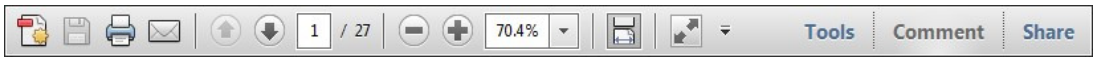
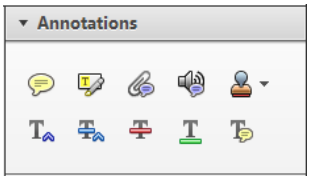
Once you have Acrobat Reader open on your computer, click on the Comment tab at the right of the toolbar:



This will open up a panel down the right side of the document. The majority of tools you will use for annotating your proof will be in the Annotations section, pictured opposite. We've picked out some of these tools below:

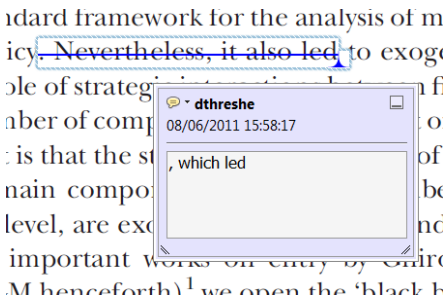


1. Replace (Ins) Tool – for replacing text.

Strikes a line through text and opens up a text box where replacement text can be entered.

How to use it

- Highlight a word or sentence.
- Click on the Replace (Ins) icon in the Annotations section.
- Type the replacement text into the blue box that appears.

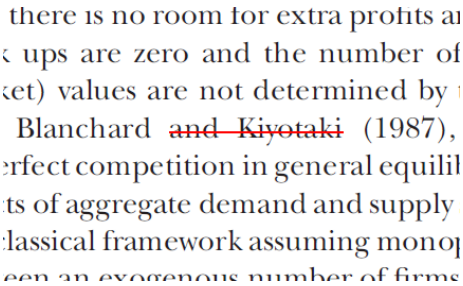


2. Strikethrough (Del) Tool – for deleting text.

Strikes a red line through text that is to be deleted.

How to use it

- Highlight a word or sentence.
- Click on the Strikethrough (Del) icon in the Annotations section.

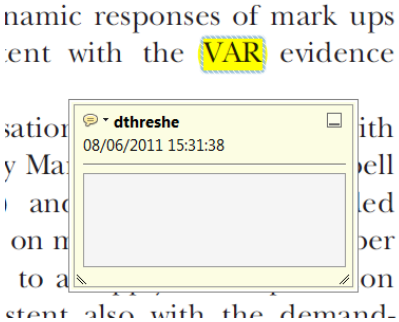


3. Add note to text Tool – for highlighting a section to be changed to bold or italic.

Highlights text in yellow and opens up a text box where comments can be entered.

How to use it

- Highlight the relevant section of text.
- Click on the Add note to text icon in the Annotations section.
- Type instruction on what should be changed regarding the text into the yellow box that appears.

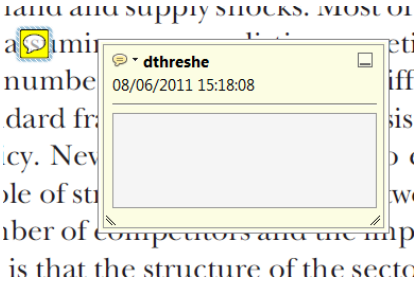


4. Add sticky note Tool – for making notes at specific points in the text.

Marks a point in the proof where a comment needs to be highlighted.

How to use it

- Click on the Add sticky note icon in the Annotations section.
- Click at the point in the proof where the comment should be inserted.
- Type the comment into the yellow box that appears.

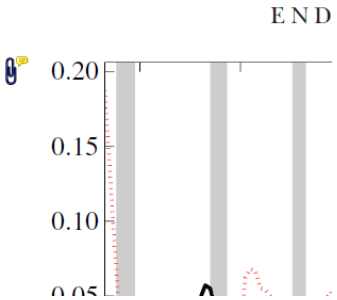


5. Attach File Tool – for inserting large amounts of text or replacement figures.

Inserts an icon linking to the attached file in the appropriate place in the text.

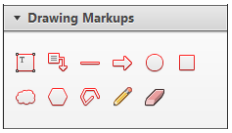
How to use it

- Click on the Attach File icon in the Annotations section.
- Click on the proof to where you'd like the attached file to be linked.
- Select the file to be attached from your computer or network.
- Select the colour and type of icon that will appear in the proof. Click OK.



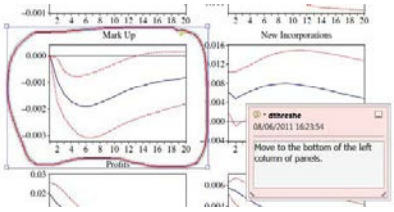
6. Drawing Markups Tools – for drawing shapes, lines and freeform annotations on proofs and commenting on these marks.

Allows shapes, lines and freeform annotations to be drawn on proofs and for comment to be made on these marks.



How to use it

- Click on one of the shapes in the Drawing Markups section.
- Click on the proof at the relevant point and draw the selected shape with the cursor.
- To add a comment to the drawn shape, move the cursor over the shape until an arrowhead appears.
- Double click on the shape and type any text in the red box that appears.



DESC	12344	WILEY	Dispatch: 21.8.15	CE: Wiley
Journal Code	Manuscript No.		No. of pages: 10	PE: Dhatchayani S

Developmental Science (2015), pp 1–10 DOI: 10.1111/desc.12344

PAPER

Effect of socioeconomic status (SES) disparity on neural development in female African-American infants at age 1 month

Laura M. Betancourt,[1] Brian Avants,[2] Martha J. Farah,[3] Nancy L. Brodsky,[1] Jue Wu,[2] Manzar Ashtari[4] and Hallam Hurt[1]

1. *Department of Neonatology, The Children's Hospital of Philadelphia, USA*
2. *Department of Radiology, University of Pennsylvania, USA*
3. *Department of Psychology, University of Pennsylvania, USA*
4. *Department of Radiology, The Children's Hospital of Philadelphia, USA*

Abstract

There is increasing interest in both the cumulative and long-term impact of early life adversity on brain structure and function, especially as the brain is both highly vulnerable and highly adaptive during childhood. Relationships between SES and neural development have been shown in children older than age 2 years. Less is known regarding the impact of SES on neural development in children before age 2. This paper examines the effect of SES, indexed by income-to-needs (ITN) and maternal education, on cortical gray, deep gray, and white matter volumes in term, healthy, appropriate for gestational age, African-American, female infants. At 5 weeks postnatal age, unsedated infants underwent MRI (3.0T Siemens Verio scanner, 32-channel head coil). Images were segmented based on a locally constructed template. Utilizing hierarchical linear regression, SES effects on MRI volumes were examined. In this cohort of healthy African-American female infants of varying SES, lower SES was associated with smaller cortical gray and deep gray matter volumes. These SES effects on neural outcome at such a young age build on similar studies of older children, suggesting that the biological embedding of adversity may occur very early in development.

Research highlights

- Utilizes a birth cohort of term, healthy, appropriate for gestational age, African-American, female infants.
- Examines relation between SES and cortical volume in infants at age 4–6 weeks.
- Lower SES associated with smaller cortical gray and deep gray matter volumes.
- Findings push back the age at which SES effects are observed, from early childhood to early infancy.

Introduction

Childhood socioeconomic status (SES) is associated with lifelong mental health and intellectual attainment, presumably through its effects on neural development. On average, poor children differ from their higher SES counterparts, achieving less success in school (Nisbett, Aronson, Blair, Dickens, Flynn *et al.*, 2012; Sirin, 2005) and suffering at a higher rate from mental disorders including ADHD, anxiety, and depression (Goodman, 1999; Kessler, Berglund, Demler, Jin, Merikangas *et al.*, 2005). In evaluations of SES effects on neurocognitive skills of school-age children the largest disparities are found in executive function, memory and language, skills that are linked to academic success (Farah, Shera, Savage, Betancourt, Giannetta *et al.*, 2006; Landry, Smith & Swank, 2002; Noble, McCandliss & Farah, 2007; Noble, Norman & Farah, 2005). Similarly, in a limited number of studies, SES effects on cognitive function in young children at toddler and preschool ages have shown differences in language (Fernald, Marchman & Weisleder, 2013; Wild, Betancourt, Brodsky & Hurt,

Address for correspondence: Laura M. Betancourt, Neonatology Research, Department of Pediatrics, 3535 Market Street, Room 1433, Philadelphia, PA 19104, USA; e-mail: betancourtl@email.chop.edu

2013) and executive function (Lipina, Martelli, Vuelta & Colombo, 2005). Assessment of SES effects on these skills is more common at these older ages; however, it is likely that SES effects on development are present earlier in infancy, during the first year of life, when development proceeds most rapidly (Holland, Chang, Ernst, Curran, Buchthal *et al.*, 2014).

Recent brain imaging studies have investigated SES effects on neural development utilizing MRI measures of gray and white matter, both of which have established associations with neurocognitive abilities. Positive relations between SES and gray matter volume have been reported in some studies (Hanson, Chandra, Wolfe & Pollak, 2011; Hanson, Hair, Shen, Shi, Gilmore *et al.*, 2013; Luby, Belden, Botteron, Marrus, Harms *et al.*, 2013) but not others (Brain-Development-Cooperative-Group, 2012). Similarly, findings regarding SES effects on white matter vary, with some investigators reporting effects (Luby *et al.*, 2013) and others reporting no effects (Brain-Development-Cooperative-Group, 2012). Analyses of repeated assessments of frontal gray matter volumes between ages 5 months and 4 years (mean age of first scan: 13.5 months) have shown a positive relationship with SES (Hanson *et al.*, 2013). Voxel-based morphometry (VBM) and region of interest (ROI) analyses have demonstrated regional gray matter correlates of SES in children. While analyses of a large cohort of children aged 4–18 years found no SES effects on specific lobar volumes (Brain-Development-Cooperative-Group, 2012), cortical thickness in certain prefrontal regions was smaller for the lower SES participants in the sample (Lawson, Duda, Avants, Wu & Farah, 2013; Noble, Houston, Kan & Sowell, 2012). Raizada *et al.* (2008) utilized scans collected from 14 5-year olds to detect a marginally significant positive relationship between SES and gray matter volume in the left inferior frontal gyrus (IFG) (Raizada, Richards, Meltzoff & Kuhl, 2008). In 5–17-year-olds, Noble and colleagues (2012) found no main effect of SES; however, they did find an interaction between SES and age with progressively larger left IFG volumes for high SES children as their age increased. Overall, ROI analyses have documented SES differences in hippocampal, amygdala, middle temporal gyri, left fusiform and right inferior occipito-temporal gyri (Hanson *et al.*, 2011; Hanson, Nacewicz, Sutterer, Cayo, Schaefer *et al.*, 2015; Jednorog, Altarelli, Monzalvo, Fluss, Dubois *et al.*, 2012; Luby *et al.*, 2013; Noble *et al.*, 2012). These studies taken together provide consensus that SES influences developing neuroanatomy in children; less is known about this relationship in younger infants and toddlers.

Given the findings in cohorts of older children, it is likely that SES influences neuroanatomy earlier in development. In the first year of life neural development is dynamic, characterized by rapidly changing and complex patterns of growth in gray matter (Gilmore, Lin, Prastawa, Looney, Vetsa *et al.*, 2007; Holland *et al.*, 2014; Knickmeyer, Gouttard, Kang, Evans, Wilber *et al.*, 2008) and of myelination and synaptic pruning in white matter tracts (Dubois, Dehaene-Lambertz, Kulikova, Poupon, Hüppi *et al.*, 2014; Uda, Matsui, Tanaka, Uematsu, Miura *et al.*, 2015). A number of investigators have shown that early neural structures are the foundation for both concurrent and later cognitive processes (Can, Richards & Kuhl, 2013; Jednorog *et al.*, 2012; Spann, Bansal, Rosen & Peterson, 2014). For example, Short, Elison, Goldman, Styner, Gu *et al.* (2013) and Deoni, O'Muircheartaigh, Elison, Walker, Doernberg *et al.* (2014) report positive associations between myelination of white matter tracts and infant working memory and language function in the first year of life. These longitudinal studies provide evidence that larger volumes of gray and white matter, assessed using MRI, are associated with better cognitive function in later years (Can *et al.*, 2013; Jednorog *et al.*, 2012; Spann *et al.*, 2014). Despite the emerging consensus that early neural development is highly responsive to environmental variation, including variation in SES levels (Hackman, Farah & Meaney, 2010), few studies have specifically examined the impact of SES on neural development and behavior at very young ages.

Although additional research is needed to firmly establish the existence of structural brain correlates of childhood SES and to identify specific patterns of areas affected, the available research supports the conclusion that SES does affect brain development in childhood. To date, however, such investigations have utilized cohorts of children who were both older than 2 years and were mostly non-poor. Given that neural development during the first year of life is rapid and dynamic (Gilmore *et al.*, 2007; Holland *et al.*, 2014), it is likely that environmental differences may shape brain development earlier than previously reported. In contrast to the question of whether SES has structural brain correlates in childhood, which has a provisional answer, two related questions remain entirely open. First, at what age are effects of SES on brain structure detectable and, second, are there differences within the lower range of SES? The present study is the first to address these two questions.

The first question, concerning the age at which effects of SES are manifest in child brain structure, is relevant to the developmental origins of morphological differences. In a cross-sectional investigation of neural development between ages 3 and 20 years, results showed that income and education were associated with increasing surface area but not cortical thickness, with the largest effects

among those at lowest income levels (Noble, Houston, Brito, Bartsch, Kan *et al.*, 2015). The youngest children analyzed for SES effects on brain structure are in a cohort of children aged 5 months to 4 years (Hanson *et al.*, 2013). Visual inspection of the growth curves for total gray matter for this sample of low, middle, and high income children shows overlap at 5 months of age and divergence only later, with the low income group separating from middle and high income groups at about 1 year. However, few subjects in this sample were 5 months old; the mean age of subjects at the first of the longitudinally collected scans was 13.5 months. In addition, curves were fit to data from multiple ages, so that the values shown at age 5 months were influenced by measurements at later ages. Presumably for these reasons, the authors did not state any conclusions regarding the age at which effects of SES emerge. There are no other reports of SES and brain structure before toddlerhood.

The second question addressed here concerns the effects of variation along the lower range of SES versus variation from low to high SES. In contrast to SES, which may refer to the full range of variation in income, education and occupational status, poverty refers to the very lowest levels of financial status with accompanying social factors including low educational attainment. For both policy and research purposes, poverty is typically gauged by the ratio of income-to-needs (ITN), with the US 'poverty line' defined as an ITN of 1. No previous study of brain structure has compared children who were poor, by this criterion, with non-poor children; indeed the largest studies to date utilize a sample that was predominantly middle class (Brain-Development-Cooperative-Group, 2012; Hanson *et al.*, 2011; Hanson *et al.*, 2013; Lange, Froimowitz, Bigler & Lainhart, 2010; Lawson *et al.*, 2013). Furthermore, stringent exclusionary criteria for this sample eliminated children disproportionately from lower SES levels (Waber, De Moor, Forbes, Almli, Botteron *et al.*, 2007), raising questions about the typicality of the lower SES children (Hanson *et al.*, 2013). An exception published by Noble *et al.* (2015) showed increased sensitivity to SES influence along the lower range of (a relatively broad distribution) of family income and education. Taken together, the samples cited above are of broader ranges of SES, with none including primarily poor and near-poor children. The sample studied for the current report is approximately half poor and half near-poor. With 22% of American children classified as poor according to the Federal standards (Canada, 2014), utilization of a cohort of primarily the lower SES participants rather than those from middle and upper ranges allows for a comparison that is both socially and scientifically relevant.

On the basis of the research reviewed above, we hypothesized an early association of SES and cortical gray matter volume in infants at 1 month of age. In addition we analyzed the association between SES and deep gray matter and white matter volumes. To limit the number of confounding variables in a small-sized cohort, we restricted gender and ethnicity to only female African-American infants.

Methods

Participant recruitment and inclusion criteria

Mothers and their infants were recruited at delivery from a single hospital for a larger study of the effects of SES on both neural and cognitive development. Mothers were eligible if they were between 18 and 45 years of age and declared that both parents were American-born African-American. Potential participants were excluded if they were non-English speaking, had significant psychiatric diagnoses, were enrolled in an alcohol or drug rehabilitation program, or had significant medical or obstetrical conditions as defined by the obstetrical service. Infants eligible for inclusion were female singletons born at 38–42 weeks gestation, with birth weights appropriate for gestational age and 5-minute Apgar scores ≥8. Infants were excluded if they were diagnosed with any condition associated with developmental delay, were hospitalized more than 3 days, failed the hearing screen, or were not discharged to their biologic mother. Target enrollment was 30 low SES infants and mothers and 30 higher SES infants and mothers. Upon enrollment all participants signed informed consent approved by the Institutional Review Board of the Children's Hospital of Philadelphia.

Socioeconomic status (SES): income-to-needs (ITN) and education

SES was indexed by ITN and maternal education. Low SES (poor group) was defined as ITN at or below government poverty line plus no more than high school education for either parent. Higher SES (near-poor) had ITN above the poverty line plus at least a high school education for both parents. The ITN variable was based on the 2013 US government official poverty definition (US Census Bureau, 2013) and was ascertained by maternal self-report of household income and composition. Mothers and infants were categorized into one of five ITN categories according to income and family size. For example, the poverty threshold for a family of two is $15,510 per year. Families making less than this amount

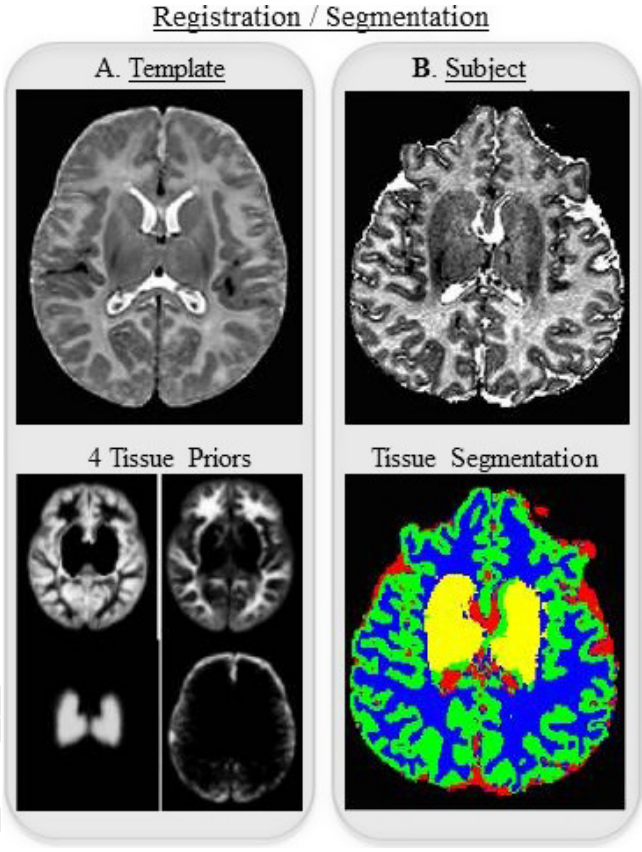
are classified as below the poverty line (ITN = 1). A family of two making $62,040 per year is classified in the higher end of the range at 400% above the poverty line (ITN = 5). The remaining three ITN categories were distributed between the low and higher income range. Education was ascertained from maternal self-report and ranged from some high school through graduate school. An SES Composite score was computed by rescaling ITN values to match the scale for values of maternal education and summing them, giving these two dimensions of SES equal weight. Because nearly two-thirds of the infants in the current cohort were living in households without their biological father, we used maternal but not paternal education in the composite (Entwislea & Astone, 1994). The current report includes neural data from the infant participants collected at age 1 month using MRI.

Image acquisition and processing

Infants underwent MRI scans at approximately 5 weeks post estimated date of confinement (EDC). No sedation was utilized. Appointments were scheduled for parent-reported infant nap times. Infants were fed, swaddled, and acclimated to the scanner room before placement in the scanner. High resolution T1- and T2-weighted and diffusion-weighted images were obtained utilizing a 3T Siemens Verio Scanner with a 32-channel head coil.

All subjects' images were converted into anonymous Neuroimaging Informatics Technology Initiative (Nifti) format. A population-specific template was built using data from 15 participants with high quality data. The final template was labeled with six spatial probability functions (priors) that defined the voxel-wise probability of six distinct tissue/anatomical classes: cortical gray (includes hippocampus and amygdala), deep gray (includes thalamus and basal ganglia), white matter, brainstem, cerebellum, and cerebrospinal fluid (Shi, Yap, Wu, Jia, Gilmore *et al.*, 2011). Our method iteratively optimized both template shape and appearance to estimate an average brain that best represented the expected anatomy in the cohort (Tustison, Cook, Klein, Song, Das *et al.*, 2014). See Figure 1 for segmentation process illustration. Estimation of hippocampal volume was not performed because variability in qualitative and quantitative aspects of existing manual segmentation protocols leads to significant disagreement in measured volumes of hippocampal and parahippocampal substructures (Yushkevich, Amaral, Augustinack, Bender, Bernstein *et al.*, 2015).

Diffeomorphic image registration (SyN algorithm, implemented in ANTs; Avants, Tustison, Stauffer, Song, Wu *et al.*, 2014; Tustison *et al.*, 2014) was used to map



LOW RESOLUTION COLOR FIG

Figure 1 *Segmentation process for images from infants at age 5 weeks. Six spatial probability functions (priors) define the voxel-wise probability of distinct tissue/anatomical classes: (1) Cortical Gray (includes the hippocampus and amygdala); (2) Deep Gray (includes thalamus and basal ganglia); (3) White Matter; (4) Brainstem; (5) Cerebellum; and (6) Cerebrospinal Fluid. Column A (left) shows the template and 4 of 6 priors used for segmentation process. Column B (right) shows the subject image before and after segmentation with the priors. Cortical Gray is shown in green. Deep gray is shown in yellow. White matter is shown in blue. CSF is shown in red. Brain stem and cerebellum not shown.*

between template and subject space. This mapping was used to transfer the six template prior probability maps into the space of the individual's T2 MRI. T1 and diffusion-weighted MRI also were mapped into the space of the T2 via a low-dimensional registration. These modalities were complemented by the Laplacian of the T2 image to form a rich feature space for basis of 6-tissue multivariate segmentation. The final segmentation procedure incorporated both T2 and T1 features with the probability maps via a Bayesian tissue segmentation algorithm, Atropos (Tustison *et al.*, 2014).

To verify quality, each segmentation was visually inspected, along with the original T1 and T2 data, and

data were reviewed for motion artifact. To assist successful 6-tissue segmentation, we first used joint label fusion to perform brain extraction (MICCAI Society, 2013; Wang, Suh, Das, Pluta, Craige *et al.*, 2013). Final tissue segmentation was performed within this brain mask defined by the labels available from the Makropoulous cohort (Makropoulos, Gousias, Ledig, Aljabar, Serag *et al.*, 2014). The full processing pipeline is publicly available (Avants *et al.*, 2014; Tustison *et al.*, 2014). MRI data for this report include cortical gray, deep gray, and white matter volumes. Examiners were masked to SES status.

Analyses

Preliminary analyses included SES group comparisons of maternal and child characteristics using *t*-tests and chi square analysis. Pearson correlations tested associations between demographic and MRI variables. Main analyses consisted of hierarchical linear regressions using the SES composite as a continuous variable to examine SES effects on neural outcomes. Covariates were birth weight and post-conception age at scan (at this age more predictive of developmental maturity than post-natal age) (Hanson *et al.*, 2013; Martin, Fanaroff & Walsh, 2011). Analyses were performed using SPSS 22.0.

Results

Of 46 scans completed, data from two subjects (both ITN of 1 and maternal high school education) were not utilized due to motion and poor resolution. Characteristics at time of enrollment and MRI are shown in Table 1 for the 44 participants with successful scans (25 Low SES, 19 Higher SES). Low SES mothers were younger than Higher SES mothers and, per enrollment criteria, reported less education. Also per enrollment criteria, ITN category for the Low SES group was 1 and for the Higher SES group was 2 or greater (74% ITN = 2, 26% ITN ≥ 3). Infant birth characteristics and age at time of MRI were similar.

Correlations between cortical gray, deep gray, and white matter volumes and participant characteristics are shown in Table 2. Cortical gray matter volume correlated with the SES Composite, ITN, maternal education, gestational age, birth weight, head circumference and length, and post-conception age at MRI. Deep gray matter volume correlated with the SES Composite, maternal education, birth weight, head circumference and length and post-conception age at MRI. White matter volume correlated with only birth weight, head circumference and post-conception age at time of MRI.

Table 1 *Infant characteristics at time of enrollment and MRI by SES group*

	Low SES group $n = 25$	Higher SES group $n = 19$	p-value
Enrollment characteristics			
Mother's age, yr	24.1 ± 4.9[a]	27.1 ± 5.6	<.001
ITN			
Below poverty line	25 (100%)	0	
Above the poverty line	0	19 (100%)	
Mother's education			<.001
1. Less than high school	16 (64%)[b]	0	
2. High school/GED	6 (24%)	3 (16%)	
3. Technical/Vocational	3 (12%)	1 (5%)	
4. Some college	0	5 (26%)	
5. Two-year degree	0	5 (26%)	
6. Four-year degree	0	4 (21%)	
7. Some graduate school	0	0	
8. MA, PhD, Professional	0	1 (5%)	
Gestational age, weeks	39.4 ± 1.0	39.6 ± 0.9	.35
Birth weight, kg	3.29 ± 0.44	3.42 ± 0.44	.36
Birth HC[c], cm	33.5 ± 1.3	34.0 ± 1.4	.33
Birth length, cm	50.2 ± 2.3	50.3 ± 2.3	.91
1-month characteristics			
Age at MRI			
Post-conception, wks	44.7 ± 0.5	45.0 ± 0.9	.17
Post-natal, wks	5.0 ± 0.9	5.0 ± 1.2	.90

[a]mean ± *SD*, [b]*n* (%); [c]Head circumference.

Table 2 *Correlations between cortical volumes and participant characteristics*

	Cortical gray matter	Deep gray matter	White matter
SES Composite	0.38 (0.01)[a]	0.34 (0.024)	0.25 (0.096)
Income-to-needs	0.37 (0.014)	0.28 (0.063)	0.11 (0.48)
Maternal education	0.41 (0.006)	0.34 (0.022)	0.22 (0.15)
Paternal education	0.13 (0.40)	0.27 (0.076)	0.22 (0.15)
Maternal age	−0.069 (0.66)	0.16 (0.29)	−0.043 (0.78)
Gestational age	0.30 (0.046)	0.19 (0.214)	0.18 (0.23)
Birth weight	0.64 (0.000)	0.47 (0.001)	0.53 (0.000)
Head circumference	0.64 (0.000)	0.46 (0.002)	0.45 (0.003)
Birth length	0.30 (0.050)	0.31 (0.047)	0.16 (0.32)
Age at MRI			
Post-conception, wks	0.49 (0.001)	0.40 (0.007)	0.48 (0.001)
Post-natal, wks	0.078 (0.61)	0.068 (0.66)	0.12 (0.46)

[a]Pearson *r* (*p*-value), $n = 44$.

To examine the relations between SES and volumes of cortical gray, deep gray, and white matter, three hierarchical linear regressions were conducted for each outcome, controlling for post-conception age and birth weight (Hanson *et al.*, 2013; Martin *et al.*, 2011). In the first step of each regression, birth weight and post-conception age at MRI were entered stepwise (Model 1). In the second step (Model 2) the SES Composite was added to the regression.

For cortical gray matter, in Model 1, birth weight, but not age at MRI, was retained in the model (R^2 = 0.38, F (1, 42) = 25.17, p < .001). Addition of the SES Composite in Model 2 resulted in a significant increase in variance accounted for by the model (ΔR^2 = 0.082, F(1, 41) = 6.21, p = .017). In the regression on deep gray matter volume, birth weight but not MRI age was retained in Model 1 (R^2 = 0.22, F(1, 42) = 1.87, p = .001). Adding SES improved the model significantly (ΔR^2 = 0.073, F(1, 41) = 4.22, p = .046). In the regression for white matter volume, birth weight and MRI age were retained after the stepwise entry in Model 1 (R^2 = 0.32, F(1, 41) = 9.85, p < .001). The addition of SES in Model 2 did not significantly improve the model (ΔR^2 = 0.015, F(1, 40) = 6.85, p = .35). Table 3 shows the regression statistics for the models for each outcome. Figure 2 illustrates the positive relationships between SES and cortical gray and deep gray matter volumes adjusted for variables retained in the final models.

We did not examine the effects of SES components, income and education on brain volumes independently of one another as these two variables were highly correlated (r = 0.86, p < .001).

Table 3 *Hierarchical linear regression analyses predicting cortical gray matter, deep gray matter and white matter volumes*

	Cortical gray matter	Deep gray matter	White matter
Model 1			
Age at MRI*	–	–	0.30 (0.044)
Birth weight	0.61 (.000)**	0.61 (0.000)	0.36 (0.019)
R^2	0.38	0.22	0.32
F (df)	25.17 (1,42)	11.87 (1,42)	9.85 (1,41)
p-value	<.001	.001	<.001
Model 2			
Age at MRI*	–	–	0.27 (0.077)
Birth weight	0.57 (0.000)	0.43 (0.003)	0.35 (0.021)
SES Composite	0.29 (0.017)	0.27 (0.046)	0.13 (0.35)
R^2	0.46	0.29	0.34
ΔR^2	0.082	0.073	0.015
F (*df*)	6.21 (1,41)	4.22 (1,41)	6.85 (1,40)
p-value	0.017	0.046	0.35

*Post-conception, wks. **Standardized regression coefficient (p-values, 2-tailed). Model 2 Predictor: SES Composite.

Discussion

In this cohort of healthy term female African-American infants, MRI showed SES-dependent differences in gray matter volume at the young age of 5 weeks with effects being present along the lower range of the distribution of SES. Both cortical gray matter, which includes the cortex of the two hemispheres and hippocampi, and deep gray matter, which includes the thalamus and basal ganglia, were significantly smaller in low SES infants. No difference was observed in white matter volume. While low SES is associated with lower birth weights and increased risk for prematurity, both of which are closely linked to brain development (Aber, Bennett, Conley & Li, 1997; Osofsky, 1974; Parker, Greer & Zuckerman, 1988), the present results are from a cohort of healthy term infants showing SES effects on brain development independent of birth weight and post-conception age.

The results reported here both add to a growing consensus that SES impacts brain development and push back the age at which such effects can be observed from early childhood to early infancy. To our knowledge no other studies have examined this relationship as early as 5 weeks of age. Two studies, however, have reported functional brain activity differences within the first year of life: Tomalski *et al.* (2013) reported EEG differences

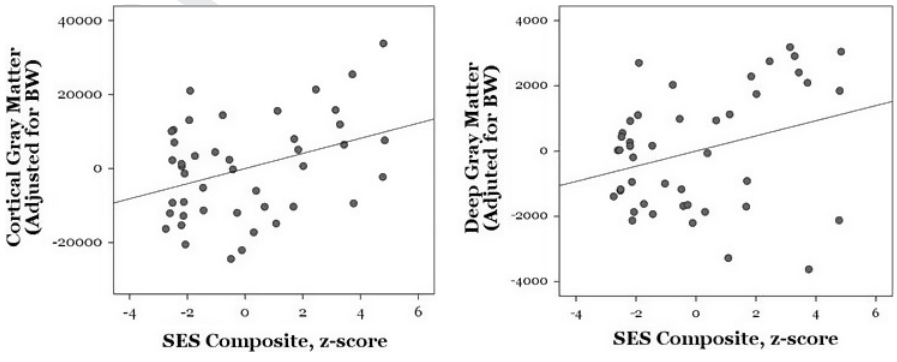


Figure 2 *SES predicts MRI volumes at age 1 month. In final models, higher levels of SES were associated with larger cortical gray and deep gray matter volumes. X-axis shows z-scores for the SES Composite. Y-axis shows residual values of each dependent variable after adjustment for birth weight.*

between low and middle SES infants between 6 and 9 months of age; Gao *et al*. (2015) reported marginal effects of SES on fMRI resting functional connectivity at 6 months of age (Gao, Alcauter, Elton, Hernandez-Castillo, Smith *et al*., 2015; Tomalski, Moore, Ribeiro, Axelsson, Murphy *et al*., 2013). The current results show that SES effects are manifest in the brain at an even earlier age. In addition, because findings are not dependent on arousal, distress, sleep deprivation or other states that affect functional measures, results reported here point more decisively to *anatomical* differences in brain development.

The timing of the emergence of SES effects can be informative as to their causes. Possible pre- or post-natal etiologies include the effects of maternal health, toxin exposure, nutrition, sleep quality or stress (Boyce & Kobor, 2015; Buss, Lord, Wadiwalla, Hellhammer, Lupien *et al*., 2007; Cordero, 1990; DiPietro, 2012; Hackman *et al*., 2010). Subjects in the present study were 5 weeks of age at time of scan, minimizing the opportunity for postnatal influence, however, such influences cannot be ruled out. Future studies utilizing MRI immediately after birth are needed to distinguish the pre- and post-natal etiologies of SES effects.

Furthermore, differences present at birth may result from prenatal factors, known to vary with SES, or from genetic factors, or from their interaction (DiPietro, Kivlighan, Costigan, Rubin, Shiffler *et al*., 2010). The influence of genes on gray matter has been reported (Knickmeyer, Wang, Zhu, Geng, Woolson *et al*., 2014); however, the relation among genes, SES influences, and neural outcome has yet to be explored. Given our results, investigations of these relations should be conducted not only for older children, but also for those at very early stages of development.

Different components of SES may impact brain development (Brito & Noble, 2014). The present study was not designed to parse the relative effects of income and education on brain structure. However, in larger samples of older children, individual effects of income and education have been evaluated and results have been mixed. For example, Hanson *et al*. (2011) reported an association between lower household income and lower total gray matter volume, with no influence of maternal education. Using a subset of the same cohort, Lawson *et al*. (2013) found an association between cortical thickness in frontal regions of interest and maternal and paternal education but not family income. Across ages 3–20, family income showed stronger associations with surface area than education in a large cohort from a broad range of SES (Noble *et al*., 2015). Studies with larger cohorts of very young infants are needed to evaluate the relative effects of SES components at young ages.

Our study, for which the long-term goal is examination of effects of SES disparity on neural and developmental outcome, joins a growing number of investigations examining brain structure and outcome of infants and young children. The relation between neural status at 1 month of age and subsequent cognitive outcome was reported by Spann *et al*. (2014) in 33 infants; associations between cerebral surface morphology and subsequent motor, language, and cognitive scores were reported. Can *et al*. (2013), in 19 infants, scanned at 7 months and evaluated at 12 months, found relations between early gray matter and white matter concentration and language skills. Amygdala volume was found to be related to language outcome in infants scanned at 6 months and evaluated at 2 years (Ortiz-Mantilla, Choe, Flax, Grant & Benasich, 2010), with another investigation showing an association of white matter microstructure and infant working memory in infants imaged at 12 months (Short *et al*., 2013). These researchers, however, did not examine SES effect on the relationship between neural development and cognitive outcome in their higher SES cohorts. Our data showing effects of SES on neural development at 1 month of age will be combined with later neural and cognitive evaluations to explore such SES effects.

Limitations of this study are several. First, our eligibility requirements, chosen to increase power by eliminating the need to control for the influential confounders gender (Giedd, Castellanos, Rajapakse, Vaituzis & Rapoport, 1997) and race/ethnicity (Bai, Abdul-Rahman, Rifkin-Graboi, Chong, Kwek *et al*., 2012), impose predictable limitations on generalizability. Regardless, findings inform for an understudied minority, and provide a template for exploration of neural outcome at very young ages in other cohorts. Second, sample size may be considered a limitation; however, a cohort of 44 infants scanned at 1 month of age without sedation in a study evaluating effect of SES disparity is, to our knowledge, unique. While motion artifact is a common challenge in infant imaging studies, only two of the 46 successful scans were excluded due to motion, a relatively high success rate (Almli, Rivkin & McKinstry, 2007; Shi *et al*., 2011). Third, we do not have a robust prenatal database for this cohort that would allow for a careful evaluation of prenatal influences on gray and deep gray matter outcomes. Finally, we do not yet have data for evaluation of whether effects on neural outcomes detected at 1 month change by 12 months, or whether there are relationships between volumetric findings and infant cognitive outcomes; however, our ongoing longitudinal follow-up will allow for these analyses.

Conclusions

In this cohort of term healthy African-American females, lower SES was associated with smaller cortical gray and deep gray matter volumes at age 4–6 weeks. These differences in neural structure are early indicators of increased risk for disadvantage in cognitive and academic skills faced by poor children (Kolb, Mychasiuk & Gibb, 2014). On the other hand, it also is well established that early intervention and enriched environments can ameliorate compromised developmental outcomes (Brooks-Gunn, Klebanov, Liaw & Spiker, 1993; Campbell, Pungello, Miller-Johnson, Burchinal & Ramey, 2001). These findings underscore the need to monitor and optimize development of our youngest through programs and policies directed at reducing impact of SES disparities (Heckman & Mastrov, 2007; Knudsen, Heckman, Cameron & Shonkoff, 2006; Shonkoff, Garner, Siegel, Dobbins, Earls *et al*., 2012). The existence of SES differences so early in life suggests that intervention cannot begin too soon in supporting families with young children (Austin, Lemon & Leer, 2005; Raikes, Green, Atwater, Kisker, Constantine *et al*., 2006; Tamis-LeMonda, Bornstein & Baumwell, 2001). Current efforts directed toward reduction of risks posed by SES disparity are focused on the preschool years, possibly well after early foundational neural growth (Spann *et al*., 2014); we suggest increased focus during infancy.

Acknowledgement

All phases of this study were supported by NIH/NICHD: R21HD072461.

Financial disclosure

The authors have no financial relationships relevant to this article to disclose.

Conflict of interest

The authors have no conflicts of interest to disclose.

References

Aber, J.L., Bennett, N.G., Conley, D.C., & Li, J. (1997). The effects of poverty on child health and development. *Annual Review of Public Health*, **18**, 463–483.

Almli, C.R., Rivkin, M.J., & McKinstry, R.C. (2007). The NIH MRI study of normal brain development (Objective-2): newborns, infants, toddlers, and preschoolers. *NeuroImage*, **35** (1), 308–325.

Austin, M.J., Lemon, K., & Leer, E. (2005). Promising practices for meeting the multiple needs of low-income families in poverty neighborhoods. *Journal of Health & Social Policy*, **21** (1), 95–117.

Avants, B.B., Tustison, N.J., Stauffer, M., Song, G., Wu, B. *et al.* (2014). The Insight ToolKit image registration framework. *Frontiers in Neuroinformatics*, **8**, 44.

Bai, J., Abdul-Rahman, M.F., Rifkin-Graboi, A., Chong, Y.-S., Kwek, K. *et al.* (2012). Population differences in brain morphology and microstructure among Chinese, Malay, and Indian neonates. *PLoS ONE*, **7** (10), e47816.

Boyce, W.T., & Kobor, M.S. (2015). Development and the epigenome: the 'synapse' of gene–environment interplay. *Developmental Science*, **18** (1), 1–23.

Brain-Development-Cooperative-Group (2012). Total and regional brain volumes in a population-based normative sample from 4 to 18 years: the NIH MRI Study of Normal Brain Development. *Cerebral Cortex*, **22** (1), 1–12.

Brito, N.H., & Noble, K.G. (2014). Socioeconomic status and structural brain development. *Frontiers in Neuroscience*, **8**, 276.

Brooks-Gunn, J., Klebanov, P.K., Liaw, F., & Spiker, D. (1993). Enhancing the development of low-birthweight, premature infants: changes in cognition and behavior over the first three years. *Child Development*, **64** (3), 736–753.

Buss, C., Lord, C., Wadiwalla, M., Hellhammer, D.H., Lupien, S.J. *et al.* (2007). Maternal care modulates the relationship between prenatal risk and hippocampal volume in women but not in men. *Journal of Neuroscience*, **27** (10), 2592–2595.

Campbell, F.A., Pungello, E.P., Miller-Johnson, S., Burchinal, M., & Ramey, C.T. (2001). The development of cognitive and academic abilities: growth curves from an early childhood educational experiment. *Developmental Psychology*, **37** (2), 231–242.

Can, D.D., Richards, T., & Kuhl, P.K. (2013). Early gray-matter and white-matter concentration in infancy predict later language skills: a whole brain voxel-based morphometry study. *Brain and Language*, **124** (1), 34–44.

Canada, G. (2014). A new war with a clear emphasis [Electronic Version]. *The New York Times*. Available at: http://www.nytimes.com/roomfordebate/2014/01/05/does-the-us-need-another-war-on-poverty/a-new-war-on-poverty-with-a-clear-emphasis.

Cordero, J.F. (1990). Effect of environmental agents on pregnancy outcomes: disturbances of prenatal growth and development. *Medical Clinics of North America*, **74** (2), 279–290.

Deoni, S.C., O'Muircheartaigh, J., Elison, J.T., Walker, L., & Doernberg, E. *et al*. (2014). White matter maturation profiles through early childhood predict general cognitive ability. *Brain Structure & Function*, 29 November. doi: PMID:25432771

DiPietro, J.A. (2012). Maternal stress in pregnancy: considerations for fetal development. *Journal of Adolescent Health*, **51** (Suppl. 2), S3–S8.

DiPietro, J.A., Kivlighan, K.T., Costigan, K.A., Rubin, S.E., Shiffler, D.E. *et al.* (2010). Prenatal antecedents of newborn neurological maturation. *Child Development*, **81** (1), 115–130.

Dubois, J., Dehaene-Lambertz, G., Kulikova, S., Poupon, C., Hüppi, P.S. *et al.* (2014). The early development of brain white matter: a review of imaging studies in fetuses, newborns and infants. *Neuroscience*, **276**, 48–71.

Entwislea, D.R., & Astone, N.M. (1994). Some practical guidelines for measuring youth's race/ethnicity and socioeconomic status. *Child Development*, **65** (6), 1521–1540.

Farah, M.J., Shera, D.M., Savage, J.H., Betancourt, L., Giannetta, J.M. *et al.* (2006). Childhood poverty: specific associations with neurocognitive development. *Brain Research*, **1110** (1), 166–174.

Fernald, A., Marchman, V.A., & Weisleder, A. (2013). SES differences in language processing skill and vocabulary are evident at 18 months. *Developmental Science*, **16** (2), 234–248.

Gao, W., Alcauter, S., Elton, A., Hernandez-Castillo, C.R., Smith, J.K. *et al.* (2015). Functional network development during the first year: relative sequence and socioeconomic correlations. *Cerebral Cortex*, **25** (9), 2919–2928.

Giedd, J.N., Castellanos, F.X., Rajapakse, J.C., Vaituzis, A.C., & Rapoport, J.L. (1997). Sexual dimorphism of the developing human brain. *Progress in Neuro-psychopharmacology & Biological Psychiatry*, **21** (8), 1185–1201.

Gilmore, J.H., Lin, W., Prastawa, M.W., Looney, C.B., Vetsa, Y.S. *et al.* (2007). Regional gray matter growth, sexual dimorphism, and cerebral asymmetry in the neonatal brain. *Journal of Neuroscience*, **27** (6), 1255–1260.

Goodman, E. (1999). The role of socioeconomic status gradients in explaining differences in US adolescents' health. *American Journal of Public Health*, **89** (10), 1522–1528.

Hackman, D.A., Farah, M.J., & Meaney, M.J. (2010). Socioeconomic status and the brain: mechanistic insights from human and animal research. *Nature Reviews Neuroscience*, **11** (9), 651–659.

Hanson, J.L., Chandra, A., Wolfe, B.L., & Pollak, S.D. (2011). Association between income and the hippocampus. *PLoS ONE*, **6** (5), e18712.

Hanson, J.L., Hair, N., Shen, D.G., Shi, F., Gilmore, J.H. *et al.* (2013). Family poverty affects the rate of human infant brain growth. *PLoS ONE*, **8** (12), e80954.

Hanson, J.L., Nacewicz, B.M., Sutterer, M.J., Cayo, A.A., Schaefer, S.M. *et al.* (2015). Behavioral problems after early life stress: contributions of the hippocampus and amygdala. *Biological Psychiatry*, **77** (4), 314–323.

Heckman, J.J., & Mastrov, D. (2007). The productivity argument for investing in young children. *Review of Agricultural Economics*, **29** (3), 446–493.

Holland, D., Chang, L., Ernst, T.M., Curran, M., Buchthal, S.D. *et al.* (2014). Structural growth trajectories and rates of change in the first 3 months of infant brain development. *JAMA Neurology*, **71** (10), 1266–1274.

Jednorog, K., Altarelli, I., Monzalvo, K., Fluss, J., Dubois, J. *et al.* (2012). The influence of socioeconomic status on children's brain structure. *PLoS ONE*, **7** (8), e42486.

Kessler, R.C., Berglund, P., Demler, O., Jin, R., Merikangas, K.R. *et al.* (2005). Lifetime prevalence and age-of-onset distributions of DSM-IV disorders in the National Comorbidity Survey Replication. *Archives of General Psychiatry*, **62** (6), 593–602.

Knickmeyer, R.C., Gouttard, S., Kang, C., Evans, D., Wilber, K. *et al.* (2008). A structural MRI study of human brain development from birth to 2 years. *Journal of Neurosciece*, **28** (47), 12176–12182.

Knickmeyer, R.C., Wang, J., Zhu, H., Geng, X., Woolson, S. *et al.* (2014). Common variants in psychiatric risk genes predict brain structure at birth. *Cerebral Cortex*, **24** (5), 1230–1246.

Knudsen, E.I., Heckman, J.J., Cameron, J.L., & Shonkoff, J.P. (2006). Economic, neurobiological, and behavioral perspectives on building America's future workforce. *Proceedings of the National Academy of Sciences of the United States of America*, **103** (27), 10155–10162.

Kolb, B., Mychasiuk, R., & Gibb, R. (2014). Brain development, experience, and behavior. *Pediatric Blood & Cancer*, **61** (10), 1720–1723.

Landry, S.H., Smith, K.E., & Swank, P.R. (2002). Environmental effects on language development in normal and high-risk child populations. *Seminars in Pediatric Neurology*, **9** (3), 192–200.

Lange, N., Froimowitz, M.P., Bigler, E.D., & Lainhart, J.E. (2010). Associations between IQ, total and regional brain volumes, and demography in a large normative sample of healthy children and adolescents. *Developmental Neuropsychology*, **35** (3), 296–317.

Lawson, G.M., Duda, J.T., Avants, B.B., Wu, J., & Farah, M.J. (2013). Associations between children's socioeconomic status and prefrontal cortical thickness. *Developmental Science*, **16** (5), 641–652.

Lipina, S.J., Martelli, M.I., Vuelta, B., & Colombo, J.A. (2005). Performance on the A-not-B task of Argentinean infants from unsatisfied and satisfied basic needs homes. *Interamerican Journal of Psychology*, **39** (1), 49–60.

Luby, J., Belden, A., Botteron, K., Marrus, N., Harms, M.P. *et al.* (2013). The effects of poverty on childhood brain development: the mediating effect of caregiving and stressful life events. *JAMA Pediatrics*, **167** (12), 1135–1142.

Makropoulos, A., Gousias, I., Ledig, C., Aljabar, P., Serag, A. *et al.* (2014). Automatic whole brain MRI segmentation of the developing neonatal brain. *IEEE Transactions on Medical Imaging*, **33** (9), 1818–1831.

Martin, R.J., Fanaroff, A.A., & Walsh, M.C. (2011). *Neonatal-perinatal medicine: Diseases of the fetus and infant* (9th edn.). St. Louis, MO: Elsevier Mosby.

MICCAI Society (2013). MICCAI Challenge Workshop on Segmentation: Algorithms, Theory and Applications ('SATA').

Nisbett, R.E., Aronson, J., Blair, C., Dickens, W., Flynn, J. *et al.* (2012). Intelligence: new findings and theoretical developments. *American Psychologist*, **67** (2), 130–159.

Noble, K.G., Houston, S.M., Brito, N.H., Bartsch, H., Kan, E. *et al.* (2015). Family income, parental education and brain

structure in children and adolescents. *Nature Neuroscience*, **18**, 773–778.

Noble, K.G., Houston, S.M., Kan, E., & Sowell, E.R. (2012). Neural correlates of socioeconomic status in the developing human brain. *Developmental Science*, **15** (4), 516–527.

Noble, K.G., McCandliss, B.D., & Farah, M.J. (2007). Socioeconomic gradients predict individual differences in neurocognitive abilities. *Developmental Science*, **10** (4), 464–480.

Noble, K.G., Norman, M.F., & Farah, M.J. (2005). Neurocognitive correlates of socioeconomic status in kindergarten children. *Developmental Science*, **8** (1), 74–87.

Ortiz-Mantilla, S., Choe, M.S., Flax, J., Grant, P.E., & Benasich, A.A. (2010). Associations between the size of the amygdala in infancy and language abilities during the preschool years in normally developing children. *NeuroImage*, **49** (3), 2791–2799.

Osofsky, H.J. (1974). Poverty, pregnancy outcome, and child development. *Birth Defects*, **10**, 37–50.

Parker, S., Greer, S., & Zuckerman, B. (1988). Double jeopardy: the impact of poverty on early child development. *Pediatric Clinics of North America*, **35** (6), 1227–1240.

Raikes, H., Green, B.L., Atwater, J., Kisker, E., Constantine, J. *et al*. (2006). Involvement in Early Head Start home visiting services: demographic predictors and relations to child and parent outcomes. *Early Childhood Research Quarterly*, **21** (1), 2–24.

Raizada, R.D., Richards, T.L., Meltzoff, A., & Kuhl, P.K. (2008). Socioeconomic status predicts hemispheric specialisation of the left inferior frontal gyrus in young children. *NeuroImage*, **40** (3), 1392–1401.

Shi, F., Yap, P.T., Wu, G., Jia, H., Gilmore, J.H. *et al*. (2011). Infant brain atlases from neonates to 1- and 2-year-olds. *PLoS ONE*, **6** (4), e18746.

Shonkoff, J.P., Garner, A.S., Siegel, B.S., Dobbins, M.I., Earls, M.F. *et al*. (2012). The lifelong effects of early childhood adversity and toxic stress. *Pediatrics*, **129** (1), e232–e246.

Short, S.J., Elison, J.T., Goldman, B.D., Styner, M., Gu, H. *et al*. (2013). Associations between white matter microstructure and infants' working memory. *NeuroImage*, **64**, 156–166.

Sirin, S.R. (2005). Socioeconomic status and academic achievement: a meta-analytic review of research. *Review of Educational Research*, **75** (3), 417–453.

Spann, M.N., Bansal, R., Rosen, T.S., & Peterson, B.S. (2014). Morphological features of the neonatal brain support development of subsequent cognitive, language, and motor abilities. *Human Brain Mapping*, **35** (9), 4459–4474.

Tamis-LeMonda, C.S., Bornstein, M.H., & Baumwell, L. (2001). Maternal responsiveness and children's achievement of language milestones. *Child Development*, **72** (3), 748–767.

Tomalski, P., Moore, D.G., Ribeiro, H., Axelsson, E.L., Murphy, E. *et al.* (2013). Socioeconomic status and functional brain development: associations in early infancy. *Developmental Science*, **16** (5), 676–687.

Tustison, N.J., Cook, P.A., Klein, A., Song, G., Das, S.R. *et al*. (2014). Large-scale evaluation of ANTs and FreeSurfer cortical thickness measurements. *NeuroImage*, **99**, 166–179.

Uda, S., Matsui, M., Tanaka, C., Uematsu, A., Miura, K. *et al.* (2015). Normal development of human brain white matter from infancy to early adulthood: a diffusion tensor imaging study. *Developmental Neuroscience*, **37** (2), 182–194.

US Census Bureau (2013, 14 February). Poverty. Available at: http://www.census.gov/hhes/www/poverty/methods/definitions.html

Waber, D.P., De Moor, C., Forbes, P.W., Almli, C.R., Botteron, K.N. *et al.* (2007). The NIH MRI study of normal brain development: performance of a population based sample of healthy children aged 6 to 18 years on a neuropsychological battery. *Journal of the International Neuropsychological Society*, **13** (5), 729–746.

Wang, H.Z., Suh, J.W., Das, S.R., Pluta, J.B., Craige, C. *et al.* (2013). Multi-atlas segmentation with joint label fusion. *IEEE Transactions on Pattern Analysis and Machine Intelligence*, **35** (3), 611–623.

Wild, K.T., Betancourt, L.M., Brodsky, N.L., & Hurt, H. (2013). The effect of socioeconomic status on the language outcome of preterm infants at toddler age. *Early Human Development*, **89** (9), 743–746.

Yushkevich, P.A., Amaral, R.S., Augustinack, J.C., Bender, A.R., Bernstein, J.D. *et al.* (2015). Quantitative comparison of 21 protocols for labeling hippocampal subfields and parahippocampal subregions in in vivo MRI: Towards a harmonized segmentation protocol. *NeuroImage*, **111**, 526–541.

Received: 6 January 2015
Accepted: 16 June 2015

Graphical Abstract

The contents of this page will be used as part of the graphical abstract of html only. It will not be published as part of main article.

4 xxxx.

Author Query Form

Journal: DESC
Article: 12344

Dear Author,
During the copy-editing of your paper, the following queries arose. Please respond to these by marking up your proofs with the necessary changes/additions. Please write your answers on the query sheet if there is insufficient space on the page proofs. Please write clearly and follow the conventions shown on the attached corrections sheet. If returning the proof by fax do not write too close to the paper's edge. Please remember that illegible mark-ups may delay publication. Many thanks for your assistance.

Query reference	Query	Remarks
1	**AUTHOR: Please confirm that given names (red) and surnames/family names (green) have been identified correctly.**	
2	**AUTHOR: Figure 1 has been saved at a low resolution of 109 dpi. Please resupply at 600 dpi. Check required artwork specifications at http://authorservices.wiley.com/bauthor/illustration.asp**	
3	**AUTHOR: Figure 2 has been saved at a low resolution of 160 dpi. Please resupply at 600 dpi. Check required artwork specifications at http://authorservices.wiley.com/bauthor/illustration.asp**	
4	**AUTHOR: The journal style is to include a graphical abstract for online publication. The graphical abstract requires a figure along with blurb content for the figure. Please designate the figure to be included as part of graphical abstract and provide 2-3 lines of text as blurb content that summarises the entire article.**	

MARKED PROOF

Please correct and return this set

Please use the proof correction marks shown below for all alterations and corrections. If you wish to return your proof by fax you should ensure that all amendments are written clearly in dark ink and are made well within the page margins.

Instruction to printer	*Textual mark*	*Marginal mark*
Leave unchanged	··· under matter to remain	New matter followed by
Insert in text the matter indicated in the margin		or (x2)
Delete	through single character, rule or underline or through all characters to be deleted	or (x2)
Substitute character or substitute part of one or more word(s)	through letter or through characters	new character or new characters
Change to italics	under matter to be changed	
Change to capitals	under matter to be changed	
Change to small capitals	under matter to be changed	
Change to bold type	under matter to be changed	
Change to bold italic	under matter to be changed	
Change to lower case	Encircle matter to be changed	
Change italic to upright type	(As above)	
Change bold to non-bold type	(As above)	
Insert 'superior' character	through character or where required	or under character e.g. or
Insert 'inferior' character	(As above)	over character e.g.
Insert full stop	(As above)	
Insert comma	(As above)	
Insert single quotation marks	(As above)	or and/or or
Insert double quotation marks	(As above)	or and/or or
Insert hyphen	(As above)	
Start new paragraph		
No new paragraph		
Transpose		
Close up	linking characters	
Insert or substitute space between characters or words	through character or where required	
Reduce space between characters or words	between characters or words affected	