# Surface Area of Human Cerebral Cortex and Its Gross Morphological Subdivisions: *In Vivo* Measurements in Monozygotic Twins Suggest Differential Hemisphere Effects of Genetic Factors

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## Abstract

■ We measured the surface area of the cerebral cortex and its gross morphological subdivisions in 10 pairs of monozygotic twins. Cortical surface area was estimated *in vivo* using magnetic resonance imaging and three-dimensional computer models of the intra- and extrasulcal pial surface. The means and standard deviations of regional (e.g., gyral), lobar, hemisphere, and total cortical surface area were tabulated for the entire population of 20 young, right-handed adults (10 females, 10 males). To determine whether genotypic differences were associated with morphometric differences, analyses of variance were carried out on each measure across unrelated twin pairs (genotype factor) and within co-twins (birth order factor). Across unrelated pairs, there was wide variation in regional cortical surface area for the left hemisphere (normalized by total cortical surface area, $p \leq 0.0001$) but not for the right hemisphere (normalized, $p = 0.12$). More variation in lobar surface area was also observed for the left hemisphere (normalized, $p = 0.05$) than for the right (normalized, $p = 0.48$). Within co-twins, no significant variation in regional surface area or lobar surface area was found for the left or right hemisphere. Although normalized regional and lobar surface area in the left hemisphere differed across unrelated pairs, overall left hemisphere surface area normalized by total cortical surface area did not ($p = 0.73$). Total cortical surface area normalized by body weight varied across unrelated pairs ($p = 0.001$) but not within co-twins ($p = 0.39$). The effects observed across unrelated pairs were not attributable to sex differences.

These results suggest: 1) both the total area and folding of the cortical surface are heavily influenced by genetic factors in humans; and 2) the cerebral hemispheres may be differentially affected by genetic influences on cortical morphogenesis, with the language-dominant left cerebral cortex under stronger genetic control than the right. ■

## INTRODUCTION

Phylogenetic increases in brain size have evolved not as a symmetrical enlargement of the entire brain, but as a disproportionate increase in the size of the cerebral hemispheres involving, in particular, the surface area of the cerebral cortex (for reviews see Kaas, 1987; Hofman, 1989; Allman, 1990; Killackey, 1990). The expansion of preexisting cortical areas and the elaboration of new ones have involved certain regions more than others. Cross-species differences in the gross morphometry of mature cortex are presaged by prenatal differences in the number and distribution of ontogenetic columns within the cortex, which in turn reflect the number and distribution of symmetrical stem cell divisions occurring early in gestation in the neuroepithelium (Rakic, 1988). Large scale phylogenetic differences in regional cortical surface area are obviously the consequence of large scale differences in genetic endowment. Yet, within a given

species, there is considerable variation in size and shape. To what extent is this intraspecies variation a consequence of genetic factors?

Inquiries into genetic influences on human cortical surface area can be addressed through studies of monozygotic twins, albeit with certain qualifications (for review see Hrubec & Rubinette, 1984). Anatomical, physiological, psychological, and pathological similarities between monozygotic twins have been quantified by many authors (for reviews see Springer & Searleman, 1980; Bouchard, Lykken, McGue, Segal, & Tellegen, 1990). While there are copious data on head measurements, handedness, and IQ, few brain measurements have been published. In light of the available data and advances in basic knowledge about cortical development at microanatomical and physiological levels (for reviews see Welker, 1990; Goodman & Shatz, 1993), it seems reasonable to hypothesize that the cortices of monozygotic twins share similarities that are detectable at the gross anatomical level. A previous *in vivo* magnetic resonance imaging (MRI) study from our laboratory demonstrated co-twin similarities in the mid-sagittal area and shape of the corpus callosum (Oppenheim, Skerry, Tramo, & Gazzaniga, 1989), which is formed by the axons of cortical neurons. A concordant delay in cortical fissuration at 19-32 weeks of gestation was noted in twin brains from the Yakovlev collection by Chi, Dooling, and Gillis, (1977). The MRI data of Weinberger and colleagues raise the possibility that normal co-twins share similarities in hippocampal size, ventricular size, and left-right asymmetries of perisylvian cortex (Suddath, Christison, Torrey, Casanova, & Weinberger, 1990; Bartley, Jones, Torrey, Zigun, & Weinberger, 1993). Significant variation within co-twins discordant for schizophrenia but not within normal co-twins has been observed for some measures.

In the present paper, we report cortical surface area measurements in 10 pairs of normal, adult, monozygotic twins. The areas of individual gyri and other gross surface structures were estimated using three-dimensional (3D) computer models of the intra- and extrasulcal surface imaged via MRI. Lobar, hemisphere, and total cortical surface area were also computed. The working hypothesis was that genetic factors contribute to interindividual differences in cortical growth, fissuration, and gyral development. The prediction was that surface area measurements would vary more across unrelated twin pairs than within co-twins.

## RESULTS

### Regional Cortical Surface Area (*r*SA)

Table 1 lists the means and standard deviations of the 64 raw *r*SA measurements for the entire population of 20 young, right-handed adults (ages 18-43 years; 5 female twin pairs, 5 male twin pairs).

A repeated measures analysis of variance (ANOVA) model was constructed to test for differences in *r*SA across unrelated twin pairs (genotype factor: Twins A vs Twins B vs . . . Twins J) and within co-twins (birth order factor: Twin $A_1$ vs Twin $A_2$, Twin $B_1$ vs Twin $B_2$ . . . Twin $J_1$ vs Twin $J_2$; Cole & Grizzle, 1966; Neter & Wasserman, 1974; Crowder & Hand, 1990). In separate series of ANOVAs, we separated the overall effect of genotype into the independent contributions of sex and genotype and removed the sex effect. ROI and hemisphere were treated as within-subject factors. The dependent variable was raw *r*SA and, in a separate series of ANOVAs, *r*SA normalized by total cortical surface area ($r_i SA \div \sum_{i=1}^{N} r_i SA$, where N = 32 or 64 depending on the particular ANOVA).

There was a highly significant interaction between ROI and hemisphere ($p \leq 0.0001$), indicating that the variation in raw *r*SA across ROIs differed in the left and right hemispheres. For normalized *r*SA, a highly significant interaction between ROI and hemisphere was again found ($p \leq 0.0001$), indicating that left-right differences in raw *r*SA could be attributed to differences in regional size and shape instead of, or in addition to, differences in the total area of the cortical surface. A highly significant three-way interaction among genotype, ROI, and hemisphere was found for both raw and normalized *r*SA (for both, $p \leq 0.0001$), but there was no interaction among birth order, ROI, and hemisphere for either dependent variable (raw, $p = 0.44$; normalized, $p = 0.26$). Thus the two-way interaction between ROI and hemisphere arose principally from variation across unrelated pairs. There was no interaction among sex, ROI, and hemisphere (raw, $p = 0.87$; normalized, $p = 0.83$), indicating that the observed interaction between ROI and hemisphere could not be attributed to sex differences. The interaction among genotype, ROI, and hemisphere remained significant when the model took into account sex differences across unrelated pairs (raw and normalized, $p \leq 0.0001$).

Data for each hemisphere were then analyzed separately. For both the left and right hemispheres, there were highly significant main effects of ROI (raw and normalized, $p \leq 0.0001$), reflecting the obvious differences in the size and shape of the 32 ROIs within each hemisphere. In the left hemisphere, there were highly significant interactions between genotype and ROI for raw and normalized *r*SA ($p \leq 0.0001$ for both), indicating that left *r*SA varied greatly across unrelated pairs. There were no left hemisphere interactions between birth order and ROI (raw, $p = 0.22$; normalized, $p = 0.46$), indicating relatively little variation in left *r*SA within co-twins. In the right hemisphere, there was an interaction between genotype and ROI for raw *r*SA ($p = 0.01$) but not normalized *r*SA ($p = 0.12$), indicating that much of the variation in right *r*SA could be attributed to variation in total cortical surface area. There was a weak interaction between birth order and ROI for raw right *r*SA ($p = 0.06$), but the evidence was even weaker for normalized right *r*SA ($p = 0.12$). Taken together, the

**Table 1.** Regional and Lobar Cortical Surface Area ($cm^2$)[a]

| *Left Hemisphere* | *M* | *SD* | *%LH* | *Right Hemisphere* | *M* | *SD* | *%RH* |
|---|---|---|---|---|---|---|---|
| Frontal lobe | 252.8 | 33.3 | 26.7 | Frontal lobe | 259.1 | 34.6 | 27.0 |
| Superior frontal g | 72.3 | 11.9 | 7.6 | Superior frontal g | 71.9 | 17.8 | 7.5 |
| Middle frontal g | 49.9 | 12.3 | 5.3 | Middle frontal g | 55.0 | 19.4 | 5.7 |
| Inferior frontal g | | | | Inferior frontal g | | | |
| Pars orbitalis | 13.2 | 3.2 | 1.4 | Pars orbitalis | 11.1 | 5.8 | 1.2 |
| Pars triangularis | 9.5 | 4.6 | 1.0 | Pars triangularis | 10.3 | 4.1 | 1.1 |
| Pars opercularis | 22.6 | 6.1 | 2.4 | Pars opercularis | 23.3 | 6.3 | 2.4 |
| Precentral g | 35.8 | 6.6 | 3.8 | Precentral g | 34.9 | 8.3 | 3.6 |
| Orbitofrontal g | 28.2 | 5.1 | 3.0 | Orbitofrontal g | 30.3 | 6.4 | 3.2 |
| Straight g | 9.5 | 1.9 | 1.0 | Straight g | 10.6 | 2.4 | 1.1 |
| Frontal pole | 11.8 | 4.6 | 1.2 | Frontal pole | 11.7 | 3.8 | 1.2 |
| Temporal lobe | 177.4 | 15.6 | 18.7 | Temporal lobe | 184.3 | 17.0 | 19.2 |
| Superior temporal g | 32.1 | 4.8 | 3.4 | Superior temporal g | 35.1 | 6.1 | 3.7 |
| Middle temporal g | 33.9 | 5.7 | 3.6 | Middle temporal g | 38.2 | 6.5 | 4.0 |
| Inferior temporal g | 29.6 | 5.5 | 3.1 | Inferior temporal g | 29.5 | 5.3 | 3.1 |
| Transverse g | 7.5 | 1.5 | <1.0 | Transverse g | 6.5 | 1.8 | <1.0 |
| Parahippocampal g | 11.4 | 2.6 | 1.2 | Parahippocampal g | 11.6 | 4.3 | 1.2 |
| Uncus | 7.5 | 2.2 | <1.0 | Uncus | 7.1 | 2.5 | <1.0 |
| Amygdala | 3.7 | 1.6 | <1.0 | Amygdala | 3.2 | 1.2 | <1.0 |
| Fusiform g | 33.1 | 3.4 | 3.5 | Fusiform g | 33.8 | 4.2 | 3.5 |
| Parainsular region | 0.8 | 0.5 | <1.0 | Parainsular region | 1.0 | 0.4 | <1.0 |
| Temporal isthmus | 1.4 | 0.7 | <1.0 | Temporal isthmus | 1.4 | 0.7 | <1.0 |
| Temporal pole | 16.4 | 3.0 | 1.7 | Temporal pole | 16.9 | 3.8 | 1.8 |
| Parietal lobe | 229.3 | 29.8 | 24.2 | Parietal lobe | 225.9 | 31.4 | 23.5 |
| Postcentral g | 45.9 | 13.5 | 4.8 | Postcentral g | 42.5 | 8.3 | 4.4 |
| Supramarginal g | 31.2 | 8.5 | 3.3 | Supramarginal g | 27.6 | 7.5 | 2.9 |
| Angular g | 37.8 | 8.6 | 4.0 | Angular g | 46.8 | 11.9 | 4.9 |
| Superior parietal lobule | 80.1 | 15.2 | 8.4 | Superior parietal lobule | 78.1 | 18.5 | 8.2 |
| Precuneus | 34.3 | 7.6 | 3.6 | Precuneus | 30.9 | 6.6 | 3.2 |
| Occipital lobe | 227.9 | 32.5 | 24.0 | Occipital lobe | 226.9 | 34.0 | 23.7 |
| Lateral occipital g | 134.9 | 34.3 | 14.2 | Lateral occipital g | 132.0 | 28.6 | 13.8 |
| Cuneus | 23.8 | 8.0 | 2.5 | Cuneus | 29.0 | 9.3 | 3.0 |
| Lingual g | 29.5 | 7.9 | 3.1 | Lingual g | 29.5 | 4.5 | 3.1 |
| Occipital pole | 39.7 | 16.7 | 4.2 | Occipital pole | 36.4 | 15.3 | 3.8 |
| Other | 60.3 | 8.7 | 6.4 | Other | 62.3 | 9.2 | 6.5 |
| Basal forebrain | 5.2 | 1.6 | <1.0 | Basal forebrain | 4.8 | 1.3 | <1.0 |
| Insula | 16.7 | 2.6 | 1.8 | Insula | 17.0 | 3.0 | 1.8 |
| Cingulate g | 38.4 | 7.3 | 4.1 | Cingulate g | 40.5 | 7.3 | 4.2 |

[a] M, mean of 20 subjects; SD, standard deviation; g, gyrus. (See methods for lobe classification.) %LH, percentage of mean left hemisphere surface area; %RH, same for right.

pattern of results for *r*SA in the left and right hemispheres was consistent with the two-way interactions between ROI and hemisphere observed for raw and normalized *r*SA across all 64 ROIs. In sum, most of the variance in the *r*SA data was attributable to genotypic differences across the 32 left hemisphere ROIs.

A significant interaction between sex and ROI was observed for normalized left *r*SA ($p = 0.007$; raw, $p = 0.07$). Since co-twins were the same sex, this interaction arose from sex differences across unrelated pairs. Still, the highly significant interaction between genotype and ROI persisted even after the effect of sex was removed (raw left *r*SA, $p \leq 0.0001$; normalized, $p = 0.0007$). In the right hemisphere, there was no interaction between sex and ROI (raw, $p = 0.66$; normalized, $p = 0.50$). There was an interaction between genotype and ROI for raw right *r*SA ($p = 0.01$) but not normalized right *r*SA ($p = 0.12$) similar to that observed before the contribution of sex differences across unrelated pairs was removed.

### Lobar Cortical Surface Area (*lob*SA)

Table 1 lists the means and standard deviations of the raw *lob*SA measurements for all 20 subjects. Repeated measures ANOVAs similar to those described above for *r*SA were carried out with raw *lob*SA and, separately, *lob*SA normalized by total cortical surface area as the dependent variable.

There was no significant interaction between lobe and hemisphere for raw *lob*SA or normalized *lob*SA (both $p = 0.19$), indicating that *lob*SA varied to a similar extent in the left and right hemispheres. No significant interactions were found for raw *lob*SA or normalized *lob*SA among genotype, lobe, and hemisphere (raw, $p = 0.15$; normalized, $p = 0.14$), birth order, lobe, and hemisphere (both $p = 0.25$), and sex, lobe, and hemisphere ($p = 0.86$, $p = 0.84$).

Data for the left and right hemispheres were then analyzed separately. Highly significant main effects of lobe for left raw, right raw, left normalized, and right normalized *lob*SA were found (all $p \leq 0.0001$), indicating that within each hemisphere *lob*SA varied greatly across the four lobes. In the left hemisphere, there were marginally significant interactions between genotype and lobe for raw *lob*SA ($p = 0.04$) and normalized *lob*SA ($p = 0.05$), indicating that left *lob*SA varied across unrelated twin pairs. No interactions between birth order and lobe were found (raw, $p = 0.38$; normalized, $p = 0.40$), indicating little variation in left *lob*SA within co-twins. There were no significant interactions between sex and lobe (raw left *lob*SA, $p = 0.17$; normalized, $p = 0.15$). When sex differences across unrelated pairs were taken into account, the interactions between genotype and lobe were only slightly weakened (raw left *lob*SA, $p = 0.05$; normalized, $p = 0.07$). In the right hemisphere, no significant interactions were observed for raw or normalized *lob*SA between genotype and lobe (raw, $p = 0.32$; normalized, $p = 0.48$), birth order and lobe ($p = 0.23$, $p = 0.19$), or sex and lobe ($p = 0.22$, $p = 0.21$). As the latter results would predict, there still was no significant interaction between genotype and lobe when the contribution of sex differences was removed (raw right *lob*SA, $p = 0.38$; normalized, $p = 0.55$).

### Hemisphere Surface Area (*hem*SA)

The means, standard deviations, and ranges of left and right *hem*SA for the entire subject population are listed in Table 2. Raw values were computed as the sum of the 32 ROIs within each hemisphere. ANOVAs similar to those described above were carried out using raw *hem*SA and, separately, *hem*SA normalized by total cortical surface area as the dependent variables.

No main effect of hemisphere was found for raw or normalized *hem*SA (respectively, $p = 0.35$, $p = 0.39$), indicating that variation in *hem*SA did not differ between the left and right hemispheres. No interactions between hemisphere and genotype (raw, $p = 0.68$; normalized, $p = 0.73$), hemisphere and birth order (both $p = 0.73$), or hemisphere and sex ($p = 0.12$, $p = 0.14$) were found for these dependent variables. No interaction between genotype and hemisphere was observed when sex differences across unrelated pairs were taken into account ($p = 0.77$, $p = 0.79$).

Data for each hemisphere were then analyzed separately. An effect of genotype was found for raw left *hem*SA ($p = 0.002$) and raw right *hem*SA ($p = 0.01$) but not for normalized left *hem*SA ($p = 0.73$) or normalized right *hem*SA ($p = 0.73$). An effect of birth order approached significance for raw left *hem*SA ($p = 0.06$), but not for raw right *hem*SA ($p = 0.22$), normalized left *hem*SA ($p = 0.73$), or normalized right *hem*SA ($p = 0.73$). No significant effects of sex were found for raw left *hem*SA ($p = 0.87$), raw right *hem*SA ($p = 0.75$), normalized left *hem*SA ($p = 0.14$), and normalized right *hem*SA ($p = 0.14$). A similar pattern of genotype effects was observed before and after the contribution of sex differences was removed (raw left *hem*SA, $p = 0.002$; raw right *hem*SA, $p = 0.01$; normalized left *hem*SA, $p = 0.79$; normalized right *hem*SA, $p = 0.79$).

The pattern of results for raw and normalized left and right *hem*SA suggests that variation across unrelated

**Table 2.** Hemisphere Surface Area and Total Cortical Surface Area ($cm^2$)[a]

| | *Range* | *M* | *SD* |
|---|---|---|---|
| L *hem*SA | 818–1136 | 948 | 86 |
| R *hem*SA | 826–1128 | 958 | 94 |
| *tot*SA | 1685–2264 | 1906 | 175 |

[a] L, left; R, right; other abbreviations as in Table 1 and the text.

pairs could largely be attributed to variation in total cortical surface area, not *hem*SA per se. The pattern of results for left *r*SA and left *hem*SA suggests that the highly significant interaction between genotype and ROI for left *r*SA can be attributed to differences in regional size and shape, not to differences in overall left *hem*SA.

### Total Cortical Surface Area (*tot*SA)

The population means, standard deviations, and ranges of *tot*SA are listed in Table 2. Raw values were computed as the sum of *r*SA for all 64 ROIs. ANOVAs similar to those described above were carried out using raw *tot*SA and, separately, *tot*SA normalized by body weight as the dependent variables.

There were highly significant effects of genotype for both raw *tot*SA and normalized *tot*SA (respectively, $p$ = 0.002, $p$ = 0.001). No significant effects of birth order were found for either measure, though raw *tot*SA did approach significance ($p$ = 0.08; normalized, $p$ = 0.39). No effects of sex were found (raw, $p$ = 0.93; normalized, $p$ = 0.41). The highly significant effects of genotype remained after the contribution of sex differences was removed ($p$ = 0.001 for both raw and normalized *tot*SA).

### Body Weight

There was a highly significant effect of genotype ($p$ = 0.001) but not birth order ($p$ = 0.72) or sex ($p$ = 0.69). The genotype effect remained after sex differences were taken into account ($p$ = 0.001).

## DISCUSSION

To summarize the main findings: In the study population as a whole, there were hemisphere effects on variation in cortical surface area that suggested left-right differences in regional size and shape but not in overall hemisphere surface area. These hemisphere differences arose principally from variation across unrelated pairs of twins (genotype factor), not from variation within cotwins (birth order factor) or between females and males (sex factor). Separate analyses within each hemisphere revealed highly significant effects of genotype on variation in regional surface area; no significant effects of birth order or sex were found. When regional surface area was normalized by total cortical surface area, a highly significant effect of genotype was observed only for the left hemisphere. Weaker but still significant effects of genotype on variation in left lobar surface area (both raw and normalized) were also found, and there were no significant effects of birth order or sex on these measures. No significant effect on raw or normalized right lobar surface area was observed for any between-subjects factor. A highly significant effect of genotype, but not of birth order or sex, was also found on raw left hemisphere and raw right hemisphere surface area; however, when total cortical surface area was taken into account, no significant effect of genotype was observed on these measures. Finally, a highly significant effect of genotype was again found on variation in total cortical surface area, and this effect remained highly significant when total cortical surface area was normalized by body weight. Consistent with the results of previous twin studies (e.g. Lauterbach 1925), a highly significant effect of genotype on body weight was also observed. No genotype effects were attributable to sex differences across unrelated pairs.

These findings suggest that the effects of genotype on variation in left regional surface area resulted not from differences in left hemisphere surface area per se, but from differences in regional size and shape that take form during fissuration and gyral development. The same applies to the results for left lobar surface area. Since regional and lobar surface area were normalized by total cortical surface area, it is unlikely that the effects of genotype on these measures arose solely from variation in total cortical surface area. At the same time, there was an effect of genotype on total cortical surface area. Thus genotypic differences contributed to variation in both the total area of the cortical surface and how it was folded.

Animal data demonstrate that developmental events occurring early in gestation critically influence cortical morphogenesis (Rakic, 1988). In humans, neurogenesis appears to be complete by mid-gestation (Rakic, 1978). The strict timetable of cortical fissuration between the eighth and forty-fourth week of gestation and the maturity of the fissuration pattern achieved by birth (Chi et al., 1977) suggest a strong genetic influence on cortical morphometry. Welker (1990) has enumerated nine sets of factors that influence the formation of gyri in mammalian cortex in general: neuronal differentiation and dendrogenesis, neuronal orientation, afferent arrival, penetration, fasciculation, and arborization, synaptogenesis, glial proliferation and myelination, laminar aggregation and segregation, plasticity, rearrangement of cell adhesion molecules and related membrane structures, and the timing of the foregoing events. Based on fissuration patterns associated with congenital malformations in humans (lissencephaly and polymicrogyria) and on predictions generated by a mechanical model of cortical folding, Richman, Stewart, Hutchinson, and Caviness (1975) proposed that the principal driving force for fissuration is the differential growth of the cortical laminae, such that folding may occur anywhere on the surface when growth of the supragranular layers exceeds that of the infragranular layers. Animal data indicate that the laminar fate of migrating neurons is determined just before the end of cortical neurogenesis (McConnell & Kaznowski, 1991), and that the formation of radial connections may be rigidly specified by activity-independent

mechanisms (for review see Goodman & Shatz, 1993). While postmitotic neurons may migrate distances that cross gross morphological boundaries (Walsh & Cepko, 1992), many do not, and the mechanisms governing postmitotic migration act at a very early developmental stage. With respect to the present results, the contrast between small within-twin variation and highly significant across-pair variation likely reflects co-twin similarities in most or all of these early developmental events.

Compared to genotype effects, birth order effects were weak. They almost reached the $p = 0.05$ level of significance for only three measures, all of them raw (right regional, left hemisphere, and total surface area). This result argues against the presence of differences in prenatal environment that might have otherwise obscured similarities within co-twins. However, we cannot directly assess the extent to which mechanical, nutritional, and other prenatal influences on cortical morphogenesis and fetal development in general (Richman, Stuart, Hutchinson, & Caviness 1975; Hofman, 1989; Young, Suidan, Antoine, Silverman, Lustig, & Wasserman 1985; Pridjian, Nugent, & Barr 1991) contributed to across-pair variation. In addition, all co-twin pairs shared similar postnatal environments. Although cortical neurogenesis is complete around mid-gestation and fissuration is near-complete by birth, total cortical surface area increases two-to-threefold after birth, and the rate and magnitude of growth vary with respect to lobes and gyri (for review see Blinkov and Glezer 1968). Still, postnatal increases in total cortical surface area are small compared to the 30-fold increase that occurs from the fourth month of gestation to birth. Moreover, comparisons between large populations of monozygotic twins reared together and those reared apart, as well as studies of twin similarities using dynamic-physiological measures (e.g. response to overfeeding, C. Bouchard et al. 1990), indicate that genetic factors account for most of the reported concordance in anthropometric measures (e.g., body mass index, Stunkard, Harris, Pederson, & McClearn (1990), neurophysiological measures (e.g., amount of 8–12 Hz activity in the resting electroencephalogram; Stassen, Lykken, Propping, & Bomben 1988), and psychological measures (e.g., IQ, personality inventories; T. Bouchard et al., 1990; Plomin & Bergeman 1991).

In light of these previous data, the neurodevelopmental studies cited above, and the pattern of results observed in the present study, we favor the interpretation that twin similarities in cortical surface area arise principally from genetic similarities. Given that neural subsystems mediating speech and language functions are largely lateralized within the left cerebral cortex in the vast majority of humans, and in view of the fact that language, at least one predicated on rules of syntax, is a uniquely human behavior, the present evidence of stronger genetic influences on left than right cortical morphogenesis is provocative with respect to neurobiological bases of human evolution.

## MATERIALS AND METHODS

### Subjects

Ten pairs of monozygotic twins were recruited through newspaper advertisements. Their age range was 18 to 43 years, with a median age of 30 years. Monozygosity was determined by analyzing 9 red blood cell surface markers and by a standardized questionnaire (Cederlof, Friberg, Johnson, & Kaij, 1961; Lee & Lebeck, 1984). Body weight was measured at the time of phlebotomy or at the MRI suite. There were 5 female pairs and 5 male pairs. The age range for females was 18–40 years and the median age was 29 years; for males, the age range was 24–43 years and the median age was 33.5 years. All subjects wrote and ate with their right hands. For females, the Edinburgh Laterality Quotient (LQ, Oldfield, 1971) ranged from 74 to 100 with a median of 87; for males, LQ ranged from 74 to 100 with a median of 89 for 9 subjects, and although the LQ was 58 for the 10th male, he strongly preferred writing with the right hand and did not prefer the use of the left hand, eye, or foot for any item. All co-twins were reared together and lived in the same locale. All had at least a secondary school education.

Subjects gave written consent for blood drawing and brain imaging after they discussed the study protocol with one of the authors during a preliminary meeting. They were paid for participation.

### Brain Image Acquisition and Analysis

MRIs were acquired using a Siemens 1.0 Magnetom system (in-plane resolution = 1.2 mm) or a General Electric 1.5 Signa system (in-plane resolution = 0.9 mm). Each subject was positioned in the scanner so that a horizontal laser marked the intercanthal line and a vertical laser intersected the midpoint of the nasion and philtrum. A mid-sagittal scout was inspected for rotational tilt and adjustments in head position were made as needed. T1-weighted sagittal sections (thickness = 5.0–8.0 mm, gap = 1.0–2.0 mm, TE/TR = 20 msec/700 msec) were then obtained, followed by T1-weighted coronal sections [thickness = 3.0 mm, no (effective) gap]. In 16 of 20 subjects, coronal sections were obtained via 3D FLASH [TE/TR = 20 msec/400 msec (Siemens) or 9/50 msec (GE)]. Before this technique became available, 4 subjects (2 female twin pairs) were imaged in serial section by interleaving two sets of 3.0 mm slices that were offset by 3.0 mm gaps. There were no obvious differences in the quality of the latter images and statistical analyses on a subset of the data taken from the 16 subjects imaged via 3D FLASH showed the same pattern

of results as those for all 20 subjects. Thus combined data are reported.

The images were printed onto X-ray film and stored on magnetic tape for transfer to a Silicon Graphics computer. Deep structures, major fissures, sulci, gyri, and other surface structures (Table 1) were identified on the coronal and sagittal images using the atlases of Matsui and Hirano (1978), Krieg (1963), and Talairach and Tournoux (1988). Detailed descriptions of surface landmarks circumscribing gross morphological subdivisions of human cerebral cortex have been provided by Ono, Kubik, and Abernathy (1990) and Rademacher, Galaburda, Kennedy, Filipek, and Caviness (1993). In practice, most of the cortical surface could be readily labeled using the aforementioned atlases on the basis of (1) the location of a coronal section along the anteroposterior axis (pole-to-pole distance on the mid-sagittal image proportional to that of each atlas brain), and (2) in that coronal section, the hemispheric quadrant in which the ROI appeared. When these indices provided no clear boundary between two possible surface structures, the relative locations of identifiable gyri and sulci, the depths and course of adjacent sulci within and across serial sections, and the gyral branching pattern within and across serial sections were used as guides. The poles were assigned as follows: frontal pole—all surfaces anterior to the coronal section in which both the superior and inferior frontal sulci were first visible; temporal pole—all surfaces anterior to the coronal section in which both the superior and inferior temporal sulci were first visible; occipital pole—all surfaces posterior to the coronal section in which the inferior occipital sulcus was last visible.

The surface area of the each lobe was computed by summing ROIs that lay within its boundaries. ROIs that span more than one lobe include the fusiform (occipitotemporal) gyrus, cingulate gyrus, and insula. The fusiform gyrus was grouped with the temporal lobe since it appeared in more temporal than occipital sections. The cingulate gyrus and insula were not included in the calculation of frontal or parietal lobe surface area as they are neither isocortical structures nor contained solely within one or the other lobe. Although part of the basal forebrain lies on the ventromedial surface of the hemisphere, it is not customary to designate the basal forebrain as cortex because it lacks characteristic cytoarchitectural features (for review see Mesulam, 1985). This small portion of the frontal lobe surface was not included in the calculation of frontal lobe surface area. Only surface area data from the lobes so defined (Table 1) were included in the ANOVAs.

To reconstruct the cortical surface via computer, each coronal MRI section was displayed on the workstation monitor and the pial surface (intra- and extrasulcal) was traced by hand with a cursor (Fig. 1). Tracings for each member of a co-twin pair were never performed by the same individual. Reference points demarcating ROIs were placed along the contours. A 3D model of the surface was generated from the contours using a triangulation algorithm (Fig. 2, Loftus, 1992; Loftus, Tramo, Thomas, Green, Nordgren & Gazzaniga, 1993). A triangle mesh was interpolated between each pair of adjacent contours, and regional surface area was computed as the sum of the triangles falling within ROI boundaries. The area for each triangle was computed by halving the cross product of two of its sides (Fraleigh & Beauregard, 1987). The resolution of the triangle sides was limited by slice thickness (3.0 mm). To preserve isotropy, the contour points were resampled to approach the resolution of slice thickness. Surface area was computed as long as one of the triangle vertices corresponded to an ROI; when an ROI appeared on one section but not the next, the sum of all triangles within the two slices was halved to estimate surface area across the two sections. To minimize error introduced by interpolation across adjacent sections, the triangulation algorithm incorporated a dynamic programming technique (Cormen, Leiserson, & Rivest, 1977) that globally minimized regional surface area (Fuchs, Kedem, & Uselton, 1977). Hence, a lower bound estimate of regional surface area was obtained without systematic foreshortening artifacts.

The present 3D method of measuring cortical surface area employs the same surface contour tracing procedure as the quasi-3D method of flat-mapping cortex that was previously developed in our laboratory (Jouandet et al., 1989). The coefficient of variation for contour length among those observers was 7.1%. For hemisphere and lobar surface area measurements, pair-wise correlations among four observers ranged from 95.9 to 99.0%; coefficients of variation across different observers and within a given observer were, respectively, 5.4 and 2.7% for hemisphere surface area, 3.5 and 2.1% for frontal lobe surface area, 2.7 and 4.4% for temporal lobe surface area, 6.4 and 3.7% for parietal lobe surface area, and 15.0 and 8.3% for occipital lobe surface area (Jouandet et al., 1990). In the present 3D method, surface area is computed automatically from the 3D reconstruction instead of manually via digitized planimetry of the flat map. Consequently, the observer-related variability associated with the quasi-3D method may be considered to represent the upper limits of that associated with the present 3D method.

### Methodological Considerations and Comparisons with Previous Studies

Problems inherent to surface area measurements of curved objects have been of longstanding concern to quantitative neuroanatomists working with postmortem tissue (for reviews see Blinkov & Glezer, 1968; Van Essen & Maunsell, 1980). Corrections for postmortem shrinkage, shrinkage during embedding, deformation during cutting, and oblique sectioning (estimated to be as high as 50% or so combined) have been proposed by a num-

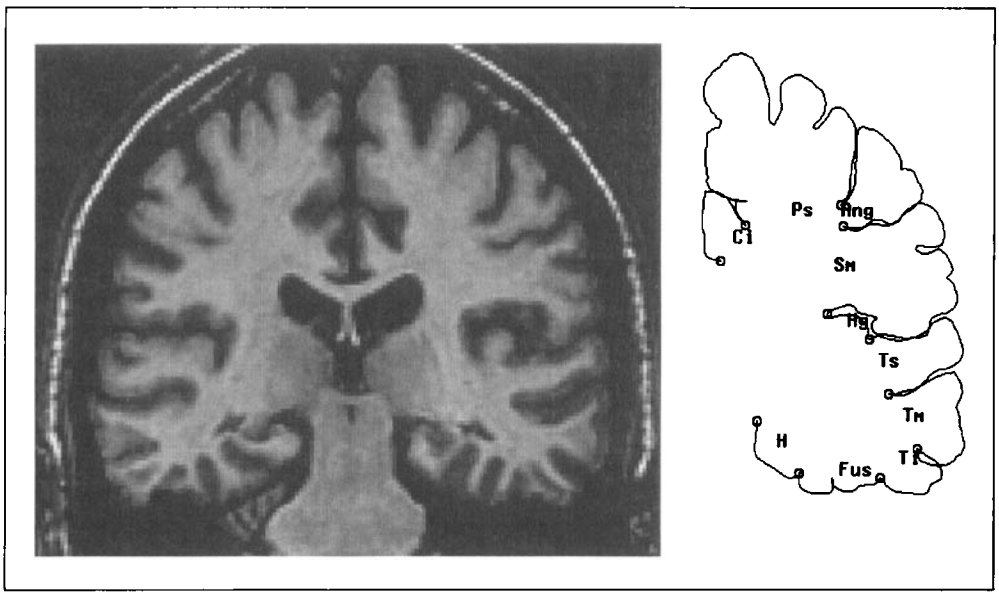


**Figure 1.** *Left.* Coronal MRI section through the parietal and temporal lobes. *Right.* Surface contour and reference points demarcating regions of interest. *Ci*, cingulate gyrus; *Ps*, superior parietal lobule; *Ang*, angular gyrus; *Sm*, supramarginal gyrus; *Ts*, superior temporal gyrus; *Tm*, middle temporal gyrus, *Ti*, inferior temporal gyrus; *Fus*, fusiform gyrus; *H*, parahippocampal gyrus/hippocampus.

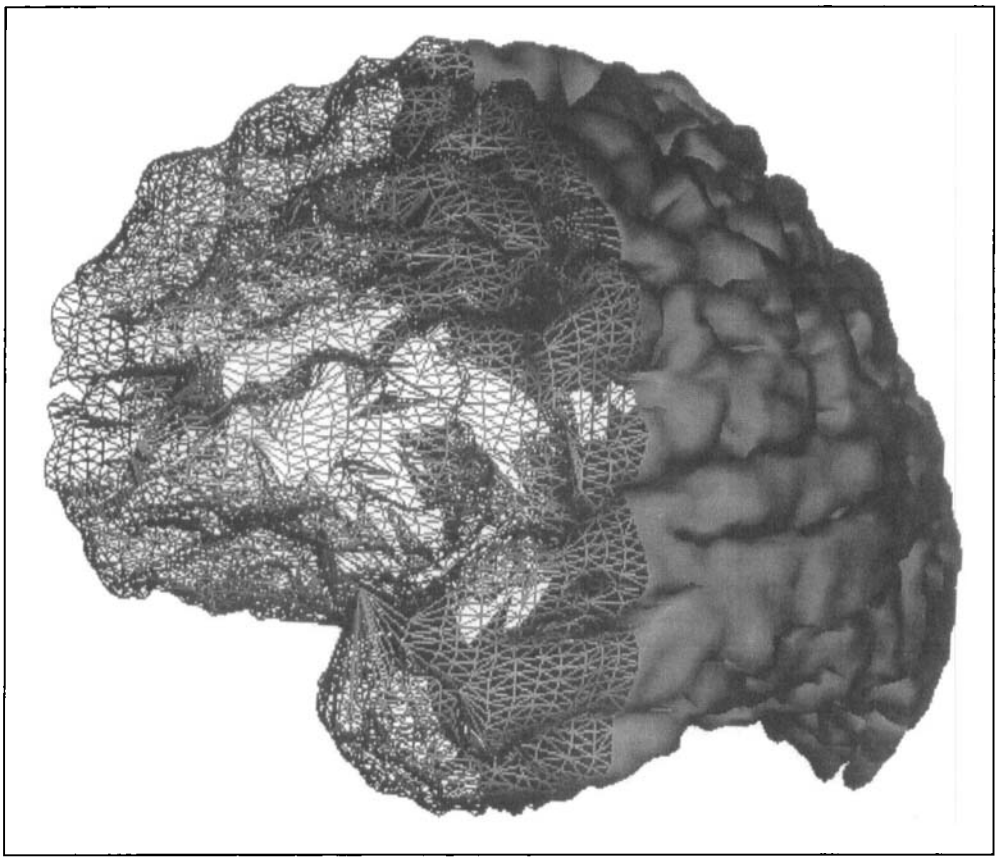

**Figure 2.** Three-dimensional computer model of the cortical surface showing unshaded (anterior) and shaded (posterior) triangle meshes.

ber of investigators since the early part of this century. For example, the postmortem studies of cortical surface area by Blinkov and Glezer incorporate a constant trigonometric correction factor that is intended to compensate for underestimations caused by the obliquity of the slice plane with respect to the cortical surface. Since the same correction factor is applied to all regions, regional differences in curvature are not taken into account. The present 3D method obviates such post hoc corrections because it explicitly reconstructs the surface between serial sections and thus compensates for local deviations in curvature and tilt. In theory, *in vivo* cortical surface area estimates based on 3D computer models of MRIs could offer greater accuracy than those based on postmortem tissue, but a standard by which to judge accuracy is lacking (e.g., a convoluted object of known

surface area). At present, the correspondence of our data with those from several different sources suggests that *in vivo* cortical surface area measurements can be at least as accurate as those obtained by postmortem analyses. For example, the present range of total cortical surface area measurements (1685–2264 $cm^2$, Table 2) lies within the range of previous postmortem studies [1468.7 $cm^2$ (Blinkov & Glezer, 1968) to 3031 $cm^2$ (Elias & Schwartz, 1971)], the methods of which vary among different authors (see also Hofman, 1985; Haug, 1987). The range and observed symmetry for hemisphere surface area in our right-handed population (Table 2) are also consistent with previous postmortem findings in subjects of uncertain handedness (Blinkov & Glezer, 1968; Elias & Schwartz, 1971). The present measurements of regional and lobar cortical surface area also correspond reasonably well with available postmortem data (Blinkov & Glezer 1968). The wide variation in *r*SA observed across the 32 ROIs within each hemisphere in our population as a whole is consistent with the wide across-section variation in gyrification index reported by Zilles, Armstrong, Schleicher, and Kretschmann (1988) in their postmortem specimens.

Because the goals of our study required sensitivity to interindividual differences in surface anatomy, ROIs could not be labeled by warping each brain to fit a stereotaxic template derived from a single, standard atlas brain, as is commonly done to group data from a number of individuals, especially when detailed anatomical information is not directly available (e.g., Fox, Perlmutter, & Raichle, 1985; for further consideration of this issue, see Rademacher, Caviness, Steinmetz, & Galaburda, 1993a). Despite the availability of high-resolution MRI sections in two planes, ROI labeling was sometimes difficult because of ambiguities in the precise location of boundaries between adjacent gyri. Even when the benefits of transforming a brain to fit a standard template are preferred over the risks of obscuring interindividual differences, gyral boundaries only sometimes overlap those of the template precisely. Given the different results presently observed for different dependent variables, different between-subject factors, and different within-subject factors, it is unlikely that the observed genotype effects for left *r*SA can be attributed to errors in boundary determination. It is even more unlikely that such errors could account for the lobe results, since only a small portion of each lobe's surface lies at a boundary. Of course, these concerns do not apply to the results for total cortical surface area, which showed a pattern of results similar to that for left regional and left lobar surface area (i.e., significant variation in normalized measures across unrelated pairs but not within co-twins). We would expect methodological errors arising from limitations in section thickness, in-plane spatial resolution, and boundary determination to decrease sensitivity. Consequently, there would be a low probability of falsely rejecting the null hypothesis. It follows that it would be unlikely that highly significant interations, such as that observed between genotype and ROI in the left hemisphere, could arise from these errors. On the other hand, any decrease in sensitivity would increase the probability of falsely accepting the null hypothesis. Thus we cannot exclude the possibility that refinements of our method would enhance the detection of effects not observed in the present study. For example, genotype effects for the right hemisphere, albeit weaker than those for the left, might reach statistical significance, or effects of birth order might emerge. Since the present study started, the technology to rapidly acquire thinner MRI sections has become more widely available and we have begun to develop new approaches to the problem of *in vivo* boundary determination. For the present, the sensitivity of our method was sufficient to detect genotype effects and hemisphere differences.

With minor modifications, the present *in vivo* method can be applied to postmortem studies of animal and human cortex. For example, the surface contour tracing procedure generalizes to MRIs of whole brain specimens, photographs of gross brain sections, or magnified images of histological sections. Quasi-3D cortical surface area measurements from histological sections of macaque brain have been carried out by Jouandet et al. (1989) using related methods of availing image data to computer analysis.

## Acknowledgments

This work was funded by awards from the National Institute of Neurological Disorders and Stroke, the National Institute on Deafness and Other Communication Disorders, the Office of Naval Research, and the National Association for Research in Schizophrenia and Depression. The authors gratefully acknowledge Dr. John Weaver for assistance in MR image acquisition, Drs. Therese Stukel and Daniel Freeman for developing statistical approaches to the data analysis, and Drs. A. Galaburda, D. Hubel, M. Livingstone, U. Drager, P. Cariani, and S. Macknik for helpful comments and criticisms. We thank P. Brown, R. Nordgren, R. Ferranti, Dr. L. Cromwell, L. Bohi, B. Baker, and Dr. B. Greenspan for their contributions.

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## REFERENCES

Allman, J. (1990). Evolution of neocortex. In E. G. Jones & A. Peters (Eds.), *Cerebral cortex, Volume 8A. Comparative structure and evolution of cerebral cortex, Part I* (pp. 269–283). New York: Plenum Press.

Bartley, A. J., Jones, D. W., Torrey, E. F., Zigun, J. R., & Weinberger, D. R. (1993). Sylvian fissure asymmetries in monozygotic twins: A test of laterality in schizophrenia. *Biological Psychiatry, 34*, 853–863.

Blinkov, S. M., & Glezer, II. (1968). *The human brain in figures and tables. A quantitative handbook.* New York: Basic Books.

Bouchard, C., Tremblay, A., Després, J.-P., Nadeau, A., Lupien, P. J., Thériault, G., Dussault, J., Moorjani, S., Pinault, S., & Fournier, G. (199). The response to long-term overfeeding in identical twins. *New England Journal of Medicine, 322,* 1477-1482.

Bouchard, T. J., Lykken, D. T., McGue, M., Segal, N., & Tellegen, A. (1990). Sources of human psychological differences: The Minnesota study of twins reared apart. *Science, 252,* 223-228.

Cederlof, R., Friberg, L., Johnson, E., & Kaij, L. (1961). Studies on similarity diagnosis in twins with aid of mailed questionnaires. *Acta Geneticae Medicae et Gemellologiae, 11,* 338-362.

Chi, J. G., Dooling, E. C., & Gilles, F. H. (1977). Gyral development of the human brain. Annals of Neurology, 1, 86-93.

Cole, J. W. L., & Grizzle, J. E. (1966). Applications of multivariate analysis of variance to repeated measurements experiments. *Biometrics, 22,* 810-828.

Cormen, T. H., Leiserson, C. E., & Rivest, R. L. (1990). *Introduction to algorithms.* Cambridge, MA: MIT Press.

Crowder, M. J., & Hand, D. J. (1990). *Analysis of repeated measures.* New York: Chapman & Hall.

Elias, H., & Schwartz, D. (1971). Cerebro-cortical surface areas, volumes, lengths of gyri and their interdependence in mammals, including man. *Zeitschrift fur Saugetierkunde, 36,* 147-163.

Fox, P. T., Perlmutter, J. S., & Raichle, M. E. (1985). A stereotactic method of anatomical localization for positron emission tomography. *Journal of Computer Assisted Tomography, 9,* 141-153.

Fraleigh, J. B., & Beauregard, R. A. (1987). *Linear algebra.* Reading, MA: Addison-Wellesley.

Fuchs, H., Kedem, K. D., & Uselton, S. P. (1977). Optimal surface reconstruction from planar contours. *Graphics Image Processing 20,* 693-702.

Goodman, C. S., & Shatz, C. J. (1993). Developmental mechanisms that generate precise patterns of neuronal connectivity. *Cell 72/Neuron 10 (Suppl),* 77-98.

Haug, H. (1987). Brain sizes, surfaces, and neuronal sizes of the cortex cerebri: A stereological investigation of man and his variability and a comparison with some mammals (primates, whales, marsupials, insectivores, and one elephant). *American Journal of Anatomy, 180,* 126-142.

Hofman, M. A. (1985). Size and shape of the cerebral cortex in mammals. I. The cortical surface. *Brain, Behavior and Evolution, 27,* 28-40.

Hofman, M. A. (1989). On the evolution and geometry of the brain in mammals. *Progress in Neurobiology, 32,* 137-158.

Hrubec, Z., & Robinette, C. D. (1984). The study of human twins in medical research. *New England Journal of Medicine, 310,* 435-441.

Jouandet, M. L., Tramo, M. J., Herron, D. M., Hermann, A., Loftus, W. C., Bazell, J., & Gazzaniga, M. S. (1989). Brainprints: Computer-generated two-dimensional maps of the human cerebral cortex in vivo. *Journal of Cognitive Neuroscience, 1,* 88-117.

Jouandet, M. L., Tramo, M. J., Thomas, C. E., Newton, C. H., Loftus, W. C., Weaver, J. B., & Gazzaniga, M. S. (1990). Brainprints: Inter- and intra-observer reliability. *Society of Neuroscience Abstracts, 16,* 1151.

Kaas, J. H. (1987). The organization and evolution of neocortex. In S. P. Wise (Ed.), *Higher brain functions: Recent explorations of the brain's emergent properties* (pp. 347-378). New York: Wiley.

Killackey, H. P. (1990). Neocortical expansion: An attempt toward relating phylogeny and ontogeny. *Journal of Cognitive Neuroscience, 2,* 1-17.

Krieg, W. J. S. (1963). *Connections of the cerebral cortex.* Evanston, IL: Brain Books.

Lauterbach, C. E. (1925). Studies in twin resemblance. *Genetics, 10,* 525-568.

Lee, C. L., & Lebeck, L. K. (1984). Estimating dizygotic/monozygotic ratio of twins by general formula. *American Journal of Clinical Pathology, 81,* 654-659.

Loftus, W. C. (1992). *Three dimensional minimal surface area reconstructions from planar contours using dynamic programming.* Office of Naval Research Technical Report No. 100.2.

Loftus, W. C., Tramo, M. J., Thomas, C. E., Green, R. L., Nordgren, R. A., & Gazzaniga, M. S. (1993). Three dimensional quantitative analysis of hemispheric asymmetry in the human superior temporal region. *Cerebral Cortex, 3,* 348-355.

Matsui, T., & Hirano, A. (1978). *An atlas of the human brain for computerized tomography.* New York: Igaku-Shoin Medical Pub.

Mesulam, M.-M. (1985). Patterns in behavioral neuroanatomy: Association areas, the limbic system, and hemispheric specialization. In M.-M. Mesulam (Ed.), *Principles of behavioral neurology* (pp. 1-70). Philadelphia: FA Davis.

McConnell, S. K., & Kaznowski, C. E. (1991). Cell cycle dependence of laminar determination in developing neocortex. *Science, 254,* 282-285.

Neter, J. & Wasserman, W. (1974). *Applied linear statistical models.* Homewood, IL: R. Irwin.

Oldfield, R. C. (1971). The assessment and analysis of handedness: The Edinburgh inventory. *Neuropsychologia,* 9, 97-113.

Ono, M., Kubik, S., & Abernathey, C. D. (1990). *Atlas of the cerebral sulci.* New York: Thieme Medical Pub.

Oppenheim, J. S., Skerry, J. E., Tramo, M. J., & Gazzaniga, M. S. (1989). Magnetic resonance imaging morphology of the corpus callosum in monozygotic twins. *Annals of Neurology, 26,* 100-104.

Plomin, R., & Bergemen, C. S. (1991). The nature of nurture: Genetic influence on "environmental" measures. *Behavioral and Brain Sciences, 14,* 373-427.

Pridjian, G., Nugent, C. E., & Barr, M. (1991). Twin gestation: Influence of placentation on fetal growth. *American Journal of Obstetrics and Gynecology, 165,* 1394-1401.

Rademacher, J., Caviness, V. S., Steinmetz, H., & Galaburda, A. M. (1993). Topographical variation of the human primary cortices: Implications for neuroimaging, brain mapping, and neurobiology. *Cerebral Cortex 3,* 313-329.

Rademacher, J., Galaburda, A. M., Kennedy, D. N., Filipek, P. A., & Caviness, V. S. (1993). Human cerebral cortex: Localization, parcellation, and morphometry with magnetic resonance imaging. Journal of Cognitive Neuroscience, 4, 352-374.

Rakic, P. (1978). Neuronal migration and contact guidance in the primate telencephalon. *Postgraduate Medical Journal, 54,* 25-40.

Rakic, P. (1988). Specification of cerebral cortical areas. *Science, 241,* 170-176.

Richman, D. P., Stewart, R. M., Hutchinson, J. W., & Caviness, V. S. (1975). Mechanical model of brain convolutional development. Pathologic and experimental data suggest a model based on differential growth within the cerebral cortex. *Science, 189,* 18-21.

Springer, S. P., & Searleman, A. (1980). Left-handedness in twins: Implications for the mechanisms underlying cerebral asymmetry of function. In J. Herron (Ed.), *Neuropsychology of lefthandedness* (pp. 139-158). New York: Academic Press.

Stassen, H. H., Lykken, D. T., Propping, P., & Bomben, G.

(1988). Genetic determination of the human EEG: Survey of recent results on twins reared together and reared apart. *Human Genetics, 80,* 165-176.

Stunkard, A. J., Harris, J. R., Pederson, N. L., & McClearn, G. E. (1990). The body-mass index of twins who have been reared apart. *New England Journal of Medicine, 322,* 1483-1487.

Suddath, R. L., Christison, G. W., Torrey, E. F., Casanova, M. F., & Weinberger, D. R. (1990). Anatomical abnormalities in the brains of monozygositic twins discordant for schizophrenia. *New England Journal of Medicine, 322,* 789-794.

Talairach, J., & Tournoux, P. (1988). *Co-planar stereotaxic atlas of the human brain.* New York: Thieme Medical Pub.

Van Essen, D. C., & Manusell, J. M. (1980). Two-dimensional maps of the cerebral cortex. *Journal of Comparative Neurology, 191,* 255-281.

Walsh, C., & Cepko, C. L. (1992). Widespread dispersion of neuronal clones across functional regions of the cerebral cortex. *Science, 255,* 434-440.

Walker, W. (1990). Why does the cerebral cortex fissure and fold? A review of determinants of gyri and sulci. In A. Peters & E. G. Jones (Eds.), *Cerebral cortex, Volume 8B. Comparative structure and evolution of cerebral cortex, Part II* (pp. 3-136). New York: Plenum Press.

Young, B. K., Suidan, J., Antoine, C., Silverman, F., Lustig, I., & Wasserman, J. (1985). Differences in twins: The importance of birth order. *American Journal of Obstetrics and Gynecology, 151,* 915-921.

Ziles, K., Armstrong, E., Schleicher, A., & Kretschmann, H.-J. (1988). The human pattern of gyrification in the cerebral cortex. *Anatomy and Embryology, 179,* 173-179.

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