

A Twin MRI Study of Size Variations in the Human Brain

Bruce F. Pennington

University of Denver

Pauline A. Filipek

University of California-Irvine

Dianne Lefly and Nomita Chhabildas

University of Denver

David N. Kennedy

Harvard Medical School

Jack H. Simon

University of Colorado

Christopher M. Filley

University of Colorado and Denver Veterans Affairs Medical Center

Albert Galaburda

Harvard Medical School

John C. DeFries

University of Colorado

Abstract

■ Although it is well known that there is considerable variation among individuals in the size of the human brain, the etiology of less extreme individual differences in brain size is largely unknown. We present here data from the first large twin sample ($N=132$ individuals) in which the size of brain structures has been measured. As part of an ongoing project examining the brain correlates of reading disability (RD), whole brain morphometric analyses of structural magnetic resonance image (MRI) scans were performed on a sample of adolescent twins. Specifically, there were 25 monozygotic (MZ) and 23 dizygotic (DZ) pairs in which at least one member of each pair had RD and 9 MZ and 9 DZ pairs in which neither member had RD. We first factor-analyzed volume data for 13 individual brain structures, comprising all of the neocortex and most of the subcortex. This analysis yielded two factors ("cortical" and "subcortical") that accounted for 64% of the variance. We next tested whether genetic and environmental influences on brain size variations varied for these two factors or by hemisphere. We computed intraclass correlations within MZ and DZ pairs in each sample for the cortical and subcortical factor scores, for left and right neocortex, and for the total cerebral volume. All five MZ correlations were substantial (r 's=.78 to .98) and

significant in both samples, as well as being larger than the corresponding DZ correlations, (r 's=0.32 to 0.65) in both samples. The MZ–DZ difference was significant for 3 variables in the RD sample and for one variable in the smaller control sample. These results indicate significant genetic influences on these variables. The magnitude of genetic influence did not vary markedly either for the 2 factors or the 2 hemispheres. There was also a positive correlation between brain size and full-scale IQ, consistent with the results of earlier studies. The total cerebral volume was moderately correlated ($r=.42$, $p<.01$, two-tailed) with full-scale IQ in the RD sample; there was a similar trend in the smaller control sample ($r=.31$, $p<.07$, two-tailed). Corrections of similar magnitude were found between the subcortical factor and full-scale IQ, whereas the results for the cortical factor ($r=.16$ and $.13$) were smaller and not significant. In sum, these results provide evidence for the heritability of individual differences in brain size which do not vary markedly by hemisphere or for neocortex relative to subcortex. Since there are also correlations between brain size and full-scale IQ in this sample, it is possible that genetic influences on brain size partly contribute to individual differences in IQ. ■

INTRODUCTION

Although it has been recognized for well over a century that there are fairly marked individual differences in brain size among normal individuals (Gould, 1981), both the etiology of such size differences and their relation, if any, to individual cognitive differences are less well understood. It is known that genetic disorders can lead to extreme variations in brain size, which are usually accompanied by mental retardation. For example, microcephaly is found in Down syndrome (Coyle, Oster-Granite, & Gearhart, 1986) and megalencephaly is found in both Fragile X syndrome (Prouty, Rogers, & Stevenson, 1988) and a subset of individuals with autism (Bailey, Phillips, & Rutter, 1996; Filipek, 1996). But the etiology of less extreme variations in human brain size is currently unknown.

There have been very few previous twin studies of the human-brain structure. All have had small sample sizes, and have mainly studied monozygotic (MZ) pairs rather than using a full-twin design, thus limiting tests of genetic and environmental influences. For instance, Tramo et al., 1995 studied 10 pairs of adult MZ twins on magnetic resonance image (MRI) measures of surface area for various cortical structures. The within-pair variance was nonsignificant for both hemispheres, whereas the between-pair variance was significant for the left hemisphere only. The authors interpreted these results as indicating greater genetic control on the left hemisphere than the right hemisphere, although they acknowledged their design could not separate the effects of genes from the effects of shared environments. Results from only MZ twins cannot definitely test for genetic influences, since MZ pairs share both all their genes and common family environmental experiences (shared environment). Hence, we do not know if the greater similarity within pairs for the left hemisphere these authors found is due to genetic influences, shared environmental influences, or some combination.

Until recently, it had been widely believed that normal size variations in the human brain were not related to cognitive ability differences (Gould, 1981; Jerison, 1989). However, several recent structural MRI studies of normal samples (Andreasen, Flaum, Swayze, et al., 1993; Raz et al., 1993; Wickett, Vernon, & Lee, 1994; Willerman, Schultz, Rutledge, & Bigler, 1991) have found significant, moderate correlations (r^2 's = .35–.43) between brain size and IQ. Of course, these correlations are open to several interpretations. Brain size could contribute in a direct way to the development of intelligence, or intelligence could lead to differential environmental influences which could increase brain size, or a third factor could influence both brain size and intelligence without there being a direct relation between the two. We also know that individual differences in IQ are moderately heritable, with about half the variation in IQ being due to the effects of

genes (Plomin, DeFries, McClearn, & Rutter, 1997). It is a fairly straightforward assumption that the genes that influence IQ act on brain development in some way, but we currently do not know which aspects of brain development mediate genetic influences on IQ. Besides size, there are other known brain correlates of cognitive differences, including brain metabolism (Haier, 1994; Haier, Siegel, Tang, Abel, & Buchsbaum, 1992) and electroencephalogram (EEG) coherence (Thatcher, 1992). It seems quite likely that there are other brain variables besides these three that relate to cognitive differences, including various aspects of neurotransmission.

A twin design provides a means of beginning to answer these questions about the etiology of nonextreme size differences in the human brain and their relation to cognitive development. Specifically, by examining both IQ and variations in size of different brain structures in twins, we can (1) test for the degree of genetic and environmental influence on different brain structures, (2) test whether the relation between brain size and IQ varies by structure, and in a sufficiently large sample, and (3) test for the overlap between the genetic and environmental influences on IQ and those acting on size variations in brain structures.

In the following report, we address these issues in a sample of adolescent and young adult twin pairs in which whole-brain morphometric analyses of structural MRI scans have been performed. Since the main goals of this MRI study were to identify brain correlates of reading disability (RD) and to examine the etiology of these brain correlates using a twin design, the MRI sample was weighted with RD twin pairs (73% of the current sample) because the appropriate twin analyses require large RD sample sizes. A separate paper (Pennington et al., in press) reports our findings for the brain correlates of RD. The twin pairs in the MRI study were selected from larger samples of RD and control same-sex twin pairs being studied in the Colorado Learning Disability Research Center (CLDRC; DeFries et al., 1997).

RESULTS

Table 1 presents data on age, gender, FSIQ, a reading composite score, and handedness in the MZ and dizygotic (DZ) twins. As can be seen, 48 of the pairs are from the RD sample, in which at least one member of the pair has RD, and 18 pairs are from the control sample, in which neither member of the pair has RD. The groups are similar in handedness, and generally similar in age and gender ratio. However, the MZ twins in the control sample were older than the RD twins ($p > .05$), and there were significantly more male pairs in the RD DZ sample than in the RD MZ sample. Because of the selection for RD, the RD twins have lower reading composite scores

Table 1. Description of the Sample

	<i>RD Sample</i>		<i>Control Sample</i>	
	<i>(MZ)</i> 25 pairs	<i>(DZ)</i> 23 pairs	<i>(MZ)</i> 9 pairs	<i>(DZ)</i> 9 pairs
Mean Age	17.10 ^a	16.82 ^a	19.41 ^b	18.71 ^{a,b}
<i>SD</i>	4.59	3.66	5.00	2.40
Gender Ratio (M:F)	12:13 ^a	16:7 ^b	4:5 ^{a,b}	4:5 ^{a,b}
FSIQ	99.98 ^a	101.70 ^a	118.72 ^b	118.17 ^b
<i>SD</i>	9.97	9.72	12.20	6.47
Mean Reading Composite*	-1.07 ^a	-0.91 ^a	1.99 ^b	2.07 ^b
<i>SD</i>	0.57	1.01	0.75	0.65
Handedness**	1.16 ^a	1.15 ^a	1.09 ^a	1.05 ^a
<i>SD</i>	0.24	0.26	0.12	0.10

Note: Values with differing superscripts are significantly different from one another ($p < .05$).

*Reading Composite is a variable reflecting degree of reading disability; a lower score indicates greater impairment.

**This variable reflects the average handedness of the sample, with 1=right-handed, 2=left-handed.

than the control twins. Because the reading composite score is correlated with FSIQ ($r = .61$, $p < .001$), they inevitably have lower FSIQ scores as well. It is also the case that the parents of the two groups differ in SES, with the RD parents having a lower SES. This difference is likewise inevitable, given the fact that RD is familial and the well-replicated result that adults with RD receive less education.

The volumes of seven structures comprising all the gray matter in the neocortex, and the volumes of six other structures comprising most of the rest of the brain were factor-analyzed using data from the entire sample.

Two factors emerged, which accounted for 64% of the total variance. These two factors were straightforwardly interpretable as a cortical factor, on which all seven neocortical structures loaded, and a subcortical factor, on which five of the remaining structures loaded, with the central gray nuclei (which includes the thalamus) cross-loading on the two factors (see Table 2). Because it is a subcortical structure, we included the central gray nuclei in factor 2. The two factor scores were computed based on the factor loadings in Table 2.

We examined whether genetic and environmental influences varied for these two factors or by hemisphere.

Table 2. Factor Loadings

	<i>Factor 1</i>	<i>Factor 2</i>
Operculum	0.917	0.058
Precallosal Cortex	0.796	0.170
Retrocallosal Cortex	0.785	0.221
Insula	0.767	0.286
Temporal Cortex	0.741	0.430
Anterior Superior Cortex	0.692	-0.030
Posterior Superior Cortex	0.507	0.338
White Matter	-0.0023	0.911
Basal Ganglia	0.163	0.809
Brain Stem	0.324	0.722
Hippocampus	0.069	0.721
Cerebellum	0.348	0.708
Central Gray Nuclei	0.538	0.553

Table 3. Intraclass Correlations (SE) in the RD Sample

	<i>MZ (25 pairs)</i>	<i>DZ (23 pairs)</i>	<i>Fisher's Z</i>
Factor 1	0.90 ^a (0.09)	0.62 ^a (0.17)	2.42 ^b
Factor 2	0.86 ^a (0.11)	0.51 ^a (0.19)	2.36 ^b
Cerebral Total	0.97 ^a (0.05)	0.39 (0.20)	5.44 ^a
Right Neocortex	0.94 ^a (0.07)	0.60 ^a (0.18)	3.38 ^a
Left Neocortex	0.96 ^a (0.06)	0.56 ^a (0.18)	4.25 ^a

^a $p < 0.01$ [alpha was set at 0.01 using Dunn's multiple comparison procedure—see text].

^b $p < 0.02$ approaches significance.

We computed intraclass correlations for MZ and DZ pairs in each sample for five brain variables: The cortical and subcortical factor scores, the left and right neocortex, and the total cerebral volume. If the MZ correlation is significant and significantly greater (by Fisher's Z test) than the DZ correlation, then there is evidence for significant heritability, b^2 , the magnitude of which can be roughly estimated by $2(MZ - DZ)$, with an upper-bound being the value of the MZ correlation (Falconer & Mackay, 1996). If the MZ correlation is greater than b^2 , then there is evidence for common environmentality (c^2). A rough estimate of c^2 is given by $2DZ - MZ$. The remaining variance ($1 - MZ$) is unique to individuals and is explained by the combination of error and non-shared environmental influences, e^2 .

Tables 3 and 4 present the intraclass correlations for MZ and DZ pairs (and their standard errors, SE) in each sample and the value of Fisher's Z test for the MZ–DZ difference for all brain variables. As can be seen, all MZ correlations in both samples are significant and range in magnitude from .78 to .98, which means that between approximately 78% and 98% of the variance in the size of these brain structures is shared by identical twins. In contrast, the proportion of brain variance shared by fraternal twins (approximately .32 to .65) was lower in every case. The values for the MZ and DZ correlations were fairly similar across samples. There was a significant difference between the MZ and DZ correlations, indicating significant heritability, for three of five variables in the RD sample (with the other two variables approaching significance) and one of five variables in the smaller

control sample. Dunn's multiple comparison procedure (or Bonferroni procedure) was utilized in the significance testing to correct for the use of multiple significance tests; therefore, a conservative alpha level of .01 was used for all tests.

This difference in MZ and DZ correlations is depicted graphically in Figures 1 and 2, which show the data for individual MZ and DZ pairs on total cerebral volume in the total sample. As can be seen, within-pair similarity is clearly greater in the MZ sample.

Tables 5 and 6 provide preliminary estimates of b^2 , c^2 , and $e^2 + \text{error}$ for all brain variables, as well as SEs for the b^2 and c^2 estimates. The SEs are quite large in the control sample because of the small sample size. As can be seen, the b^2 values did not vary markedly either for the two factors or the two hemispheres in either sample. These b^2 estimates were substantial (.56 to .97), indicating that well over 50% of the variance in size is attributable to genetic influences. In contrast, the estimates of c^2 (0 to .37) and $e^2 + \text{error}$ (.03 to .22) were smaller, suggesting that a smaller proportion of the variance in brain size is attributable to either shared or non-shared environmental influences. However, given the size of the SEs, the confidence intervals for the b^2 and c^2 estimates overlap in all cases except for the cerebral total in the RD sample. Therefore, the differences between the b^2 and c^2 estimates are not significant except in this one case. (Parenthetically, the reliabilities of these five brain measures in both samples are all high, given the uniformly high MZ correlations, which provide a minimum estimate of reliability. In other words, the

Table 4. Intraclass Correlations (SE) in the Control Sample

	<i>MZ (9 pairs)</i>	<i>DZ (9 pairs)</i>	<i>Fisher's Z</i>
Factor 1	0.78 ^a (0.24)	0.34 (0.36)	1.20
Factor 2	0.84 ^a (0.20)	0.32 (0.36)	1.54
Cerebral Total	0.98 ^a (0.08)	0.58 (0.31)	2.84 ^a
Right Neocortex	0.93 ^a (0.14)	0.60 (0.30)	1.67
Left Neocortex	0.93 ^a (0.14)	0.65 (0.29)	1.53

^a $p < 0.01$ [alpha was set at 0.01 using Dunn's multiple comparison procedure].

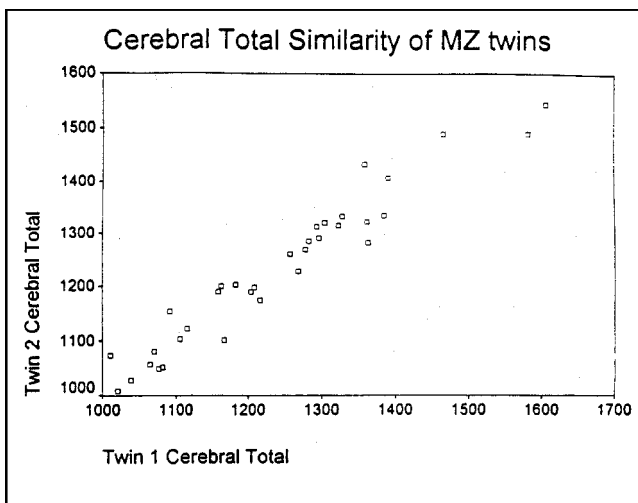


Figure 1. Cerebral volume similarity in MZ twins.

reliabilities are at least 0.86 to 0.97 in the dyslexic sample and 0.78 to 0.98 in the control sample.)

The final results concern the relation between brain size and IQ. The correlations between Wechsler FSIQ and brain variables are given in Table 7. The total cerebral volume was moderately correlated ($r = .42$, $p < .01$, two-tailed) with full-scale IQ in the RD sample; there was a similar trend in the smaller control sample ($r = .31$, $p < .07$, two-tailed). Correlations of similar magnitude were found between the subcortical factor and full-scale IQ, whereas the results for the cortical factor ($r = .16$ and $.13$) were smaller and not significant. In the RD sample, the difference between the correlation of Factor 1 and Factor 2 with FSIQ approached significance ($Z = 1.88$, $p < .06$). These results replicate previous studies that have found correlations between brain size and IQ and they also suggest the relation may be stronger for subcortical structures than for neocortex.

DISCUSSION

To our knowledge, this study is the first examination of genetic and environmental influences on individual differences in human brain size using a full twin design. The main finding is that genes play a substantial role in influencing brain size. Our results did not differ markedly for the cortical vs. the subcortical factor or by hemisphere. In this larger sample, we did not replicate the findings of Tramo et al. (1995), who found that genetic influence may be greater on the left neocortex than the right. While our high MZ correlations are similar to their results, as is our finding that the b^2 value for the left neocortex (0.80) was greater than that for the right neocortex (0.68) in the larger RD sample, this difference in b^2 is not significant in a sample this size. While Tramo et al. (1995) found significant results for the left hemisphere and not the right, they were unable to test whether the results for the two hemispheres were significantly

different from each other because of their small sample size. Tests of differential heritability require very large twin samples to have adequate power. It will take a much larger twin sample than the present one to test clearly whether heritability varies by hemisphere or for cortical vs. subcortical structures.

Similar to four previous studies (Andreasen et al., 1993; Raz et al., 1993; Wickett et al., 1994; Willerman et al. 1991), we found positive correlations between brain size and IQ. The largest correlations were found between IQ and both the subcortical factor and total cerebral volume. In contrast, the correlations between the cortical factor and IQ were relatively small and not significant in either sample. This is a somewhat counterintuitive result, which needs to be replicated in another sample.

Since variations in overall size were highly heritable, the brain-IQ correlations are consistent with the hypothesis that genetic influences on IQ act in part by influencing the size of brain structures. In a larger twin sample, this hypothesis can be explicitly tested by calculating the genetic correlation between brain volume and IQ. To briefly illustrate how this may be done, we can calculate the intraclass cross-correlations between total cerebral volume and FSIQ in the total sample. This cross-correlation is simply the average of the correlations between Twin 1's cerebral volume and Twin 2's FSIQ, and Twin 1's FSIQ and Twin 2's cerebral volume. The average MZ cross-correlation is .315, and the average DZ cross-correlation is .155 in the total sample. The MZ cross-correlation approaches significance ($p < .08$). If the MZ value were significant and significantly greater than the DZ value, that would indicate significant bivariate heritability or shared genetic influences on the relation of the two variables. In this example, the value for bivariate heritability ($b^2_{x,y}$) would be $0.32 [2(MZ - DZ)]$, which is lower than the univariate heritabilities for either total cerebral volume (about 0.90) or FSIQ (about 0.50, based on previous studies). From these values, we may calcu-

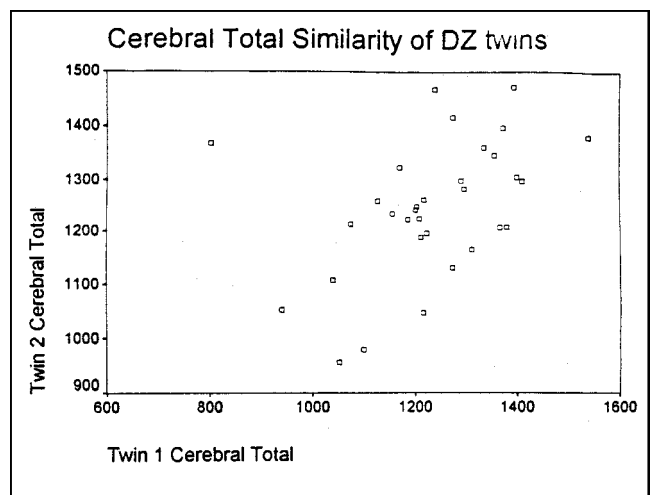


Figure 2. Cerebral volume similarity in DZ twins.

Table 5. Estimates of b^2 , c^2 , and e^2 +error in the RD Sample

Region	b^2	c^2	e^2 +error
Factor 1	0.56 (0.39) ^a	0.34 (0.35)	0.10
Factor 2	0.70 (0.43)	0.16 (0.39)	0.14
Cerebral Total	0.97 (0.42)	0	0.03
Right Neocortex	0.68 (0.38)	0.26 (0.36)	0.06
Left Neocortex	0.80 (0.38)	0.16 (0.37)	0.04

^aValues in parentheses are the standard error.

late the genetic correlation, r_g , between total cerebral volume and FSIQ (Falconer and Mackay, 1996).

The formula for r_g is

$$r_g = \frac{b_{x,y}^2}{\sqrt{b_x^2} \sqrt{b_y^2}}$$

In other words, the genetic correlation between two variables (x and y) is their bivariate heritability divided by the product of the square roots of their univariate heritabilities. In this example, $r_g = .48$. This value means that about half of the genetic influences on either cerebral volume or FSIQ are common to both cerebral volume and FSIQ. We may also calculate the proportion of the phenotypic overlap that is accounted for by these shared genetic influences. That proportion (PG) is simply the bivariate heritability (0.32) divided by the phenotypic correlation (about .40), which gives a proportion of 0.80. This value means that approximately 80% of the phenotypic correlation between total cerebral volume and FSIQ would be attributable to genetic influences acting on both phenotypes. So these preliminary results support the hypothesis that the relation between brain size and IQ is mediated in part by genes.

Our finding of smaller environmental than genetic influences on brain-size variations needs to be interpreted carefully. Environmental influences on brain development have been divided into those that are largely species-typical, called experience-expectant, and

those that are more individual-specific, called experience-dependent (Greenough, Black, & Wallace, 1987). Behavior genetic analyses only detect environmental and genetic influences that differ between individuals, so these analyses do not tell us about species-typical environmental (or genetic) influences on these brain structures, which almost undoubtedly influence brain size. Moreover, though we found substantial heritability for the size of brain structures, this does not mean that genes “hardwire” the brain. Other aspects of brain structure, especially microstructure, are likely to be less heritable than size. There is not enough information in a genome with approximately 10^5 genes to specify the approximately 10^{15} synaptic connections in the mature human brain. We also know that axonal branching patterns differ among clonal animals, who are genetically identical; therefore, such patterns must depend on epigenetic interactions (Changeux, 1985).

Nonetheless, our findings of substantial heritability for individual differences in brain size converge with similar results from twin studies of electrophysiology, both of the resting EEG and of event-related potentials (ERPs). In a recent review of previous adult-twin studies, Van Beijsterveldt and Boomsma (1994) found evidence of significant genetic influence on both EEG and ERP measures. Two more recent ERP twin studies with adults support this conclusion. O’Conner, Morzorati, Christian, and Li (1994) found significant heritability of the ERP in an auditory oddball paradigm. Young, Waldo, Rutledge, and Freedman (1996) reported a heritability of 0.44 for inhibitory gating of the P50 auditory ERP, which has

Table 6. Estimates of b^2 , c^2 , and e^2 +error in the Control Sample

Region	b^2	c^2	e^2 +error
Factor 1	0.78 (0.85) ^a	0	0.22
Factor 2	0.84 (0.83)	0	0.16
Cerebral Total	0.80 (0.63)	0.18 (0.62)	0.02
Right Neocortex	0.66 (0.67)	0.27 (0.62)	0.07
Left Neocortex	0.56 (0.64)	0.37 (0.59)	0.07

^aValues in parentheses are the standard error.

Table 7. Correlations Between Brain Regions and Full-Scale IQ

<i>Brain Region</i>	<i>RD Sample</i>	<i>Control Sample</i>
Factor 1	0.161	0.134
Factor 2	0.413 ^a	0.339
Cerebral Total	0.423 ^a	0.309
Right Neocortex	0.293 ^a	0.204
Left Neocortex	0.293 ^a	0.243

^aCorrelation is significant at the 0.01 level (two-tailed) [alpha was set at 0.01 using Dunn's multiple comparison procedure].

been found to be deficient in schizophrenia. Similar evidence for the heritability of EEG and ERP measures is now emerging in twin studies of children and adolescents. Van Baal, De Geus, and Boomsma (1996) studied EEG power spectra in 209 five-year-old twin pairs and found heritabilities in the range of 0.63–0.76 for relative power. This sample was also studied with a visual odd-ball ERP paradigm and the heritability of P3 latency was examined both at age 5 and age 7 (Van Baal et al., 1996). Heritabilities ranged from 0.13 to 0.78 at age 5 and from 0.36 to 0.99 at age 7, with similar genetic influences acting on the ERP at most scalp locations at both ages. Another study of a sample of 119 adolescent twin pairs found median heritabilities of 0.75 and 0.71 for the absolute and relative power spectra of the spontaneous EEG (McGuire, Katsanis, Iacono, & McGue, 1998).

In sum, there is considerable evidence for the heritability of EEG and ERP measures of brain function across development. If cognitive measures are available in these twin samples, the genetic correlation between electrophysiology and cognitive processing could be evaluated. To our knowledge, the heritability of other measures of brain function, such as positron emission tomography (PET) or functional magnetic resonance imaging (fMRI), has not been examined using a twin design.

There are some limitations to the current study. First, the bulk of the twin sample consists of dyslexic twins, so it is possible that these results may not generalize. But our results were quite similar in both the dyslexic and control samples. So, it is unlikely that the main results reported here are specific to a dyslexic sample, although this possibility needs to be tested further as our sample increases. Second, the estimates of heritability and environmentality are preliminary, and need to be confirmed in larger samples in which more sophisticated quantitative behavior genetic modeling can be employed. For instance, the method used here to estimate heritability (b^2) will overestimate b^2 if there are non-additive genetic influences operating. One indication of such nonadditive genetic influences is a MZ correlation that is considerably greater than twice the DZ correlation. In this study, the MZ correlation was, in fact, *smaller* than twice the DZ correlation for all but one

of the five variables in the RD sample (cerebral total) and all but two in the control sample (Factor 1 and Factor 2). For these three variables, the estimate of b^2 was just the MZ correlation, not $2(MZ - DZ)$, thus avoiding an overestimate of b^2 .

Despite these limitations, these results do provide new information about the possible neurobiological bases of individual differences in cognitive development.

METHODS

The research presented here is part of the CLDRC, in which a large sample of RD and control twins are being studied at the genetic, neurological, and cognitive levels of analysis. The MRI project involves a collaboration among four different sites: (1) the Institute for Behavioral Genetics (IBG) which recruits twins for the MRI study from the larger twin samples; (2) the MRI facility at UCHSC which performs the scans; (3) Dr. Filipek's Morphometric Analysis Laboratory at UCI which performs the morphometric analysis of these scans without knowledge of the subject's RD, zygosity, or co-twin status; and (4) Dr. Pennington's lab at DU, which performs the statistical analyses. In what follows, the methods used across these sites will be described in three sections dealing respectively with subject recruitment and characteristics, MRI image acquisition and morphometric analyses, and statistical analyses.

Subjects

CLDRC Twin Sample

The RD and control twin pairs were recruited from the larger population sample of twins who had already participated in other studies of the CLDRC, and who are recruited (ongoing) in the following manner. In order to minimize the possibility of referral bias (Vogel, 1990), twin pairs are systematically ascertained through cooperating school districts. Administrators and school personnel in a total of 27 different school districts within the state of Colorado currently participate in this study. Without regard to reading status, all twin pairs within each district are identified, and

permission is then sought from parents to review the school records of both members of each pair for evidence of reading problems. If either member of a twin pair manifests a positive history of reading problems (for example, low reading achievement test scores, referral to a reading therapist because of poor reading performance, reports by classroom teachers or school psychologists, and so on), both members of the pair are invited to complete an extensive battery of tests in the laboratories of the CLDRC. A comparison group of control twin pairs is also recruited, who are matched to probands on the basis of age, gender, and school district. In order for a twin pair to be included in the control sample, both members of the pair must have a negative school history for reading problems.

In the laboratory of J. C. DeFries, the RD and control twins are administered a psychometric test battery that includes the Wechsler Intelligence Scale for Children—Revised (WISC-R) (Wechsler, 1974) (or the Wechsler Adult Intelligence Scale—Revised (WAIS-R) (Wechsler, 1981) and the Peabody Individual Achievement Test (PIAT) (Dunn & Markwart, 1970). Employing discriminant weights estimated from an analysis of PIAT Reading Recognition, Reading Comprehension, and Spelling data obtained from an independent sample of 140 reading-disabled and 140 control nontwin children (DeFries, 1985), a discriminant function score (“reading discriminant score”) is then computed for each subject. In order for an individual to be diagnosed as being reading disabled in this component of the program project, he or she must have a positive school history for reading problems and also be classified as affected by the discriminant score. Likewise, for an individual to be included in the control sample, he or she must have a negative school history and be classified as unaffected by the discriminant score. Additional diagnostic criteria include an IQ score of at least 90 on either the Verbal or Performance Scale of the WISC-R or WAIS-R; no evidence of neurological problems; and no uncorrected visual or auditory acuity deficits. Children with major psychiatric problems were also excluded.

Selected items from the Nichols and Bilbro (1966) questionnaire are used to determine zygosity of twin pairs. In ambiguous cases, zygosity of the pair is confirmed by standard genotyping analysis of blood samples. In the RD proband cohort, a total of 198 pairs of identical (MZ) twins, 148 pairs of same-sex fraternal (DZ) twins, and 97 pairs of opposite-sex DZ twins meet criteria for inclusion (that is, at least one member of the pair of twins is reading disabled). In addition, a total of 157 pairs of MZ twins, 107 pairs of same-sex DZ twins, and 61 pairs of opposite-sex DZ twins comprise the current CLDRC control sample. These twins were all reared in English-speaking, middle-class homes.

MRI Twin Sample

The MRI project was approved by the Institutional Review Board of the University of Colorado. For subjects who agreed to participate, the nature of the study and the MRI imaging procedure were verbally explained to subjects. They then read and signed a written consent form, and were paid US\$50 for their participation.

The following selection criteria were used to select same-sex twin pairs for the MRI study from the larger CLDRC twin sample: (1) 12 years of age or greater; (2) for RD pairs, a discriminant score greater than 2.5 standard deviations (*SD*) below the control mean; and (3) sex and ethnicity representative of the overall sample.

We also controlled for handedness, which was measured by an 11-item questionnaire regarding hand preference for everyday activities (for example, write, throw, and hammer). On each item, a right-hand preference was scored as a 1 and a left-hand preference as a 2; the handedness score was the average score across the 11 items and ranged from 1.0 (exclusively right-handed) to 2.0 (exclusively left-handed). As can be seen in Table 1, the mean handedness scores did not differ by group.

MR Image Acquisition and Morphometric Procedures

MR Image Acquisition

All MRI scans were performed on the *General Electric 1.5 Tesla Signa MR System* (5×) located at UCHSC. After standard sagittal scout and coronal T₂-weighted sequences, a coronal T₁-weighted 3-D spoiled gradient echo SPGR pulse sequence was performed with the following parameters: TR/TE=40/8 msec, TE=8 msec, flip angle=40°, field of view=24 cm, slice thickness=contiguous 3.0 mm, matrix=256×256, averages=1; imaging time=10.5 min.

All scans were read clinically by co-author JHS, and a clinical interpretation for each was provided by co-author CMF. Only three of the scans exhibited minor anomalies on clinical reading; the remainder were read as clinically normal. Two of these individuals were controls and one was RD. The RD subject had an 8–10 mm left frontal white matter lesion. One control had a 3–4 mm mass along the base of the skull near the right fifth and sixth cranial nerves, presumably a benign tumor. The other control had an 8-mm lesion in the left posteroinular region at the grey/white matter junction. These three subjects were excluded from the analyses presented here.

Morphometric Analyses

All 3-D-SPGR scans were analyzed blind to diagnosis or twin/sibling status according to the standard mor-

phometric protocol, which includes *positional normalization*, *image segmentation*, and "*pericallosal*" *Hemispheric Regional partitioning* (Filipek, Kennedy, & Caviness, 1991; Filipek, Richelme, Kennedy, & Caviness, 1994; Filipek et al., 1997). On each normalized T₁-weighted 3D MRI slice, anatomic segmentation was performed using *intensity contour mapping* and *differential intensity contour algorithms*, which identify, classify and create a continuous outline corresponding only to those voxel locations constituting the specified anatomic borders. These algorithms have been described in greater detail elsewhere (Filipek et al., 1989, 1991; Kennedy, Filipek, & Caviness, 1989; Kennedy, Meyer, Filipek, & Caviness, 1994; Kennedy & Nelson, 1987).

The following anatomic substructures were segmented: cerebral cortex, white matter, basal ganglia (caudate, putamen, and pallidum), central gray nuclei (diencephalon), hippocampus, brainstem, and cerebellum (see Filipek et al., 1994 for definitions). The cerebral hemispheres were then partitioned into eight subdivisions, based on the Hemispheric Regional segmentation methods to include: *Precallosal*, *Anterior-Superior*, *Inferior* and *-Temporal*, *Posterior-Superior*, *Inferior*, and *-Temporal*, and *Retrocallosal Regions* (see Filipek et al., 1997 for definitions and figure). *Operculum* was then defined as the combined cortex located within the anterior- and posterior-superior regions extending from the tip of the superior circular insular sulcus laterally to the external border of the hemisphere. *Insula* was defined as the combined cortex located within the anterior- and posterior-inferior regions extending from the tip of the superior to the tip of the inferior circular sulci.

Analyses

Because left and right hemispheric measures were highly correlated, we combined the structural volumes (in cm³) from each hemisphere for each structure. Thirteen of the segmented substructures were subsequently used for the data analyses. These structures comprise virtually the whole brain: cerebral cortex (five structures described below), operculum, insula, white matter, total basal ganglia, central gray nuclei, hippocampus, brainstem, and cerebellum. The cerebral cortex (but not other structures) was divided into five structures in the present analyses: *Precallosal*, *Anterior-Superior*, *Posterior-Superior*, *Retrocallosal*, and *Temporal Regions* (with *Anterior-* and *Posterior-Temporal* cortices combined as *Temporal*).

The analyses consisted of a factor analysis of the 13 brain variables, followed by computation of intraclass correlations, and a test for the difference between the MZ and DZ correlations by Fisher's Z test of two independent correlations. The rationale for the factor analysis and the methods used to compute the SE of *b*² and *c*² follow.

Factor Analysis

Since the morphometric analyses yield a very large number of variables per subject, we needed a data reduction strategy that fit with the overall goal of exploring the etiology of individual differences in the size of major brain structures. There were two steps to this strategy: (1) selecting a reasonably small set of composite variables that were both comprehensive and meaningful; and (2) factor analyzing the composite variables. To arrive at the 13 composite variables discussed earlier, we (1) picked the major subcortical structures identified by the anatomic segmentation algorithms, (2) reduced the set of possible cortical variables by combining some of the pericallosal partitions as described earlier, and (3) tested whether it was justifiable to collapse across hemispheres. In the total sample, there was a high degree of correlation (median *R* = .93, range = .82–.99) between the right and left sides of any given structure; it thus seemed reasonable to collapse across hemispheres in creating composites. We next factor-analyzed the 13 brain variables in the total sample of 132 subjects, using Principal Components factor analysis with Varimax rotation (Maxwell & Delaney, 1990). The criteria for a significant factor was an eigenvalue > 1.0, with at least two variables loading on the factor.

SE of *b*² and *c*²

The SE of *b*² and *c*² are a function of the SE of both the MZ and DZ intraclass correlations (Falconer & Mackay, 1996). The formula used for the SE of the MZ and DZ intraclass correlations was

$$SE = \sqrt{\frac{1 - r^2}{N - 2}} \quad (1)$$

The formula used for the SE of *b*² was

$$SE_{b^2} = \sqrt{4(SE_{MZ})^2 + 4(SE_{DZ})^2} \quad (2)$$

The formula for the SE of *c*² was

$$SE_{c^2} = \sqrt{4(SE_{DZ})^2 + SE_{MZ}^2} \quad (3)$$

Acknowledgments

This research was supported by two NICHD Center grants: Learning Disability Research Center grant P50 HD27802 and a Mental Retardation Research Center (MRRC) grant P30 HD04024, and by two NIMH grants: K02 MH00419 (RSA) and R37 MH38820 (MERIT). Thanks go to Terry Goldhammer for help in recruiting and scheduling subjects.

Reprint requests should be sent to Bruce F. Pennington at the Department of Psychology, University of Denver, 2155 S. Race St., Denver, CO 80208.

REFERENCES

- Andreasen, N. C., Flaum, M., Swayze, V., et al. (1993). Intelligence and brain structure in normal individuals. *American Journal of Psychiatry*, *150*, 130–134.
- Bailey, A., Phillips, W., & Rutter, M. (1996). Autism: Towards an integration of clinical, genetic, neuropsychological, and neurobiological perspectives. *Journal of Child Psychology and Psychiatry*, *37*, 89–126.
- Changeux, J. P. (1985). *Neuronal man*. New York: Oxford University Press.
- Coyle, J. T., Oster-Granite, M. L., & Gearhart, J. D. (1986). The neurobiologic consequences of Down Syndrome. *Brain Research Bulletin*, *16*, 773–787.
- DeFries, J. C. (1985). Colorado Reading Project. In D. B. Gray & J. F. Kavanaugh (Eds.), *Biobehavioral measures of dyslexia* (pp. 123–135).
- DeFries, J. C., Filipek, P. A., Fulker, D. W., Olson, R. K., Pennington, B. F., Smith, S. D., & Wise, B. W. (1997). Colorado Learning Disabilities Research Center. *Learning Disabilities: A Multidisciplinary Perspective*, *8*, 7–19.
- Dunn, L. M., & Markwardt, F. C. (1970). *Examiner's manual: Peabody Individual Achievement Test*. Circle Pines, American Guidance Service, MN.
- Falconer, D. S., & Mackay, T. F. C. (1996). *Introduction to quantitative genetics* (4th ed.), Longman Group, London.
- Filipek, P. A. (1996). Structural variations in measures of developmental disorders. In R. W. Thatcher, G. R. Lyon, J. Rumsey & N. Krasnegor (Eds.), *Developmental neuroimaging: Mapping the development of brain and behavior* (pp. 169–186). San Diego, CA: Academic Press.
- Filipek, P. A., Kennedy, D. N., Caviness, V. S., Jr. (1991). Volumetric analysis of central nervous system neoplasm based on MRI. *Pediatric Neurology*, *7*, 347–351.
- Filipek, P. A., Kennedy, D. N., Caviness, V. S., Rossnick, S. L., Spraggins, T. A., & Starewicz, P. M. (1989). MRI-based brain morphometry: Development and application to normal subjects. *Annals of Neurology*, *25*, 61–67.
- Filipek, P. A., Richelme, C., Kennedy, D. N., & Caviness, V. S. (1994). The young adult human brain: An MRI-based morphometric analysis. *Cerebral Cortex*, *4*, 344–360.
- Filipek, P. A., Semrud-Clikeman, M., Steingard, R. J., Renshaw, P. F., Kennedy, D. N., & Biederman, J. (1997). Volumetric MRI analysis comparing Attention-Deficit Hyperactivity Disorder and normal controls. *Neurology*, *48*, 589–601.
- Gould, S. J. (1981). *The Mismeasure of Man*. New York: WW Norton.
- Greenough, W. T., Black, J. E., & Wallace, C. S. (1987). Experience and brain development. *Child Development*, *58*, 539–559.
- Haier, R. J. (1994). Cerebral glucose metabolism and intelligence. In A. Vernon (Ed.), *Biological Approaches to the Study of Human Intelligence*. Norwood, NJ: Ablex.
- Haier, R. J., Siegel, B., Tang, C., Abel, L., & Buchsbaum, M. A. (1992). Intelligence and changes in regional cerebral glucose metabolic rate following learning. *Intelligence*, *16*, 415–426.
- Jerison, H. J. (1989). *Brain Size and the Evolution of Mind*. New York: American Museum of Natural History.
- Kennedy, D. N., Filipek, P. A., & Caviness, V. S. (1989). Anatomic segmentations and volumetric calculations in nuclear magnetic resonance imaging. *IEEE Transactions on Medical Imaging*, *8*, 1–7.
- Kennedy, D. N., Meyer, J. W., Filipek, P. A., & Caviness, V. S. (1994). MRI-based topographic segmentation. In R. W. Thatcher, M. Hallet, T. Zeffiro, E. R. John & M. Huerta (Eds.), *Functional Neuroimaging: Technical Foundations* (pp. 201–208). San Diego: Academic Press.
- Kennedy, D. N., & Nelson, A. C. (1987). Three-dimensional display from cross-sectional tomographic images: An application to magnetic resonance imaging. *IEEE Transactions on Medical Imaging*, *TMI-6*, 134–140.
- Maxwell, W. E., & Delaney, H. D. (1990). *Designing experiments and analyzing data: A model comparisons approach*. Belmont, CA: Wadsworth.
- McGuire, K. A., Katsanis, J., Iacono, W. G., & McGue, M. (1998). Genetic influences on the spontaneous EEG: An examination of 15-year-old and 17-year-old twins. *Developmental Neuropsychology*, *14*, 7–18.
- Nichols, R. C., & Bilbro, W. C. (1966). The diagnosis of twin zygosity. *Acta Genetica*, *16*, 265–275.
- O'Connor, S., Morzorati, S., Christian, J. C. & Li, T. K. (1994). Heritable features of the auditory oddball event-related potential: Peaks, latencies, morphology and topography. *Electroencephalography and Clinical Neurophysiology Evoked Potential*, *92*, 115–125.
- Pennington, B. F., Filipek, P. A., Lefly, D. L., Churchwell, J., Kennedy, D. N., Simon, J. H., Filley, C. M., Galaburda, A., Alarcon, M., & DeFries, J. C., (in press). Brain morphometry in reading-disabled twins.
- Plomin, R., DeFries, J. C., McClearn, G. E., & Rutter, M. (1997). *Behavioral genetics* (3rd ed.). New York: W.H. Freeman.
- Prouty, L. A., Rogers, R. C., Stevenson, R. E., Dean, J. H., Palmer, K. K., Simensen, R. J., Coston, G. N., Schwartz, C. E. (1988). Fragile X syndrome: Growth development and intellectual function. *American Journal of Medical Genetics*, *30*, 123–142.
- Raz, N., Torres I, Spencer, W. E., Millman, D., Baertschi, J. C., & Sarpel, G. (1993). Neuroanatomical correlates of age-sensitive and age-invariant cognitive abilities: An in vivo MRI investigation. *Intelligence*, *17*, 407–422.
- Thatcher, R. W. (1992). Cyclic cortical reorganization during early childhood. *Brain Cognition*, *20*, 24–50.
- Tramo, N. J., Loftus, W. C., Thomas, C. E., Green, R. L., Mott, L. A., & Gazzaniga, M. J. (1995). Surface area of human cerebral cortex and its gross morphological subdivision: In vivo measurements in monozygotic twins suggest differential hemisphere effects of genetic factors. *Journal of Cognitive Neuroscience*, *7*, 292–301.
- Van Baal, G. C. M., De Geus, E. J. C., & Boomsman, D. I. (1996). Genetic architecture of EEG power spectra in early life. *Electroencephalography and Clinical Neurophysiology*, *98*, 502–514.
- Van Beijsterveldt, C. E. M., & Boomsma, D. I. (1994). Genetics of the human electroencephalogram (EEG) and event-related brain potentials (ERPs): A review. *Human Genetics*, *94*, 319–330.
- Vogel, S. A. (1990). Gender differences in intelligence, language, visual-motor abilities, and academic achievement in males and females with learning disabilities: A review of the literature. *Journal of Learning Disabilities*, *23*, 44–52.
- Wechsler, D. (1974). *Examiner's manual: Wechsler Intelligence Scale for Children—Revised (WISC-R)*. New York: Psychological Corporation.
- Wechsler, D. (1981). *Examiner's manual: Wechsler Adult Intelligence Scale—Revised (WAIS-R)*. New York: Psychological Corporation.
- Wickett, J., Vernon, P., & Lee, D. (1994). In vivo brain size, head perimeter, and intelligence in a sample of healthy adult females. *Personality and Individual Differences*, *16*, 831–838.
- Willerman, L., Schultz, R., Rutledge, J. N., & Bigler, E. D. (1991). In vivo brain size and intelligence. *Intelligence*, *15*, 223–228.
- Young, D. A., Waldo, M., Rutledge, J. H., III, & Freedman, R. (1996). Heritability of inhibitory gating of the P50 auditory-evoked potential in monozygotic and dizygotic twins. *Neuropsychobiology*, *33*, 113–117.

Copyright of Journal of Cognitive Neuroscience is the property of MIT Press and its content may not be copied or emailed to multiple sites or posted to a listserv without the copyright holder's express written permission. However, users may print, download, or email articles for individual use.