



White matter in infancy is prospectively associated with language outcomes in kindergarten

Jennifer Zuk^{a,b,*}, Xi Yu^{b,c}, Joseph Sanfilippo^b, Michael Joseph Figuccio^d, Jade Dunstan^b, Clarisa Carruthers^b, Georgios Sideridis^{b,e}, Ted K. Turesky^{b,e}, Borjan Gagoski^{e,f}, Patricia Ellen Grant^{e,f}, Nadine Gaab^{b,e,g}

^a Department of Speech, Language & Hearing Sciences, Boston University, Boston, MA, 02215, USA

^b Laboratories of Cognitive Neuroscience, Division of Developmental Medicine, Department of Medicine, Boston Children's Hospital, Boston, MA, 02115, USA

^c State Key Laboratory of Cognitive Neuroscience and Learning, Beijing Normal University, Beijing, China

^d Department of Psychology, Farmingdale State College, East Farmingdale, NY, 11735, USA

^e Harvard Medical School, Boston, MA, 02115, USA

^f Fetal-Neonatal Neuroimaging and Developmental Science Center, Boston Children's Hospital, Boston, MA, 02115, USA

^g Harvard Graduate School of Education, Cambridge, MA, 02138, USA

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ABSTRACT

Language acquisition is of central importance to child development. Although this developmental trajectory is shaped by experience postnatally, the neural basis for language emerges prenatally. Thus, a fundamental question remains: do structural foundations for language in infancy predict long-term language abilities? Longitudinal investigation of 40 children from infancy to kindergarten reveals that white matter in infancy is prospectively associated with subsequent language abilities, specifically between: (i) left arcuate fasciculus and phonological awareness and vocabulary knowledge, (ii) left corticospinal tract and phonological awareness, and bilateral corticospinal tract with phonological memory; controlling for age, cognitive, and environmental factors. Findings link white matter in infancy with school-age language abilities, suggesting that white matter organization in infancy sets a foundation for long-term language development.

1. Introduction

The neural basis for language has been postulated to emerge prenatally, suggesting that the rudimentary foundation for language has already been established in the brain by the time an infant is born (Dehaene-Lambertz and Spelke, 2015). Yet the complex trajectory of language acquisition extends well beyond childhood (Owens, 2008). Environmental input and experience over time play a crucial role in shaping the trajectory of language acquisition (Rowe, 2012; Weisleder and Fernald, 2013), but the first two years of life signify a particularly rapid period of language development and brain maturation (Pujol et al., 2006) and are increasingly recognized to set an important foundation for long-term development (Gilmore et al., 2018). Thus, it remains unknown whether the neural scaffold for language established in infancy is prospectively associated with the protracted trajectory of language development.

While environmental experience is essential for language

acquisition, biological determinants also make an integral contribution during prenatal and early postnatal development (Graham and Fisher, 2013). White matter encompasses one critical component of neuroarchitecture underlying efficient signal transmission and corresponding skill acquisition, and undergoes a robust developmental period within the first two years of life (Geng et al., 2012; Gilmore et al., 2018). Structural organization of white matter as indicated by fractional anisotropy (FA), the degree of directionality in water diffusion within white matter pathways, has been shown to increase throughout early childhood and beyond (Barnea-Goraly et al., 2005; Lebel and Beaulieu, 2011). Yet, this process of white matter maturation is characterized by reorganization and plasticity that builds upon the structural organization established during infancy (Gilmore et al., 2018). The most rapid production of myelin (which forms around axons that comprise white matter to increase signal transmission efficiency) occurs during prenatal and early postnatal development (Lenroot and Giedd, 2006), in conjunction with dynamic changes in gene expression in brain tissue

* Corresponding author at: Department of Speech, Language & Hearing Sciences, Boston University 635 Commonwealth Avenue, Boston, MA, 02215, USA.
E-mail address: jzuk@bu.edu (J. Zuk).

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(Naumova et al., 2013). Whole-genome expression studies in the developing brain reveal that temporal dynamics of the transcriptome are more robust prenatally than at any postnatal stage (Johnson et al., 2009). Moreover, candidate susceptibility genes for speech/language disorders show an especially prominent window of expression prenatally in perisylvian cortex, which comprises the neural basis of language (Abrahams et al., 2007). Thus, an important neural foundation is established in infancy that sets children on a path for subsequent development.

Although the first two years of life signify a peak white matter developmental rate, longitudinal studies tracking long-term language development from infancy have predominantly employed behavioral and electrophysiological methods. Behavioral and functional neural responses to tones and speech syllables in infancy are prospectively associated with the number of words a child understands and produces as a toddler (Benasich and Tallal, 2002; Tsao et al., 2004; Friedrich and Friederici, 2006; Kuhl et al., 2008), and subsequent phonological awareness and oral comprehension abilities in preschool (Molfese and Molfese, 1997; Guttorm et al., 2005, 2010; Leppanen et al., 2010, 2012). These findings illuminate functional foundations in infancy predictive of subsequent language outcomes, yet what is the role of underlying white matter?

While the relation between white matter organization and function is complex and subject to several contributing factors, emerging evidence suggests that white matter organization may precede functional development (Mahon and Caramazza, 2011; Hannagan et al., 2015; Saygin et al., 2016). Crucial for language, the arcuate fasciculus connects inferior frontal and superior temporal regions responsible for language production and comprehension, respectively (Catani et al., 2005; Brauer et al., 2011; Brown et al., 2014; Maffei et al., 2015). The arcuate fasciculus, along with the superior longitudinal fasciculus, encompass key dorsal pathways for language (Saur et al., 2008; Brauer et al., 2011), which together with ventral pathways comprise a comprehensive network of white matter underlying the integration of language comprehension, formulation, and expression (Hickok and Poeppel, 2004; Dick et al., 2014). One prominent language-related ventral pathway is the inferior longitudinal fasciculus, which connects anterior temporal and posterior occipitotemporal regions (Catani et al., 2003). Accordingly, this ventral pathway is suggested to play an important role in facilitating semantic and lexical access (for a recent review see Herbert et al., 2018), particularly in mapping visual information about words to meaning (Yeatman et al., 2012a; Wandell and Yeatman, 2013; Cummine et al., 2015). Methodological advances to conduct infant neuroimaging reveal that these dorsal and ventral pathways emerge within the first year of life (Dubois et al., 2009; Leroy et al., 2011; Brauer et al., 2013; Dubois et al., 2016; Kostović et al., 2019). Longitudinal investigations of prematurely born infants reveal that properties of the left arcuate fasciculus, left inferior longitudinal fasciculus, and left superior temporal gyrus at full-term equivalent-age relates to subsequent toddler-age language abilities (Aeby et al., 2013; Salvan et al., 2017; Dubner et al., 2020). This line of work has also importantly identified that alterations in language networks of the brain are associated with premature birth (Thomason et al., 2017), which further illuminates the need for developmental studies of full-term infants to serve as a model for typical development.

Despite a prominent focus on brain and language development in the cognitive neuroscience field, scarcely any studies have examined the typical trajectory of white matter in relation to language development from infancy to school age. While the typical trajectory of white matter development in relation to general cognitive abilities from birth to age five has been specified (Deoni et al., 2016), the only study to link brain structure in infancy with subsequent long-term language outcomes up to the preschool age exclusively focused on gray matter volume associated with the amygdala (Ortiz-Mantilla et al., 2010). Furthermore, one longitudinal study examined white matter organization among seven-month-old infants and subsequent language outcomes, but

language was measured only five months later, at twelve months of age (Deniz Can et al., 2013). Given the limited language capacities at this age, it remains unclear what this means for the ultimate trajectory of language acquisition. Thus, although the neural basis for language is evident in infancy, the extent to which white matter organization relates to long-term language abilities (e.g., by school age) along a typical trajectory of development remains largely unspecified.

From a developmental perspective, brain-behavior links between white matter and language among typically developing children have predominantly been characterized cross-sectionally among preschool-age children (Reynolds et al., 2019), specifically indicating positive associations between structural organization of the left arcuate fasciculus and language constructs such as phonological awareness and vocabulary knowledge (Lebel and Beaulieu, 2009; Saygin et al., 2013; Skeide et al., 2016; Reynolds et al., 2019). The left arcuate fasciculus has also been associated with language exposure (via parent-child conversational turns) independent of socioeconomic status (Romeo et al., 2018). Moreover, properties of the left arcuate fasciculus were observed to mediate the well-known link between language exposure and language skills among four- to six-year-old children (Romeo et al., 2018). Therefore, language-related white matter organization has been indicated as a specific mechanism underlying language abilities; however, this has yet to be examined from a developmental perspective among healthy, full-term infants.

Here we examine prospective longitudinal associations between white matter in infancy and subsequent language abilities in kindergarten. The present investigation initially acquired neuroimaging with full-term, healthy infants who were then re-invited for longitudinal follow-up several years later (at the kindergarten age). As a first step to address this missing link in the field, we focused on structural organization of the arcuate fasciculus as one of the most prominent dorsal white matter pathways important for language, and incorporated the inferior longitudinal fasciculus as a ventral pathway of interest for comparison. One non-language-dominant pathway, the corticospinal tract, was also included as a control to examine the potential specificity of language-related pathways. Structural organization of these pathways in infancy was quantified and then examined in relation to children's subsequent language abilities at school age in the areas of phonological awareness, vocabulary knowledge, and oral comprehension. We hypothesized that left-hemispheric white matter organization in infancy within language-related pathways, especially the arcuate fasciculus, would be prospectively associated with subsequent language abilities in kindergarten. Findings carry implications for white matter within the first two years of life as an important foundation underlying the developmental trajectory of language development.

2. Materials and methods

2.1. Participants and design

Forty children (20 female) were included in the present study as part of a larger NIH-funded investigation of children from infancy to school-age, tracking brain and language/pre-literacy development longitudinally (NIH-NICHD R01 HD065762). Families were initially recruited from 2010 to 2014 from the Greater Boston area through the Research Participant Registry within the Division of Developmental Medicine at Boston Children's Hospital, as well as through ads and flyers disseminated in local newspapers, schools, community events, and through social media. We invited families for initial participation when children were infants (mean age at infancy: 10 months; age range: 4–18 months), and children were re-invited for longitudinal follow-up when they reached the preschool/kindergarten age (mean age: 5.5 years, age range: 4–6.5 years). The majority of children completed follow-up assessment at the start of formal schooling (i.e., in kindergarten); three children were in first grade at the time of their follow-up. Participating children completed an MRI scan session at the infant

time point and then, at follow-up, a standardized battery of cognitive-linguistic assessments and repeat MRI session. Here we focus on the standardized language assessments at follow-up from the first cohort within our larger longitudinal investigation (Cohort 1 of 2 cohorts), which follows children from infancy to early elementary school. Within this cohort, 47 infants have since reached school age and returned for follow-up assessment. Of these 47, 40 children yielded both DTI data of sufficient quality for analyses and met all criteria for inclusion at the follow-up time point, and thus were included in the present study.

Children were screened at both time points for psychiatric, neurological, or sensory illness/impairment, contraindications for MRI evaluation (i.e., metal implants), premature birth, and psychotropic medication treatment (except for one child with a diagnosis of ADHD reported at the follow-up time point). All children included were from American English-speaking families and were born at gestational week 37 or later. Parent report of race/ethnicity were as follows: 75% White/Caucasian, 3% Black/African American, 8% Asian, 7% Multiracial, and 7% Hispanic (with one non-response). Of these 40 children in the present study, 29 were right-handed, eight were left-handed, and three had unknown handedness at follow-up. Per parent report, 14 children had a first-degree family history of reading difficulties (developmental dyslexia). In addition, all children demonstrated nonverbal cognitive abilities within one standard deviation of the mean or above, as indicated by the Matrix Reasoning subtest of the Kaufman Brief Intelligence Test: 2nd Edition (KBIT-2, Kaufman and Kaufman, 2004). The same criterion was applied in terms of oral comprehension and vocabulary knowledge abilities as well to ensure that no language delays or developmental language disorder impact the associations investigated. As a result, among the initial 47 children with longitudinal follow-up, one child characterized by standardized phonological awareness and vocabulary knowledge performance below one standard deviation of the mean at follow-up was excluded from the present analysis. Other participants were excluded due to primary exposure to a language other than English at home ($n = 1$), and lack of usable DTI acquisitions to be included in the analysis ($n = 5$). This study was approved by the Institutional Review Board of Boston Children's Hospital. Informed written consent was provided by a parent/legal guardian for each participating child, and written assent was provided by each child at follow-up, beginning at age four.

2.2. Environmental characteristics

Children were either enrolled in or had completed preschool at the time of follow-up participation. Of the 40 children in the sample, 30 participated in extracurricular activities (e.g., music, art, sports) for an average of two hours per week, with minimal variation overall. Minimal variation in socioeconomic status was also evident among families in the present sample, as indicated by parent report in a questionnaire adapted from the MacArthur Research Network (<http://macses.ucsf.edu/default.php>). Specifically, families were characterized by middle to high socioeconomic backgrounds, as 36 children in the present sample had at least one parent who had at least a Bachelor's degree, and 25 families reported an annual income of \$100,000 or more (note: one family did not provide socioeconomic information).

Another environmental factor measured included the home literacy environment (HLE), in which parents completed a questionnaire at the infant time point characterizing children's exposure to literacy (Powers et al., 2013). Two key variables characterizing HLE were included in the present analysis: (1) the number of children's books in the home, known to be related to children's language and literacy-related skills (van Bergen et al., 2017), and (2) the amount of time a parent spends reading to their child, as shared parent-infant book reading has been linked with language acquisition in infancy (Karrass and Braungart-Rieker, 2005), and has been prospectively associated with subsequent language abilities and school readiness (Raikes et al., 2006). Distributed representation of binned responses was provided for each variable (for detailed

overview, see Table 1).

2.3. Language measures at longitudinal follow-up

Subsequent language outcomes at longitudinal follow-up were characterized by several key language constructs, including phonological awareness, phonological working memory, oral comprehension, and vocabulary knowledge. These measures comprise a subset of the battery from the larger investigation tracking language and pre-literacy development for the purposes of the specific research questions in the present study. Thus, the following language assessments were investigated at longitudinal follow-up:

2.3.1. Phonological awareness and phonological working memory

Phonological awareness was measured by two standardized subtests from the Woodcock-Johnson Tests of Oral Language (WJ-IV OL; Mather et al., 2014): Segmentation and Sound Blending. The Segmentation subtest measures the ability to break words down into their basic speech sounds by requiring children to listen to words (compound, multi-syllabic, and monosyllabic) and identify the parts (at the level of compound words, syllable sounds, and individual speech sounds). The Sound Blending subtest measures the ability to synthesize speech sounds through an auditory processing task, by requiring children to listen to a sequence of syllables or speech sounds and combine the sounds into a word. Phonological working memory was measured by the Memory for

Table 1
Group Demographics.

General Information	
Overall n	40 (20 f / 20 m)
Infant age (in months)	10.38 \pm 3.81
Age at follow-up (in months)	65.74 \pm 6.52
Time elapsed from infancy to follow-up (in years)	4.61 \pm 0.65
Nonverbal IQ	107.23 \pm 11.66
White Matter Organization in Infancy (overall mean FA)	
Left arcuate fasciculus ($n = 34$) [*]	0.35 \pm 0.05
Right arcuate fasciculus ($n = 13$) [*]	0.33 \pm 0.04
Left inferior longitudinal fasciculus	0.32 \pm 0.03
Right inferior longitudinal fasciculus ($n = 38$) [*]	0.32 \pm 0.02
Left corticospinal tract	0.53 \pm 0.05
Right corticospinal tract ($n = 37$) [*]	0.54 \pm 0.05
Language Abilities at Follow-up	
Oral Comprehension	117.03 \pm 12.38
Segmentation ($n = 39$)	115.49 \pm 13.67
Blending ($n = 39$)	104.41 \pm 12.19
Memory for Digits (Forward)	102.4 \pm 13.64
Vocabulary Knowledge	123.31 \pm 9.57
Environmental Characteristics	
Children's books in the home ($n = 36$) [*]	
0 – 50	27.78%
51 – 100	30.56%
101 – 200	22.22%
201 – 300	11.11%
301 or more	8.33%
Time spent reading with child per week ($n = 34$) [*]	
Less than 1 hr	9.38%
Less than 2 hr	18.75%
Less than 3 hr	21.87%
Less than 4 hr	12.5%
4–5 hrs	21.87%
6 or more hrs	15.63%

Note: Nonverbal cognitive and language measures at follow-up reported in standard scores (mean = 100, SD = 15). Environmental characteristics summarize percent represented within each category/bin option provided via parent questionnaire.

^{*} White matter tracts were not able to be reconstructed for all infants, and not all families provided responses to questionnaires (see Methods for details).

Digits subtest of The Comprehensive Test of Phonological Processing (CTOPP-2; Wagner et al., 2013), in which children are required to repeat strings of numbers, which vary in length from two to eight digits.

2.3.2. Oral comprehension and vocabulary knowledge

The Oral Comprehension subtest from the WJ-IV OL was administered, which measures listening, reasoning, and vocabulary abilities by requiring children to listen to a brief audio-recorded passage and then provide the omitted word through the use of syntactical and semantic clues. The Peabody Picture Vocabulary Test (PPVT-4, Dunn and Dunn, 2007) measures vocabulary knowledge, in which the examiner reads a single word and children are asked to select the picture (out of four possible choices) that best reflects the target word.

These assessments were administered and scored by a trained psychoeducational evaluator, and then double-scored by an additional evaluator to ensure accuracy. Raw scores for each of the assessments were converted to standard scores, and standard scores were utilized in subsequent analyses.

2.4. Neuroimaging acquisition

Neuroimaging was acquired while the infants were naturally sleeping, utilizing a previously established pediatric neuroimaging protocol (Raschle et al., 2012). Diffusion-weighted and structural T1-weighted images were acquired on a 3.0T Siemens TrioTim MRI scanner with a standard Siemens 32-channel radio frequency head coil. One parent remained in the MRI room with the infant for the duration of the scan, in addition to a researcher who stood by the bore to monitor changes in the infant's sleeping state and potential motion. Structural T1-weighted whole-brain multi-echo magnetization-prepared rapid gradient-echo sequences with prospective motion correction (moco-MEMPRAGE) were acquired for each participant (acquisition parameters: TR = 2270 ms; TE = 1450 ms; TA = 4.51 min; TI = 1450 ms; flip angle = 7°; field of view = 220 × 220 mm; voxel resolution = 1.1 × 1.1 × 1.0 mm (176 slices) with an in-plane acceleration factor of 2). Diffusion-weighted echo planar images were acquired using 64 slices from 30 gradient directions with 10 non-diffusion-weighted volumes (acquisition parameters: slice thickness = 2.0 mm; b = 1000s/mm²; field of view = 256 × 256 mm²; TE = 88 ms; TR = 8320 ms; TA = 5:59 min; flip angle = 90°).

2.5. Diffusion-weighted image processing

Diffusion-weighted images were processed with the same approach taken in a previous study from our lab with infants from this cohort (Langer et al., 2017). A brain mask was generated for each infant by separating brain tissue from non-brain tissue from the structural T1-weighted image utilizing the Brain Extraction Tool (BET, Smith, 2002). Raw DWI data were converted from DICOM to NRRD format through the DicomToNRRDConverter software of Slicer4 (www.slicer.org). The DWI images were aligned with structural T1 images via the b0 images. Quality control of diffusion images was evaluated utilizing QCTool, a MATLAB-based toolbox that detects artifacts based on Kullback–Leibler divergence calculations for each gradient in the sequence relative to a brain intensity mask for each individual subject. A combination of the tool's recommendations and visual inspection was implemented to detect motion artifacts, and gradients containing motion artifacts were removed prior to tract estimation. At most, 30% of gradients were removed for one participant for whom data quality greatly improved following outlier removal. Otherwise, no motion outliers were detected for 15 participants, 16 participants had only 1–2 poor gradients, and up to 25% of gradients were removed for the rest of the sample (see Supplementary Information for a full summary of poor gradients by participant and overall relation to age). Among the five additional infants who had to be excluded due to unusable DTI acquisitions, three infants had either more than 30% poor gradients or unusable head

rotations from their sleeping position, and two infants did not manage to sleep through the DTI acquisition sequence. Following quality assurance, DWI data were processed with the VISTALab mrDiffusion toolbox and diffusion MRI software suite (www.vistalab.com), including eddy current correction and tensor-fitting estimations with a linear least-squares (LS) fit for fitting the diffusion tensors.

2.6. Automated Fiber Quantification

The Automated Fiber Quantification (AFQ) software package was used to quantify specific white matter tracts in the current study, in accordance with previous procedures and parameters employed in our laboratory (Langer et al., 2017). For a brief summary, whole brain tractography was computed using a deterministic streamline tracking algorithm (Basser et al., 1994; Mori et al., 1999), with a fractional anisotropy (FA) threshold of 0.1 (as previously employed with this age range, Langer et al., 2017). Fiber tracking was terminated in instances where the minimum angle between the last path segment and the next step direction was > 40°. Region of interest (ROI)-based fiber tract segmentation and subsequent fiber-tract cleaning were then employed (using a statistical outlier rejection algorithm). The individual infant brains were registered/warped to a standard space template, which encompasses corresponding ROIs. The inverse transformation was then applied to the ROIs to bring them from standard to native space (for further details, please refer to github.com/jyeatman/AFQ; Yeatman et al., 2012b). Thereafter, quantification of FA was conducted along the trajectory of each tract based on eigenvalues from the diffusion tensor estimation (Basser et al., 1994). FA of each fiber was sampled to 100 equidistant nodes. For each tract, the characterization of 100 nodes was resampled to 50 nodes, thereby discarding the portion of the tract where individual fibers separate from the core fascicle toward their cortical destination, capturing the core of the tract. While there are currently several approaches to characterization of FA within white matter tracts (for a detailed methodological review, see Turesky et al., 2021), the present analysis examined FA within 50 nodes along the trajectory of each tract, modeling one approach previously employed in closely related literature (Langer et al., 2017; Wang et al., 2016), since it is known to improve normalization and co-registration of each tract for group comparisons (Yeatman et al., 2012a). For the AF, resampling of nodes was furthermore employed utilizing an alignment approach previously established in the AFQ literature (Yeatman et al., 2012a; Langer et al., 2017). Specifically, the trajectories of FA among all participants were aligned to the lowest point of FA along the trajectory of each tract to co-register anatomically analogous nodes of the tract across subjects and ensure comparison of comparable regions in the principal arc of the tract, in accordance with the previously established protocol (Yeatman et al., 2012a).

2.7. White matter pathways of interest

Building on converging evidence linking the arcuate fasciculus and language abilities among preschool/school-age children and adults (Lebel and Beaulieu, 2009; Saygin et al., 2013; Reynolds et al., 2019), the present investigation focused on the arcuate fasciculus as a primary dorsal pathway important for language. In addition, the inferior longitudinal fasciculus was investigated as a corresponding ventral pathway implicated in language, suggested to play a role in facilitating semantic and lexical access (for a recent review see Herbet et al., 2018), especially in mapping visual information from words to meaning (Yeatman et al., 2012a; Wandell and Yeatman, 2013; Cummine et al., 2015). Lastly, the corticospinal tract (CST) was incorporated as a control tract to examine whether potential effects are specific to language-related tracts or may be reflected in this pathway known to play a primary role in motor control (non-language-specific). White matter pathways of interest were examined bilaterally to examine hemispheric specificity of associated effects.

Since AFQ utilizes an automated approach for tract identification, tracts of interest could not be reliably identified among all infants. Following automated tractography, visual inspection of individual white matter tracts verified that tractography of the left-hemispheric ILF and CST was successful for all infants, and visual inspection of right-hemispheric correlates confirmed the inclusion of 38 for the right ILF and 37 out of 40 infants for the right CST. As for the arcuate, the left arcuate was successful for 34 out of 40 infants, and the right arcuate could be reconstructed for only 13 infants. Although the right arcuate was unable to be identified among a large number of infants, this is in line with prior studies that were unable to identify a right arcuate, even among adults (e.g., Catani and Mesulam, 2008). This observation is consistent with the previously established left > right asymmetry in the arcuate shown to emerge in early childhood (Reynolds et al., 2019), which may at least in part explain the absence of a trackable right arcuate. Thus, the limited sample size for the right arcuate was insufficient to be included in group-level analyses. Although we initially set out to also investigate the superior segment of the arcuate, the superior longitudinal fasciculus, we were unable to reliably reconstruct the tract utilizing this automated approach in a sufficient number of infants to warrant inclusion in the present analysis.

2.8. Statistical analyses

Multiple regression models were constructed for each white matter pathway of interest to examine the extent to which white matter in infancy (as indicated by FA) is prospectively associated with subsequent language outcomes in kindergarten. Age at the neuroimaging session in infancy constituted a control predictor to account for potential age effects in white matter organization, and language outcome measures were defined as the outcome variables (using standardized performance on each measure to account for age at the time of follow-up assessment). Socioeconomic status was controlled by minimal variation in the present sample, but key factors characterizing the home literacy environment were included in the model (amount of time spent reading to the child, number of children's books in the home), as shared parent-infant book reading has been linked with language acquisition in infancy (Karras and Braungart-Rieker, 2005) and prospectively associated with subsequent language abilities (Raikes et al., 2006).

Thus, multiple regression analyses were employed for each white matter pathway bilaterally in infancy as predictors with key characteristics of the home literacy environment in infancy (number of children's books in the home, amount of time spent reading with the child at home) and infant age as simultaneous control variables, and subsequent language measures as outcome variables. In addition, to assess the linguistic specificity of potential associations, a non-linguistic outcome variable was applied with the same approach to evaluate whether white matter pathways in infancy show prospective associations with nonverbal cognitive abilities (as indicated by KBIT-2) in kindergarten. Multiple regression analyses were conducted for FA of each white matter tract of interest along 50 individual nodes produced from AFQ utilizing MATLAB (<https://www.mathworks.com/products/matlab.html>) and R (<https://www.r-project.org/>). Subsequent visual inspection of resultant relationships was conducted to check for potential outliers in each distribution and systematically defined by values outside 1.5 times the interquartile range (above or below, as indicated via box plot). Any outliers identified were then removed in a comparative analysis to verify the stability of effects indicated.

In a final step, the average FA for significant nodes identified within each tract was estimated to determine prediction estimates by tract for each language outcome, while accounting for control predictors (number of children's books in the home, amount of time spent reading with the child per week (i.e., shared book reading time), infant age, and nonverbal cognitive abilities assessed at follow-up). Two additional control predictors addressed at this stage included (a) the number of gradients removed within diffusion-weighted processing, and (b) sex.

These factors were incorporated in provisional models for each tract, and remained in the final model only if they significantly contributed to the prediction of each outcome and/or impacted the overall prediction estimates.

Power analysis was employed to ensure a sufficient sample size to observe potential effects. Sample size for the final multivariate linear regression model (including the average of significant nodes identified within each tract) was estimated using four covariates and two independent variables for the prediction of language achievement. For power levels equal to 80 and predicted R-square equal to 40% the required sample size was $n = 25$. Interestingly, the multivariate model explained more than 61% of the total variance in phonological awareness, which in terms of effect size indicators is in excess of a large effect. Thus, the multivariate model was originally powered for a large effect and the observed effect much exceeded the 40% explained variance threshold, representing a salient prediction.

To further ensure that final results were not impacted by the number of poor gradients removed per participant based on our quality assurance process, two additional steps were taken. First, correlation analyses revealed the following: although the number of gradients removed per participant is negatively correlated with infant age at time of scan ($r = -0.350$, $p = 0.027$), indicating that older infants tended to have more motion outliers, there were no significant associations observed between the number of poor gradients and subsequent language outcomes ($p > 0.366$). This step allowed us to verify that the number of poor gradients within diffusion-weighted images in infancy does *not* in itself show any relation with the outcome variables of interest. In addition, final multiple regression analyses were replicated in a subset of the sample which included only participants with five or less poor gradients removed (excluding $n = 5$ participants), verifying that effects remain consistent with those observed in the full sample.

In addition to the classical procedure of correcting for multiple comparisons utilizing the Family-Wise Error (FWE) correction method, parametric bootstrapping was adopted to simulate the sampling distribution in the general population in order to ensure proper evaluation of the significance of the present effects. This approach involved simulating the population distribution under the null hypothesis based on partial beta values using sampling with replacement from the original sample. Specifically, multiple regression analyses (as described above) were performed for each white matter tract, with each subsequent language outcome randomly assigned to each participant. A simulation of 5000 replicated samples of the same size was programmed in MATLAB and replicated in Mplus (version 8). Variance estimates of the correlation coefficients (up to eight decimal places) derived from these samples were then utilized to simulate the population distribution under the null hypothesis, using mean and variance estimates of the sample's correlation coefficient. These distributions were tested for normality using the Kolmogorov-Smirnov test and across all bivariate comparisons they did not deviate from normality besides the expected nominal alpha level. Thus, symmetric 95% confidence intervals were utilized. Final indicators of significance for all analysis models for the actual participant-specific language performance within the present sample was then determined based on corresponding null distribution derived from this parametric bootstrapping procedure. In addition, the Family-Wise Error (FWE) correction method was employed with resultant p -values in order to account for the 50 nodes of each white matter tract. Once significant nodes for each white matter pathway were established via bootstrapping and FWE correction for multiple comparisons, the average FA across significant nodes identified for each tract was incorporated into final summative multiple regression models for overall estimation of variance explained by each model (R^2) and corresponding effect sizes (f^2 , determined in accordance with Cohen, 1988).

3. Results

With 40 children who completed diffusion-weighted imaging in

infancy and also returned for longitudinal follow-up (4–5 years later), the present study investigated longitudinal associations between white matter in infancy and subsequent follow-up language measures characterizing phonological skills, oral comprehension, and vocabulary knowledge (for an overview of participant demographics, see Table 1). Multiple regression models were constructed for each white matter tract in infancy with age at time of scan and home literacy variables as predictors, and subsequent language measures in kindergarten as outcome variables. The significant effects indicated are those that survive thresholds for both parametric bootstrapping and Family-Wise Error (FWE) rate adjustment to correct for multiple comparisons with each model. For each language outcome, additional provisional models incorporated (a) the number of gradients removed within the diffusion-weighted image processing for each participant, and (b) sex. These models verified that the prediction estimates did not change with either of these predictors included in the models for any outcome of interest, and neither of these control predictors significantly contributed to the prediction of language outcomes. Therefore, for parsimony, these factors were not included in final models.

3.1. Prospective associations between language-related white matter organization in infancy and subsequent language abilities

With the left arcuate fasciculus as a predictor while controlling for the effects of infant age and key factors related to the home literacy environment, white matter organization of the posterior segment of this tract in infancy is prospectively associated with several of the language outcome measures. Specifically, structural organization in the posterior segment of the left arcuate in infancy (as indicated by fractional anisotropy, FA) is prospectively associated with subsequent phonological awareness abilities, as characterized by Segmentation and Sound Blending measures (all significant effects FWE-corrected, $p < 0.005$) from the Woodcock-Johnson Oral Language Assessment (WJ-OL IV, Fig. 1). On average across all significant nodes identified within the posterior segment of this tract in relation to Segmentation (nodes 38–50) with infant age, home literacy factors, and nonverbal cognitive abilities as control predictors, this model explains 42.2% of the variance in kindergarten-age segmentation skills with a large effect size ($f^2 = 0.73$). For the model with Sound Blending as the outcome variable with predictors including significant nodes in the left arcuate identified in association (nodes 48–50) and infant age, home literacy factors, and nonverbal cognitive abilities as control predictors, the model explains 50.6% of the variance in sound blending abilities with a large effect size ($f^2 = 1.02$). Significant prospective associations were also identified between structural organization of the posterior segment of the left arcuate in infancy and subsequent vocabulary knowledge (FWE-

corrected, $p < 0.006$, FA in nodes 48–50, see Fig. 1), as measured by the Peabody Picture Vocabulary Test (PPVT-IV). With the average of these significant nodes implicated, the model explains 21.7% of the variance in kindergarten-age vocabulary knowledge with a medium effect size ($f^2 = 0.27$; including infant age, home literacy factors, and nonverbal cognitive abilities as control predictors). See Supplementary Information for full summary of these regression models.

No prospective associations were identified between FA of the inferior longitudinal fasciculus (bilaterally) in infancy and kindergarten-age language abilities when controlling for the effects of infant age and key factors related to the home literacy environment. In addition, no significant associations were identified between the arcuate or inferior longitudinal fasciculus in infancy and subsequent non-linguistic cognitive abilities in kindergarten (as indicated by standardized performance on matrix reasoning).

3.2. Corticospinal tract in infancy is prospectively associated with subsequent phonological abilities

To determine whether these findings point towards language domain-specific or possibly more domain-general effects, we investigated the corticospinal tract as well. To our surprise, the present findings indicated that the bilateral corticospinal tract is prospectively associated with subsequent phonological skills at the school age. For the left-hemispheric corticospinal tract, structural organization of this pathway was prospectively associated with phonological awareness (as indicated by Segmentation; significant for FA in nodes 1–12, FWE-corrected, $p < 0.006$), and phonological working memory skills (as indicated by the Memory for Digits subtest from the Comprehensive Test of Phonological Processing, CTOPP-2; FWE-corrected, $p < 0.007$, significant effects with FA in nodes 10–15, see Fig. 2), with infant age and key factors related to the home literacy environment as control predictors. On average across all significant nodes identified within this tract for each outcome measure respectively with infant age, home literacy factors, and nonverbal cognitive abilities as control predictors, each model explains 49.9% of the variance in kindergarten-age segmentation skills with a large effect size ($f^2 = 0.99$), and 19.8% of the variance in phonological memory abilities with a medium effect size ($f^2 = 0.25$). See Supplementary Information for full summary of these regression models.

The right-hemispheric corticospinal tract revealed a significant association with phonological skills as well, in the domain of phonological memory. Specifically, structural organization of the right corticospinal tract in infancy (as indicated by significant effects with FA among nodes 27–36, FWE-corrected, $p < 0.009$) is prospectively associated with phonological memory skills in kindergarten, with infant age and key

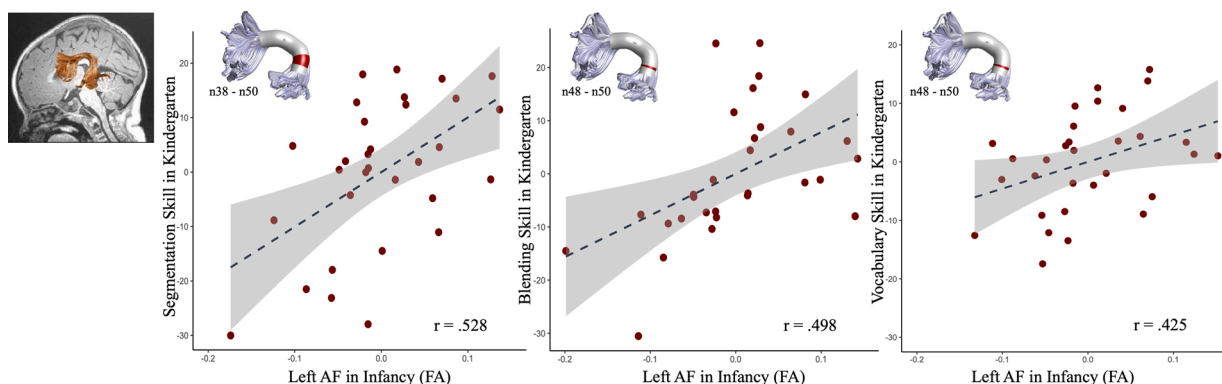


Fig. 1. Structural organization of the left arcuate fasciculus in infancy is prospectively associated with phonological awareness and vocabulary knowledge skills in kindergarten. *Note:* Significant associations between FA of the posterior segment of the left arcuate in infancy and language skills in kindergarten are displayed in terms of the centered residuals produced from partial correlations with the average FA across significant nodes indicated. Nodes in the posterior segment of the left arcuate that show significant effects are indicated on the rendered tract displayed in the upper left panel of each plot ($p < 0.05$, FWE-corrected).

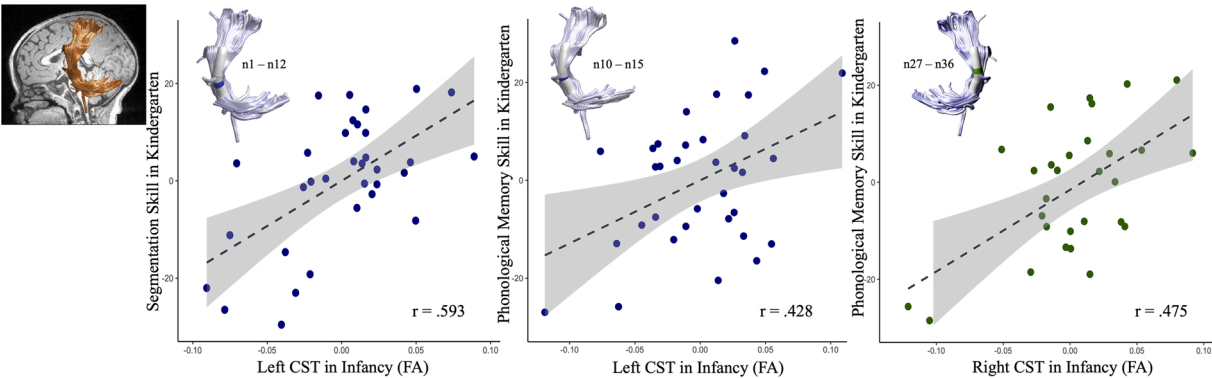


Fig. 2. Structural organization of the corticospinal tract in infancy is prospectively associated with subsequent phonological awareness and phonological memory in kindergarten. *Note:* FA of the left corticospinal tract in infancy is prospectively associated with subsequent phonological awareness, and both left and right (bilateral) corticospinal tract in infancy are prospectively associated with phonological memory in kindergarten. Significant associations between FA of bilateral corticospinal tract in infancy and phonological skills in kindergarten are displayed in terms of the centered residuals produced from partial correlations with the average FA across significant nodes indicated. Nodes of the corticospinal tract that show significant effects are indicated on the rendered tract displayed in the upper left panel of each plot ($p < 0.05$, FWE-corrected).

factors related to the home literacy environment as control predictors. On average across all significant nodes identified within this tract with infant age, home literacy factors, and nonverbal cognitive abilities as control predictors, the model explains 23.3% of the variance in phonological memory abilities with a medium effect size ($f^2 = 0.30$). Upon visual inspection and subsequent outlier analysis, two outliers were identified within this distribution. Removal of these outliers and subsequent re-analysis revealed that structural organization of the right corticospinal tract in infancy is still prospectively associated with kindergarten-age phonological memory in overlapping and additional significant nodes (nodes 27–39, FWE-corrected, $p < 0.009$). No significant effects were identified between the right corticospinal tract in infancy and subsequent phonological awareness or broad language skills. Lastly, no significant associations were identified between the corticospinal tract (bilaterally) in infancy and subsequent non-linguistic cognitive abilities in kindergarten (as indicated by standardized performance for matrix reasoning).

3.3. Contributions of the arcuate fasciculus and corticospinal tract in infancy to the prediction of kindergarten-age phonological awareness abilities

Based on our findings within the left arcuate and corticospinal tract in association with kindergarten-age phonological awareness abilities (as indicated by Segmentation), we built a final model incorporating the average FA of significant nodes identified in each tract while also accounting for infant age, key factors related to the home literacy environment, and nonverbal cognitive abilities as control predictors. For the average FA across significant nodes identified in previous analysis models for each tract, both the left arcuate ($\beta = .388$, $p = 0.01$) and left corticospinal tract ($\beta = .601$, $p = 0.002$) are prospectively associated with kindergarten-age segmentation skills. Overall, the model explains 61.6% of the variance in segmentation skills with a large effect size ($f^2 = 1.60$; see Table 2 for an overview). Relative importance analysis reveals that the left arcuate contributes 24.67% of the variance, the left corticospinal tract contributes 41.04% of the variance, and the remaining is explained by the non-significant control predictors (age, nonverbal cognitive abilities, and home literacy environment variables).

4. Discussion

The present study identifies prospective associations between white matter in infancy and subsequent language skills in kindergarten. Findings suggest that structural organization of the posterior portion of the left arcuate fasciculus in infancy is prospectively associated with

Table 2
Longitudinal Prediction of Phonological Awareness (Segmentation).

	<i>B</i>	<i>SE B</i>	β	<i>t</i>	<i>p</i>
White Matter Pathways in Infancy					
Left arcuate fasciculus (nodes 38–50)	72.785	26.805	0.388	2.715	0.012
Left corticospinal tract (nodes 1–12)	160.241	44.660	0.601	3.588	0.002
Control Predictors					
Infant age	−0.046	0.022	−0.356	−2.072	0.050
Number of children's books	2.241	1.581	0.191	1.401	0.175
Shared book reading time	0.051	1.188	0.006	0.043	0.966
Nonverbal IQ (at follow-up)	0.307	0.150	0.267	2.047	0.052
Intercept	−15.306	24.092		−0.635	0.531

Note. $R^2 = 0.616$; adjusted $R^2 = 0.516$.

core components of language: phonological awareness and vocabulary knowledge. By contrast, no significant associations were indicated with the left inferior longitudinal fasciculus in infancy. Yet, structural organization of the bilateral corticospinal tract in infancy was found to be prospectively associated with subsequent phonological skills. All prospective longitudinal relationships between white matter in infancy and subsequent language outcomes indicated remain significant when controlling for nonverbal cognitive abilities. Furthermore, no significant longitudinal associations between white matter in infancy and subsequent nonverbal cognitive abilities were observed. Taken together, tract-specific and language domain-specific longitudinal associations between neuroanatomical properties of white matter in infancy and subsequent language abilities were evident even while controlling for age, home literacy environmental factors, and nonverbal cognitive abilities. These findings suggest that the neural foundation for language established in infancy may underlie the subsequent developmental trajectory of language acquisition.

Findings in the arcuate fasciculus build upon previous literature implicating this pathway as crucial for language and extend beyond in revealing that this tract significantly contributes to the prediction of school-age language skills from as early as infancy. The left arcuate fasciculus has been repeatedly linked with language skills among pre-school and school-age children (Lebel and Beaulieu, 2009; Yeatman et al., 2011; Saygin et al., 2013; Reynolds et al., 2019). Limited evidence has pointed towards contributions of the arcuate from early childhood,

but without long-term longitudinal links among full-term infants due to short-range longitudinal designs (Deniz Can et al., 2013; Reynolds et al., 2019). Here we demonstrate that these links between the left arcuate fasciculus and language are not only present in childhood, but that structural organization in the posterior portion of this tract in infancy is prospectively associated with specific school-age language outcomes. Importantly, not all follow-up language measures of interest were linked with the left arcuate in infancy. The present findings specifically link structural organization of the arcuate fasciculus in infancy with subsequent phonological awareness and vocabulary knowledge, which directly aligns with previously reported characterization of the arcuate fasciculus at the preschool/school-age in relation to phonological awareness (Lebel and Beaulieu, 2009; Saygin et al., 2013; Reynolds et al., 2019) as well as vocabulary knowledge (Su et al., 2018; López-Barroso et al., 2013). Our findings suggest that specific associations between the arcuate and phonological awareness as well as vocabulary knowledge are not only evident at the preschool/school age but are further revealed in association with structural foundations for the arcuate established in infancy.

The present longitudinal associations between structural organization of the posterior segment of the left arcuate in infancy and subsequent language outcomes in kindergarten also account for aspects of the home environment, specifically factors pertaining to home literacy exposure. Broadly speaking, this is in line with the previously identified role of the left arcuate fasciculus in mediating the association between environmental language exposure (via conversational turns) and language skills among four- to six-year-olds (Romeo et al., 2018). Findings indicate that these links may be, in part, explained by pre-existing brain structure from as early as infancy, consistent with the notion that white matter organization precedes functional development (Mahon and Caramazza, 2011; Hannagan et al., 2015; Saygin et al., 2016). Moreover, the specificity of findings within the posterior segment of the left arcuate fasciculus anatomically aligns with the specific posterior portion of this tract implicated among preschool-age children (Romeo et al., 2018). While the arcuate is known to be receptive to environmental input and fractional anisotropy in white matter is known to increase throughout early childhood (Barnea-Goraly et al., 2005), the present findings suggest that the structural organization of the arcuate established within the first two years of life (during the most robust period for white matter development) provides an important foundation upon which it is hypothesized that ongoing experience builds and refines over time. Taken together, findings suggest that the structural foundation for the arcuate fasciculus established in infancy is prospectively associated with subsequent language abilities four to five years later, and therefore likely contributes to cross-sectional links between the brain and language observed at preschool, school age, and later stages of development.

In addition to findings in the primary dorsal pathway implicated in language, this investigation also points towards early contributions of the corticospinal tract as foundational for subsequent phonological skills. Notably, however, we did not find a comparable association with the ventral pathway investigated: no significant associations were indicated between the inferior longitudinal fasciculus in infancy and subsequent language skills. Although this is an earlier-developing ventral pathway in infancy (Dubois et al., 2008) and is known to ultimately contribute to the comprehensive network of white matter subserving language (Dick et al., 2014), the present findings suggest this may be linked to automatic retrieval of foundational language skills once acquired, or may be prospectively associated with specific facets of higher-order language skills that were not addressed in the present study (e.g., semantics). Another noteworthy consideration pertains to the role of the inferior longitudinal fasciculus in language, for the precise contributions of this tract to language remain underspecified (Mandonnet et al., 2007). That said, recent longitudinal neuroimaging investigation among prematurely born infants observed that structural organization of the left inferior longitudinal fasciculus at the time of neonatal hospital discharge (within the first month of life, in a modest sample of 30

infants) is associated with subsequent toddler-age composite language abilities (Dubner et al., 2020). Therefore, it is possible that non-significant effects for the left ILF may be attributed to a lack of variation in structural organization of this tract within the present sample, which could be at least partly due to investigation at a later stage in infancy (mean: 9 months) with typically developing infants born full-term, relative to the focus on newborn brain structure among infants born prematurely in the study by Dubner and colleagues. These findings call for further investigation in early childhood development to specify the role of the inferior longitudinal fasciculus as well as additional ventral pathways that support language functions. By contrast, our incorporation of the corticospinal tract as a non-language-dominant tract, given its primary role in motor control, did reveal significant associations.

Although the corticospinal tract served as our control, findings suggest that structural organization of the left corticospinal tract in infancy is prospectively associated with phonological awareness and bilaterally with phonological memory skills in kindergarten. Although the majority of evidence implicates language-dominant pathways in association with phonological awareness (Lebel and Beaulieu, 2009; Saygin et al., 2013; Reynolds et al., 2019), associations between the corticospinal tract and specifically phonological skills among preschool-age children have been reported (Walton et al., 2018). From a developmental perspective, the left corticospinal tract matures earlier than arcuate and inferior longitudinal fascicles (Dubois et al., 2008). Therefore, it is conceivable that higher indices of myelination within the corticospinal tract in infancy may facilitate early cognitive-linguistic development, in line with the proposition that cognitive development arises from domain-general interactions sub-served by multiple pathways (Johnson, 2011). While the corticospinal tract is known to be an earlier-developing tract, it remains unclear whether myelination in the corticospinal tract may truly be the source of variation in long-term language outcomes. Alternatively, one possibility is that the tractography approach employed to define the corticospinal tract may have also captured corticobulbar fibers, which are known to innervate cranial nerves associated with speech production and therefore play a known role in speech and language (Northam et al., 2019). Corticospinal and corticobulbar pathways are closely aligned as they pass through the internal capsule towards their subcortical projections, which can make distinction between these two tracts challenging using tractography (e.g., Pan et al., 2012). Therefore, future work is needed to clarify the potential role of the corticospinal tract from infancy in facilitating language development.

The present findings carry implications for language development as a process of refinement that builds upon pre-existing structural scaffolds in infancy, but also brings forth a multitude of questions regarding respective contributions of genetics and environmental experience. Results support notions of the early impact of genetic susceptibility, as temporal dynamics of the transcriptome are known to be most robust prenatally (Johnson et al., 2009); further reflected in the rapid developmental trajectory of white matter within the first two years of life (Gilmore et al., 2018). Yet, this also coincides with an especially rich time for environmental input - with an average age of 10 months among infants in the present sample, it remains unclear to what extent these findings reflect experience-driven effects, as an infant's first year encompasses a rapid time of learning and development (Dehaene-Lambertz and Spelke, 2015). Therefore, it is possible that the present characterization of white matter organization in infancy inherently reflects environmental influences in pathways highly susceptible to environmental input, such as the arcuate fasciculus. This supports the working hypothesis that the brain is especially sensitive to environmental input within the first two years of life, coinciding with the most robust period of white matter development (Gilmore et al., 2018). Although infant age and home literacy environment were accounted for, future studies are needed from a narrower and earlier starting point to further disentangle biological determinants from environmental experience.

The present findings are to be interpreted in the context of limitations. It is important to recognize the modest sample size acquired due to attrition in our extended longitudinal design (4–5 years), and the wide infant age range due to flexible age criteria in our initial efforts to establish a protocol for successful neuroimaging acquisition with naturally sleeping infants. On another note, selected measures utilized to characterize language abilities at school age are non-exhaustive in providing a first indicator of these longitudinal relationships among a subset of language constructs; further investigation will be necessary to examine white matter in relation to additional facets of language, particularly in expressive domains such as sentence formulation. In addition, recognizing the constraints of our current approach to white matter tractography in infancy that necessitated both participant and tract-specific quality control procedures, methodological advancement of infant neuroimaging analysis continues to be needed in future work to optimize tract definition and reconstruction within the first two years of life (Turesky et al., 2021). That said, the medium-to-large effect sizes associated with significant effects presently identified suggest that, despite necessary exclusions due to careful quality control, the present sample size was sufficient to capture the magnitude of potential effects. The present analysis further provides an initial indicator of prospective associations between white matter in infancy and subsequent language based on our selected white matter pathways of interest; therefore, future research with a larger sample size may be warranted to investigate whether longitudinal relationships are evident with additional white matter pathways (such as the understudied role of the middle longitudinal fasciculus in language; Dick and Tremblay, 2012; Conner et al., 2018). For an additional consideration beyond infancy, it is important to recognize that the present findings do not suggest static white matter organization established solely in infancy, for white matter development undergoes a process of maturation and refinement that continues throughout early childhood into adulthood (Barnea-Goraly et al., 2005). Therefore, it will also be important in future work to investigate the relative contributions of white matter in infancy versus developmental changes throughout early childhood in relation to language outcomes.

Another consideration pertains to additional environmental factors that shape language development. Present associations between white matter in infancy and subsequent language account for aspects of home environment, yet factors involving parent-child interactions have also been linked with subsequent language skills (Karrass and Braungart-Rieker, 2005; Raikes et al., 2006). Findings are to be interpreted in the context of the rich body of evidence implicating the important role of the environment in shaping language skills, and modest prediction estimates in the present models suggest contributions from additional factors, such as the quantity and quality of language input/exposure (Rowe, 2012; Weisleder and Fernald, 2013). Minimal socioeconomic variation controlled the present sample; therefore, future work is necessary to characterize these relationships among a sample with varied socioeconomic representation and examine whether white matter organization mediates previously documented relationships between home environmental factors and language/literacy from as early as infancy, as reported among preschool-age children (Romeo et al., 2018; Ozernov-Palchik et al., 2019). Nonetheless, putative influences of the home environment must also be interpreted in the context of heritability, as parents contribute both environmental and genetic influences on children's outcomes (Hart et al., 2019). It is also conceivable that associations between white matter and language in early childhood are particularly essential for language within a certain developmental stage (e.g., infancy, toddlerhood) and less evident among long-range associations due to dynamic and rapidly evolving environmental contributions. Taken together, findings illuminate the importance of investigating neurobiological foundations from infancy in conjunction with environmental factors when examining language development.

Overall, findings suggest that white matter organization established in infancy sets one important foundation underlying the trajectory of

language acquisition. This study supports the working hypothesis that certain genes and their temporal dynamics, critical for brain development, give rise to white matter that is highly receptive to environmental input, and these factors together establish a structural foundation for language in the brain that is characterized by the most robust period of development within the first two years of life. Thereafter, it is hypothesized that the neural foundation established in infancy is then further built upon and refined by experience over time, and ultimately shapes language outcomes. Thus, the present findings point towards the significance of brain structure from as early as infancy in providing a scaffold upon which ongoing experience can build throughout development.

Data Statement

The data utilized in the present study have not been made available on a permanent third-party archive due to Institutional Review Board regulations at Boston Children's Hospital; requests may be sent via email to the corresponding author.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.dcn.2021.100973>.

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