Behavioral/Cognitive

An Investigation into the Origin of Anatomical Differences in Dyslexia

Anthony J. Krafnick,[1] D. Lynn Flowers,[1,2] Megan M. Luetje,[1] Eileen M. Napoliello,[1] and Guinevere F. Eden[1]

[1]Center for the Study of Learning, Georgetown University Medical Center, Washington, DC 20057, and [2]Wake Forest University Baptist Medical Center, Winston-Salem, North Carolina 27157

Studies have converged in their findings of relatively less gray matter volume (GMV) in developmental dyslexia in bilateral temporoparietal and left occipitotemporal cortical regions. However, the interpretation of these results has been difficult. The reported neuroanatomical differences in dyslexia may be causal to the reading problems, following from, for example, neural migration errors that occurred during early human development and before learning to read. Alternatively, less GMV may represent the consequence of an impoverished reading experience, akin to the experience-dependent GMV differences attributed to illiterate compared with literate adults. Most likely, a combination of these factors is driving these observations. Here we attempt to disambiguate these influences by using a reading level-matched design, where dyslexic children were contrasted not only with age-matched controls, but also with younger controls who read at the same level as the dyslexics. Consistent with previous reports, dyslexics showed less GMV in multiple left and right hemisphere regions, including left superior temporal sulcus when compared with age-matched controls. However, not all of these differences emerged when dyslexics were compared with controls matched on reading abilities, with only right precentral gyrus GMV surviving this second analysis. When similar analyses were performed for white matter volume, no regions emerged from both comparisons. These results indicate that the GMV differences in dyslexia reported here and in prior studies are in large part the outcome of experience (e.g., disordered reading experience) compared with controls, with only a fraction of the differences being driven by dyslexia per se.

Key words: **anatomy; dyslexia; MRI; reading; VBM**

Introduction

Developmental dyslexia, a common reading disability that occurs in 5–12% of the population, has been attributed to a language-based deficit in phonological processing (Lyon et al., 2003). The earliest evidence of anatomical differences in dyslexia came from postmortem studies that revealed an absence of the typical leftward asymmetry of the planum temporale observed in nondyslexic brains (Galaburda and Kemper, 1979), as well as cortical anomalies (ectopias) indicative of neuronal migration errors during development, primarily in left hemisphere perisylvian regions (Galaburda et al., 1985; Humphreys et al., 1990). Following significant advances in both imaging technology and standardization of analysis tools for brain morphometric measurement, there have been many *in vivo* examinations of gray matter volume (GMV) using voxel-based morphometry (VBM) in the last decade. Such studies in dyslexia have found reduced GMV in several brain regions, usually including left hemisphere perisylvian cortex, thought to be involved in written language. Recent meta-analyses report convergence to left superior temporal sulcus and right superior temporal gyrus (Richlan et al., 2013); and to bilateral supramarginal gyrus and cerebellum, left fusiform gyrus, and right superior temporal gyrus (Linkersdörfer et al., 2012).

While these reports speak to consistency across studies, an unresolved question is whether these reductions in GMV are the cause of the reading problems or a consequence of the disordered reading experience of those with dyslexia relative to their peers (Linkersdörfer et al., 2012). There is evidence to suggest that anatomical anomalies may precede the reading problems encountered by dyslexics. Microstructural differences observed in dyslexia at postmortem have been attributed to anomalies in early development (Galaburda et al., 1985). These in turn may give rise to gross anatomical differences, reflected in reduced GMV in studies of dyslexia. At the same time, GMV is known to change following skill acquisition (May and Gaser, 2006), including reading, as evidenced by greater GMV in posterior perisylvian cortex in individuals who learn to read as adults compared with illiterates (Carreiras et al., 2009). These studies in adults suggest that when development is taken out of the equation, GMV growth can unambiguously be attributed to learning experiences.

Received May 16, 2013; revised Nov. 22, 2013; accepted Nov. 28, 2013.

Author contributions: A.J.K., D.L.F., and G.F.E. designed research; A.J.K., D.L.F., M.M.L., E.M.N., and G.F.E. performed research; A.J.K., M.M.L., E.M.N., and G.F.E. analyzed data; A.J.K., D.L.F., M.M.L., E.M.N., and G.F.E. wrote the paper.

This work was supported by the National Institutes of Child Health and Human Development (P50 HD40095, R01 HD056107). We thank Corina Moore, Emily Curran, Iain DeWitt, Alison Merikangas, Ashley Wall-Piche, Robert Twomey, and Jenni Rosenberg for their assistance in MRI data collection; Emma Cole, Martha Miranda, and Gina Smith for collection of behavioral psychometric measures; and Diana Alkire for editing the manuscript. We especially thank our subjects for their participation and the students, parents, and staff at the Jemicy School, Owings Mills, Maryland, for their involvement.

The authors declare no competing financial interests.

Correspondence should be addressed to Guinevere Eden, DPhil, Professor, Department of Pediatrics, Georgetown University Medical Center, BOX 571406, Suite 150, Building D, 4000 Reservoir Road, NW, Washington, DC 20057. E-mail: edeng@georgetown.edu.

DOI:10.1523/JNEUROSCI.2092-13.2013

 0270-6474/14/330901-08$15.00/0

Table 1. Participant characteristics and group matching

	Dyslexics	Control AGE	Control READ	*p* values: dyslexics versus control AGE	*p* values: dyslexics versus control READ
N	15	15	15		
Age	9.8 years (SD, 1.5 years)	9.9 years (SD, 2.7 years)	7.4 years (SD, 0.9 year)	0.8374	3.49×10^{-5}
Gender	6 boys, 9 girls	9 boys, 6 girls	8 boys, 7 girls	0.2893	0.4814
Performance IQ: standard score	101.3 (SD, 12.3)	102.3 (SD, 8.2)	109.2 (SD, 14.0)	0.7960	0.1137
Word identification: standard score	77.4 (SD, 7.6)	118.2 (SD, 8.3)		3.48×10^{-14}	
Word identification: reading age	7.6 (SD, 0.9)		8.1 (SD, 0.5)		0.0621
Passage comprehension: standard score	78.3 (SD, 11.0)	110.3 (SD, 10.0)		3.54×10^{-8}	
Passage comprehension: reading age	7.3 (SD, 1.0)		7.6 (SD, 0.6)		0.3400

Distinguishing those characteristics causal to dyslexia from those that are the consequence of disordered reading experience is critical in identifying its correct etiology and treatment. For example, dyslexic readers show a relative deficit in phonological processing that not only has been attributed to their impoverished reading experience, but also has been shown to be worse than the phonological processing skills exhibited by younger children who read at the same level. This latter observation was derived from reading level-matched studies (Goswami and Bryant, 1989) and demonstrates that the weakness in phonological processing in dyslexia is specific to the reading deficit and not simply a reflection of lower reading levels in dyslexia (Goswami and Bryant, 1989). Here we apply this approach to an investigation into anatomical differences in dyslexia, which, like phonological skills, are thought to be affected by dyslexia but also modulated by reading experience.

Materials and Methods

Subjects and subject-testing procedures. Participants were 15 children with dyslexia (six boys, nine girls), most recruited from a private school that specializes in teaching students with dyslexia ($n = 13$), but some from a public school ($n = 2$), and 30 typically reading children (17 boys, 13 girls) recruited from the general population to serve as controls. Inclusion criteria for the dyslexic children were as follows: (1) a documented history of dyslexia, as reported by the school; (2) single real-word reading standard score of <92 (Woodcock-Johnson III Tests of Achievement, Letter-Word Identification subtest; Woodcock et al., 2001); (3) Full Scale IQ >80 (Wechsler Abbreviated Scale of Intelligence; Wechsler, 1999); (4) monolingual English speaker; and (5) no significant medical, neurological, or psychiatric illness. For the control children, the inclusion criteria were identical except that they had no history of learning disabilities and their real-word reading standard scores were >92. All children were screened before entering the study to ensure they had no history of neurodevelopmental disability, congenital or acquired neurological disorder (such as a traumatic brain injury, disease affecting brain function, or known history of birth complications), or a diagnosis of specific language or hearing impairment. They also had no contraindication to MRI scanning, such as metallic implants, plates or pins, or claustrophobia.

Study design. To replicate previous investigations into GMV and white matter volume (WMV) in dyslexics compared with age-matched controls, we used experimental and analytical methods consistent with prior studies. In addition, we conducted a comparison of the same dyslexics with a younger, reading level-matched control group. Analyses of the first between-group comparison revealed differences driven by factors related to dyslexia per se as well as reading experience. The second comparison was conducted to remove the influence of reading experience and revealed differences more likely to be attributed to dyslexia. Half of the typically reading children ($n = 15$) constituted the chronological age-matched control group (control AGE; $n = 15$) by which to compare the dyslexic group. The other half of the typically reading children ($n = 15$) formed the younger, reading level-matched control group (control READ; $n = 15$). This group was therefore equivalent to the dyslexic group in their reading performance (matched using the single real-word reading-age equivalent; Table 1). The dyslexic group was matched on performance IQ to the age-matched controls and also to the reading level-matched controls (Table 1).

The study was approved by the Institutional Review Board of Georgetown University Medical Center. Parent consent and child assent were obtained. Subjects received book vouchers and choices of prizes for their participation.

Behavioral testing. Psychoeducational tests were administered to evaluate single-word reading accuracy and reading comprehension. All measures of reading described here provide age-referenced standardized scores with a mean of 100 and an SD of 15. Standard score averages are reported for the chronological age-matched comparisons of dyslexics versus controls in Table 1. However, for the reading level-matched comparisons of dyslexics and controls, reading age was used to ensure groups were matched for single real-word reading. Average reading ages are reported in the portion of the table describing the dyslexic sample and their reading level-matched control group.

Single real-word reading entailed untimed, out-loud reading of single real words of increasing difficulty and was assessed using the Letter-Word Identification subtest from either the Woodcock-Johnson III Tests of Achievement (Woodcock et al., 2001) or the Woodcock Reading Mastery Tests—Revised (Woodcock, 1987). Reading comprehension was assessed via the Passage Comprehension subtest (Woodcock-Johnson III Tests of Achievement; Woodcock et al., 2001) and required subjects to fill in the missing word of a sentence. As can be seen in Table 1, the dyslexics and controls matched on age differed in their standardized scores of out-loud reading of single words and comprehension of silently read text. However, the younger controls were deliberately matched to the dyslexics on these skills using measures of reading age.

Handedness was determined in all subjects using the Edinburgh Handedness Inventory (Oldfield, 1971). Twenty-one subjects were determined to be right-handed (laterality quotient, >33), three were left-handed (laterality quotient, <−33; one age-matched control, one reading level-matched control, and one dyslexic), and six were not strongly lateralized (two age-matched controls, three reading level-matched controls, and one dyslexic).

Imaging procedures. Anatomical MRI scans were acquired on a 3.0 tesla Siemens Trio whole-body MRI system. High-resolution T1-weighted 3D MPRAGE images were acquired for each subject: TR/TE, 1600/4.38 ms; 256 × 256 field of view; 160 mm slab thickness; 256 × 256 × 160 matrix (effective resolution is 1.0 mm^3); one excitation; 15° flip angle. All subjects participated in functional MRI studies as part of the protocol. For most subjects, three structural MRI scans were acquired, with some subjects receiving fewer or more scans, depending on time and subject compliance. In a population that is susceptible to head motion, multiple scan acquisition is one way to increase chances of obtaining an artifact-free image, given that a single, short head movement during the scan's acquisition cannot be removed and can significantly degrade image quality. All images were inspected and rated by two research assistants blind to the subjects' diagnostic group. For each subject, the scan with the least motion artifact was used for analysis. Retrospective analysis of the raters' average scores revealed no significant differences in the mean rating scores for the dyslexic versus control groups for both the age-matched and reading level-matched comparisons.

Preprocessing and analysis. Structural MRI scans were preprocessed using VBM in SPM8 (Wellcome Department of Imaging Neuroscience,

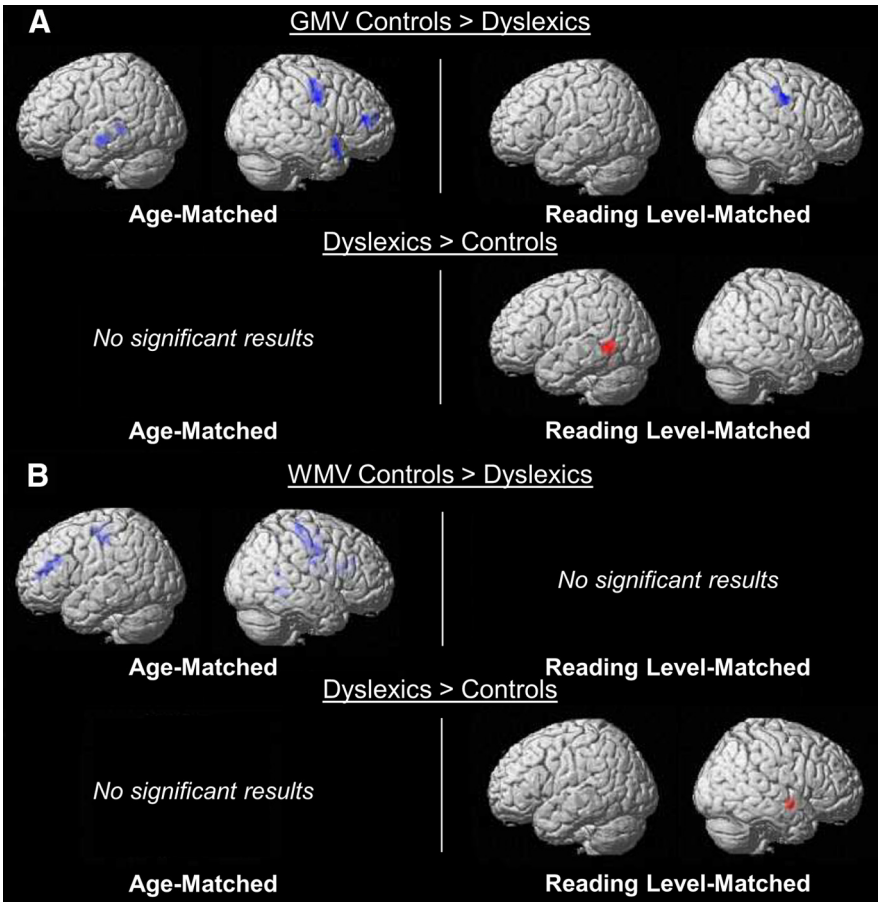

Figure 1. Whole-brain matter volume differences in dyslexics when compared with either age-matched or reading level-matched controls. ***A***, GMV differences. Whole-brain renderings for the GMV comparisons between dyslexics with both age-matched and reading level-matched groups at a height threshold of $p < 0.01$ uncorrected, and a cluster level threshold of $p < 0.01$ FWE corrected. Age-matched controls showed greater GMV than dyslexics in left middle temporal gyrus, left anterior cingulate gyrus, right precentral gyrus, right middle frontal gyrus, and right anterior superior temporal gyrus. No significant results were found for the dyslexics > controls contrast when matched on age. Reading level-matched controls showed greater GMV than dyslexics in right precentral gyrus. Dyslexics showed greater GMV than the reading level-matched controls in left middle temporal gyrus. See Table 2 for details on all clusters. ***B***, WMV differences. The same whole-brain renderings as in ***A***, but this time for WMV comparisons of dyslexics and the two control groups. Age-matched controls showed greater WMV than dyslexics in left paracentral lobule, left middle frontal and superior frontal gyrus, right middle frontal gyrus, right precentral gyrus, right WM anterior to the thalamus, and right subgyral temporal WM. No significant results were found for the dyslexics > controls contrast when matched on age. Also, no significant results were found for the controls > dyslexics contrast for the reading level-matched comparison. Dyslexics showed greater WMV than reading level-matched controls in right WM just lateral to the putamen. See Table 3 for details on all clusters.

London, UK), with the modulation option selected such that resulting analyses represent tissue volume as opposed to density. Because the analyses requires the generation of a study-specific template that is an average of all (and only those) participants who are included in the specific statistical analysis (see below), separate preprocessing was performed for the analyses using age-matched groups and for the analyses using reading level-matched groups. The following processing steps were completed: (1) each subject's image was manually aligned to the anterior commissure to decrease variability and coregistered to the SPM8 white matter template; (2) images were segmented into gray matter, white matter, and CSF using the New Segment toolbox (Ashburner and Friston, 2005); (3) DARTEL (Diffeomorphic Anatomical Registrations Through Exponentiated Lie Algebra) was used to register each structural image to a custom, study-specific template derived from the subject's images; (4) the template file generated by DARTEL was affine registered to more closely align and spatially normalize the images to Montreal Neurological Institute (MNI) space; and (5) the resulting images were smoothed using an 8 mm full-width at half-maximum Gaussian kernel, and an intensity threshold of 0.2 was used to remove voxels of low intensity from the analysis and to prevent possible edge effects.

Between-group differences for GMV and WMV contrasting the dyslexics with both the age-matched and reading level-matched groups were generated using two-sample *t* tests in SPM8. Height thresholds of $p < 0.01$ uncorrected and extent thresholds of $p < 0.01$ corrected were applied. Analyses were conducted using both the typical familywise-error (FWE) correction as well as a nonstationary cluster correction (Hayasaka et al., 2004). Previous studies on dyslexia have used both of these types of cluster-level corrections; we have included both here for greater ease of comparison with the existing literature and as a way to abide by the current trend of a more stringent analysis approach. Our sample sizes are also consistent with previous studies of GMV in dyslexia, again to provide consistency with the literature. Peak coordinates as reported by SPM8 were converted from MNI to Talairach space. Anatomical labels were assigned using the anatomy toolbox included with SPM8 and verified by two independent investigators using the Talairach Atlas (Talairach and Tournoux, 1988). Total intracranial volume (TIV) was calculated by adding WMV, GMV, and CSF (after intensity thresholding) for each subject. There were no differences in TIV between the dyslexic and control groups for the age-matched comparison or reading level-matched comparison. Importantly, no differences were observed in total gray matter or total white matter between groups. This is consistent with previous studies of dyslexia (Vinckenbosch et al., 2005; Evans et al., 2013) and, just as in these studies, we did not include these variables in the analysis presented. However, to address any possible concerns about the role of total GMV (Peelle et al., 2012), we also conducted the analysis with it as a regressor of no interest and found the central findings of our study to be the same.

In addition to conducting whole-brain between-group comparisons of dyslexics with controls (both age-matched and reading level-matched), we used any areas resulting from the comparisons of dyslexics with age-matched controls as regions of interest (ROIs) in the analyses contrasting the dyslexics with the reading level-matched controls. As such, this strategy uses one control group to define the regions and another to test for differences, while including the same dyslexic group in both, consistent with the reading level-matched design, and as implementation in functional MRI studies of dyslexia (Hoeft et al., 2007). Examining these specific ROIs provides another opportunity to discover subtle differences that may not have survived the whole-brain analysis. Extraction of GMV and WMV signal from these clusters was performed using the MarsBaR toolbox (Brett et al., 2002). This process extracts the average intensity from the voxels within the region identified for each subject. Two-sample *t* tests were performed on the extracted intensity for each ROI, and *p* values were Bonferroni corrected for multiple comparisons.

Results

GMV whole-brain comparisons

For the controls > dyslexics chronological age-matched comparison, five clusters were found (height threshold $p < 0.01$ uncorrected, FWE cluster corrected $p < 0.01$; Fig. 1*A*; Table 2). Two were located in the left hemisphere: midposterior temporal lobe (BA 21), located mostly in middle temporal gyrus, extending into superior temporal gyrus; and anterior cingulate gyrus (BA 24).

Table 2. Peak coordinates and cluster details of GMV differences

Hemisphere	Talairach coordinates (*X*, *Y*, *Z*)	Cluster size	*Z* score	Cluster *p* value	Peak anatomical location
Age-matched comparison					
Controls > dyslexics					
Left[a]	−45, −15, −12	1289	3.79	4.37×10^{-7}	Middle temporal gyrus, BA 21
Left	−16, −18, 40	1026	3.17	7.79×10^{-6}	Cingulate gyrus, BA 24
Right[a]	42, −10, 33	1545	5.33	3.18×10^{-8}	Precentral gyrus, BA 6
Right	34, 38, 12	711	4.66	3.49×10^{-4}	Middle frontal gyrus, BA 10
Right	51, 9, −13	518	3.29	0.005	Superior temporal gyrus, BA 38
Dyslexics > controls					
No significant results					
Reading level-matched comparison					
Controls > dyslexics					
Right	42, −13, 32	694	3.72	0.001	Precentral gyrus, BA 6
Dyslexics > controls					
Left	−63, −53, 1	672	5.04	0.001	Middle temporal gyrus, BA 21

[a]Clusters that survive both FWE and nonstationary cluster corrections.

Table 3. Peak coordinates and cluster details of WMV differences

Hemisphere	Talairach coordinates (*X*, *Y*, *Z*)	Cluster size	*Z* score	Cluster *p* value	Peak anatomical location
Age-matched comparison					
Controls > dyslexics					
Left	−4, −24, 52	784	4.53	4.78×10^{-6}	Paracentral lobule
Left	−39, 43, 13	1051	4.05	9.94×10^{-8}	Middle frontal gyrus
Left	−30, −13, 45	918	3.49	6.53×10^{-7}	Middle frontal gyrus
Left	−20, 44, 32	356	3.54	0.007	Superior frontal gyrus
Right	48, 26, 24	1063	4.47	8.42×10^{-8}	Middle frontal gyrus
Right[a]	42, −8, 36	3465	4.30	3.54×10^{-19}	Precentral gyrus
Right	40, −42, −5	878	3.52	1.17×10^{-6}	Subgyral temporal lobe
Right	10, −3, 17	1021	3.38	1.51×10^{-7}	Anterior to thalamus
Dyslexics > controls					
No significant results					
Reading level-matched comparison					
Controls > dyslexics					
No significant results					
Dyslexics > controls					
Right	27, −4, −9	386	3.80	0.004	Lateral to putamen

[a]Clusters that survive both FWE and nonstationary cluster corrections.

Three right-hemisphere clusters were identified: precentral gyrus (BA 6), middle frontal gyrus (BA 10) extending into inferior frontal gyrus (BA 46), and anterior superior temporal gyrus (BA 38). This contrast was repeated using a nonstationary cluster-level correction ($p < 0.01$ corrected), as this has been suggested to better account for VBM data (see Materials and Methods). When this correction is applied, only the left middle temporal gyrus (BA 21) and right precentral gyrus (BA 6) clusters survived.

The reverse contrast (dyslexics > controls) revealed no significant results using either the FWE or nonstationary cluster corrections.

For the controls > dyslexics reading level-matched comparison, one cluster was identified (height threshold $p < 0.01$ uncorrected, FWE cluster corrected $p < 0.01$; Fig. 1*A*; Table 2): right precentral gyrus (BA 6) extending into postcentral gyrus. The location of this cluster is very close to one found in the age-matched comparison above and is the only cluster that emerged from both the age-matched and reading level-matched comparisons. When the nonstationary cluster correction ($p < 0.01$ corrected) was applied, there were no statistically significant results.

The reverse contrast (dyslexics > controls) revealed one cluster in left middle temporal gyrus (BA 21), but nothing survived the nonstationary cluster correction. Hence, there were no regions in which dyslexics consistently showed more GMV.

WMV whole-brain comparisons

Parallel analyses were performed for white matter VBM data. For the controls > dyslexics age-matched comparison, eight clusters were identified (height threshold $p < 0.01$ uncorrected, FWE cluster corrected $p < 0.01$; Fig. 1*B*; Table 3). The four left-hemisphere clusters were all located in frontal cortex: paracentral lobule extending into medial frontal gyrus, white matter underlying the middle frontal, precentral, and the superior frontal gyri. Four right-hemisphere clusters were identified: middle frontal gyrus, an area medial to the precentral gyrus, subgyral temporal (medial to mid/posterior middle/superior temporal cortex), and a region just anterior to the thalamus. When the nonstationary cluster correction ($p < 0.01$ corrected) was applied here, only the area around the right precentral gyrus remained significant.

The reverse contrast (dyslexics > controls) revealed no significant results for either the FWE or nonstationary cluster corrections.

For the reading level-matched comparison, the controls > dyslexics contrast revealed no significant results (height threshold $p < 0.01$ uncorrected, FWE cluster corrected $p < 0.01$). The reverse contrast (dyslexics > controls) revealed a single cluster just posterior to the right putamen. However, there were no significant results when applying the nonstationary cluster correction (Fig. 1*B*; Table 3). Together there were no regions where

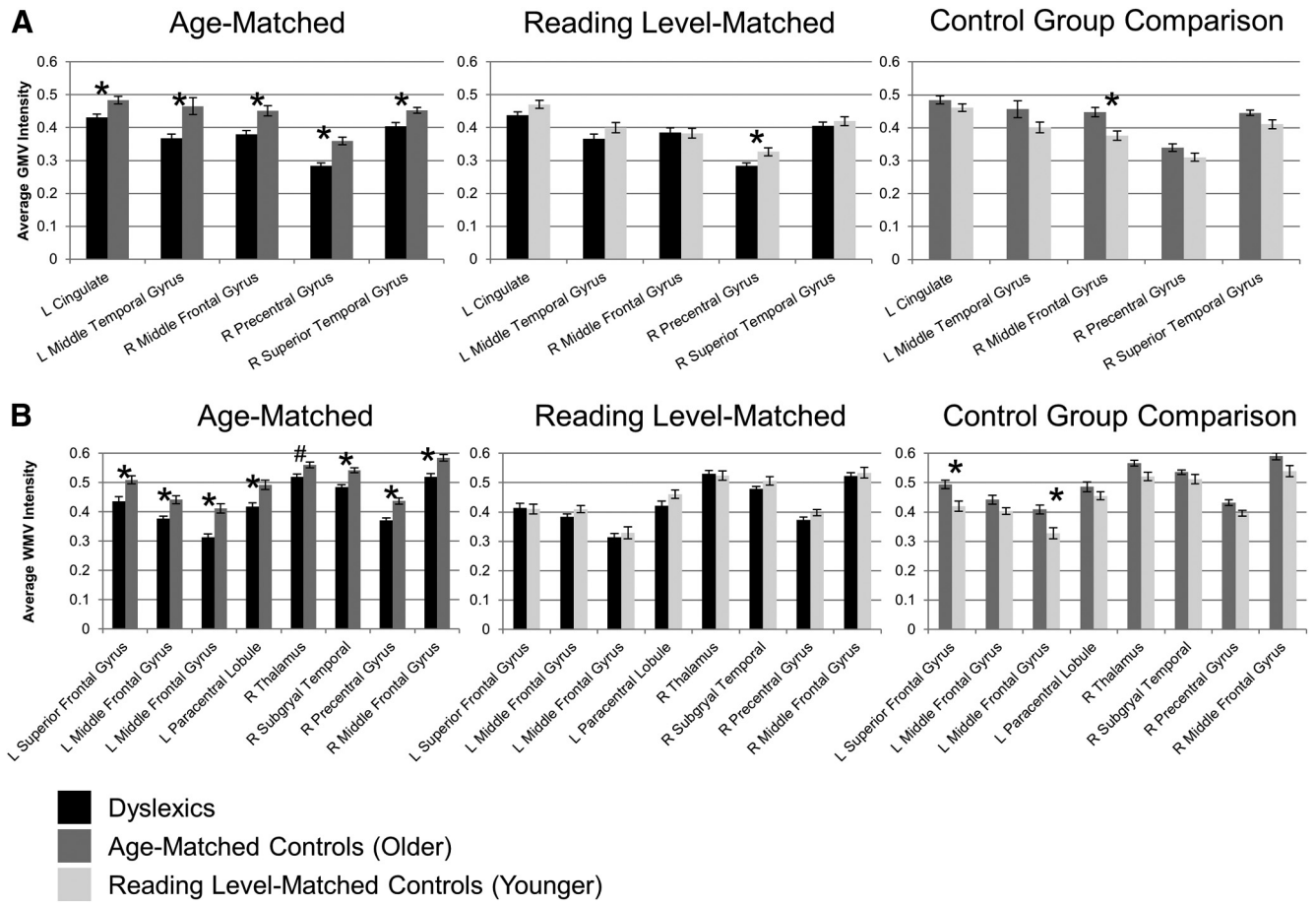

Figure 2. Gray and white matter intensities in ROIs. ***A***, Average GMV intensity within ROIs (identified in the whole-brain, age-matched controls > dyslexics GMV comparison). For the dyslexic versus control comparisons in groups matched on age, all ROIs were, as expected, significantly different. However, when comparing the same dyslexics with a younger control group matched on reading level, most areas failed to show a difference. To probe for a possible role for age-related changes in the typical readers, the right chart shows between-group differences in GMV in the younger versus older control group in these same ROIs. ***B***, Average WMV intensity within ROIs (identified in the whole-brain, age-matched controls > dyslexics WMV comparison). As above, all ROIs were significant in the comparisons of dyslexics and controls matched on age, as expected. There were no significant differences in these ROIs when comparing the dyslexics with controls matched on reading level. Again, the right chart gauges differences that exist between younger and older typical readers, this time in WMV. Like above, there was a trend for age-related differences in these regions, with left middle frontal gyrus and superior frontal gyrus being statistically significant. *$p < 0.05$ Bonferroni corrected for multiple comparisons. #$p = 0.05$ Bonferroni corrected for multiple comparisons.

WMV differences were consistently observed between dyslexics and both of the control groups.

Gray matter ROI comparisons

Regions shown to differ in GMV between the dyslexics and their age-matched controls (Table 2, top) were applied to the images from the comparison of the dyslexics with the reading level-matched controls, and statistics were performed on the data extracted from these regions. This provided an additional opportunity to probe for between-groups differences between dyslexics and controls when matched for reading ability in brain regions where such differences should be most likely (because of the outcome from the dyslexic vs age-matched comparison).

Of the five total clusters showing significant differences in GMV between dyslexics and controls from the age-matched group, only the right precentral gyrus showed a significant difference between the reading level-matched groups (two-sample *t* tests, $p < 0.05$ Bonferroni-corrected for multiple comparisons; Fig. 2). As noted above, the right precentral gyrus was significant for the between-group analyses using both types of control groups at the level of the whole brain, so this ROI approach did not yield any additional regions. Using a non-Bonferroni-corrected *p* value of $p < 0.05$ did not result in any additional significant clusters.

White matter ROI comparisons

Of the eight clusters showing significant differences in WMV between dyslexics and controls from the age-matched group, none showed significant differences between the reading level-matched groups ($p < 0.05$, Bonferroni-corrected for multiple comparisons; Fig. 2). Using a non-Bonferroni-corrected *p* value of <0.05 here did not result in any additional significant clusters.

Gray and white matter ROI comparisons in the younger and older control groups to test for age-dependent differences

Our results suggest that while less GMV and WMV is observed in dyslexia when compared with an age-matched control group, most of these do not manifest when the dyslexics are compared with a reading level-match control group. In fact, the results in the control groups in Figure 2 suggests an age-dependent growth in some of these regions and therefore give rise to the possibility that dyslexics differ due to absence of this kind of GMV or WMV growth, rather than being different in dyslexia to begin with. To directly test for age-dependent increases in GMV in our two typ-

ically reading control groups, we interrogated the same ROIs resulting from the comparisons of dyslexics with age-matched controls in the two control groups. These between-group analysis procedures were the same as above. Preprocessing was conducted for just the two control groups (to generate a study-specific template, which is why the data are replotted on the right side of Fig. 2). All other aspects of the analysis were identical to those for the above described comparisons between the dyslexic and reading level-matched control group. Again, total intracranial volume, total gray matter, or total white matter did not differ between the two groups and was not entered into the analysis. We found that all five ROIs trended toward greater GMV in the older control group (i.e., the group previously serving as the age-matched group for the dyslexics) compared with the younger group (reading level-matched control for the dyslexics). The right middle frontal gyrus showed a significant difference (two-sample *t* tests, $p < 0.05$ Bonferroni-corrected for multiple comparisons; Fig. 2) and right anterior superior temporal gyrus was significantly different between groups when using a non-Bonferroni-corrected *p* value of $p < 0.05$.

As with the GMV ROIs, the eight WMV ROIs were used to conduct comparisons of the two control groups. Again, each of the ROIs showed a trend for greater WMV in the older control group compared with the younger control group. Left superior frontal gyrus and left middle frontal gyrus proved to be significantly different between the two control groups ($p < 0.05$ Bonferonni-corrected for multiple comparisons; Fig. 2). Right thalamus, precentral gyrus, and middle frontal gyrus also survived significance testing using a non-Bonferonni-corrected *p* value of $p < 0.05$.

Discussion

The goal of this study was to gain insights into the source of the differences in GMV and WMV in dyslexia, previously reported in anatomical MRI studies using VBM. Specifically, we asked whether these neuroanatomical anomalies can be attributed to dyslexia per se, or are due in part to the altered and likely impoverished reading experience that is concomitant with dyslexia. The reading level-matched design has been used to gauge differences between dyslexics and nondyslexics while at the same time taking their lower reading level into consideration. This approach addresses the concern that reading itself may bolster some of these same features that are associated with reading disability, in this case GMV or WMV. As such, these anatomical measures could be relatively different in dyslexics compared with their peers as a consequence (and not a cause) of their reading disability; in other words, as a function of growth in typically reading children, but not in dyslexic children.

In the first analysis, we compared dyslexic children with age-matched controls to establish consistency with prior publications. The second analysis provided the novel contribution, comparing the same dyslexic group to younger controls matched on reading ability while using the same whole-brain VBM approach used in prior publications. The results revealed that most regions identified in the age-matched analysis did not emerge as different when we controlled for reading level. We conclude that the previously observed GMV and WMV differences in dyslexia assessed with VBM are in large part a reflection of their lower reading level.

A notable example of consistency between the first analysis and the published literature is less GMV in dyslexics in left middle temporal gyrus extending into left superior temporal sulcus, as previously reported in studies of children (Hoeft et al., 2007) and adults (Brown et al., 2001; Silani et al., 2005; Vinckenbosch et al., 2005), and in a meta-analysis (Richlan et al., 2013). The proximity of this area to regions that support phonological processing (Pugh et al., 2000, 2001; Jobard et al., 2003; Frost et al., 2009) has always been considered logical, given that phonological processing is a principal dysfunction in dyslexia (for review, see Peterson and Pennington, 2012). Regarding WMV, the comparison of dyslexics with age-matched controls revealed several bilateral frontal regions, as well as clusters located in the right temporal lobe and anterior to the thalamus. This is consistent with previous VBM studies showing lesser left frontal WMV in dyslexics compared with controls (Eckert et al., 2005; Silani et al., 2005).

While the results from the age-matched comparison confirm the previous literature, they do not speak to the nature of the relationship between reading, dyslexia, and brain anatomy. The reading level-matched control group in the second analysis offers insight and demonstrates that many of the differences are, in part, likely related to reading experience. Only the right precentral gyrus GMV difference was replicated in our reading level-matched comparison. Our result of only a single brain region surviving across both control group comparisons is not unlike the single focus (left inferior parietal lobule) revealed by Hoeft et al. (2007) when using functional MRI data to determine the ROIs. The right precentral gyrus has been shown to differ in prior studies of dyslexia using age-matched controls in children (Hoeft et al., 2007; Jednoróg et al., 2013) and an ROI analysis in adults (Menghini et al., 2008). Menghini et al. (2008) discussed the precentral gyrus anomaly in the context of motor system and motor learning in dyslexia (Nicolson et al., 2001; Menghini et al., 2006, 2008), while Jednoróg et al. (2013) focus their explanation on problems with articulatory feedback. Other findings observed in the age-matched comparison, including reduced left middle/superior temporal GMV in the dyslexics, did not replicate in the reading level-matched comparison. Our data suggest that GMV differences in dyslexia are to some degree a product of the increase that occurs in typical readers during reading acquisition, and this may not occur in dyslexia because of their degraded reading experience. This impoverished reading experience likely includes both spending less time reading, and reading qualitatively different due to difficulties in naming and decoding of words.

The notion that learning to read bolsters GMV is supported by studies demonstrating GMV increases following experience-dependent behavioral improvement (Draganski et al., 2004, 2006; Boyke et al., 2008; Driemeyer et al., 2008; Ilg et al., 2008; Krafnick et al., 2011). Specific to reading, work in illiterate populations suggests that functional and anatomical changes are associated with reading experience (Castro-Caldas et al., 1998, 1999; Carreiras et al., 2009; Dehaene et al., 2010). In particular, adults who were illiterate but then learned to read as adults showed greater GMV in temporoparietal regions (including posterior middle temporal gyrus) compared with illiterates (Carreiras et al., 2009), suggesting a learning-induced increase due to reading.

It is noteworthy that the majority of dyslexia studies have been conducted in adults, which means the control groups in these studies have experienced many years of reading, presumably leading to experience-dependent neuroanatomical change. It is this anatomical outcome that could be driving the results reported between adult dyslexics and controls. Pediatric studies, of which there have only been three (matching on age but not on reading level), have the advantage of minimizing this impact (i.e., experience-dependent growth in GMV in the controls will not be

as large in children). The ROI analysis between our two control groups revealed an age-dependent increase in GMV in the control groups (statistically significant in right middle frontal gyrus, an observation that holds when the analyses are conducted at the whole-brain level), and that this was in part (but not entirely) driving the significant between-group differences in the dyslexic versus age-matched control comparison. Interestingly, the apparent growth in these areas in our control subjects occurs during a time when there is a general decrease in GMV in the brain (Giedd et al., 1999; Wilke et al., 2007), suggesting an experience-dependent change in the opposite direction of age-specific changes. Indeed, cortical thickness studies have shown a decrease with age in the majority of the brain, while perisylvian regions involved in language show growth (Sowell et al., 2004), and these increases correlate with improving phonological skill (Lu et al., 2007). The developmental trends observed in our ROIs might be more pronounced in older children who have had relatively more opportunity to exercise their reading skills. Longitudinal studies of dyslexia will be critical in addressing this question of cause versus consequence, but are unavailable at this time.

The observations from the current study inform the ongoing conversation on the developmental scenario of dyslexia. It has been suggested that genetically driven microstructural changes thought to arise during prenatal cortical development (Galaburda et al., 1985, 2006) give rise to gross anatomical differences, measured here as less GMV. However, altered neuronal migration (leading to altered cell distribution across the layers of cortex) would not necessarily lead directly to differences in GMV measured in VBM; rather, they could disrupt the local circuitry in those regions. As such, ectopias (or another factor) may hinder normal acquisition of phonological processing skills, resulting in the impoverished reading experience in dyslexia and with it an absence of experiential growth of GMV. Critically, it is in large part consequential to poorer reading that dyslexics have less growth of GMV when compared with their peers. Hence, the relationship between gross and microstructural differences may or may not be direct, but is in part mediated by reading experience. Notably, there is compelling work using a mouse model that shows that the induction of ectopias results in deficits in auditory temporal processing, a critical prerequisite for phonological skills (Fitch and Tallal, 2003). This also raises the issue that our findings (and many of the prior reports discussed above) are based on a voxel-based, volumetric approach, and we cannot exclude the possibility that other methods, such as those involving connectivity, cortical thickness, or cortical pattern analyses, may reveal differences that are consistent across both the age-level and reading-level comparisons. Certainly our results provide an important context by which to interpret the increasingly large literature on GMV and WMV voxel-based neuroanatomical differences in dyslexia.

Our approach may also need to be applied to other learning disabilities and developmental disorders where differences in brain volume are often interpreted as causal. In reading specifically, there is a growing literature suggesting that literacy acquisition has wide-ranging effects on behavior (Szwed et al., 2012), neuroanatomy (Castro-Caldas et al., 1998, 1999; Carreiras et al., 2009), and brain function (Dehaene et al., 2010). When considering the use of brain structure for diagnostic purposes, it is important to recognize that some of the differences associated with dyslexia may not be revealed until experience-dependent discrepancies have taken effect.

References

Ashburner J, Friston KJ (2005) Unified segmentation. Neuroimage 26:839–851. CrossRef Medline

Boyke J, Driemeyer J, Gaser C, Büchel C, May A (2008) Training-induced brain structure changes in the elderly. J Neurosci 28:7031–7035. CrossRef Medline

Brett M, Valabregue R, Poline J (2002) Region of interest analysis using an SPM toolbox. In: 8th International Conference on Functional Mapping of the Human Brain, Sendai, Japan.

Brown WE, Eliez S, Menon V, Rumsey JM, White CD, Reiss AL (2001) Preliminary evidence of widespread morphological variations of the brain in dyslexia. Neurology 56:781–783. CrossRef Medline

Carreiras M, Seghier ML, Baquero S, Estévez A, Lozano A, Devlin JT, Price CJ (2009) An anatomical signature for literacy. Nature 461:983–986. CrossRef Medline

Castro-Caldas A, Petersson KM, Reis A, Stone-Elander S, Ingvar M (1998) The illiterate brain. Learning to read and write during childhood influences the functional organization of the adult brain. Brain 121:1053–1063. CrossRef Medline

Castro-Caldas A, Miranda PC, Carmo I, Reis A, Leote F, Ribeiro C, Ducla-Soares E (1999) Influence of learning to read and write on the morphology of the corpus callosum. Eur J Neurol 6:23–28. CrossRef Medline

Dehaene S, Pegado F, Braga LW, Ventura P, Nunes Filho G, Jobert A, Dehaene-Lambertz G, Kolinsky R, Morais J, Cohen L (2010) How learning to read changes the cortical networks for vision and language. Science 330:1359–1364. CrossRef Medline

Draganski B, Gaser C, Busch V, Schuierer G, Bogdahn U, May A (2004) Changes in grey matter induced by training. Nature 427:311–312. CrossRef Medline

Draganski B, Gaser C, Kempermann G, Kuhn HG, Winkler J, Büchel C, May A (2006) Temporal and spatial dynamics of brain structure changes during extensive learning. J Neurosci 26:6314–6317. CrossRef Medline

Driemeyer J, Boyke J, Gaser C, Büchel C, May A (2008) Changes in gray matter induced by learning—revisited. PLoS One 3:e2669. CrossRef Medline

Eckert MA, Leonard CM, Wilke M, Eckert M, Richards T, Richards A, Berninger V (2005) Anatomical signatures of dyslexia in children: unique information from manual and voxel based morphometry brain measures. Cortex 41:304–315. CrossRef Medline

Evans TM, Flowers DL, Napoliello EM, Eden GF (2013) Sex-specific gray matter volume differences in females with developmental dyslexia. Brain Struct Funct. Advance online publication. Retrieved Dec. 5, 2013. Medline

Fitch RH, Tallal P (2003) Neural mechanisms of language-based learning impairments: insights from human populations and animal models. Behav Cogn Neurosci Rev 2:155–178. CrossRef Medline

Frost SJ, Landi N, Mencl WE, Sandak R, Fulbright RK, Tejada ET, Jacobsen L, Grigorenko EL, Constable RT, Pugh KR (2009) Phonological awareness predicts activation patterns for print and speech. Ann Dyslexia 59:78–97. CrossRef Medline

Galaburda AM, Kemper TL (1979) Cytoarchitectonic abnormalities in developmental dyslexia: a case study. Ann Neurol 6:94–100. CrossRef Medline

Galaburda AM, Sherman GF, Rosen GD, Aboitiz F, Geschwind N (1985) Developmental dyslexia: four consecutive patients with cortical anomalies. Ann Neurol 18:222–233. CrossRef Medline

Galaburda AM, LoTurco J, Ramus F, Fitch RH, Rosen GD (2006) From genes to behavior in developmental dyslexia. Nat Neurosci 9:1213–1217. CrossRef Medline

Giedd JN, Blumenthal J, Jeffries NO, Castellanos FX, Liu H, Zijdenbos A, Paus T, Evans AC, Rapoport JL (1999) Brain development during childhood and adolescence: a longitudinal MRI study. Nat Neurosci 2:861–863. CrossRef Medline

Goswami U, Bryant P (1989) The interpretation of studies using the reading level design. J Lit Res 21:413–424. CrossRef

Hayasaka S, Phan KL, Liberzon I, Worsley KJ, Nichols TE (2004) Nonstationary cluster-size inference with random field and permutation methods. Neuroimage 22:676–687. CrossRef Medline

Hoeft F, Meyler A, Hernandez A, Juel C, Taylor-Hill H, Martindale JL, McMillon G, Kolchugina G, Black JM, Faizi A, Deutsch GK, Siok WT, Reiss AL, Whitfield-Gabrieli S, Gabrieli JD (2007) Functional and morpho-

metric brain dissociation between dyslexia and reading ability. Proc Natl Acad Sci U S A 104:4234–4239. CrossRef Medline

Humphreys P, Kaufmann WE, Galaburda AM (1990) Developmental dyslexia in women: neuropathological findings in three patients. Ann Neurol 28:727–738. CrossRef Medline

Ilg R, Wohlschläger AM, Gaser C, Liebau Y, Dauner R, Wöller A, Zimmer C, Zihl J, Mühlau M (2008) Gray matter increase induced by practice correlates with task-specific activation: a combined functional and morphometric magnetic resonance imaging study. J Neurosci 28:4210–4215. CrossRef Medline

Jednoróg K, Gawron N, Marchewka A, Heim S, Grabowska A (2013) Cognitive subtypes of dyslexia are characterized by distinct patterns of grey matter volume. Brain Struct Funct. Advance online publication. Retrieved Dec. 5, 2013. Medline

Jobard G, Crivello F, Tzourio-Mazoyer N (2003) Evaluation of the dual route theory of reading: a metanalysis of 35 neuroimaging studies. Neuroimage 20:693–712. CrossRef Medline

Krafnick AJ, Flowers DL, Napoliello EM, Eden GF (2011) Gray matter volume changes following reading intervention in dyslexic children. Neuroimage 57:733–741. CrossRef Medline

Linkersdörfer J, Lonnemann J, Lindberg S, Hasselhorn M, Fiebach CJ (2012) Grey matter alterations co-localize with functional abnormalities in developmental dyslexia: an ALE meta-analysis. PLoS One 7:e43122. CrossRef Medline

Lu L, Leonard C, Thompson P, Kan E, Jolley J, Welcome S, Toga A, Sowell E (2007) Normal developmental changes in inferior frontal gray matter are associated with improvement in phonological processing: a longitudinal MRI analysis. Cereb Cortex 17:1092–1099. Medline

Lyon GR, Shaywitz SE, Shaywitz BA (2003) Defining dyslexia, comorbidity, teachers' knowledge of language and reading. A definition of dyslexia. Ann Dyslexia 53:1–14. CrossRef

May A, Gaser C (2006) Magnetic resonance-based morphometry: a window into structural plasticity of the brain. Curr Opin Neurol 19:407–411. CrossRef Medline

Menghini D, Hagberg GE, Caltagirone C, Petrosini L, Vicari S (2006) Implicit learning deficits in dyslexic adults: an fMRI study. Neuroimage 33:1218–1226. CrossRef Medline

Menghini D, Hagberg GE, Petrosini L, Bozzali M, Macaluso E, Caltagirone C, Vicari S (2008) Structural correlates of implicit learning deficits in subjects with developmental dyslexia. Ann N Y Acad Sci 1145:212–221. CrossRef Medline

Nicolson RI, Fawcett AJ, Dean P (2001) Developmental dyslexia: the cerebellar deficit hypothesis. Trends Neurosci 24:508–511. CrossRef Medline

Oldfield RC (1971) The assessment and analysis of handedness: the Edinburgh inventory. Neuropsychologia 9:97–113. CrossRef Medline

Peelle JE, Cusack R, Henson RNA (2012) Adjusting for global effects in voxel-based morphometry: Gray matter decline in normal aging. Neuroimage 60:1503–1516. CrossRef Medline

Peterson RL, Pennington BF (2012) Developmental dyslexia. Lancet 379: 1997–2007. CrossRef Medline

Pugh KR, Mencl WE, Jenner AR, Katz L, Frost SJ, Lee JR, Shaywitz SE, Shaywitz BA (2000) Functional neuroimaging studies of reading and reading disability (developmental dyslexia). Ment Retard Dev Disabil Res Rev 6:207–213. Medline

Pugh KR, Mencl WE, Jenner AR, Katz L, Frost SJ, Lee JR, Shaywitz SE, Shaywitz BA (2001) Neurobiological studies of reading and reading disability. J Commun Disord 34:479–492. CrossRef Medline

Richlan F, Kronbichler M, Wimmer H (2013) Structural abnormalities in the dyslexic brain: a meta-analysis of voxel-based morphometry studies. Hum Brain Mapp 34:3055–3065. CrossRef Medline

Silani G, Frith U, Demonet JF, Fazio F, Perani D, Price C, Frith CD, Paulesu E (2005) Brain abnormalities underlying altered activation in dyslexia: a voxel based morphometry study. Brain 128:2453–2461. CrossRef Medline

Sowell ER, Thompson PM, Leonard CM, Welcome SE, Kan E, Toga AW (2004) Longitudinal mapping of cortical thickness and brain growth in normal children. J Neurosci 24:8223–8231. CrossRef Medline

Szwed M, Ventura P, Querido L, Cohen L, Dehaene S (2012) Reading acquisition enhances an early visual process of contour integration. Dev Sci 15:139–149. CrossRef Medline

Talairach J, Tournoux P (1988) Co-planar stereotaxic atlas of the human brain: 3-dimensional proportional system—an approach to cerebral imaging. New York: Thieme Medical Publishers.

Vinckenbosch E, Robichon F, Eliez S (2005) Gray matter alteration in dyslexia: converging evidence from volumetric and voxel-by-voxel MRI analyses. Neuropsychologia 43:324–331. CrossRef Medline

Wechsler D (1999) Wechsler abbreviated scale of intelligence. San Antonio, TX: Psychological.

Wilke M, Krägeloh-Mann I, Holland SK (2007) Global and local development of gray and white matter volume in normal children and adolescents. Exp Brain Res 178:296–307. CrossRef Medline

Woodcock RW (1987) Woodcock reading mastery tests—revised. Circle Pines, MN: American Guidance Service.

Woodcock RW, McGrew K, Mather N (2001) The Woodcock-Johnson tests of achievement, third edition. Itasca, IL: Riverside.