSD Autism Center of Excellence, San Diego, USA

Abstract

A pivotal period in the development of language occurs in the second year of life, when language comprehension undergoes rapid acceleration. However, the brain bases of these advances remain speculative as there is currently no functional magnetic resonance imaging (fMRI) data from healthy, typically developing toddlers at this age. We investigated the neural basis of speech comprehension in this critical age period by measuring fMRI activity during passive speech comprehension in 10 toddlers (mean ± SD; 21 ± 4 mo) and 10 3-year-old children (39 ± 3 mo) during natural sleep. During sleep, the children were presented passages of forward and backward speech in 20-second blocks separated by 20-second periods of no sound presentation. Toddlers produced significantly greater activation in frontal, occipital, and cerebellar regions than 3-year-old children in response to forward speech. Our results suggest that rapid language acquisition during the second year of life may require the utilization of frontal, cerebellar, and occipital regions in addition to classical superior temporal language areas. These findings are consistent with the interactive specialization hypothesis, which proposes that cognitive abilities develop from the interaction of brain regions that include and extend beyond those used in the adult brain.

Introduction

The toddler years mark a dramatic increase in cognitive capacity, with remarkable advances observed across a wide range of abilities (Bates, Thal & Janowsky, 1992). One of the most striking advances is in a child's language development. For example, the average 24-month-old understands more than 10 times as many words as the average 16 month old, and more than 200 times as many words as the average 8-month-old (Fenson, Dale, Reznick, Bates, Thal & Pethick, 1994). Equally remarkable is that by 16 to 19 months of age, toddlers are able to learn the meaning of new words in as little as one trial and without explicit reference to the object being named (Baldwin, 1991; Heibeck & Markman, 1987).

The neural bases of this incredible advance in language ability, including other cognitive advances, remain speculative as little neurofunctional data exist during this age. By one account, the same regions used in the mature adult brain are also used during infancy, and the dramatic increases in language comprehension capacity are attributable to significant advances in the capacity of these particular structures (Dehaene-Lambertz, Hertz-Pannier & Dubois, 2006a). Support for this hypothesis comes from neuroimaging studies of 2–3-month-old infants and neonates showing similar neural and electrophysiological responses to speech stimuli in infants as in adults (Dehaene-Lambertz, Dehaene & Hertz-Pannier, 2002; Dehaene-Lambertz & Gliga, 2004; Pena, Maki, Kovacic, Dehaene-Lambert, Koizumi, Bouquet & Mehler, 2003; Dehaene-Lambertz, Hertz-Pannier, Dubois, Meriaux, Roche, Sigman & Dehaene, 2006b).

A second explanation is that in early childhood, language development depends on a broader network of neural systems, including those mediating social, cognitive, memory, sequence tracking, and novelty detection functions (Bates, 1992; Johnson, 2001; Muller & Basho, 2004; Neville & Mills, 1997; Quartz & Sejnowski, 1997). This hypothesis is consistent with current theories suggesting that language in the prelinguistic child is not language *per se*, but rather a combination of attention, perception, social processing, imitation and symbolic processing, to name a few (Bates & Dick, 2002). Supporting evidence comes from event-related potential (ERP) (Mills, Coffey-Corina & Neville, 1993, 1994, 1997; Mills & Neville, 1997) and lesion studies (Bates, 1997; Thal, Marchman, Stiles, Aram, Trauner, Nass & Bates, 1991; Vicari, Albertoni, Chilosi, Cipriani, Cioni & Bates, 2000) suggesting that frontal and right hemisphere regions may be critical for early language

Address for correspondence: Elizabeth Redcay, 8110 La Jolla Shores Drive, Suite 201, La Jolla, CA 92037, USA; e-mail: eredcay@ucsd.edu

9600 Garsington Road, Oxford OX4 2DQ, UK and 350 Main Street, Malden, MA 02148, USA.

comprehension, rather than classic adult language comprehension regions in left posterior superior temporal cortex. ERP differences to known vs. unknown words are distributed over anterior and posterior scalp electrode sites in 13–17-month-olds, but become increasingly focused to electrodes over left temporal and parietal regions with increasing age and language skill (Mills *et al.*, 1994). Similarly patterns of left frontal and occipital scalp-recorded EEG coherence at 14 months of age predict later language ability at 24 months (Mundy, Fox & Card, 2003).

These different hypotheses regarding neural substrates remain speculative because there have been no fMRI studies of speech processing in healthy normal toddlers during this pivotal period in language development. The only existent fMRI studies of speech processing in healthy, typically developing children have been with 2–3-month-old infants (Dehaene-Lambertz *et al.*, 2002; Dehaene-Lambertz *et al.*, 2006b), an age prior to the initial burst of language comprehension capacities, and with children 5 years and older (Ahmad, Balsamo, Sachs, Xu & Gaillard, 2003; Balsamo, Xu & Gaillard, 2006; Karunanayaka, Holland, Schmithorst, Solodkin, Chen, Szaflarski & Plante, 2007; Plante, Holland & Schmithorst, 2006; Schapiro, Schmithorst, Wilke, Byars, Strawsburg & Holland, 2004; Szaflarski, Schmithorst, Altaye, Byars, Ret, Plante & Holland, 2006), an age after the burst of language comprehension capacities. Two groups have examined the fMRI response to speech presentation in patient populations under sedation. However, these studies have utilized very broad age ranges (e.g. 2 months–9 years) (Souweidane, Kim, McDowall, Ruge, Lis, Krol & Hirsch, 1999; Altman & Bernal, 2001; Bernal & Altman, 2003) and have not systematically examined age-related changes between 1 and 3 years of age.

To test directly whether toddlers recruit different regions to process speech than older children who already have a base of lexical knowledge, we recorded fMRI (BOLD) activity from 10 healthy, typically developing toddlers and 10 3-year-old children during presentation of forward and backward speech stories during natural sleep. fMRI activity was recorded during natural sleep because reliable fMRI data acquisition requires subjects to limit movement for an extended period of time, which is currently not feasible for awake toddlers. Numerous ERP studies of basic sensory and cognitive processing in infants, toddlers and children indicate similar neurophysiological responses to various stimuli when presented during sleep and wake states (Cheour, Ceponiene, Leppänen, Rinne, Alho, Lehtokoski, Kujala, Renlund, Fellman & Näätänen, 2002a; Cheour, Martynova, Näätänen, Erkkola, Sillanpää, Kero, Raz, Kaipo, Hiltunen, Aaltonen, Savela & Hämäläinen, 2002b; Martynova, Kirjavainen & Cheour, 2003). We chose 3-year-olds for a contrast group because children in this age group are no longer experiencing the initial early 'burst' of growth in vocabulary (Bates, Thal, Finlay & Clancy, 2003; Bates *et al.*, 1992) but are young enough to still be studied with the same natural sleep fMRI method.

Materials and methods

Participants

Twenty-eight healthy children (ages 13–44 months) with no known neurological diseases or psychological disorders participated in this experiment. All children were recruited through community parents' magazines or flyers. Eight children were not included in the analysis due to an inability to fall or stay asleep (five), experimenter error (one), or repeated failure to show up for scheduled appointments (two). Eighteen of the 20 participants received the Mullen Scales of Early Learning assessment (Mullen, 1995) within 2 months of fMRI data acquisition (mean .43 ± .94 months) (see Table 1 for participant description). All research was approved by the Institutional Review Board of the Children's Hospital, and the University of California, San Diego (UCSD) approved this study. Informed written consent was obtained from the parents and they were compensated monetarily for participation.

Stimuli

Three classes of auditory stimuli were used: (1) simple forward speech (Fw:s), (2) complex forward speech

Table 1 *Participant information*

ID	Age	Sex	Mullen Composite	Receptive Language T score	Receptive age equivalent	CDI words produced
Toddler						
T1	13	M	89	35	9	2
T2	14	M	80	31	8	1
T3	20	F	*	54	13	15
T4	21	M	87	56	23	26
T5	22	M	124	65	28	350
T6	22	M	105	58	25	146
T7	23	F	110	59	25	124
T8	24	M	85	32	18	90
T9	24	F	105	63	30	170
T10	24	M	*	47	23	268
Mean (stdev)	21(4)	7M 3F	98(15)	50(13)	20(8)	119(119)
3-year-old						
C1	35	M	111	56	39	*
C2	36	M	123	68	47	446
C3	37	M	118	56	39	653
C4	37	M	82	47	36	610
C5	37	F	121	58	44	658
C6	39	M	116	58	65	615
C7	40	F	138	76	59	*
C8	40	M	113	50	41	*
C9	41	M	131	72	55	478
C10	44	F	*	*	*	669
Mean (stdev)	39(3)	7M 3F	117(16)	60(10)	47(10)	589(90)

* Indicates data from within 2 months of fMRI testing was not available. CDI refers to the MacArthur Bates Communicative Developmental Inventory. Comprehension vocabulary measures only are given for children 8–16 months of age on the CDI.

(Fw:c), and (3) backward speech (Bw). Each stimulus block was separated by a 20-second silent rest (R) condition during which time no stimulus was presented. The simple speech was an excerpt from a book for children at a comprehension level between 12 and 36 months. The complex speech was an excerpt taken from a book at a comprehension level over age 48 months. The books used were determined to be unfamiliar to the children by asking the parents if they had ever read this book to their child. All auditory stimuli were generated using Cool Edit 2000 digital audio software (Syntrillium Software Corp., Scottsdale, AZ). The backward speech was generated by reversing the simple speech and, thus, had similar acoustic complexity as the speech but it was not comprehensible as speech stimuli. All story excerpts and rest periods with no sound presentation were 20 seconds in length. Stimulus blocks were repeated three times in a partially counterbalanced order (see Supplementary Figure S1). All stimuli were read by the same female voice and recorded as binaural WAV files with 44 kHz sampling with 16-bit resolution. The stimuli were pre-recorded onto a CD and presented through a pneumatic headphone system with approximately 30dB of noise reduction.

Procedure

All children were imaged during natural sleep without the use of sedation. Prior to the night of scanning, families were asked to play CDs with the sounds of the MRI scanner while their child fell asleep at home. Children arrived at the scanner between 9:00 and 10:00 pm. They were allowed to fall asleep naturally either in the waiting room or the scanner room. When the child was placed on the scanner bed, earplugs and headphones were placed on the child. Earplugs were used with the intention of reducing startle responses with the onset of speech sounds and filtering out background scanner noise. The child's parent and researcher remained in the scanner during the entire scan session. A mirror was placed over the head coil to allow the researcher to observe the child during scanning. If the child awoke, data acquisition was halted and the child was removed from the scanner.

FMRI acquisition

Functional MR images were acquired on a 1.5 Tesla Siemens Symphony system at the UCSD MR Center at Hillcrest Hospital. Whole brain axial slices were collected with a gradient-recalled echo-planar imaging (EPI) pulse sequence (TR (repetition time) = 2500 ms; TE (echo time) = 35 ms; flip angle = 90 deg; field of view (FOV) = 256 mm; 64 × 64 matrix (4 mm^2 in-plane resolution), number of slices = 30; slice thickness = 4 mm; 154 volumes acquired). A T1-weighted anatomical image in the coronal plane using an MPRAGE sequence was collected prior to the fMRI scanning for co-registration with the functional images (FOV = 228 mm; matrix = 256 × 256; 128 slices (.89 mm^2 in-plane resolution); slice thickness = 1.5 mm).

Individual data analyses

Analyses were performed with Analysis of Functional NeuroImages software package (AFNI, release version 3/21/06) (Cox, 1996). Motion correction and three-dimensional registration of each participant's functional images were done using an automated alignment program (3dvolreg), which co-registered each volume in the time series to the middle volume acquired in that run using an iterative process (Cox & Jesmanowicz, 1999). Data points containing head motion that were not correctable by co-registration were removed from analysis. Motion was considered uncorrectable if the summed distance value of the translational (mm) and rotational (deg) parameters was greater than 0.3. Data points with excessive motion were removed from the analysis for one toddler (10% of the run) and two 3-year-olds (2.5% and 3.9% of the run). None of the censored volumes overlapped in time across these three subjects, which suggests that motion due to onset or offset of a stimulus was not a problem. Additionally, the first two volumes in each data series were removed to compensate for T1 equilibration effects. Images were then smoothed with a Gaussian filter (full-width half-maximum = 6 mm).

Multiple linear regression analyses of time series data were conducted using the program 3dDeconvolve. Nine input stimulus time series were modeled; forward simple, forward complex, and backward speech, and six motion parameters accounting for translation and rotation in three dimensions which were extracted from the 3dvolreg output. Because stimuli were presented in a block design, a gamma variate function was used to model the shape of the hemodynamic response. The gamma function was convolved with the time series and a multiple regression analysis was conducted to determine the 'goodness of fit' coefficients (or linear contrast weight). The regression included terms to remove both the global mean and linear trend. A general linear test was performed that included both types of forward speech (Fw:s and Fw:c) which provided the forward speech (Fw) condition used in the remaining analyses. The Fw speech condition allows for a main effect of forward speech across stimuli of slightly varying levels of complexity.

Group data analyses

Data were registered into a standard space based on the Talairach template (Talairach & Tournoux, 1988). Previous studies from within our own lab have shown that spatial normalization to the Talairach template produced a similar range of spatial deviation within the central sulcus in toddlers and 3-year-old children when compared to adults (see Supplementary Information and Figure S3). However, while alignment within groups is comparable to that seen with adult co-registration, the regional and Brodmann area labels assigned to the Talairach coordinates do not necessarily correspond to those of the adult brain. For this reason, all reported regions of activation

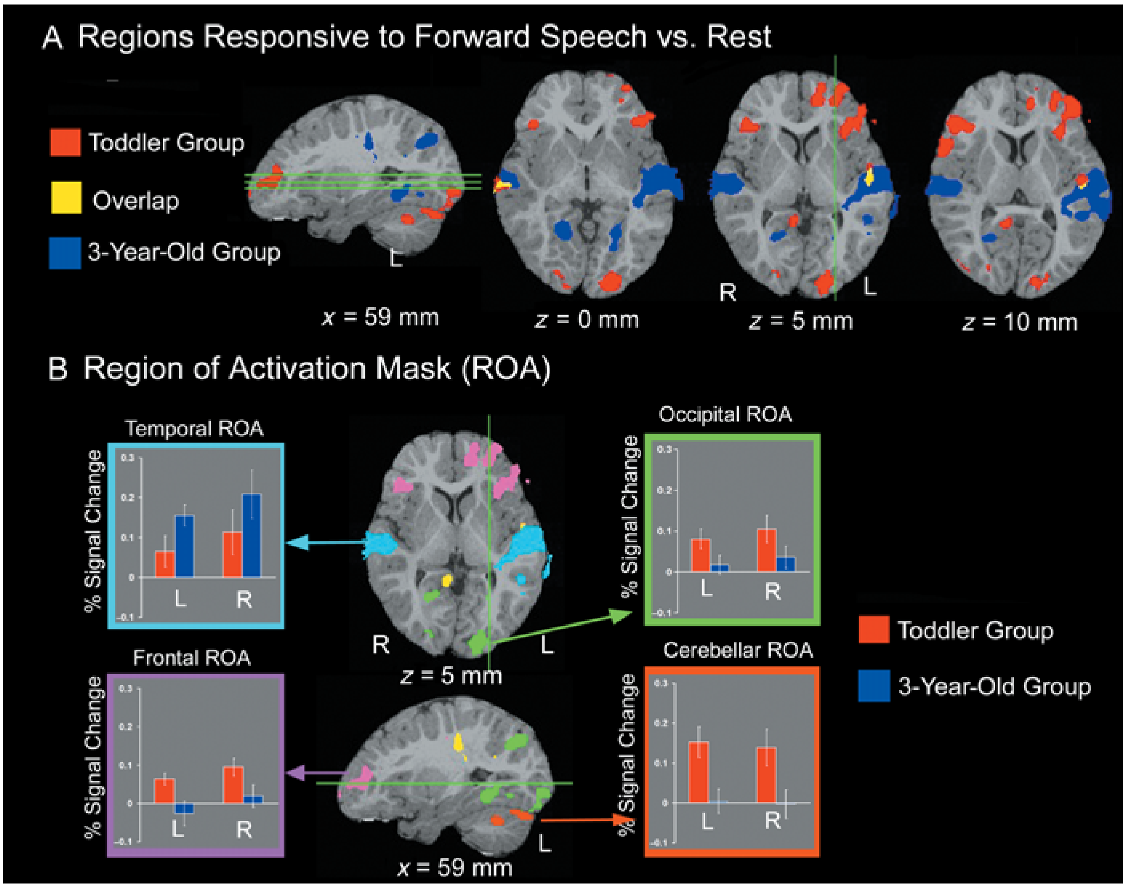

Figure 1 *Regions of Significant Activation to Forward Speech in Toddlers and 3-year-olds. (A) Map of regions significantly responsive to forward speech (*p *< .05, 13 voxel minimum cluster). Regions of significant activation in response to the forward speech condition for both the toddler (displayed in red) and 3-year-old (displayed in dark blue) groups were combined to determine the overlapping regions of significant activity (displayed in yellow). (B) The combined regions of significant activation for both age groups identified in (A) were used as a Region of Activation (ROA) mask to extract percent signal change values from each individual. The ROA mask was separated based on lobar and hemispheric boundaries. Plotted are mean percent signal change from left (L) and right (R) hemispheres from temporal (light blue), frontal (pink), occipital (green), and cerebellar (orange) regions. In the graphs, values from the toddlers are displayed in red and values from the 3-year-olds are displayed in blue. Error bars represent standard error of the mean. Activation maps are displayed on a representative structural image from a single subject for all figures.*

were determined by using anatomical landmarks identified in the combined anatomical datasets of both the toddler and 3-year-old group, separately (see Figure S3 for images of these group anatomical datasets).

The linear contrast coefficient for each condition was converted to percent signal change by calculating the percent change from the baseline parameter of the regression model. In order to determine the response to each condition as compared to rest for each group separately, two separate one-way repeated measures ANOVAs were run with the percent signal change values from the four conditions (Fw:s, Fw:c, Bw, Fw) as the repeated measures. In order to compare activation between groups, a two-way repeated measures analysis was run using the percent signal change values from each condition as the dependent variable and age group as the between-subjects variable (toddler and 3-year-old). Within the ANOVA, within-group contrasts were run to compare forward speech with backward speech (Fw vs. Bw) and between-group contrasts were run to compare the response to forward (Fw) and backward (Bw) speech between toddlers and 3-year-olds. The ANOVA was performed using the matlab package within AFNI. Unless otherwise noted, all group comparisons are set at an intensity threshold of $p < .01$ and cluster corrected for an overall alpha value of $p < .05$ (cluster volume = 960 mm^3; connectivity radius = 6.0).

Results

Regions of significant activation to forward speech vs. rest and backward speech vs. rest for both groups are listed in Supplementary Information Table S1. The response to forward and backward speech versus rest did not reach statistical significance within superior temporal lobes at $p < .01$, corrected in the toddler group. When the threshold was relaxed to $p < .05$ (corrected for 13 voxel minimum), bilateral STG activation was seen in both conditions (Table S1, Figure 1A). For this reason,

Table 2 *Within-group comparisons*

Region	Side	Brodmann Area	Talairach Coordinates (x,y,z)	*t*-value
		Toddler		
Forward > Backward				
Frontal				
Cingulate Gyrus	R	31	(2, 4, 32)	3.76
Temporal				
None				
Parietal				
Angular Gyrus	L	39	(−46, −62, 36)	3.88
Posterior Cingulate	L	29	(−10, −50, 8)	2.15
Posterior Cingulate	R	29	(10, −49, 12)	3.63
Precuneus	L	31	(−2, −69, 32)	3.51
Occipital				
Middle Occipital Gyrus	R	19	(39, −82, 15)	3.66
Subcortical				
Anterior Cerebellar Vermis	R		(1, −45, 0)	3.5
Backward > Forward				
Frontal				
None				
Temporal				
Anterior Middle Temporal Gyrus	L	28	(−39, −5, −16)	−4.33
		3-Year-Old		
Forward > Backward				
Frontal				
None				
Temporal				
Fusiform Gyrus	L	37	(−18, −65, −4)	3.09
Parahippocampal Gyrus	L	36	(−26, −49, −4)	4.31
Superior Temporal Gyrus	L	42	(−46, −9, 4)	3.25
Superior Temporal Gyrus	L	22	(−46, −13, 0)	3.30
Superior Temporal Gyrus	R	42/22	(57, −17, 8)	3.35
Transverse Temporal Gyrus	R	41	(46, −18, 12)	3.87
Transverse Temporal Gyrus	L	41	(−37, −35, 16)	3.12
Parietal				
Precuneus	L	7	(−21, −65, 36)	4.15
Precuneus	R	31	(18, −52, 24)	3.54
Occipital				
Calcarine Gyrus	R	17	(19, −80, 4)	3.95
Cuneus	L	30	(−18, −68, 12)	3.55
Lingual Gyrus	L	18	(−27, −47, −4)	4.31
Middle Occipital Gyrus	L	17	(−21, −87, 0)	3.24
Backward > Forward				
Frontal				
Middle Frontal Gyrus	L	46	(−26, 42, 12)	−3.43
Middle Frontal Gyrus	R	46	(30, 51, 11)	−4.23

Data are given at a threshold of $p < .01$, corrected. For all tables, t-values represent the peak t-value for the region identified within each significant cluster. Multiple regions may be reported for each cluster of activation if the cluster spans multiple gyri and/or Brodmann areas. For all Talairach tables, subregions are listed alphabetically.

information in Table S1 and Figure 1 is given at a threshold of $p < .05$, corrected for both the toddler and 3-year-old groups.

Differential response to speech

To examine whether toddlers and 3-year-olds showed a differential neural response to forward as compared to backward speech during sleep, blood oxygenation level dependent (BOLD) activation to forward speech was contrasted with backward speech in toddlers and 3-year-olds separately. When presented with forward speech, toddlers recruited primarily parietal regions including left angular gyrus (AG) ($t = 3.88$), bilateral posterior cingulate (PCC) (R: $t = 3.63$; L: $t = 2.15$), and left precuneus (Pre) ($t = 3.51$) to a greater extent than when presented with backward speech (see Table 2 for full list; Figure 2).

In 3-year-olds, forward speech elicited greater temporal lobe activity as compared to backward speech primarily within regions of temporal cortex, including bilateral superior temporal gyri (STG) (BA 42) (R: $t = 3.35$; L: $t = 3.3$), left STG (BA 22) ($t = 3.3$), and bilateral transverse temporal gyri (TTG) (BA 41) (R: $t = 3.87$; L: $t = 3.12$) (Figure 2). Three-year-olds also engaged parietal and occipital regions to a greater extent during processing of forward as compared to backward speech (see Table 2 for full list).

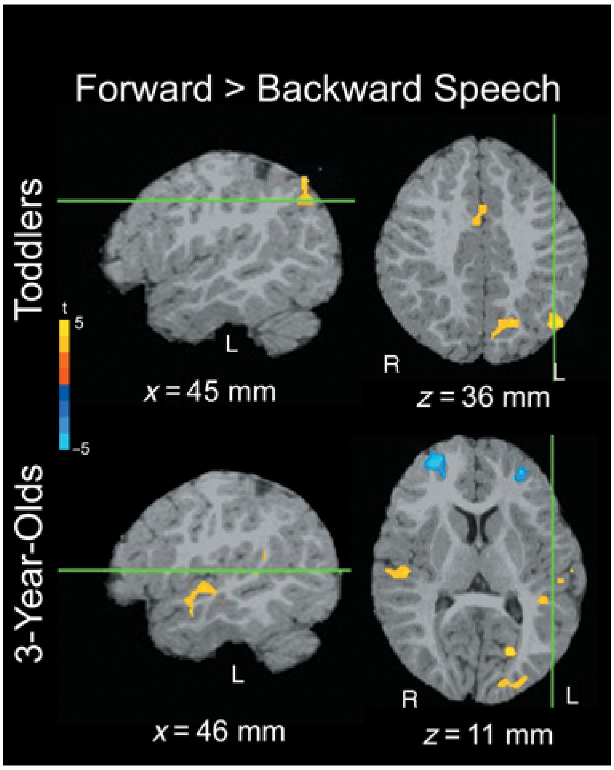

Figure 2 *Forward vs. Backward Speech in Toddlers and 3-year-olds. Both groups showed a differential response to speech in parietal regions. However, 3-year-olds, but not toddlers, also engaged superior temporal regions to a greater extent for processing forward than backward speech. Positive* t*-values represent regions in which forward speech elicited a significantly greater response than backward speech while negative* t*-values represent regions in which backward speech elicited a significantly greater response than forward speech.*

Age-related effects

To examine directly whether the BOLD activation patterns to speech presentation change with age, activity to forward and backward speech as compared to no sound presentation was compared between toddlers and 3-year-olds (Figure 3). Toddlers showed greater activation to forward speech than 3-year-olds in numerous brain regions within frontal, parietal, occipital, cerebellar, and subcortical structures (see Table 3 for a full list). The regions showing the greatest significant difference in intensity (i.e. $t \geq 5$) included bilateral medial frontal gyri (MedFG) (R BA 10: $t = 5$; L BA 10: $t = 5.38$), right superior frontal gyrus (SFG) (BA 9; $t = 6.2$), left middle frontal gyrus (MFG) (BA 9; $t = 7.75$), left orbitofrontal gyrus (OFG) (BA 11/47; $t = 5.15$), left lingual gyrus (LG) (BA 18; $t = 6.81$), and bilateral cerebellar hemispheres (L: $t = 5.85$; R: $t = 5.26$). Of note also is the bilateral activation of the inferior frontal gyrus, particularly the left inferior frontal gyrus (IFG) at the pars opercularis (i.e. Broca's area) ($t = 4.29$).

In comparison to toddlers, 3-year-olds showed greater BOLD activation to forward speech primarily within temporal and parietal regions. In addition, some regions within frontal, occipital, and insular cortex showed greater BOLD activation to a lesser degree (Table 3). With a significance of $t \geq 5$, 3-year-olds showed greater activation within a region of left STG (BA 22; $t = 4.92$), left MTG (BA 37; $t = 5.36$), TTG (BA 41; $t = 5.23$), inferior parietal lobule (BA 40; $t = 5.98$), superior parietal lobule (BA7; $t = 5.75$), paracentral lobule (BA 7; $t = 5.12$), and left MFG (BA 8; $t = 5.26$).

Age-related differences in the backward speech condition were not seen in frontal or superior temporal cortices. Rather, toddlers showed greater activation than 3-year-olds within left fusiform gyrus (FG) and bilateral LG. Three-year-olds showed greater activation than toddlers to backward speech in cingulate gyrus, paracentral lobule and precuneus. In contrast to the age-related differences seen in the forward speech condition, no age-related differences reached a t-value greater than or equal to 5.0 for the backward speech condition.

Discussion

The current study is the first to examine language processing in non-sedated toddlers. It is also the first to test whether there may be developmental differences in brain activation patterns to language between toddlers, who are at an early stage of language learning, and 3-year-olds, who have far more advanced language capacity. We found that toddlers recruited an extended network of brain regions, primarily within frontal, occipital, and cerebellar cortices, as compared to 3-year-olds during passive perception of forward speech in sleep (Figures 1, 3). Additionally, we found a differential neural response to forward and backward speech in both toddlers and 3-year-olds during sleep (Figure 2). Our findings support previous ERP and lesion studies which have suggested that in early development, speech perception may recruit a more broadly distributed network of neural systems and functions than that which is recruited in more language skillful older children and adults (Bates, 1997; Mills *et al.*, 1994; Mills & Neville, 1997; Neville & Mills, 1997). Our fMRI findings extend and complement these ERP and lesion studies by providing a more localized neuroanatomical substrate that is responsive to language at this key period in language development.

We suggest that the use of fMRI during natural sleep could offer a number of advantages to the study of developmental cognitive neuroscience. For instance, sleep fMRI can provide specific neuroanatomical information about cortical and subcortical regions involved in language functions in very young children without motion confounds. However, an inherent difficulty in studying cognition during natural sleep is the potential influence that different sleep stages might have on neural and blood flow factors affecting observed brain activity. A first question is whether the sleeping brain detects

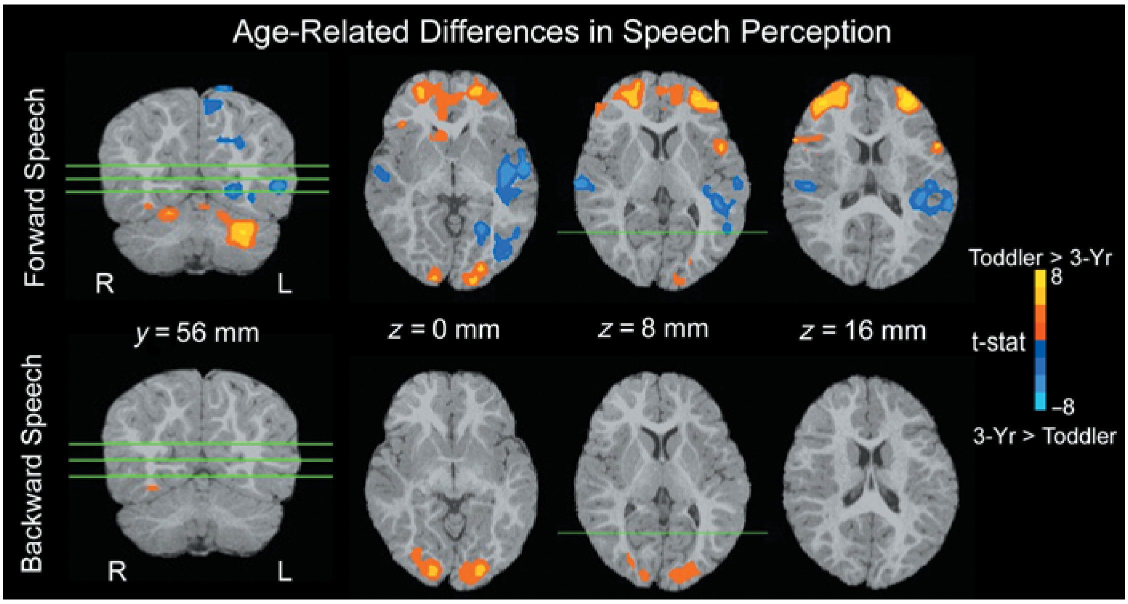

Figure 3 *Age-Related Differences in Responses to Forward and Backward Speech versus Rest. To process forward speech, toddlers recruited bilateral frontal, occipital, and cerebellar regions to a greater extent than 3-year-olds while 3-year-olds recruited superior temporal and parietal regions to a greater extent than toddlers. To process backward speech, toddlers recruited occipital and cerebellar regions to a greater extent than 3-year-olds while 3-year-olds recruited parietal and cingulate regions (See Table 3.4 for precise locations) to a greater extent than toddlers. Positive* t-*values represent regions in which toddlers showed a greater response to speech than 3-year-olds while negative* t-*values represent regions in which 3-year-olds showed a greater response to speech than toddlers.*

Table 3 *Between-group comparisons*

Region	Side	Brodmann Area	Talairach Coordinates (x,y,z)	*t*-value
		Forward Speech		
Toddler > 3-year-old				
Frontal				
Anterior Cingulate Gyrus	R	24	(6, 31, 4)	4.44
Anterior Cingulate Gyrus	R	32	(2, 3, 32)	3.89
Inferior Frontal Gyrus	L	44	(–54, 11, 20)	4.45
Inferior Frontal Gyrus, Pars Opercularis	L	44	(–46, 11, 8)	4.29
Inferior Frontal Gyrus	R	44	(38, 7, 26)	4.55
Inferior Frontal Gyrus	R	44	(56, 18, 22)	4.13
Medial Frontal Gyrus	L	10	(–14, 51, 4)	5.38
Medial Frontal Gyrus	R	10	(18, 59, 8)	5.00
Medial Frontal Gyrus	R	32	(2, 35, 27)	4.40
Medial Frontal Gryus	R	32	(5, 51, –8)	3.90
Middle Frontal Gyrus	L	9	(–30, 47, 16)	7.75
Middle Frontal Gyrus	R	10	(26, 47, –4)	4.88
Orbitofrontal Cortex	L	11/47	(–22, 27, –8)	5.15
Superior Frontal Gyrus	R	9	(38, 43, 16)	6.20
Superior Frontal Gyrus	R	10	(14, 47, 28)	4.78
Temporal				
None				
Parietal				
Posterior Cingulate	R	30	(14, –45, 16)	3.73
Precuneus	L	31	(–10, –69, 36)	4.34
Occipital				
Cuneus	L	18	(–10, –99, 16)	3.53
Lingual Gyrus	L	18	(–18, –89, –12)	6.81
Lingual Gyrus	R	18	(30, –77, –12)	4.39
Subcortical				
Caudate	R		(8, 19, 0)	3.75
Cerebellar Hemisphere	L		(–27, –57, –24)	5.85
Cerebellar Hemisphere	R		(14, –65, –12)	5.26
Globus Pallidus	R		(23, –1, –7)	4.05

Table 3 *Continued*

Region	Side	Brodmann Area	Talairach Coordinates (x,y,z)	*t*-value
3-year-old > Toddler				
Frontal				
Middle Frontal Gyrus	L	8	(−30, 19, 48)	−5.26
Precentral Gyrus	R	6	(26, −17, 58)	−4.24
Superior Frontal Gyrus	L	8	(−18, 31, 32)	−4.40
Superior Frontal Gyrus	R	6	(10, −1, 44)	−3.27
Temporal				
Fusiform Gyrus	L	36	(−34, −23, −12)	−4.07
Inferior Temporal Gyrus	L	20	(−38, −5, −20)	−6.95
Middle Temporal Gyrus	L	37	(−50, −57, 4)	−5.36
Middle Temporal Gyrus	R	22	(62, −33, 0)	−3.63
Parahippocampal Gyrus	L	36	(−22, −53, 0)	−4.81
Parahippocampal Gyrus	R	36	(22, −49, 0)	−4.31
Superior Temporal Gyrus	L	22	(−54, −5, 0)	−6.06
Superior Temporal Gyrus	L	22	(−42, −33, 4)	−4.30
Superior Temporal Gyrus	R	22/42	(62, −17, 8)	−4.92
Superior Temporal Sulcus	L	21/22	(−54, −21, −4)	−4.25
Superior Temporal Sulcus	R	21/22	(58, −9, −4)	−4.13
Transverse Temporal Gyrus	L	41	(−42, −9, 0)	−5.23
Transverse Temporal Gyrus	R	41	(50, −21, 12)	−4.18
Parietal				
Cingulate Sulcus	L	31	(−18, −25, 32)	−4.95
Inferior Parietal Lobule	L	40	(−66, −33, 16)	−5.98
Paracentral Lobule	L	7	(−10, −37, 56)	−5.12
Paracentral Lobule	R	7	(10 −29, 48)	−4.28
Postcentral Gyrus	R	4	(38, −21, 56)	−4.46
Superior Parietal Lobule	L	7	(−22, −41, 56)	−5.75
Occipital				
Middle Occipital Gyrus	L	19	(−42, −73, 0)	−4.08
Insula				
Posterior Insula	L		(−38, −33, 16)	−4.51
Insula	R		(42, 7, −4)	−4.39
		Backward Speech		
Toddler > 3-year-old				
Frontal				
None				
Temporal				
Fusiform Gyrus	L	37	(26, −69, −8)	4.20
Fusiform Gyrus	L	19	(−17, −73, −15)	4.04
Parietal				
None				
Occipital				
Lingual Gyrus	L	18	(−22, −86, 0)	4.91
Lingual Gyrus	L	19	(−17, −73, −15)	4.04
Lingual Gyrus	R	18	(14, −85, 0)	4.92
Subcortical				
Cerebellar Hemisphere	L		(−27, −53, −24)	3.85
3-year-old > Toddler				
Frontal				
Cingulate Gyrus	R	31	(10, −1, 40)	−4.49
Temporal				
None				
Parietal				
Paracentral Lobule	L	7	(−10, −22, 48)	−3.68
Precuneus	L	7	(−10, −41, 44)	−3.42
Occipital				
None				

Data are given at a threshold of $p < .01$, corrected. *T*-values represent the peak *t*-value for the region identified.

and discriminates between external stimuli. There is a growing corpus of brain activity findings in children that indicate that the sleeping brain may perform complex cognitive and language processing, and that in some cases activation patterns during sleep and awake states may share significant similarities. For example, fMRI data was recorded during story presentation from a 6-year-old boy who fell asleep during scan acquisition. The authors reported strikingly similar patterns of brain activation from both the asleep and awake states and when compared to a control group of children (Wilke, Holland & Ball, 2003). Additionally, ERP studies of infants and toddlers have documented similar brain responses during waking and sleep to speech and tones (Cheour *et al.*, 2002a; Cheour *et al.*, 2002b; Martynova *et al.*, 2003; Pena *et al.*, 2003). One particularly compelling ERP study has shown that the simple presentation of vowels to neonates during sleep actually enhanced auditory discrimination abilities while awake (Cheour *et al.*, 2002b), suggesting that active cognitive processing and learning can occur during sleep, at least at young ages.

In the present study, a differential response to forward versus backward speech was found in both toddlers and 3-year-olds during sleep, suggesting that the brain may be able to discriminate speech from speech-like stimuli during sleep. Three-year-olds showed a greater response within superior temporal regions to forward than to backward speech, consistent with a pattern seen in adults, in addition to parietal and occipital regions. Toddlers showed differential activity within the left parietal lobe (left AG and Pre) and cingulate gyrus. These parietal regions were the same regions that showed a differential response to forward versus backward speech in the study of 2–3-month-old infants (Dehaene-Lambertz *et al.*, 2002). Our finding of a differential neural response between forward versus backward speech offers additional support for some degree of cognitive processing during sleep in young children.

A second question is whether the broadly distributed activation seen in the toddlers, but not 3-year-olds, is a sleep rather than a developmental phenomenon. Previous work using PET techniques suggests that regional blood flow is altered across different sleep stages in adults (Maquet, 2000). Although concurrent EEG recording was deemed infeasible in this study, we attempted to mute the potential confound of sleep stage by initiating testing of each child at the same time after the onset of sleep (approximately 35–45 minutes) so that children would be in NREM sleep. While more data are needed, reports suggest that variability in REM latencies after sleep onset may be larger within the toddler and 3-year-old group than between groups. For 3–5-year-old children, REM onsets are reported at approximately 90 minutes into sleep (range 28–234 minutes) (Montgomery-Downs, O'Brien, Gulliver & Gozal, 2006). A longitudinal study reports mean REM latencies between 53 and 70 minutes for 1–2-year-old children and at 90 minutes between 1 and 2 years of age for the one child whose data are given individually (Louis, Cannard, Bastuji & Challamel, 1997). Therefore, the fMRI activation effects we observed seem unlikely to have been due to systematic between-group differences in the sleep stage during which activations were recorded.

Between-subject variability (whether due to sleep stage or other factors) may have been a source of measurement noise in each group and as such could have decreased the power to detect within-group activations. In fact, we noted between-subject variability in BOLD response within the bilateral STG region of the ROA mask (see Figure 1B): six of the 10 toddlers showed positive mean activity to forward speech as compared to rest in this region but four did not (Supplementary Figure S2). Variability in the sign of the BOLD response in STG has been noted in a previous study examining BOLD response to tones in sleeping neonates (Anderson, Marois, Colson, Peterson, Duncan, Ehrenkranz, Schneider, Gore & Ment, 2001). Furthermore, fMRI studies of adults during sleep show a reduced percent signal change (Tanaka, Fujita, Takanashi, Hirabuki, Yoshimura, Abe & Nakamura, 2003) or area of activation (Czisch, Wetter, Kaufmann, Pollmacher, Holsboer & Auer, 2002) within auditory cortices, suggesting that auditory studies of the sleeping brain may require more statistical power than during the wake state to achieve reliable activation within temporal cortex. A potential source of variability may result from the relatively broad range in age and language capacity within the toddler group. Further studies examining a narrow age range, containing a larger sample size, and controlling for sleep stages should be able to give insight into the specific sources of this variability.

As this is the first fMRI study to examine age-related changes between the second and fourth year of life, and given that sleep stage was not directly recorded, the reason for the greater number of frontal, occipital, and cerebellar regions of activation in toddlers remains uncertain. In adults, these frontal and cerebellar regions are involved in some linguistic (Demonet, Thierry & Cardebat, 2005) as well as non-linguistic behaviors that may be critical to language learning such as working memory, novelty detection, attention, extraction of patterns, and socio-emotional processing. Thus, one hypothesis is that speech perception in toddlers requires the participation of other neural systems in addition to classical language systems. The neural substrate for these processes may be tightly linked early in development such that they are recruited even during a passive state such as sleep. After the initial gains in language comprehension (i.e. after 2 to 3 years of age), these 'other systems' may no longer be recruited to the same degree during passive language listening, as the child would then already have a base of semantic knowledge with which to process familiar words and to incorporate newly learned words. In other words, in the language-skilled person, during passive listening superior temporal 'receptive language' regions alone may be sufficient to add to the base of linguistic knowledge formed in the second year of life.

It is premature to conclude that the age-related change from a pattern of more widespread activation in response to speech in toddlers to more limited activation in young children is unique to either the domain of language or the current age range. For instance, in older children, a body of developmental imaging work reveals a pattern of diffuse to focal brain activity with age, experience, and performance in domains outside of language (see Durston, Davidson, Tottenham, Galvan, Spicer, Fossella & Casey, 2006, for review). Further, EEG/ERP studies with infants reveal distributed activity in response to other complex stimulus types (e.g. faces) at early ages and increasing neural specialization with increasing age and experience (Courchesne, Ganz &

Norcia, 1981; de Haan, Pascalis & Johnson, 2002; Halit, de Haan & Johnson, 2003). Johnson proposed an 'interactive specialization framework' of functional brain development in which regions of cortex become specialized to respond to a particular stimulus set through interactions between a broad number of brain regions and networks early on (Johnson, 2000, 2001; Johnson, Griffin, Csibra, Halit, Farroni, de Haan, Baron-Cohen & Richards, 2005). Given the greater response of frontal and other neural systems to language during a period of rapidly increasing language capacity, this framework may be applicable to language acquisition in the second year of life as well.

The importance of being able to record fMRI data from infants and toddlers during natural sleep may extend beyond the topic of language development. The second year of life is a remarkable age in a child's cognitive and neural development, not limited to the realm of language development (Bates *et al.*, 2003; Courage & Howe, 2002). Between an infant's first and second birthdays, he or she becomes able for the first time to express a multitude of words (Fenson *et al.*, 1994), initiate episodes of joint attention (Carpenter, Nagell & Tomasello, 1998), achieve self-recognition (Amsterdam, 1972), and understand a speaker's intention (Diesendruck, Markson, Akhtar & Reudor, 2004). In other words, during this short period of life the child is rapidly transforming into an active and capable communicator, learner, and social participant of his or her world. The present study raises the possibility that the neural bases for these behavioral achievements could be probed through the use of auditory paradigms during natural sleep. To facilitate the interpretation of results from natural sleep fMRI analyses, studies could be designed to test for correlations between the former and behavioral performance in the same child while awake. The ability to study children at this unique stage in life with fMRI has the potential to lead to considerable advances in the relatively young field of developmental cognitive neuroscience.

Acknowledgements

The authors gratefully acknowledge the parents and children who participated in this study. We also thank members of our research staff for help with data collection (Anne Erickson, Vera Grindell, Cindy Hsu, Grace Kim, Lindsey Schubert, and Weifang Zhou) and clinical testing (Dr Natacha Akshoomoff & Dr Cynthia Carter). We thank Dr Ruth Carper, Graham Wideman, and Dr Natalia Kleinhans for their contributions to the Talairach validation study in Supplementary Information. Additionally, we are grateful to Dr Jeff Elman, Dr Donna Thal, Dr Greg Allen, and Dr Dan Kennedy for helpful comments on the manuscript. This research was supported by the National Institute of Health Grants ROI-NS-19855 and MH36840 awarded to E.C.

References

Ahmad, Z., Balsamo, L.M., Sachs, B.C., Xu, B., & Gaillard, W.D. (2003). Auditory comprehension of language in young children: neural networks identified with fMRI. *Neurology*, **60** (10), 1598–1605.

Altman, N.R., & Bernal, B. (2001) Brain activation in sedated children: auditory and visual functional MR imaging. *Pedriatic Imaging*, **221**, 56–63.

Amsterdam, B. (1972). Mirror self-image reactions before age two. *Developmental Psychobiology*, **5** (4), 297–305.

Anderson, A.W., Marois, R., Colson, E.R., Peterson, B.S., Duncan, C.C., Ehrenkranz, R.A., Schneider, K.C., Gore, J.C., & Ment, L.C. (2001). Neonatal auditory activation detected by functional magnetic resonance imaging. *Magnetic Resonance Imaging*, **19** (1), 1–5.

Baldwin, D.A. (1991). Infants' contribution to the achievement of joint reference. *Child Development*, **62** (5), 875–890.

Balsamo, L.M., Xu, B., & Gaillard, W.D. (2006). Language lateralization and the role of the fusiform gyrus in semantic processing in young children. *NeuroImage*, **31** (3), 1306–1314.

Bates, E. (1992). Language development. *Current Opinion in Neurobiology*, **2** (2), 180–185.

Bates, E. (1997). Origins of language disorders: a comparative approach. *Developmental Neuropsychology*, **13** (3), 447–476.

Bates, E., & Dick, F. (2002). Language, gesture, and the developing brain. *Developmental Psychobiology*, **40** (3), 293–310.

Bates, E., Thal, D., Finlay, B., & Clancy, B. (2003). Early language development and its neural correlates. In S.J. Segalowitz & I. Rapin (Eds.), *Child neuropsychology, Part II* (2 edn., Vol. 8, pp. 525–592). New York: Elsevier.

Bates, E., Thal, D., & Janowsky, J.S. (1992). Early language development and its neural correlates. In S.J. Segalowitz & I. Rapin (Eds.), *Child neuropsychology (Part 2)* (Vol. 7, pp. 69–110). New York: Elsevier.

Bernal, B., & Altman, N.R. (2003) Speech delay in children: a functional MR imaging study. *Pediatric Imaging*, **229**, 651–658.

Carpenter, M., Nagell, K., & Tomasello, M. (1998). Social cognition, joint attention, and communicative competence from 9 to 15 months of age. *Monographs of the Society for Research in Child Development*, **63** (4), i–vi, 1–143.

Cheour, M., Ceponiene, R., Leppänen, P., Rinne, T., Alho, K., Lehtokoski, A., Kujala, T., Renlund, M., Fellman, V., & Näätänen, R. (2002a). The auditory sensory memory trace decays rapidly in newborns. *Scandinavian Journal of Psychology*, **43** (1), 33–39.

Cheour, M., Martynova, O., Näätänen, R., Erkkola, R., Sillanpää, M., Kero, P., Raz, A., Kaipio, M.-L., Hiltunen, J., Aaltonen, O., Savela, J., & Hämäläinen, H. (2002b). Speech sounds learned by sleeping newborns. *Nature*, **415** (6872), 599–600.

Courage, M.L., & Howe, M.L. (2002). From infant to child: the dynamics of cognitive change in the second year of life. *Psychological Bulletin*, **128** (2), 250–277.

Courchesne, E., Ganz, L., & Norcia, A.M. (1981). Event-related brain potentials to human faces in infants. *Child Development*, **52** (3), 804–811.

Cox, R.W. (1996). AFNI: software for analysis and visualization of functional magnetic resonance neuroimages. *Computers and Biomedical Research*, **29**, 162–173.

Cox, R.W., & Jesmanowicz, A. (1999). Real-time 3D image registration for functional MRI. *Magnetic Resonance in Medicine*, **42** (6), 1014–1018.

Czisch, M., Wetter, T.C., Kaufmann, C., Pollmacher, T., Holsboer, F., & Auer, D.P. (2002). Altered processing of acoustic stimuli during sleep: reduced auditory activation and visual deactivation detected by a combined fMRI/EEG study. *NeuroImage*, **16** (1), 251–258.

de Haan, M., Pascalis, O., & Johnson, M.H. (2002). Specialization of neural mechanisms underlying face recognition in human infants. *Journal of Cognitive Neuroscience*, **14** (2), 199–209.

Dehaene-Lambertz, G., Dehaene, S., & Hertz-Pannier, L. (2002). Functional neuroimaging of speech perception in infants. *Science*, **298** (5600), 2013–2015.

Dehaene-Lambertz, G., & Gliga, T. (2004). Common neural basis for phoneme processing in infants and adults. *Journal of Cognitive Neuroscience*, **16** (8), 1375–1387.

Dehaene-Lambertz, G., Hertz-Pannier, L., & Dubois, J. (2006a). Nature and nurture in language acquisition: anatomical and functional brain-imaging studies in infants. *Trends in Neuroscience*, **29** (7), 367–373.

Dehaene-Lambertz, G., Hertz-Pannier, L., Dubois, J., Meriaux, S., Roche, A., Sigman, M., & Dehaene, S. (2006b). Functional organization of perisylvian activation during presentation of sentences in preverbal infants. *Proceedings of the National Academy of Sciences, USA*, **103** (38), 14240–14245.

Demonet, J.F., Thierry, G., & Cardebat, D. (2005). Renewal of the neurophysiology of language: functional neuroimaging. *Physiological Reviews*, **85** (1), 49–95.

Diesendruck, G., Markson, L., Akhtar, N., & Reudor, A. (2004). Two-year-olds' sensitivity to speakers' intent: an alternative account of Samuelson and Smith. *Developmental Science*, **7** (1), 33–41.

Durston, S., Davidson, M.C., Tottenham, N., Galvan, A., Spicer, J., Fossella, J.A., & Casey, B.J. (2006). A shift from diffuse to focal cortical activity with development. *Developmental Science*, **9** (1), 1–8.

Fenson, L., Dale, P.S., Reznick, J.S., Bates, E., Thal, D.J., & Pethick, S.J. (1994). Variability in early communicative development. *Monographs of the Society for Research in Child Development*, **59** (5), 1–173; discussion 174–185.

Halit, H., de Haan, M., & Johnson, M.H. (2003). Cortical specialisation for face processing: face-sensitive event-related potential components in 3- and 12-month-old infants. *NeuroImage*, **19** (3), 1180–1193.

Heibeck, T.H., & Markman, E.M. (1987). Word learning in children: an examination of fast mapping. *Child Development*, **58** (4), 1021–1034.

Johnson, M.H. (2000). Functional brain development in infants: elements of an interactive specialization framework. *Child Development*, **71** (1), 75–81.

Johnson, M.H. (2001). Functional brain development in humans. *Nature Reviews Neuroscience*, **2** (7), 475–483.

Johnson, M.H., Griffin, R., Csibra, G., Halit, H., Farroni, T., de Haan, M., Baron-Cohen, S., & Richards, J. (2005). The emergence of the social brain network: evidence from typical and atypical development. *Development and Psychopathology*, **17** (3), 599–619.

Karunanayaka, P.R., Holland, S.K., Schmithorst, V.J., Solodkin, A., Chen, E.E., Szaflarski, J.P., & Plante, E. (2007). Age-related connectivity changes in fMRI data from children listening to stories. *NeuroImage*, **34** (1), 349–360.

Louis, J., Cannard, C., Bastuji, H., & Challamel, M.J. (1997). Sleep ontogenesis revisited: a longitudinal 24-hour home polygraphic study on 15 normal infants during the first two years of life. *Sleep*, **20** (5), 323–333.

Maquet, P. (2000). Functional neuroimaging of normal human sleep by positron emission tomography. *Journal of Sleep Research*, **9** (3), 207–231.

Martynova, O., Kirjavainen, J., & Cheour, M. (2003). Mismatch negativity and late discriminative negativity in sleeping human newborns. *Neuroscience Letters*, **340** (2), 75–78.

Mills, D.L., Coffey-Corina, S.A., & Neville, H.J. (1993). Language acquisition and cerebral specialization in 20-month-old infants. *Journal of Cognitive Neuroscience*, **5** (3), 317–344.

Mills, D.L., Coffey-Corina, S.A., & Neville, H.J. (1994). Variability in cerebral organization during primary language acquisition. In G. Dawson & K.W. Fischer (Eds.), *Human behavior and the developing brain* (pp. 427–455). New York: Guilford.

Mills, D., Coffey-Corina, S., & Neville, H.J. (1997). Language comprehension and cerebral specialization from 13 to 20 months. *Developmental Neuropsychology*, **13** (3), 397–445.

Mills, D.L., & Neville, H.J. (1997). Electrophysiological studies of language and language impairment. *Seminars in Pediatric Neurology*, **4** (2), 125–134.

Montgomery-Downs, H.E., O'Brien, L.M., Gulliver, T.E., & Gozal, D. (2006). Polysomnographic characteristics in normal preschool and early school-aged children. *Pediatrics*, **117** (3), 741–753.

Mullen, E.M. (1995). *Mullen Scales of Early Learning* (AGS edn.). Minnesota, MN: American Guidance Service Inc.

Muller, R.A., & Basho, S. (2004). Are nonlinguistic functions in 'Broca's area' prerequisites for language acquisition? FMRI findings from an ontogenetic viewpoint. *Brain and Language*, **89** (2), 329–336.

Mundy, P., Fox, N., & Card, J. (2003). EEG coherence, joint attention and language development in the second year. *Developmental Science*, **6** (1), 48–54.

Neville, H., & Mills, D. (1997). Epigenesis of language. *Mental Retardation and Developmental Disabilities Research Reviews*, **3**, 282–292.

Pena, M., Maki, A., Kovacic, D., Dehaene-Lambertz, G., Koizumi, H., Bouquet, F., & Mehler, J. (2003). Sounds and silence: an optical topography study of language recognition at birth. *Proceedings of the National Academy of Sciences, USA*, **100** (20), 11702–11705.

Plante, E., Holland, S.K., & Schmithorst, V.J. (2006). Prosodic processing by children: an fMRI study. *Brain and Language*, **97** (3), 332–342.

Quartz, S.R., & Sejnowski, T.J. (1997). The neural basis of cognitive development: a constructivist manifesto. *Behavioral and Brain Sciences*, **20** (4), 537–556; discussion 556–596.

Schapiro, M.B., Schmithorst, V.J., Wilke, M., Byars, A.W., Strawsburg, R.H., & Holland, S.K. (2004). BOLD fMRI signal increases with age in selected brain regions in children. *NeuroReport*, **15** (17), 2575–2578.

Souweidane, M.M., Kim, K.H.S., McDowall, R., Ruge, M.I., Lis, E., Krol, G., & Hirsch, J. (1999) Brain mapping in sedated infants and young children with passive-functional magnetic resonance imaging. *Pediatric Neurosurgery*, **30**, 86–92.

Szaflarski, J.P., Schmithorst, V.J., Altaye, M., Byars, A.W., Ret, J., Plante, E., & Holland, S.K. (2006). A longitudinal functional magnetic resonance imaging study of language development in children 5 to 11 years old. *Annals of Neurology*, **59** (5), 796–807.

Talairach, J., & Tournoux, P. (1988). *Co-planar stereotaxic atlas of the human brain*. New York: Thieme Medical Publishers.

Tanaka, H., Fujita, N., Takanashi, M., Hirabuki, N., Yoshimura, H., Abe, K., & Nakamura, H. (2003). Effect of stage 1 sleep on auditory cortex during pure tone stimulation: evaluation by functional magnetic resonance imaging with simultaneous EEG monitoring. *American Journal of Neuroradiology*, **24** (10), 1982–1988.

Thal, D.J., Marchman, V., Stiles, J., Aram, D., Trauner, D., Nass, R., & Bates, E. (1991). Early lexical development in children with focal brain injury. *Brain and Language*, **40** (4), 491–527.

Vicari, S., Albertoni, A., Chilosi, A.M., Cipriani, P., Cioni, G., & Bates, E. (2000). Plasticity and reorganization during language development in children with early brain injury. *Cortex*, **36** (1), 31–46.

Wilke, M., Holland, S.K., & Ball, W.S., Jr. (2003). Language processing during natural sleep in a 6-year-old boy, as assessed with functional MR imaging. *American Journal of Neuroradiology*, **24** (1), 42–44.

Received: 10 January 2007
Accepted: 2 May 2007

Supplementary information

Region of activation analyses

Individual percent signal change data were extracted from a region of activation (ROA) mask (Figure 1B). This mask was created by combining the map of regions significantly responsive to forward speech ($p < .05$, 13 voxel minimum cluster) in the toddler group with that of the 3-year-old group ($p < .05$, 13 voxel minimum cluster) (Figure 1A). Nine separable clusters were identified within this ROA mask. The specific regions within this mask are detailed in Table S1 under both the toddler and 3-year-old response to forward speech vs. rest. More than nine regions are listed because data are reported based on all regions activated within the clusters, rather than simply the center of a cluster. Individual unthresholded percent signal change data from within the forward speech condition were then extracted from each of the nine clusters within the combined ROA mask for each participant.

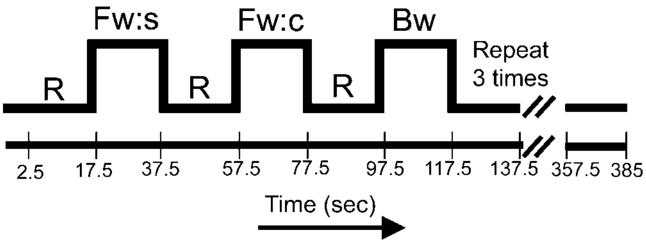

Figure S1 *Experimental design: Stimuli were presented in a block design with alternating 20-second 'on' blocks during which time one of three speech conditions (Forward simple (Fw:s); Forward complex (Fw:c); and Backward (Bw) speech) were presented and 'off' blocks during which time no stimuli were presented (Rest (R)). This design was repeated three times in a partially counterbalanced order.*

Exploratory correlations

Correlation analyses were used to examine whether each of the nine clusters within the ROA mask showed significant correlations of the BOLD response with behavioral age equivalent scores from the Mullen Scales of Early Learning for both receptive and expressive language. These analyses were exploratory as the wide age range made it difficult to disentangle effects of age from effects of skill. Percent data from the forward speech condition for each subject from each of the nine clusters of the ROA mask were correlated with the age equivalent scores for receptive and expressive language. All statistical analyses were carried out in SPSS v12.0 (SPSS, Chicago, IL).

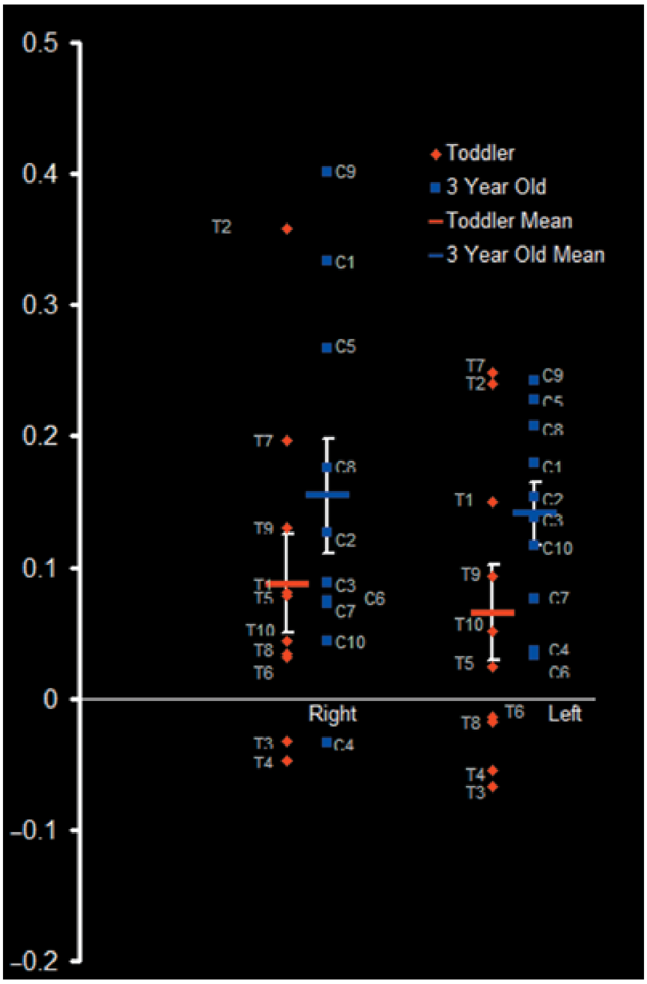

Figure S2 *Individual variability within STG. Percent signal change data from each individual in the toddler and 3-year-old group were extracted from the left and right STG regions of the ROA mask. The ROA mask is depicted in Figure 1B.*

Table S1 *Forward and Backward Speech versus Rest*

	Talairach Coordinates					Talairach Coordinates			
	Side	BA	(x,y,z)	*t*-value		Side	BA	(x,y,z)	*t*-value
Region	Toddler				Region	3-Year-Old			
Forward Speech > Rest									
Frontal					Frontal				
Medial Frontal gyrus	L	32	(–8, 44, 10)	3.73	Middle Frontal Gyrus	L	8	(–28, 18, 47)	3.84
Middle Frontal Gyrus	L	9	(–38, 42, 11)	5.01	Temporal				
Middle Frontal Gyrus	R	9	(46, 31, 13)	4.82	Fusiform Gyrus	R	20	(30, –38, –17)	5.10
Inferior Frontal Gyrus	L	45	(–31, 23, 7)	4.91	Inferior Temporal Gyrus	L	20	(–38, –6, –20)	4.49
Inferior Frontal Gyrus	R	9	(37, 8, 22)	3.70	Middle Temporal Gyrus	L	37	(–46, –53, 3)	2.53
Inferior Frotnal Gyrus	R	44	(54, 15, 10)	3.82	Middle Temporal Gyrus	R	21	(61, –9, –11)	3.79
Inferior Frontal Gyrus	R	47	(41, 28, 3)	3.47	Parahippocampal Gyrus	L	37	(–23, –49, –10)	3.45
Superior Frontal Gyrus	L	10	(–26, 47, 3)	3.60	Parahippocampal Gyrus	R	35	(30, –21, –17)	3.48
Temporal					Superior Temporal Gyrus	L	22	(–55, –9, –1)	6.20
Inferior Temporal Gyrus	L	20	(–51, –33, –18)	5.40	Superior Temporal Gyrus	L	22	(–41, –41, 15)	3.26
Superior Temporal Gyrus	L	22	(–48, –2, 2)	3.09	Superior Temporal Gyrus	R	22	(58, –15, 7)	3.87
Superior Temporal Gyrus	R	22	(57, –17, –2)	2.78	Superior Temporal Sulcus	L	21/22	(–51, –41, 7)	2.97
Superior Temporal Gyrus	R	38	(46, 11, –13)	3.60	Superior Temporal Sulcus	R	21/22	(49, –9, –10)	2.84
Transverse Temporal Gyrus	L	41	(–50, –13, 7)	2.90	Transverse Temporal Gyrus	L	41	(–42, –23, 7)	3.08
Parietal					Transverse Temporal Gyrus	R	41	(46, –21, 11)	3.40
Angular Gyrus	L	39	(–34, –60, 34)	3.57	Parietal				
Angular Gyrus	R	39	(50, –65, 31)	4.35	Cingulate Gyrus	L	31	(–18, –26, 32)	2.92
Inferior Parietal Lobule	L	40	(–44, –61, 43)	3.94	Posterior Cingulate	R	30	(21, –55, 8)	3.11
Posterior Cingulate	R	29/30	(–14, –45, 12)	2.60	Precuneus	L	19	(–25, –71, 34)	3.51
Occipital					Precuneus	L	31	(–15, –54, 31)	3.17
Cuneus	L	18	(–11, –89, 7)	2.90	Occipital				
Cuneus	R	19	(18, –89, 7)	3.31	Lingual Gyrus	L	19	(–23, –64, –5)	4.83
Lingual Gyrus	L	18	(–18, –85, –5)	3.24	Lingual Gyrus	R	19	(14, –53, –6)	3.99
Lingual Gyrus	R	18	(17, –78, –10)	5.42	Insula				
Subcortical					Insula	L	13	(–39, –24, 22)	2.71
Caudate	R		(10, 10, 10)	3.80					
Cerebellum	L		(–31, –57, –25)	3.94					
Cerebellum	R		(18, –56, –14)	3.13					
Insula									
Insula	L	13	(–31, 3, 3)	3.49					
Rest > Forward Speech									
Frontal					Frontal				
Superior Frontal Gyrus	L	8/9	(–18, –8, 6)	–4.62	Precentral Gyrus	R	4	(45, –10, 27)	–3.86
Parietal					Superior Frontal Gyrus	R	10	(25, 56, 10)	–3.37
Paracentral Lobule	L	5	(–18, –42, 55)	–3.48	Subcortical				
Postcentral Gyrus	L	40	(–35, –33, 51)	–3.12	Thalamus	L		(–10, –18, 4)	–2.56
Subcortical					Thalamus	R		(14, –21, 8)	–2.64
Thalamus	L			–3.32					
Thalamus	R			–4.05					

Table S1 *Continued*

	Talairach Coordinates					Talairach Coordinates			
	Side	BA	(x,y,z)	*t*-value		Side	BA	(x,y,z)	*t*-value
Region	Toddler				Region	3-Year-Old			
				Backward Speech > Rest					
Frontal					Frontal				
Inferior Frontal Gyrus	R	9	(57, 3, 22)	3.6	Anterior Cingulate	R	24	(2, 26, 12)	4.13
Inferior Frontal Gyrus	R	44	(49, 2, 19)	3.43	Medial Frontal Gyrus	L	10	(–15, 35, 10)	4.66
Middle Frontal Gyrus	L	9	(–39, 39, 15)	3.94	Middle Frontal Gyrus	L	10	(–26, 46, –5)	4.22
Middle Frontal Gyrus	R	46	(38, 31, 22)	3.37	Middle Frontal Gyrus	R	6	(44, 5, 45)	2.9
Middle Frontal Gyrus	R	6	(37, 7, 52)	2.61	Middle Frontal Gyrus	R	9	(44, 30, 27)	7.06
Superior Frontal Gyrus	L	10	(–22, 50, 15)	3.02	Superior Frontal Gyrus	R	8	(18, 34,47)	4.08
Temporal					Temporal				
Fusiform Gyrus	R	19	(30, –64, –10)	3.79	Middle Temporal Gyrus	L	21	(–51, –54, 8)	2.69
Inferior Temporal Gyrus	L	20	(–54, –20, –16)	5.2	Parahippocampal Gyrus	R	35	(27, –33, –17)	2.96
Middle Temporal Gyrus	R	22	(57, –45, 2)	3.77	Superior Temporal Gyrus	L	21/22	(–57, –10, –2)	5.95
Superior Temporal Gyrus	L	22	(–51, –13, 6)	3.2	Superior Temporal Gyrus	L	21/22	(–57, –29, 8)	5.43
Superior Temporal Gyrus	R	22	(46, –4, –7)	3.73	Transverse Temporal Gyrus	L	41	(–45, –26, 10)	7.8
Superior Temporal Gyrus	R	38	(50, –1, –6)	4.64	Parietal				
Transverse Temporal Gyrus	L	41	(–50, –13, 7)	3.2	Angular Gyrus	L	39	(–43, –65, 32)	2.47
Parietal					Insula				
Inferior Parietal Lobule	R	40	(55, –49, 51)	3.69	Anterior Insula	L	13	(–25, 28, 5)	2.58
Occipital									
Lingual Gyrus	L	17	(–22, –89, –3)	3.97					
Middle Occipital Gyrus	L	18	(–27, –81, –8)	3.09					
Middle Occipital Gyrus	R	18	(33, –84, 0)	2.68					
Subcortical									
Cerebellum	L		(–31, –52, 30)	3.21					
Insula									
Insula	R	13	(35, –1, 14)	4.83					
				Rest > Backward Speech					
Frontal					Occipital				
Cingulate Gyrus	R	24	(12, –4, 39)	–3.19	Lingual Gyrus	L	19	(–27, –77, –9)	–3.33
Medial Frontal Gyrus	L	6	(–10, –24, 59)	–4.26	Middle Occipital Gyrus	L	18	(–27, –81, 4)	–3.51
Parietal					Subcortical				
Cingulate Gyrus	L	34	(–10, –16, 43)	–6.02	Thalamus	L		(–14, –21, 10)	–2.85
Paracentral Lobule	R	5	(2, –33, 55)	–4.34	Thalamus	R		(14, –22, 15)	–3.50
Precentral Gyrus	L	4	(–25, –24, 52)	–3.58					
Precuneus	L	7	(–6, –44, 52)	–3.55					
Subcortical									
Cerebellar Vermis	B		(–6, –45, –9)	–4.94					
Thalamus	L		(–15, –17, 7)	–3.44					
Thalamus	R		(18, –21, 7)	–3.35					

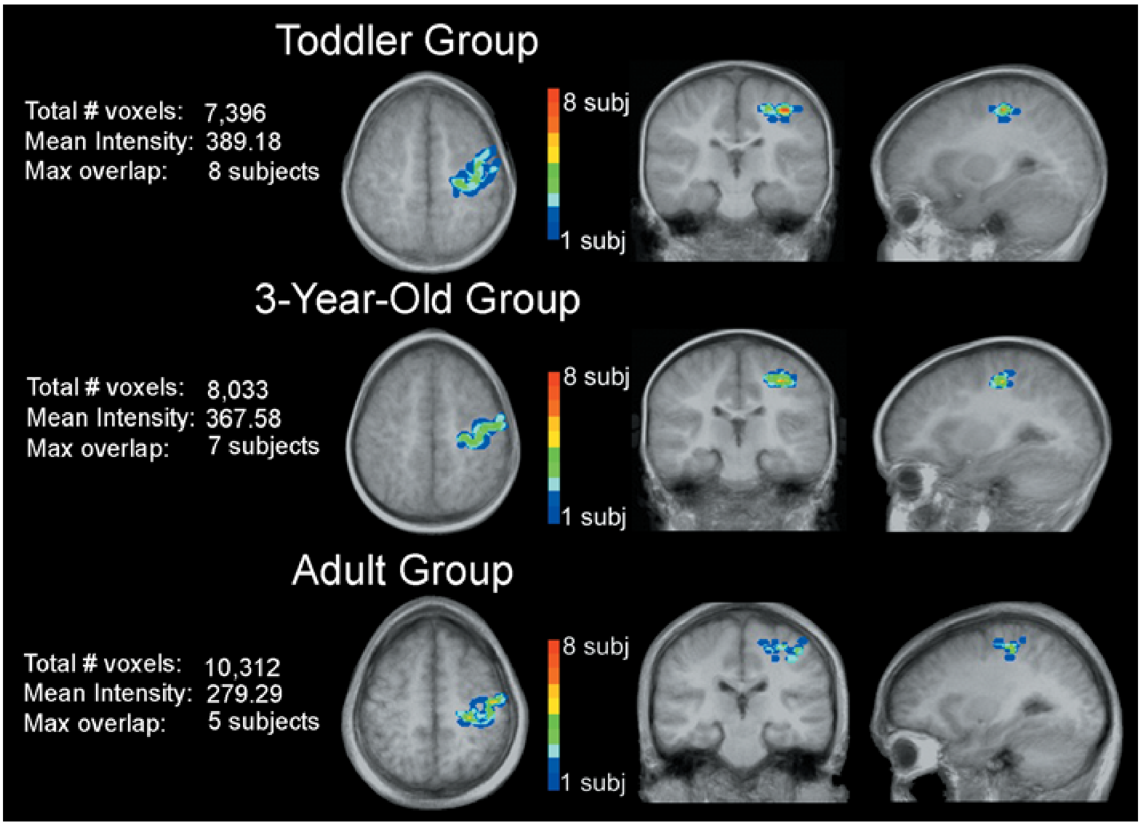

Figure S3 *Talairach spatial normalization: Individual tracings of CS were summed for each group (toddlers, 3-year-olds and adults). Color indicates the degree of overlap between subjects. Total number of of voxels indicates the number of voxels in the combined CS tracings within each group, such that fewer voxels indicates a greater degree of within-group spatial overlap. Mean intensity indicates the average amount of overlap within the group CS tracing (individual CS tracings have a value of 200). Each group tracing is displayed on an anatomical dataset created by averaging the mean intensities of all 10 anatomical datasets from that group.*

No clusters showed significant correlations with receptive or expressive language age equivalent at a conservative bonferroni correction ($p < .0156$). At a more lenient significance threshold of $p < .05$, the left frontal cluster (left inferior, middle, superior, and medial frontal gyrus) showed a negative correlation with receptive and expressive language age. Additionally, bilateral cerebellar clusters showed significant negative correlations with expressive language age equivalent scores.

Nonparametric analyses

Due to the wide age range and variability in language skill across and within groups, a nonparametric test (Wilcoxon signed-rank) was run to take into account a possible deviation from the assumption of normal distribution. The Wilcoxon signed-rank test showed similar results to the ANOVA. For forward speech, left MFG (peak coordinate: (–26, 42, 15); $t = -2.8$) and left cerebellar hemisphere (peak coordinate: (–28, –56, –23); $t = -2.8$) showed greater activation to forward speech in the toddlers, whereas left temporal pole ((–38, –1, –21); $t = 2.8$) showed greater activation in the 3-year-olds. For backward speech, a region of left STG (peak: (–52, 17, 2); $t = 2.8$) and left ITG (peak: (–50, –19, –21); $t = 2.8$) showed greater activation in the 3-year-olds and no region showed significantly greater activation in the toddlers.

Talairach validation study

Unpublished studies from within our laboratory have examined the degree of anatomical overlap when using Talairach space to co-register pediatric brains. In one such study (Redcay, Carper, Wideman, Kleinhans & Courchesne, unpublished observations) one plane of the central sulcus (CS) of the 10 toddlers and 10 3-year-olds from this study and a group of 10 adults (23–27 years) were traced. The CS was chosen for reasons unrelated to the current fMRI study; however, it offers a good examination of spatial normalization because it is on the superior edge of the brain and thus susceptible to alignment errors in spatial normalization with Talairach. Additionally, it allows for a consistent anatomical landmark from which to choose the slice in the axial plane to trace. Specifically, the precentral gyrus contains a distinct region that constitutes the motor 'hand area' and has a knob-like appearance extending toward the postcentral gyrus. This region can also be identified in the sagittal plane by a hook-like appearance (for details see Yousry, Schmid, Alkadhi, Schmidt, Peraud, Buettner & Winkler, 1997).

Individual measures from the CS tracings were obtained in order to determine the degree of individual variability in CS location within each group. The center of each individual CS tracing was identified in each of the three planes (axial, sagittal, and coronal). A Levene test for homogeneity of variances was run to determine if the variances in the midpoint of the traced central sulcus within groups was different between groups. No significant difference was seen in a Levene test for homogeneity of variances between the three groups in any plane of the central sulcus except one. In the axial plane, greater variance was seen in the adults as compared to the toddlers ($p < .04$). Analysis of the degree of overlap of these structures revealed comparable or even better registration among the toddlers and children in the 3-year-old group than among the adults (see Figure S3).

Supplementary reference

Yousry, T.A., Schmid, U.D., Alkadhi, H., Schmidt, D., Peraud, A., Buettner, A., & Winkler, P. (1997). Localization of the motor hand area to a knob of the precentral gyrus: a new landmark. *Brain*, **120** (Pt1), 141–157.