Tracking the Roots of Reading Ability: White Matter Volume and Integrity Correlate with Phonological Awareness in Prereading and Early-Reading Kindergarten Children

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Developmental dyslexia, an unexplained difficulty in learning to read, has been associated with alterations in white matter organization as measured by diffusion-weighted imaging. It is unknown, however, whether these differences in structural connectivity are related to the cause of dyslexia or if they are consequences of reading difficulty (e.g., less reading experience or compensatory brain organization). Here, in 40 kindergartners who had received little or no reading instruction, we examined the relation between behavioral predictors of dyslexia and white matter organization in left arcuate fasciculus, inferior longitudinal fasciculus, and the parietal portion of the superior longitudinal fasciculus using probabilistic tractography. Higher composite phonological awareness scores were significantly and positively correlated with the volume of the arcuate fasciculus, but not with other tracts. Two other behavioral predictors of dyslexia, rapid naming and letter knowledge, did not correlate with volumes or diffusion values in these tracts. The volume and fractional anisotropy of the left arcuate showed a particularly strong positive correlation with a phoneme blending test. Whole-brain regressions of behavioral scores with diffusion measures confirmed the unique relation between phonological awareness and the left arcuate. These findings indicate that the left arcuate fasciculus, which connects anterior and posterior language regions of the human brain and which has been previously associated with reading ability in older individuals, is already smaller and has less integrity in kindergartners who are at risk for dyslexia because of poor phonological awareness. These findings suggest a structural basis of behavioral risk for dyslexia that predates reading instruction.

Introduction

Developmental dyslexia, an unexplained difficulty in learning to read, affects ~10% of children in the United States (Lyon et al., 2003). Dyslexia is associated with atypical brain function for reading, especially reduced activation in left temporoparietal regions (Gabrieli, 2009) that is independent of current reading ability (Hoeft et al., 2007) or IQ (Tanaka et al., 2011). Dyslexia is also associated with structural differences in white matter as measured by diffusion weighted imaging (DWI), specifically lower fractional anisotropy (FA) in the left hemisphere (for review, see Vandermosten et al., 2012a) and reduced white matter volume (Silani et al., 2005). These differences may reflect weakened white matter connectivity among left-hemisphere areas that support reading. This interpretation is bolstered by evidence that similar DWI measures correlate with reading skill (Klingberg et al., 2000; Beaulieu et al., 2005; Deutsch et al., 2007; Dougherty et al., 2007; Hoeft et al., 2011).

A fundamental question is whether white matter structural differences are a cause or a consequence of poor reading in dyslexia. Prior DWI studies have not answered this question because they examined children and adults with extensive reading experience, and children with reading difficulty tend to read far less than typically reading peers (Cunningham and Stanovich, 1998) and appear to develop alternative reading pathways (e.g., enhanced right-hemisphere activation; Shaywitz et al., 2002). The most direct way to evaluate whether white matter differences contribute to the etiology of dyslexia is to examine kindergartners for whom reading instruction and experience is unlikely to influence brain structure.
Prereaders and early readers cannot exhibit dyslexia per se, but their prereading skills that predict later reading abilities can be evaluated. In English, such predictors are phonological awareness (PA; awareness of the sound structure of spoken words, which must be mapped onto letters to read), rapid automatized naming (RAN), and letter knowledge (Schatschneider et al., 2004). In children ages 7–11, PA correlated negatively with FA in callosal fibers connecting the temporal lobes (Dougherty et al., 2007). In adults, PA correlated positively with volume of the left superior longitudinal fasciculus (SLF; Frye et al., 2011) and with higher FA of the left arcuate fasciculus (Vandermosten et al., 2012b). The left arcuate is part of the SLF and connects temporo-parietal and inferior frontal regions that are core constituents of the language network.

We hypothesized that if white matter differences are related to the cause of dyslexia, then such differences ought to be related to prereading skills that predict future reading ability. We examined two aspects of the left SLF (the arcuate fasciculus and SLFp), the frontoparietal aspect of the SLF because of their importance in language and phonological awareness (Rauschecker et al., 2009; Yeatman et al., 2011; Vandermosten et al., 2012b), and the left inferior longitudinal fasciculus (ILF) because of its involvement in visual word-form processing (Cohen et al., 2003; Yeatman et al., 2012). We investigated whether these tracts were related to PA, RAN, and letter knowledge skills in prereading and early reading kindergartners. Finally, a whole-brain analysis examined the anatomic specificity of the tract-based findings.

Materials and Methods

Overview

As part of a larger study, children completed a short battery of psychoeducational assessments in their schools in New England in the spring of prekindergarten or fall of kindergarten before formal word reading instruction. Children with varying prereading skills were invited to take part in brain imaging. This study was approved by the institutional review boards at the Massachusetts Institute of Technology and Boston Children’s Hospital. Parents gave written consent and children gave verbal assent to participate.

Participants

DWI data were collected from 52 children. Of these, 12 children had excessive motion (determined by visual inspection) and were excluded from subsequent analysis. Analyses included 40 children (21 females/19 males, 28 white/12 nonwhite; demographic information and scores are provided in Table 1). All children met eligibility criteria including: native speaker of American English; born after at least 36 weeks gestation; no sensory or perceptual difficulties other than corrected vision; no history of head or brain injury or trauma; no neurological, neuropsychological, or developmental diagnoses; no medications affecting the nervous system; and standard scores >80 on measures of nonverbal and verbal IQ (Kaufman Brief Intelligence Test [KBIT-2], Kaufman and Kaufman, 2004 Matrices; Peabody Picture Vocabulary Test [PPVT-4], Dunn and Dunn, 2007).

Table 1. Demographic information and scores for participants (n = 40)

<table>
<thead>
<tr>
<th>Measure</th>
<th>Mean (SD)</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years;months)</td>
<td>5.6 (0.5)</td>
<td>4.10 – 6.4</td>
</tr>
<tr>
<td>KBIT-2 matrices SS</td>
<td>100.9 (10.3)</td>
<td>85 – 120</td>
</tr>
<tr>
<td>PPVT-4 vocabulary SS</td>
<td>116.2 (12.8)</td>
<td>83 – 160</td>
</tr>
<tr>
<td>PA composite SS</td>
<td>10.3 (1.8)</td>
<td>7.3 – 15.1</td>
</tr>
<tr>
<td>RAN composite SS</td>
<td>97.5 (13.5)</td>
<td>68.3 – 122.3</td>
</tr>
<tr>
<td>LK composite SS</td>
<td>106.8 (10.2)</td>
<td>87.0 – 132.0</td>
</tr>
<tr>
<td>Word ID raw score</td>
<td>12.8 (19.3)</td>
<td>0 – 67</td>
</tr>
</tbody>
</table>

Lk, Letter knowledge; SS, Standard Score, in which standardized mean is 100 except for PA (CTOPP), in which it is 10.

Behavioral measures

Phonological awareness. Three subtests from the Comprehensive Test of Phonological Processing (CTOPP; Wagner et al., 1999) were given to assess phonological skills: (1) Elision: the child repeats a word after removing a given sound (e.g., “say boat without saying /b/” = “oat”); (2) Blending Words: the child lists to a recorded series of sounds and blends them together to derive a real word (e.g., /ma/-/f/-/l/ = “mat”); (3) Nonword Repetition: the child listens to a recorded made-up word (e.g., “sart”) and repeats it. A composite standard score was created from the mean standard score for the three subtests (mean for each subtest is 10, SD is 3). The range of standard scores for the CTOPP is limited for this age range because age 5 is the youngest age the test is normed for; a child who scores 0 on the Elision or Blending Words subtests receives a standard score of 7, which is considered to be in the average range.

Letter knowledge. The letter identification subtest from the Woodcock Reading Mastery Test, Revised/ Normative Update (WRMT-R/NU; Woodcock, 1998) was given to assess letter name knowledge. The Letter Sound Knowledge subtest from the York Assessment of Reading for Comprehension (YARC; Snowling et al., 2010) was given to assess knowledge of letter sounds and phoneme isolation skills. A composite was created from the mean standard scores for the YARC and WRMT-R subtests.

RAN. The Rapid Automated Naming/Rapid Alternating Stimulus (RAN/RAS) tests (Wolf and Denckla, 2005) were given to measure the speed and efficiency of processes by which a series of randomly arranged stimuli are named. Subtests given were Object, Color, and Letter Naming. A composite was created from the mean standard scores for each subtest. For children who could not identify the letters during practice and thus did not receive a score for RAN Letters, a composite was created from the Objects and Colors subtests.

Word ID. The WRMT-R/NU (Woodcock, 1998) Word ID subtest was given to assess single word reading skills. In this test, the child is asked to read aloud single words of increasing difficulty. Raw scores were used to determine prereader versus reader status. Prereaders were defined as children who correctly read 0 words or 1 word on this test.

Image acquisition and processing

Data were acquired on a 3T Siemens Trio Tim MRI scanner with a standard Siemens 32-channel phased array head coil. A whole-head, high-resolution T1-weighted multiecho MPRAGE (van der Kouwe et al., 2008) anatomical volume was acquired (acquisition parameters: TR = 2550 ms, TE = 1.64 ms, TI = 1400 ms, flip angle = 7°, FOV = 192 × 192, 176 slices, voxel resolution = 1.0 mm³, acceleration = 4). An online prospective motion correction algorithm was implemented to reduce the effect of motion artifacts during the structural scan, and 10 selective reacquisition time points were acquired and included to replace time points that were affected by motion (Tisdall et al., 2012). The diffusion-weighted scan (5 min total) included 10 non-diffusion-weighted volumes (b = 0) and 30 diffusion-weighted volumes acquired with non-collinear gradient directions (b = 700 s/mm²), all at 128 × 128 base resolution and isotropic voxel resolution of 2.0 mm³.

Structural MRI data were processed using a semiautomated processing stream using the default parameters in FreeSurfer v5.1.0 (Dale et al., 1999; Fischl et al., 2002, 2004; Desikan et al., 2006; http://surfer.nmr.mgh.harvard.edu/), which includes motion and intensity correction, surface coregistration, spatial smoothing, subcortical segmentation, and cortical parcellation based on spherical template registration. Diffusion-weighted images were checked for motion artifacts and processed using FreeSurfer’s TRACULA and FSL’s FDT software (http://www.fmrib.ox.ac.uk/fsl/fdt/index.html).

Tract-of-interest analyses

We defined the tracts of interest using FreeSurfer’s TRACULA (Yendiki et al., 2011), an automated method that reconstructs probabilistic distributions of major white matter tracts from each participant’s native diffusion images. This method has been shown to accurately reconstruct tracts in an individual subject (compared with manual labeling) using anatomical priors. It uses a tract atlas of manually labeled tracts and FreeSurfer anatomical segmentations from a separate group of individuals to

Saygin, Norton et al. • White Matter in Prereaders
obtain the probability of each tract traversing or neighboring the anatomical segments along its trajectory. These anatomical priors are incorporated in a probabilistic framework to guide tractography in each novel subject, which allows for individual variation while reconstructing tracts that are anatomically consistent across individuals.

Preprocessing included registering diffusion-weighted images to the \( b = 0 \) images to compensate for motion and eddy-current distortions. A registration transform was computed using FreeSurfer’s bregistere (Greve and Fischl, 2009) for mapping each participant’s \( b = 0 \) image to the native structural scan. Images were checked for registration errors and no corrections were necessary. Cortical parcellations and subcortical segmentations from each individual’s FreeSurfer reconstruction were mapped to his/her DWI using the above transform. FSL’s DTIFIT estimated the tensor fits at each voxel, which produced FA images. FSL’s bedpostX applied the ball-and-stick model of diffusion to each individual’s DWI per voxel (Behrens et al., 2007). FSL’s FLIRT computed a registration transform of each participant’s structural scan to the Montreal Neurological Institute 152 (MNI152) template. This, combined with the first transformation (DWI-to-structural), was used to map the anatomical priors from the tract atlas to the individual’s native diffusion images. The priors were then combined with each individual’s local diffusion orientations obtained from the ball-and-stick model, as well as the individual’s anatomical segmentations, to estimate probability distributions for each of the major pathways in each individual’s native DWI space. After visual inspection, tract volumes were extracted and FA values averaged over the tract per individual. Tensor fits were only used to calculate the FA measures and not in the tractography algorithm itself; the latter uses the ball-and-stick fit, which, unlike the tensor fit, can model multiple (here, up to two) diffusion directions at each voxel. Although the anatomical priors in the tract atlas are computed in MNI space, the information encoded in these priors consists of the anatomical labels that each tract traverses and neighbors in \( x, y, \) and \( z \) along its trajectory, and not the exact spatial location of the tract in MNI space. Therefore, this method is less sensitive to misalignment than voxel-based approaches that rely on exact spatial correspondence between subjects.

We examined a priori three white matter tracts that connect critical components of language and reading networks and that have most often been associated with variation in reading ability: the left ILF and two separate components of the left SLF, the arcuate fasciculus and SLP. The left ILF is important for visual processing of words (Cohen et al., 2003; Yeatman et al., 2012) and damage to this tract can result in alexia (Eipelbaum et al., 2008), presumably due to its role in connecting low-level visual regions to the visual word form area (a fusiform region involved in processing word/letter strings; Delaene et al., 2002). The SLF connects frontal regions to both the parietal cortices (via the SLFP) and to temporal cortices (via the arcuate fasciculus). In particular, FA measures of the left arcuate and SLFP, FA values for the right hemisphere homologs were included as predictors in the multilinear regression and terms were removed or added based on model fit (both removal and entry significances were defined as \( p < 0.05/3 \), corrected for each behavioral metric). For the whole-brain analyses, FSL’s Randomize tool with threshold-free cluster-enhancement (Smith and Nichols, 2009) was used to perform 5000 permutation tests with a significance threshold set at \( p < 0.05 \), FWE corrected for multiple comparisons across voxels.

**Results**

**Behavioral results**

Mean scores and score ranges are reported in Table 1. We also characterized children as “at risk” for dyslexia based on scores in each of the three constructs of interest to provide context for the degree of variation in our sample. Although there are no definitive behavioral criteria for identifying which kindergarten children will go on to have dyslexia, longitudinal research indicates that classifying the lowest 30% of children as at-risk yields the optimal balance between sensitivity and specificity (Catts et al., 2001; Torgesen, 2004). Considering a cutoff of below the 30th percentile for age-based standard scores, the number of children from the sample of 40 who would be considered at risk in each category is 9 for PA, 13 for RAN, and 5 for letter knowledge. There is some overlap of these categories: 22 children were not at risk in any of the 3 categories, 11 in only 1 category, 5 in 2 categories, and 2 in all 3 categories. There were no differences between boys and girls on any behavioral measure (age, KBIT, PPVT, or composites of PA, RAN, or letter knowledge composites; independent samples \( t \) tests, two-tailed, all \( p > 0.08 \)).
Brain analyses
We performed correlations of volume and FA for the ILF, arcuate, and SLFp with behavioral predictors of dyslexia (Fig. 1). The volume of the left arcuate was significantly and positively correlated with individual composite scores on phonological awareness ($r = 0.38, p = 0.014$). The average FA values of the left arcuate were also positively correlated with phonological awareness scores overall ($r = 0.37, p = 0.020$), but did not pass significance for 2 of 40 cross-validation loops. Neither volume nor FA of the left ILF or SLFp correlated significantly with phonological awareness measures. Of the six tracts (bilateral ILF, arcuate, and SLFp), only the volume of the left arcuate was found to be a significant predictor of dyslexia, poor phonological awareness, and the left arcuate fasciculus was corroborated by an independent whole-brain analysis. The discovery that such a relation exists before formal reading instruction and substantial reading behavior predicts of dyslexia. These results were observed in the whole group of 40 children with varied reading abilities in the first half of kindergarten and also in the subset of 18 children who were prereaders. The specific relation between phonological awareness and the left arcuate fasciculus is classically posited to facilitate communication between Broca’s and Wernicke’s areas.

Discussion
In kindergarten children, we found a correlation between phonological awareness for spoken language and indices of white matter organization of the left arcuate fasciculus, specifically volume and FA. This relationship was both anatomically and behaviorally specific; it was not observed in other tracts (left ILF, left SLFp, or right hemisphere homologs) or for other behavioral predictors of dyslexia. These results were observed in the whole group of 40 children with varied reading abilities in the first half of kindergarten and also in the subset of 18 children who were prereaders. The specific relation between phonological awareness and the left arcuate fasciculus is classically posited to facilitate communication between Broca’s and Wernicke’s areas. Overall, these findings support the expected importance of a white matter tract that
connects critical anterior and posterior language areas that are, in turn, essential for mapping language onto print. Consistent with the present findings, greater FA in left arcuate fasciculus has been associated with superior phonological awareness in adults (Rolheiser et al., 2011; Vandermosten et al., 2012b). In contrast, a study in children ages 7–11 found that greater FA in left arcuate fasciculus was associated with inferior phonological awareness (Yeatman et al., 2011). In a longitudinal study of children ages 7–15 examining the left arcuate fasciculus, superior readers initially had lower FA that increased across 3 years.

Figure 2. Greater volume and FA of the left arcuate fasciculus are associated with superior phonological awareness. a. Volume of the left arcuate fasciculus plotted against individual raw scores of the Blending Words subtest. b. Phonological awareness, illustrated by Blending Words raw scores, was significantly correlated with average FA of the left arcuate fasciculus. In a and b, thick lines represent the best fits and thin outer lines represent the 95% confidence intervals. c. To illustrate the relation between the behavioral phonological awareness scores with left arcuate volume and FA, this tract was rendered from example participants (filled red circles in a and b) and colored according to FA. The tracts are ordered by Blending Words raw score increasing from left to right.

Figure 3. Greater volume and FA of the left arcuate fasciculus are associated with superior phonological awareness in prereading children. In prereading children (who could read 0 or 1 word), the Blending Words subtest scores were significantly correlated with the volume (a) and the average FA (b) of the left arcuate.

Figure 4. Whole-brain voxelwise analyses of the relation between FA and behavioral predictors of dyslexia. TBSS analyses revealed that FA values were associated only with phonological awareness scores and only in a cluster of voxels (red) at the bend of the SLF near Wernicke’s area, where the arcuate elbows toward the temporal lobe (p < 0.05 corrected; cluster size = 22). All statistics were run on the standard skeleton obtained using TBSS and displayed on thickened skeletons for ease of viewing. (L, left; P, posterior).

Consistent with the present findings, greater FA in left arcuate fasciculus has been associated with superior phonological awareness in adults (Rolheiser et al., 2011; Vandermosten et al., 2012b). In contrast, a study in children ages 7–11 found that greater FA in left arcuate fasciculus was associated with inferior phonological awareness (Yeatman et al., 2011). In a longitudinal study of children ages 7–15 examining the left arcuate fasciculus, superior readers initially had lower FA that increased across 3 years,
whereas inferior readers initially had higher FA that decreased over 3 years (Yeatman et al., 2012). Only a longitudinal study spanning prereading and reading children can offer strong evidence on this topic, but the combined findings suggest a dynamic developmental pattern such that in early (prereading) and late (later childhood and adult) stages there is a positive relation between left arcuate microstructure and language or reading ability, but in a middle stage of intensive reading acquisition, this relation temporarily reverses.

Reduced indices of white matter organization could reflect several aspects of white matter tracts, such as the axonal properties of the arcuate. Alternatively, there may be more crossing fibers in children with lower phonological awareness, which may lead to lower measures of FA. Measures of anisotropy change with age (Lebel and Beaulieu, 2011), but this developmental difference was not observable in the arcuate for the narrow (1.5-year) age range of our participants.

Variation in left arcuate fasciculus volume and microstructure among prereading and early-reading children was associated specifically with phonological awareness, and not with other reading-related skills such as naming fluency or letter knowledge. The absence of any correlation between behavioral measures and properties of the left ILF, a white matter tract that connects visual and temporal cortices, may reflect the minimal reading experience of children in the present study. Indeed, other studies of children and adults with extensive reading experience have reported relations between measures of the left ILF and reading ability (Steinbrink et al., 2008; Yeatman et al., 2012).

There are several limitations in the present study. First, we did not observe DWI correlations with RAN or letter knowledge, which is consistent with a prior study reporting correlations specific to phonological awareness and not to other reading-related measures (Yeatman et al., 2011). Studies with greater statistical power may observe brain-behavior correlations for other predictors of poor reading. Alternatively, other structural or functional brain measures may offer more sensitive measures for the other predictors. Second, the availability of sensitive measures of phonological awareness for kindergarten children is limited. We used the CTOPP, a widely used phonological awareness measure, but because the test is designed for such a wide age range, its sensitivity is limited for such young children. Future studies may be able to better characterize other subcomponents of phonological awareness using measures that elicit a wider variety of scores in order to better characterize other subcomponents of phonological awareness. For example, variation in language experience in home environments may influence phonological skill development (Burgess, 2002) that, in turn, influences white matter development before kindergarten. Evidence that experience alters white matter comes from a study reporting that reading interventions in older children who were poor readers was associated with white matter plasticity in a different brain region (Keller and Just, 2009). Therefore, the present study does not define what experiential or genetic factors influence the development of the left arcuate fasciculus before reading instruction in school, but it does indicate that such variation in brain-behavior relations precedes learning to read.

A clinical and educational goal of reading research is to improve the accuracy with which children at risk for dyslexia are identified so that they can receive early, preventive intervention rather than intervention that follows years of reading failure (Strickland, 2002). Although behavioral measures of phonological awareness, RAN, and letter knowledge in kindergartners predict reading ability years later (Catts et al., 2001; Schatschneider et al., 2004), the sensitivity and specificity of these behavioral measures is modest (Pennington and Lefly, 2001). There is some evidence that brain measures substantially enhance the accuracy of predicting reading ability across a school year (Hoef et al., 2007; Rezaie et al., 2011) or across multiple years (Maurer et al., 2009; Hoef et al., 2011). The present study indicates that DWI measures of white matter organization reveal a specific structural risk factor for reading difficulty that, in combination with behavioral and other brain measures, may improve the identification of prereaders at risk for dyslexia.

The present study documents a relation between phonological awareness and the left arcuate fasciculus. Phonological awareness is thought to be an essential precursor for learning to read because it allows for the mapping of previously learned sound units of oral language onto print (Wagner and Torgesen, 1987). Accordingly, variation in phonological awareness is one of the strongest predictors of later reading ability, and weakness in phonological awareness is common in dyslexia (Torgesen et al., 1999; Melby-Lervag et al., 2012). In the present study, one test of phonological awareness, the Blending Words subtest measuring phoneme blending, was most strongly related to the characteristics of the left arcuate fasciculus. Phoneme blending may be a particularly sensitive predictor at this age among the many phonological awareness subskills that vary in their predictive power over development (Paris, 2005).

More generally, the present findings support the view that brain differences can make learning to read difficult before the commencement of substantial reading instruction and experience. Dyslexia is strongly heritable (Pennington, 1990), and studies have found brain differences in individuals at familial risk for dyslexia. Newborns at familial risk exhibit differences in event-related potentials to language sounds within hours or days of birth (Guttorm et al., 2001), and longitudinal studies report correlations between such event-related potential differences during infancy and later language and reading abilities (Molfese, 2000; Guttorm et al., 2005). Prereading children at familial risk for dyslexia also exhibit reduced gray-matter volume in left posterior cortex (Raschle et al., 2011) and reduced functional activation for phonological processing (Raschle et al., 2012). Prereading children at behavioral risk based on poor letter knowledge also show reduced activation in bilateral posterior areas for processing letters relative to false fonts (Yamada et al., 2011).

The present study cannot identify specific genetic or experiential factors (home or school environments) that influence the prereading development of phonological awareness and the white matter pathways that support such awareness. For example, variation in language experience in home environments may influence phonological skill development (Burgess, 2002) that, in turn, influences white matter development before kindergarten. Evidence that experience alters white matter comes from a study reporting that reading interventions in older children who were poor readers was associated with white matter plasticity in a different brain region (Keller and Just, 2009). Therefore, the present study does not define what experiential or genetic factors influence the development of the left arcuate fasciculus before reading instruction in school, but it does indicate that such variation in brain-behavior relations precedes learning to read.

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