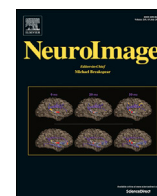


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Sex differences associated with corpus callosum development in human infants: A longitudinal multimodal imaging study



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ARTICLE INFO

Keywords:

Corpus callosum
Sexual dimorphism
Development
Brain imaging
Infants

ABSTRACT

The corpus callosum (CC) is the largest connective pathway in the human brain, linking cerebral hemispheres. There is longstanding debate in the scientific literature whether sex differences are evident in this structure, with many studies indicating the structure is larger in females. However, there are few data pertaining to this issue in infancy, during which time the most rapid developmental changes to the CC occur. In this study, we examined longitudinal brain imaging data collected from 104 infants at ages 6, 12, and 24 months. We identified sex differences in brain-size adjusted CC area and thickness characterized by a steeper rate of growth in males versus females from ages 6–24 months. In contrast to studies of older children and adults, CC size was larger for male compared to female infants. Based on diffusion tensor imaging data, we found that CC thickness is significantly associated with underlying microstructural organization. However, we observed no sex differences in the association between microstructure and thickness, suggesting that the role of factors such as axon density and/or myelination in determining CC size is generally equivalent between sexes. Finally, we found that CC length was negatively associated with nonverbal ability among females.

1. Introduction

The corpus callosum (CC) is the primary anatomical connection between brain hemispheres, consisting of several hundred million fibers (Tomasch, 1954). The structure is unique to eutherian mammals (Mihreshahi, 2006) and has been studied in detail in human and nonhuman primates (Hofer et al., 2008; Sakai et al., 2017a). The CC forms in utero (Kostović and Jovanov-Milošević, 2006) and develops across the

lifespan. The most dramatic changes to this structure occur during the first years of life, followed by a period of growth that extends through childhood before plateauing post-adolescence (Rauch and Jinkins, 1994; Luders et al., 2010; Tanaka-Arakawa et al., 2015). While the majority of existing work has been based on cross-sectional data, there are longitudinal studies substantiating that CC growth rates vary by region and follow a rostral-caudal progression during later childhood (Giedd et al., 1999; Thompson et al., 2000; Westerhausen et al., 2016). Abnormalities

Abbreviations: CC, corpus callosum; DTI, diffusion tensor imaging; ELC, Early Learning Composite score; FoV, field of view; MP-RAGE, magnetization prepared rapid acquisition gradient echo; MSEL, Mullen Scales of Early Learning; RD, radial diffusivity; TBV, total brain volume.

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<https://doi.org/10.1016/j.neuroimage.2020.116821>

Received 3 December 2019; Received in revised form 21 February 2020; Accepted 27 March 2020

Available online 7 April 2020

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in CC development have been associated with numerous disorders that manifest during early childhood (Paul, 2011a), including autism (Wolff et al., 2015), epilepsy (Schneider et al., 2014), and many genetic syndromes (Edwards et al., 2014).

There is a longstanding debate in the scientific literature as to whether sex differences are evident in CC development. There is evidence of sexual dimorphism from cross-sectional in-vivo brain imaging and post-mortem studies of older children and adults (Rauch and Jinkins, 1994; Schmithorst et al., 2008), with the majority indicating that the structure is relatively larger in females (Johnson et al., 1994; Mitchell et al., 2003; Ardekani et al., 2013; Smith, 2005; De Lacoste-Utamsing and Holloway, 1982; Allen et al., 1991), though this trend is not consistent (Clarke et al., 1989; Elster et al., 1990). However, other studies have found no sex differences (Bastianello et al., 1994; Hasan et al., 2009; Bishopaand and Wahlsten, 1997), including when total brain size is accounted for (Bruner et al., 2012; Luders et al., 2014). These disparate findings may be attributed in part to whether and how overall brain size was accounted for as well as age ranges of study samples. More recent reports further indicate that sexual dimorphism in CC development may vary by subregion and, importantly, by age (Luders et al., 2010; Tana-ka-Arakawa et al., 2015; Schmithorst et al., 2008).

While CC growth has been well-characterized in older children and adults, relatively few brain imaging studies have examined its development in infants. Early cross-sectional studies suggest that the CC develops from a uniformly thin structure to an adult-like shape by about 8 months (Barkovich, Kjos). In a cross-sectional study of children ranging from neonates to age 18 years, Vannucci and colleagues (Vannucci et al., 2017) reported that the CC expanded significantly across infancy, including regions of the genu, body, and splenium. Regarding sex differences, the authors reported the only difference being splenium length-height ratio, which was larger in females under age 2. Using ultrasonography in a sample of 1- to 6-month olds, Chang and colleagues (Chang et al., 2018) found that female infants had a significantly thicker corpus callosum when measured prior to age 2 months, with differences between male and female infants no longer evident after this time. Both cross-sectional and longitudinal data indicate that CC size and white matter structural connectivity increase in approximately linear fashion through the first two years of life (Geng et al., 2012a; Kinney et al., 1988a; Sakai et al., 2017b). While CC development during infancy entails robust axon elimination (Lamantia and Rakic, 1990), net growth during this period may be attributed to axon caliber enlargement and rapid myelination (Kinney et al., 1988b), with the latter process reflected by previous MRI studies of infants and toddlers (Geng et al., 2012b; O'Muircheartaigh et al., 2014).

Understanding how the CC develops over the first years of life is likely to provide key insights given the unique pace and plasticity of neurodevelopment during this period. Moreover, several neurological disorders have differing prevalence in males and females, and have been associated with abnormalities in early CC development (Paul, 2011b). Thus, more detailed mapping of CC growth processes is required to create a normative reference overall and with regard to sex differences that may be apparent in CC growth trajectories. In this study, our primary objectives were (a) to characterize and compare trajectories of midsagittal CC structural development between female and male infants, and (b) examine the association between CC microstructural development, as measured by diffusion tensor imaging, and macrostructural development over the 6–24 month age interval. As a secondary aim, we conducted a limited set of analyses to determine whether features of CC development were associated with early cognitive development, and whether these associations differed by sex.

2. Materials & methods

2.1. Participants

Participating infants were drawn from the low familial risk for autism group of the NIH Infant Brain Imaging Study, an ongoing longitudinal

study of children at low- and high-familial risk for autism spectrum disorder. The parent study enrolled infants at either 6- or 12- months of age, and behavioral and brain imaging data were collected longitudinally at these time points and again at 24 months. Clinical data collection sites included the Children's Hospital of Philadelphia, University of North Carolina at Chapel Hill, University of Washington, and Washington University in St. Louis. All study procedures were approved by the respective institutional review boards at each clinical data collection site and carried out in accordance with the Declaration of Helsinki, with informed written consent obtained from parents or legal guardians.

All infants in the present study had completed cognitive and behavioral assessments at age 24 months and at least one MRI scan at 6, 12, or 24 months age. Exclusion criteria for this sample included: (a) evidence of a genetic, medical, or neurological condition known to affect brain development; (b) significant sensory impairment; (c) low birth weight (<2000 g) or premature birth (<36 weeks); (d) significant perinatal adversity or prenatal exposure to specific medications or neurotoxins; (e) contraindication for MRI; (f) predominant home language other than English; (g) children who were adopted, half siblings, or twins; and (h) first degree relative with a developmental disorder, psychosis, schizophrenia, or bipolar disorder; (i) did not meet diagnostic criteria for autism spectrum disorder at age 2 years. Given these criteria, our study sample comprised 104 typically developing children including 42 females and 62 males. Scan complement by sex at each time point is provided in the Supplementary Information (see Inline Supplementary Table 1).

General cognitive development was assessed by expert clinicians at age 24-months using the Mullen Scales of Early Learning (Mullen, 1995). The MSEL is a standardized developmental assessment that includes t-scores for five scales (gross motor, visual reception, fine motor, expressive language, and receptive language) and is appropriate for children 0–68 months of age. The measure provides an Early Learning Composite (ELC) score, which reflects overall cognitive/motor development.

2.2. Image acquisition

Magnetic resonance imaging scans were acquired during natural sleep using identical 3T Siemens TIM Trio scanners equipped with 12-channel head coils. The imaging protocol included: sagittal T1 MP-RAGE (TR = 2,400 ms, TE = 3.16 ms, slice thickness = 1 mm, FoV = 256 mm, 256x160 matrix), three-dimensional T2 fast spin echo (TR = 3,200 ms, TE = 499 ms, slice thickness = 1 mm, FoV = 256 mm, 256x160 matrix), and 25-direction ep2d_diff with FoV = 190 mm (6 and 12 months) or FoV = 209 mm (24 months), 75–81 transversal slices, slice thickness = 2 mm isotropic, 2x2x2mm3 voxel resolution, TR = 12800–13300 ms, TE = 102 ms, variable b-value 0–1000 s/mm². All scan data were reviewed by a pediatric neuroradiologist for clinically relevant abnormalities. Intra- and inter-site reliability was initially established and maintained across sites throughout the study by traveling human phantoms (Gouttard et al., 2008).

2.3. Image processing

2.3.1. Brain tissue segmentation

Grey and white tissue volumes were obtained using a framework of atlas-moderated expectation-maximization with co-registration of T1 and T2 weighted MRI images, skull stripping, bias correction, and multimodal tissue classification using AutoSeg (Wang et al., 2014). Population average templates and corresponding probabilistic brain tissue priors for grey and white matter were created for ages 6, 12, and 24 months. Grey and white matter tissue volumes for the cerebrum and cerebellum were summed to generate an estimate of total brain tissue volume (TBV).

2.3.2. CC area segmentation

Corpora callosa were cross-sectionally aligned with the midsagittal plane in normative atlas space. Sagittal slices within 2 mm of the

midsagittal plane were averaged to create a single 2-D image, with segmentation performed via the CCseg tool (Vachet et al., 2012), which employs a prior, statistical model of contour shape and image appearance of the C. Starting from average shape, CC contour is iteratively deformed to match image intensities while restricting deformations to the model shape statistics. Lastly, the contour is deformed without restrictions but only within a close neighborhood. The model used here was trained with data from an independent pediatric imaging study (Cascio et al., 2006). Through model deformation, this approach provided point-to-point correspondence of CC boundaries across subjects and time. Contours were visually inspected by a blind rater (T.S.) for quality of segmentation and manually corrected through re-initialization or insertion of a repulsion point (Vachet et al., 2012). Details on quality control procedures used for CC segmentations can be found in the Supplementary Materials.

2.3.3. CC length and thickness

These features were derived using a previously described framework (Wolff et al., 2015). Contour parameterization is transformed into a Process-Induced Symmetric Axis where CC length is represented by the medial axis between end points of the genu and splenium, with thickness (or width) attributed to each medial axis point (Sun et al., 2007). After resampling of the medial axis into 100 equidistant length intervals, we computed 5 regions for thickness based on the segmentation scheme proposed by Hofer and Frahm (2006). These projection regions were comprised of: 1) prefrontal; 2) pre-/supplementary motor; 3) motor; 4) sensory; and 5) parietal/temporal/occipital. Additional details on the procedures for creating CC boundaries, medial axis definition and location, and thickness measurements across age intervals have been previously described (Paul, 2011a).

2.3.4. Diffusion-weighted imaging

To investigate the microstructure underlying CC thickness, we generated radial diffusivity values (RD). This measure was selected over others (e.g. mean diffusivity), given greater putative sensitivity to key factors including, but not limited to, axon caliber and packing density (Klawiter et al., 2011). Deterministic tractography was performed using 5 label maps based on Hofer and Frahm (2006) and mirroring the segmentations used for CC thickness regions (Fig. 1, panel B). An image showing the entirety of an extracted corpus callosum from the

study-specific atlas using deterministic tractography is presented as an exemplar in Supplementary Materials, Supplementary Figure 1. Fiber track definitions were refined and processed with DTIAtlasFiberAnalyzer following a publicly available pipeline (Verde et al., 2014), with RD values reflecting a mean of the centermost 3 sagittal slices. See Supplementary Materials, for additional details related to diffusion data processing.

2.4. Statistical analysis

Longitudinal trajectories of CC morphology across ages 6, 12, and 24 months were analyzed using mixed-effects models for repeated measures with unstructured covariance matrices. Initial dependent measures included total CC area, thickness, and length. Independent variables of interest included sex, age, and the interaction of sex by age. Given its known relation to CC size, we controlled for TBV at each corresponding scan age (6, 12, and 24 months). Mother's education and MSEL Early Learning Composite score were evaluated as potential covariates but ultimately excluded as they did not improve model fit. Following the initial set of analyses, we next examined properties of CC thickness across five mutually exclusive regions to provide additional information on morphological features contributing to observed differences associated with sex, age, and the interaction thereof. Linear, quadratic, and log age terms were assessed for the base model of each CC measure. Based on fit statistics, a linear model was selected for total area, length, thickness, and thickness in regions 1, 2, and 5. A quadratic model was fit for thickness regions 2 and 4. In a final set of exploratory analyses, we examined MSEL composite scores at age 24 months in relation to concurrent CC area, thickness, and length using separate partial correlations (controlling for total brain size) for males and females. Analyses were performed using SPSS 23 (Chicago, IL) and RStudio (version 1.1.423).

3. Results

Descriptive and demographic data for study participants are presented in Table 1. Early Learning Composite standard scores from the MSEL at age 24 months were higher for females ($M = 116.1$, $SD = 14.9$) than males ($M = 108.1$, $SD = 13.9$), $t(102) = 2.8$, $p = 0.006$, $g = 0.55$. Proportion of mothers with a college degree or higher was equivalent between males and females, $\chi^2(1, N = 104) = 0.02$, $p = 0.89$. As anticipated, brain volume was significantly higher for males at all three visits, $p < 0.001$, $g \geq 1.3$.

3.1. Total CC area, length, and thickness

The first set of analyses concerned longitudinal change in CC area from 6 to 24 months age, with a primary focus on possible sex differences during this interval. Linear mixed-effects model results for longitudinal area data are presented in Table 2. There were significant effects for age for CC total area ($t(78.1) = 11.3$, $p < 0.001$) and for the interaction of sex \times age ($t(55.4) = 3.0$, $p = 0.004$). This age by sex interaction was characterized by relatively equivalent CC area between males and females at age 6 months followed by a steeper increase for males such that by age 24 months, males had significantly larger brain-size adjusted CC area, $g = 0.55$ (Fig. 2).

To further investigate the early morphological development of the CC, its length and thickness were examined longitudinally. As shown in Table 2, for CC length the main effect of sex was significant ($t(105.1) = 2.0$, $p = 0.048$), with females having a longer brain-size adjusted CC, $g = 0.41$. There was no significant main effect for age or the interaction of sex \times age. For CC thickness, there was a main effect for age at $p < 0.001$ and no significant main effect for sex. The interaction of sex \times age was significant ($p = 0.005$), with males showing a steeper increase over the interval. As with area, thickness was relatively equivalent between sexes at age six months but significantly greater in males by age 24 months, $g = 0.72$. When examining CC thickness for five mutually exclusive regions,

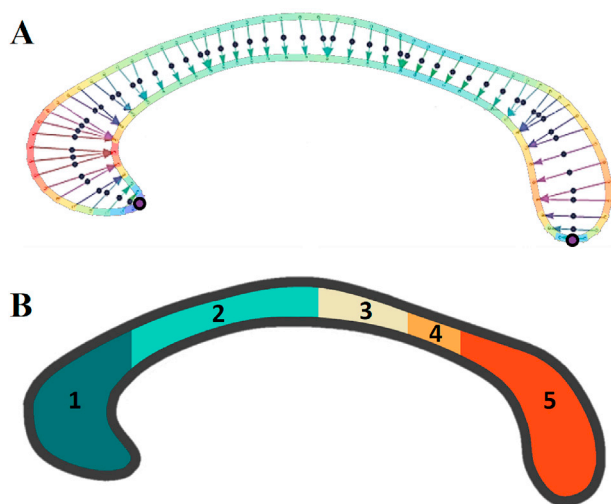


Fig. 1. Illustrations of corpus callosum (CC) measurement. Panel A represents CC boundary with medial axis points and corresponding thickness measurements. Length was based on distance along medial axis from genu to splenium endpoints. Panel B represents CC segmentation based on Hofer and Frahm (Vachet et al., 2012). Numbered CC segments refer to putative connectivity for the following regions: 1) prefrontal; 2) pre-/supplementary motor; 3) motor; 4) sensory; and 5) parietal/temporal/occipital.

Table 1
Descriptive and demographic data for study sample 24 months.

Statistical descriptor		Females	Males	Total
Number of subjects	N	42	62	104
Age at MRI 6 months	N	33	54	87
	Mean	6.7	6.8	6.7
	SD	.7	.7	.7
	Range	5.8–8.9	5.5–9.0	5.5–9.0
12 months	N	33	48	81
	Mean	12.9	12.6	12.7
	SD	.8	.6	.7
	Range	12.0–15.9	11.8–14.6	11.8–15.9
24 months	N	26	30	56
	Mean	24.6	24.9	24.8
	SD	.8	1.0	.9
	Range	23.6–27.8	23.8–28.0	23.6–28
Maternal education	% College degree or higher	83.3	82.3	82.7
Race/Ethnicity				
Asian	%	2.3	0	1.0
Black	%	7.1	4.8	5.8
White	%	78.6	88.7	84.6
Mixed race	%	9.5	6.5	7.7
Not reported	%	2.3	0	1.0
Hispanic (any race)	%	10.0	3.3	5.9
Early Learning Composite ^a	N	42	62	104
	Mean	116.1	108.1	111.3
	SD	14.9	13.9	14.8
	Range	89–141	82–140	82–141
Brain tissue volume ^b	N	33	54	87
	Mean	711.8	787.6	758.8
	SD	56.7	59.5	68.9
	Range	569.3–808.2	640.7–954.2	569.3–954.2
12 months	N	33	48	81
	Mean	905.6	984.7	952.5
	SD	61.7	71.0	77.5
	Range	744.2–1072.5	815.9–1124.2	744.2–1124.2
24 months	N	26	30	56
	Mean	1029.2	1143.1	1090.2
	SD	88.8	81.6	101.9
	Range	820.4–1211.9	969.4–1285.6	820.4–1285.6

^a Mullen Scales of Early Learning at age 2 years.

^b Total grey and white matter in cm³.

there was a main effect for sex in regions 1 ($t(102.5) = 2.0, p = 0.046, g = 0.34$) and 3 ($t(87.6) = 2.4, p = 0.002, g = 0.86$), with males having higher thickness. The main effect of age was significant for all five regions of thickness with this property increasing over the age interval. The interaction of sex \times age was significant for region 2 ($t(64.9) = 2.9, p = 0.005$) and region 4 ($t(61.6) = 2.0, p = 0.048$), with males showing a faster increase in model adjusted thickness.

To account for the potential of a non-isometric association between

Table 2
Mixed-effect model results for CC total area, total length, and thickness segmentations.

	Age				Sex				Sex \times Age			
	Estimate	SE	F	p	Estimate	SE	F	p	Estimate	SE	F	p
Total CC area	4.97	.441	129.4	<.001	12.0	8.57	2.0	.166	.973	.324	9.0	.004
Total CC length	.072	.056	2.9	.094	4.2	2.1	4.0	.048	.021	.049	0.2	.671
Total CC thickness	.122	.010	66.9	<.001	.050	.193	0.1	.795	.023	.007	8.6	.005
CC thickness segmentation												
Region 1	.087	.017	28.2	<.001	.901	.448	4.1	.046	.018	.014	1.6	.212
Region 2	.146	.014	116.8	<.001	.034	.219	0.0	.879	.032	.011	8.5	.005
Region 3	.172	.014	176.6	<.001	.584	.241	10.3	.002	.026	.013	2.9	.097
Region 4	.121	.015	78.3	<.001	.483	.265	3.3	.071	.024	.012	4.1	.048
Region 5	.091	.014	45.1	<.001	.339	.265	1.6	.204	.019	.011	3.3	.074

brain size and CC size (Bishopaand and Wahlsten, 1997), we conducted a follow-up set of analyses accounting for allometric scaling, which represents an alternative approach to account for brain size. Results from this follow-up analysis were consistent with those from our primary model, suggesting that sex differences are not fully explained by disproportionality of CC to brain size (Supplementary Materials, Methods & Inline Supplementary Table 2 & Fig. 2).

3.2. Corpus callosum RD and thickness

To examine the microstructure underlying CC development, we also evaluated relations of CC thickness to RD across CC regions in infants. Longitudinal mixed-effects model analysis indicated a significant effect for age on RD for all five CC regions (1, 2, 4, and 5, $p < 0.001$; and 3, $p = 0.03$), with RD decreasing across the 6–24 month interval for all regions. Coefficient estimates indicated that RD decreased between approximately 7×10^{-6} to 1.1×10^{-5} for each one month increase in age, with slope steepest for anterior regions. There was no effect of sex on the relation of RD to CC development. To illustrate the association between midsagittal RD and CC thickness over time, we generated heat maps based on Pearson correlation coefficients, controlling for TBV, for both sexes (Fig. 3). As a follow-up to the analysis of RD, we also examined fractional anisotropy (FA). There were significant effects for age for region 1 ($t(62.7) = 12.2, p < 0.001, \beta = 0.007$), region 2 ($t(54.3) = 15.7, p < 0.001, \beta = 0.007$), region 3 ($t(59.1) = 15.8, p < 0.001, \beta = 0.008$), and region 4 ($t(67.5) = 4.1, p < 0.001, \beta = 0.003$). There were no effects for age on region 5 ($t(77.2) = 0.3, p = 0.782, \beta = 0.0004$), for which FA trajectories were relatively flat. There were no effects for sex or sex \times age on any region. A heatmap showing relations of regional FA values to thickness is presented in Supplementary Figure 3.

3.3. Relation of CC morphology to cognitive ability

For females, MSEL ELC at age 24 months was significantly associated with CC length ($r = -.47, p = 0.019$) but not total area ($r = -.30, p = 0.15$) or thickness ($r = 0.04, p = 0.84$). For males, no significant relations were observed for CC area ($r = -.10, p = 0.61$), length ($r = -.13, p = 0.50$) or thickness ($r = 0.03, p = 0.89$). To further investigate the relation of CC length to cognitive ability in females, we examined nonverbal and verbal developmental quotient derived from the MSEL (derived from subscales comprising ELC composite). We found that CC length was significantly associated nonverbal developmental quotient ($r = -.57, p = 0.003$) but not verbal developmental quotient ($r = -.14, p = 0.51$). While we considered this set of tests for targeted brain-behavior variables exploratory, note that only the test of CC length in relation to NVNQ survives Bonferroni correction.

4. Discussion

In this multi-modal imaging study, we characterized trajectories of corpus callosum (CC) development in 104 typically developing infants from 6 to 24 months of age using a longitudinal design. Our first set of

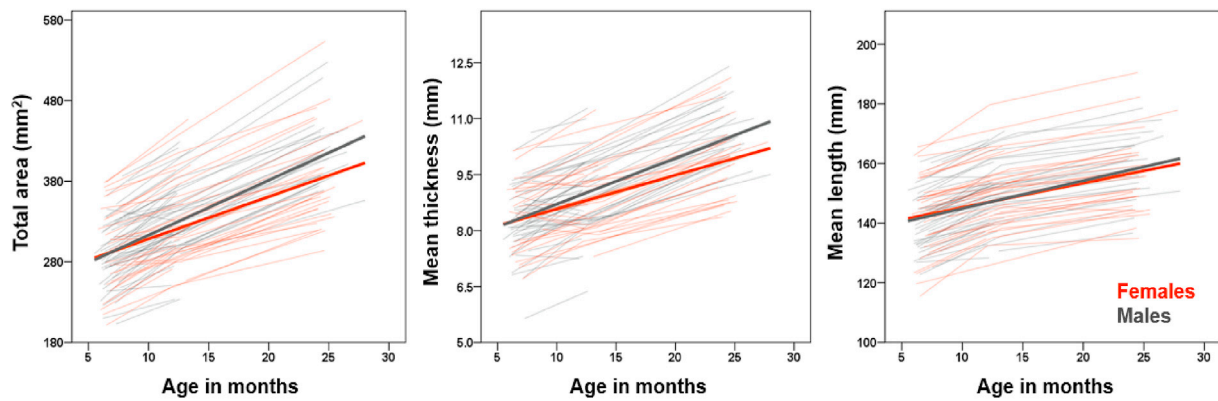


Fig. 2. Trajectories for morphological features of the corpus callosum by sex. Bold lines represent model group means. Thin lines represent individual trajectories.

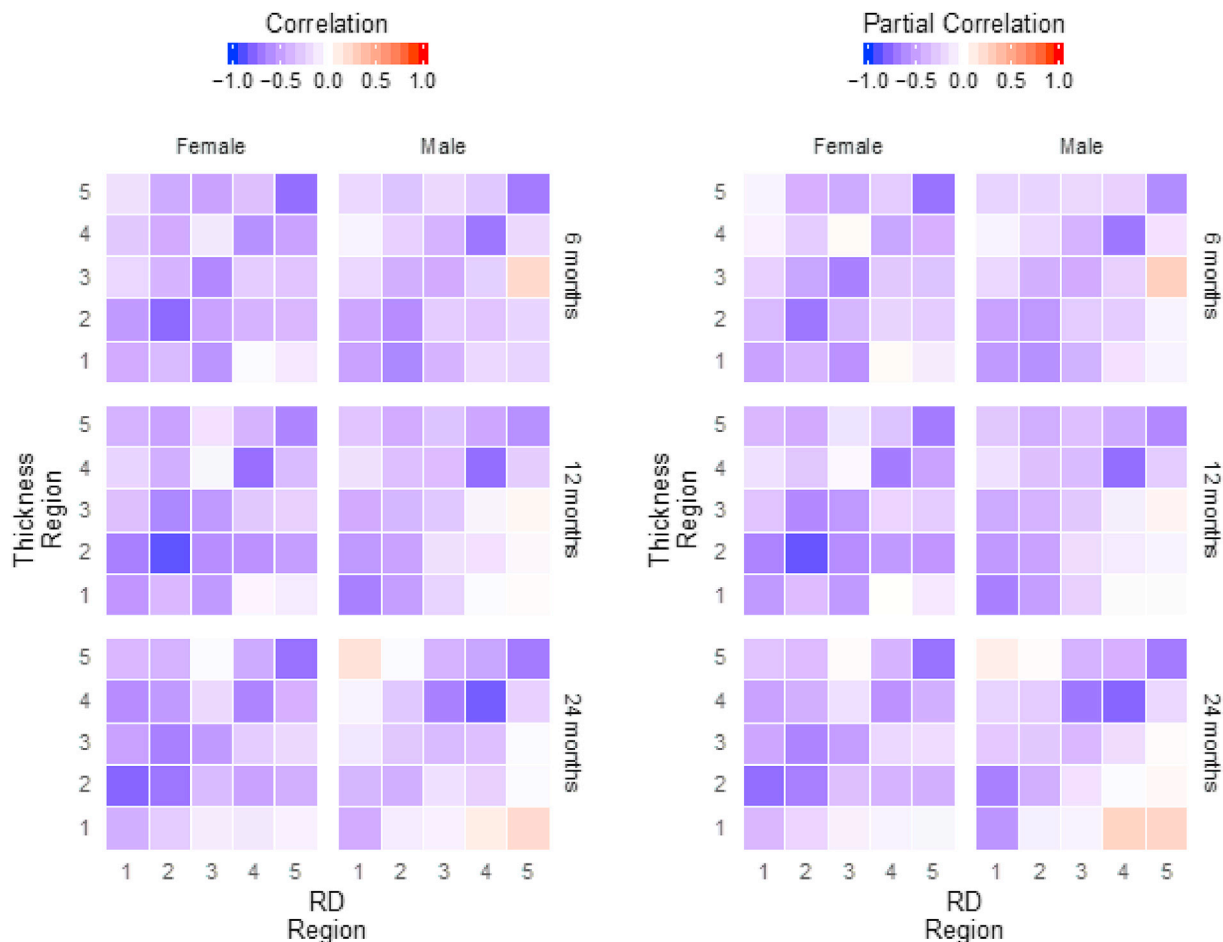


Fig. 3. Relations of CC thickness to radial diffusivity across CC regions and time in female and male infants. Panels display Pearson correlations. Left side displays unadjusted data. Right side displays data adjusted for total brain volume. Upper panels, 6 months (left, females N = 28; right, males N = 45); middle panel, 12 months (left, females N = 25; right, males N = 44); lower panel, 24 months (left, females N = 24; right, males N = 25). CC regions refer to: 1) pre-/supplementary motor; 2) motor; 3) sensory; and 5) parietal/temporal/occipital.

analyses examined trajectories for midsagittal area, length, and thickness between female and male infants, controlling for total brain volume. We found significant age by sex interactions for CC area and thickness, suggesting differences in growth trajectories between male and female infants. Specifically, males were equivalent to females in terms of CC area and thickness at age 6 months, but exhibited a significantly higher rate of growth controlling for brain size from 6 to 24 months of age. This result was apparent with and without adjustment for total brain size, including a model accounting for allometric scaling (Supplementary Information,

Table S2; Fig. S1). Morphometric differences in the CC were most pronounced in the pre- and supplementary motor and primary sensory regions. As differences in growth rates have not been reported in studies of older children (Giedd et al., 1999; Westerhausen et al., 2016), we expect the effects observed in our sample are likely unique to the first years of life.

Reports of sex differences in CC structure extend back over a century. The earliest of these works relied on gross pathological examination of postmortem brains, seemingly with the goal of corroborating biological

determinism or scientific racism (Bean, 1906). In 1982, De Lacoste-Utamsing & Holloway published a landmark paper in *Science* reporting sex differences in the CC characterized by a larger splenium in females (Smith, 2005). While this paper generated some minor controversy at the time (Holloway, 2017), it reads now as prescient given renewed interest in biological sex differences (Prager, 2017). The 1982 paper was followed by numerous others, with some supporting the finding of increased size in females (Johnson et al., 1994; Mitchell et al., 2003; Ardekani et al., 2013; Smith, 2005; De Lacoste-Utamsing and Holloway, 1982; Allen et al., 1991) and others reporting no differences between the sexes (Bastianello et al., 1994; Hasan et al., 2009; Bishopaand and Wahlsten, 1997; Bruner et al., 2012; Luders et al., 2014). These inconsistencies may be attributed to several factors: power to detect a modest effect, age ranges of samples, and whether and how total brain size is accounted for (Ardekani et al., 2013; O'Muircheartaigh et al., 2014). Relevant to the present study, nearly all previous work has focused on the adult brain. In notable contrast to studies of older individuals, our finding of sex differences in infants and toddlers was characterized by a higher rate of growth in males.

Our results indicate that male and female infants appeared relatively equivalent in terms of CC size at age 6 months, with differences emerging from late infancy through toddlerhood. It is likely that the finding of increased growth rate in males reflects differences in one or more developmental processes unique to infancy. Changes in CC area and thickness during this period are likely due to a combination of factors, such as increases in myelination and axon caliber, as well as factors that attenuate its growth, such as pruning. One possibility is that female infants undergo more vigorous developmental axon elimination given the known relation of CC size to axon complement across species (Olivares et al., 2001). The possibility that the timing of growth and refinement of the CC differs by sex is consistent with findings from a recent ultrasonographic study indicating that the CC was significantly thicker in female infants prior to age 2 months, with no sex differences observed between 2 and 6 months (Chang et al., 2018). The regressive process of rapid axonal pruning is unique to infancy, with primate studies suggesting that upwards of 70% of axons present at birth are removed during the first year of life (Geng et al., 2012a). There is some evidence from animal models that this process of developmental axon elimination may differ by sex (Kim and Juraska, 1997). As such, a smaller brain-size adjusted CC early in life may reflect more advance development of white matter connectivity through the refinement of axon structure. This interpretation is further supported by our brain-behavior results, wherein cognitive abilities were *negatively* associated with CC length in females, as well as previous work pertaining to infant neurodevelopment wherein smaller volumes or slower progression may in some circumstances reflect a developmental advantage (Swanson et al., 2017; Deoni et al., 2016).

In a second set of analyses, we examined the microstructural properties of midsagittal CC regions using diffusion tensor imaging. This set of analyses was performed to determine whether: 1) CC size may be explained by white matter microstructural composition, and 2) whether this relation differs between female and male infants. We found that CC thickness was strongly associated with white matter structure across all subdivisions. Further, we noted no effect of sex on this relation, suggesting that the underlying mechanisms driving CC size, including factors such as axon density and myelination, may be relatively equivalent for female and male infants. This finding aligns with previous work examining the CC fiber composition in post-mortem adult brains, wherein CC size has been found to be determined by absolute number, but not density, of axons (Aboitiz et al., 1992). Of note, that study found no differences in axon density between males and females. Together this suggests that our finding of a higher CC size and growth rate in males may not be explained by differences in underlying microstructure (e.g., size or density of axons). However, we must also consider a plausible alternative explanation: that the negative relations between radial diffusivity and CC thickness are illusory and the product of partial volume effects (Westhausen et al., 2011; Vos et al., 2011). A thinner corpus callosum would

be more vulnerable to such effects, and thus the relation of RD to CC size may have more to do with artifact than with axon composition. While our tract-based processing and analysis should be robust to such effects (Verde et al., 2014) (e.g., refinement procedures that remove areas showing partial volumes), we cannot rule out this potential confound. Given this consideration as well as the limited capability of DTI to directly interrogate discrete aspects of white matter structure, follow-up work with alternative approaches is necessary.

The rate and timing of CC development may be governed in part by endogenous sex hormones. To date, there is mixed evidence from MRI studies of humans regarding the effects of these on CC morphology (Holloway, 2017; Prager, 2017), and we are aware of no such data from infants. However, studies of non-human animals strongly suggest that axonal development is mediated by sex hormones. In a microscopy study of juvenile rats, Pesarisi and colleagues (Pesarisi et al., 2015) found that androgens explained observed increases in axon caliber and the ratio of axon size to myelin thickness (i.e., g-ratio) in males. At the cellular level, multiple and substantial sex differences in glial cells essential to axon growth have been observed in rats (Cerghet, 2006). Among those findings two are of particular relevance to the present study: 1) female rats generated glia in the CC at twice the rate of males, but also underwent increased apoptosis; and 2) males had a higher density of oligodendrocytes, which was most pronounced early in their life cycle. Glial cells and their rate of proliferation have been directly implicated in the developmental pruning of axons (Watts et al., 2004; Awasaki and Ito, 2004). It is likely that sex differences in glial cells and subsequent axonal growth and development may be attributed to the differential role of sex hormones on the expression or suppression of neurotrophins. While the present data cannot speak to these issues directly, it is plausible that the higher brain-size adjusted area and thickness of the CC in males observed in our sample may be a function of these sex-mediated effects. The specific mechanism(s) underlying the differences we observed herein remains an area for further study in both human and non-human models of early neurodevelopment (Hines, 2011).

Finally, we also examined relations between CC development and performance on cognitive measures. We found that in females, overall CC length was negatively associated with Mullen ELC score, which is an index of general cognitive ability. This suggests that a longer brain-size adjusted CC may be associated with less advanced cognitive performance. However, this particular relation did not survive correction for multiple comparisons and should be considered with the potential for a type-1 error in mind. Examination of verbal and nonverbal domain scores for females suggested that nonverbal cognitive ability, comprised of fine motor ability and visual reception, drives the relation of cognition to CC development. While the direction of the correlation values were negative in both females and males, we observed no significant associations in the latter group. It may be that the brain-behavior relations observed in females emerge later in males as CC development catches up with that of females. We hypothesize this would be borne out if differences in developmental axon elimination do indeed underlay sex differences in early CC trajectories. Our findings partially align with previous reports indicating that differences in CC morphology are associated with cognitive factors such as IQ (Hutchinson et al., 2009; Luders et al., 2011) or language function (O'Muircheartaigh et al., 2014). Further study will be needed to clarify whether and how early corpus callosum morphology is related to specific aspects of cognitive development.

4.1. Limitations

There are several limitations that merit consideration. First, the sample of children included in this study were predominantly White, with a higher percent of parents with a college education or greater compared to the general population of the United States. Whether these findings generalize to other populations of children is unknown, and studies of more diverse samples are necessary to address this empirical question. Our longitudinal data comprised imaging data collected at

three time points over the first two years of life. While a linear trajectory adequately fit these data, additional time points (i.e. greater than 3 repeated measures) would allow for more detailed characterization of CC growth over this period. Furthermore, additional longitudinal data at later ages would directly inform whether and when female growth trajectories converge with, and possibly outpace, that of males. Such data could potentially resolve the discontinuity between our findings in infants and reports of larger female CC measures in older children and adults. Statistical models used to compare CC size were adjusted for total brain volume, which included the cerebellum. Adjustment based on cerebral volume or other parcellations more specific to CC function may increase precision. Our segmentation of the CC was based work by Hofer and Frahm (Vachet et al., 2012), which itself was a tractography-based update of a longstanding segmentation scheme. Because the segmentation was derived from MRI scans of adults, it is possible that it inaccurately reflects regional parcellation in very young children due to developmental effects, such as morphological changes in infancy (Simpson et al., 2019). Age-specific or more fine-grained approaches to parcellation (Luders et al., 2010) are likely to further inform infant CC development.

5. Conclusion

The CC has been a common and longstanding target for studies of neurobiological sex differences. However, its longitudinal development during infancy between male and female has not been examined despite the unique changes that occur during this developmental epoch. Moreover, the CC represents a particularly vulnerable target for early adversity related to a multitude of endogenous and exogenous factors (Paul, 2011a; Sánchez et al., 1998). In the present study, we found that male and female human infants were similar in CC area and thickness at age 6 months, followed by diverging trajectories into toddlerhood characterized by a higher growth rate for males. This pattern was observed regardless of approach to adjustment for total brain size. In addition to sex differences observed in macrostructural development, we also found that CC length was significantly associated with cognitive ability in females, though the direction of the effect was equivalent for both sexes. Further large-scale developmental studies interrogating brain structure and behavior are needed to evaluate the cognitive, emotional, and social differences among sexes (Grabowska, 2017).

Funding

This research was supported by grants from the National Institutes of Health (K01-MH101653, R01-HD055741, HD055741-S1, P30-HD03110), a student fellowship award from the American Academy of Child and Adolescent Psychiatry, and the National Alliance for Medical Image Computing, funded by the NIH Roadmap for Medical Research (U54-EB005149). The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Data availability statement

Datasets analyzed for the current study are publicly available through the National Institute of Mental Health Data Archive (NDA; <https://nda.nih.gov>).

Declaration of competing interest

None.

CRediT authorship contribution statement

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Writing - review & editing. **Guido Gerig:** Software, Resources, Investigation, Data curation, Writing - review & editing. **Martin Styner:** Software, Resources, Investigation, Data curation, Writing - review & editing. **Meghan R. Swanson:** Software, Resources, Investigation, Data curation, Writing - review & editing. **Jed T. Elison:** Investigation, Writing - review & editing. **Mark D. Shen:** Investigation, Writing - review & editing. **Robert C. McKinstry:** Investigation, Writing - review & editing. **John R. Pruett:** Investigation, Writing - review & editing. **Kelly N. Botteron:** Investigation, Project administration. **Annette M. Estes:** Investigation, Project administration. **Stephen R. Dager:** Investigation, Project administration. **Heather C. Hazlett:** Investigation, Project administration. **Robert T. Schultz:** Investigation, Project administration. **Joseph Piven:** Conceptualization, Methodology, Investigation, Supervision, Funding acquisition, Writing - review & editing. **Jason J. Wolff:** Conceptualization, Methodology, Investigation, Supervision, Funding acquisition, Writing - review & editing.

Acknowledgements

We thank the IBIS families for their ongoing participation. We also thank Rachel G. Smith for assisting with data processing and quality control.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.neuroimage.2020.116821>.

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