ORIGINAL ARTICLE

Hippocampal Volumes in Schizophrenic Twins

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Context: The effects of genes and environment on brain abnormalities in schizophrenia remain unclear.

Objective: To examine the contributions of genes and environment to hippocampal volume reduction in schizophrenia.

Design: Population-based twin cohort study.

Setting: Finland.

Participants: Seven monozygotic (MZ) twin pairs concordant for schizophrenia and 16 MZ and 32 dizygotic (DZ) twin pairs discordant for schizophrenia, ascertained so as to be representative of all such probands in a Finnish birth cohort, along with 28 MZ and 26 DZ healthy comparison twin pairs without a family history of psychosis.

Main Outcome Measures: Hippocampal volume measurements taken from high-resolution magnetic resonance images.

Results: Hippocampal volumes of probands were smaller than those of their nonschizophrenic MZ and DZ co-twins and healthy twins. Hippocampal volumes of probands' non-ill co-twins were smaller than those of healthy twins, but those of non-ill MZ and DZ co-twins of schizophrenic patients were similar. The intraclass correlations for hippocampal volumes among healthy and discordant MZ pairs were larger than those among the respective DZ pairs. The intraclass correlation for healthy MZ pairs was larger than that for discordant MZ pairs, and the variance component estimate for additive genetic effects was lower in discordant twins than in healthy twins.

Conclusions: Although hippocampal volume in healthy individuals is largely affected by genetic factors, it is subject to substantially greater modulation by environmental factors in schizophrenic patients and their relatives. The results are discussed in view of assumptions underlying classic twin methods.

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HIPPOCAMPAL VOLUME REduction is a robust correlate of schizophrenia,[1] but the etiology of the deficit remains unclear.[2] Several studies[3-11] of children and siblings of schizophrenic patients have suggested that genetic predisposition and environmental risk factors may contribute to hippocampal volume reduction in schizophrenia. Twin studies can be used to separate genetic from shared and unique environmental effects. Genetic effects would be suggested by the observation of smaller hippocampal volumes in the healthy monozygotic (MZ) compared with dizygotic (DZ) co-twins from pairs discordant for schizophrenia who share on average 100% and 50% of their genes with affected individuals, respectively, and by higher intraclass correlations (ICCs) for MZ than for DZ pairs discordant for schizophrenia. Shared environmental effects would be suggested by equivalent hippocampal volumes in the healthy MZ compared with DZ co-twins of schizophrenic patients and by equivalent ICCs for hippocampal volume in MZ and DZ pairs discordant for schizophrenia. Unique environmental (or possibly epigenetic) effects would be implicated if probands were observed to have smaller hippocampal volumes than their healthy MZ co-twins and if the ICCs were lower in discordant twin pairs than in healthy twin pairs. If sample sizes are sufficient, variance components indicating the proportion of the variance in hippocampal volumes explained by additive genetic factors and by shared and unique environmental factors can be calculated.

To date, 7 articles and 1 abstract from 4 independent twin samples examining hippocampal volumes in schizophrenia

have been published.[12-19] Their results are equivocal on the question of etiology, with some studies[12,14,15] implicating a major role for environmental factors and other studies[16,17] suggesting that genetic, shared environmental, or both factors are involved. Furthermore, Weinberger and colleagues[18] reported no difference in the hippocampal volumes of probands from concordant and discordant pairs, suggesting that a similar etiology may underlie hippocampal volume reductions in both proband types.

Neuropsychological studies[20-22] have shown reduced performance in probands compared with their healthy MZ co-twins on tests thought to be sensitive to temporal lobe functioning, suggesting that medial temporal lobe structures are at least in part affected by environmental factors. One such study[23] has even shown strong associations between difference (proband–co-twin) left hippocampal volumes and difference verbal memory test scores of MZ probands discordant for schizophrenia. Cannon and colleagues[22] showed reduced ICCs for verbal episodic memory in discordant compared with healthy twin pairs. In that study, the ICCs for discordant MZ pairs were larger than those for DZ pairs, suggesting that genetic (or shared environmental) factors may also play a role.

We have now completed magnetic resonance imaging and quantitation on our full series of 16 MZ and 32 DZ twin pairs discordant for schizophrenia, 7 MZ pairs concordant for schizophrenia, and matched groups of healthy twins (28 MZ and 26 DZ pairs). Based on the findings reviewed previously herein, we hypothesize that (1) the mean hippocampal volumes of probands from pairs concordant and discordant for schizophrenia will be similar; (2) the hippocampal volumes of healthy MZ co-twins of schizophrenic patients will be smaller than those of DZ co-twins, whose volumes will be smaller than those of healthy twins, and the ICCs for hippocampal volume in MZ discordant pairs will be larger than those in DZ discordant pairs (implicating a genetic contribution); and (3) the mean hippocampal volumes of probands will be smaller than those of their MZ co-twins, the ICCs for hippocampal volume in twin pairs discordant for schizophrenia will be lower than those in healthy twin pairs and pairs concordant for schizophrenia, and the variance component for additive genes will be higher in healthy twins than in twin pairs discordant for schizophrenia (consistent with a larger unique environmental effect in schizophrenia).

METHODS

The study protocol was reviewed and approved by the institutional review boards of the University of California (Los Angeles), the University of Pennsylvania (Philadelphia), and the National Public Health Institute of Finland (Helsinki), and all participants signed institutional review board–approved informed consent forms.

SAMPLE ASCERTAINMENT

Participants were drawn from a twin cohort consisting of all same-sex twins born in Finland between 1940 and 1957 (N=9562 pairs) identified through the Finnish national twin registry. Questionnaire-based classification identified 2495 MZ twins, 5378 DZ twins, and 1689 twins of unknown zygosity.[24] This cohort was screened, for 1969 to 1991, for a history of hospitalization, medicine prescription, and work disability due to psychiatric indication in 3 national computerized databases: the Hospital Discharge Register, the Free Medicine Register, and the Pension Register.[25] These searches identified 348 index twin pairs with at least 1 co-twin with a diagnosis of schizophrenia or schizoaffective disorder and 9214 healthy pairs with no schizophrenia diagnosis in either co-twin according to any of the 3 sources. After exclusion because of death or emigration, a total of 260 twins consisting of 60 (27 MZ and 33 DZ) index pairs were chosen randomly from the available index pairs (n=229: 50 MZ, 121 DZ, and 58 unknown zygosity), along with 70 (34 MZ and 36 DZ) demographically balanced healthy pairs. Index pairs in which, on direct interview, either the proband had a diagnosis of schizoaffective disorder, affective type, or the co-twin had a psychotic disorder diagnosis were excluded (n=1 concordant MZ pair). Healthy pairs were excluded if there was a history of psychosis-related treatment or work disability in any of their first-degree relatives or if either co-twin was found, on direct interview, to meet diagnostic criteria for a psychotic disorder or schizotypal, paranoid, or schizoid personality disorder (n=15 pairs: 6 MZ and 9 DZ). The selected sample of 114 pairs (n=228) consisted of 8 pairs concordant for schizophrenia (7 MZ and 1 DZ), 51 pairs discordant for schizophrenia (19 MZ and 32 DZ), and 55 healthy pairs (28 MZ and 27 DZ). High-resolution magnetic resonance images were acquired on 252 of the 260 twins. Two images were excluded because of technical problems with the magnetic resonance imaging, and 1 was excluded because of a large frontal lobe lesion, leaving 249 images on which hippocampal volumes were measured. After exclusions, the sample (n=219) on which hippocampal volumes were gathered comprised 16 twins concordant for schizophrenia (7 MZ pairs and 1 DZ pair), 94 twins discordant for schizophrenia (16 MZ and 28 DZ pairs and 2 and 4 additional MZ co-twins and DZ probands, respectively), and 56 MZ (28 pairs) and 53 DZ (26 pairs) healthy comparison individuals.

DIAGNOSTIC EVALUATION

Each co-twin was interviewed using the *Structured Clinical Interview for* DSM-III-R *Disorders,* patient or nonpatient edition,[26] by an examiner who was blind to the zygosity and diagnostic status of their co-twin, and the twins were assigned diagnoses according to *DSM-IV*.[27] Co-twins and healthy individuals were also interviewed and rated on the cluster A items from the *Personality Disorder Examination*.[28] Diagnostic reliability was excellent (ie, mean±SD $\kappa = 0.94 \pm 0.02$)[29]; final diagnoses were made by consensus among 3 independent raters (T.D.C., M.H., and J.L.) after review of written case reports. Individuals with a psychotic condition were also rated using the *Scale for the Assessment of Positive Symptoms*[30] and the *Scale for the Assessment of Negative Symptoms*.[31] Of the 64 probands, 58 (26 MZ and 32 DZ) were diagnosed as having schizophrenia and 8 as having schizoaffective disorder (3 MZ and 5 DZ). Five MZ and 2 DZ co-twins of schizophrenic patients had a cluster A personality disorder (**Table 1**). Substance disorder was rated as present when participants were actively abusing alcohol, sedatives, cannabis, stimulants, opioids, cocaine, hallucinogens, or a multitude of other substances as scored by the *Structured Clinical Interview for* DSM-III-R *Disorders*.

ZYGOSITY

Zygosity was determined by DNA analysis using the following markers: DIS80 (20 alleles), DI7S30 (13 alleles), apoB (20 al-

Table 1. Demographic Characteristics of the Twin Sample

Characteristic	CC MZ Probands (n = 14)	DC MZ Probands (n = 16)	DC DZ Probands (n = 32)	MZ Co-twins (n = 18)	DZ Co-twins (n = 28)	Control Twins (n = 109)	F or χ^2*	*P* Value*
Age, mean (SD), y	42.9 (8.2)	48.5 (4.4)	48.1 (4.7)	48.5 (4.9)	48.1 (5.0)	49.2 (4.1)	4.3	<.001
Age at onset, mean (SD), y	21.1 (2.5)	26.0 (6.9)	24.0 (5.2)	NA	NA	NA	3.2	.05
Men, No. (%)	8 (57)	8 (50)	16 (50)	9 (50)	15 (54)	58 (53)	0.3	.99
Parental social class, mean (SD)†	7.0 (1.8)	5.6 (1.4)	6.9 (1.1)	5.6 (1.4)	6.9 (1.1)	6.4 (1.4)	2.0	.09
Right handed, No. (%)	13 (93)	15 (94)	29 (91)	15 (83)	25 (89)	103 (95)	3.3	.66
Cohabitation, mean (SD), y	19.7 (4.4)	20.5 (2.9)	18.8 (3.5)	20.6 (2.9)	19.1 (3.5)	20.5 (4.3)	1.3	.27
Substance disorder, No. (%)	3 (21)	3 (19)	8 (25)	3 (17)	3 (11)	5 (5)	13.5	.02
Other Axis I disorder, No. (%)	1 (7)	0	1 (3)	4 (22)	5 (18)	10 (9)	8.8	.12
Cluster A disorder, No. (%)‡	NA	NA	NA	5 (28)	2 (7)	0	36.8	.001
Paranoid personality disorder, No. (%)				3 (17)	2 (7)			
Schizoid personality disorder, No. (%)				3 (17)	1 (4)			
Schizotypal personality disorder, No. (%)				2 (11)	0			
Intracranial volume, mean (SD), mL	1248 (168)	1242 (137)	1227 (145)	1247 (154)	1205 (124)	1242 (110)	0.44	.82

Abbreviations: CC MZ, concordant monozygotic; DC DZ, discordant dizygotic; DC MZ, discordant monozygotic; NA, not applicable.
*F, χ^2, and *P* values are based on all groups except the DC DZ probands, who are not included in the analyses because of small sample size.
†Socioeconomic status on the Rauhala scale (9 indicates lowest and 1, highest).[32]
‡Some individuals had multiple personality disorders.

leles), COL2A1 (10 alleles), vWA (9 alleles), and HUMTH01 (6 alleles). Assuming an average heterozygosity rate of 70% per marker, we estimate that this procedure will falsely classify a DZ pair as MZ in approximately 1 of 482 cases.

IMAGING PROCEDURES

Acquisition

Magnetic resonance images were acquired on a 1.0-T scanner (Siemens Medical Systems, Iselin, NJ) in the Department of Radiology, University of Helsinki, using a standard magnetization-prepared rapid gradient echo sequence, with a repetition time of 10 milliseconds, an echo time of 4 milliseconds, a number of excitations equal to 1, and a flip angle of 12°. The images comprised 128 sagittal slices, with 1.2-mm slice thickness and no interslice gap. The matrix size was 256×256 pixels, corresponding to a field of view of 25 cm^2 and an in-plane resolution of 0.9766×0.9766 mm.

Segmentation and Reslicing

After deleting nonbrain voxels using a conservative automated procedure followed by manual removal of nonbrain tissue, the images were segmented into gray matter, white matter, and cerebrospinal fluid using an adaptive, 3-dimensional, Bayesian algorithm[33] previously validated for this purpose.[34] To control for differences in head tilt during acquisition, images were resliced parallel to the anterior commissure–posterior commissure plane and saved in sagittal view.

Anatomical Tracings

A method for outlining the hippocampal region of interest was developed by 2 of us (P.A.S. and T.G.M.vE.) and is described and depicted in detail in another publication.[10] A previous publication using the same method reported lower hippocampal volumes in patients compared with non-ill siblings and in non-ill siblings compared with healthy individuals, and the range of the volumes in that study[10] is very consistent with that in this study. Briefly, tracings were started on the most lateral slice on which the hippocampus was first visible, in the most lateral extent of the temporal horn of the lateral ventricle. The inferior and superior borders were determined by drawing a line through the white matter separating the hippocampus from the parahippocampal and fusiform gyri and the alveus separating the hippocampus from the lateral ventricles, respectively. More medially, the anterior hippocampus was separated from the amygdala by a thin line of white matter between the 2 structures. The last slice on which the hippocampus was clearly distinct from the amygdala formed the medial border. This roughly corresponds to the second slice medial to the slice on which the parahippocampal gyrus separates or 2 slices before the midbrain forms in the temporal horn of the lateral ventricle. The hippocampal volume measures include the cornu Ammonis, the gyrus dentatus, the prosubiculum, and the subiculum proper (see **Table 2** for raw hippocampal volumes). Only voxels in the region of interest that were classified as gray matter were counted. Tracings were performed blindly to diagnosis, birth history, and hemisphere. Interrater and intrarater reliabilities based on 10 cases were excellent (ICCs >0.95).

STATISTICAL ANALYSES

Before analysis, data were checked for normality[35] and homogeneity of variance.[36] Data were analyzed using the general linear mixed model with repeated measures (SAS version 6.12; SAS Institute Inc, Cary, NC), correcting for dependency (ie, correlation) among co-twins by treating twin pair as a random variable and adjusting the model error terms accordingly (Satterthwaite option). Hypotheses pertaining to the mean comparisons were tested by modeling risk group (probands from MZ concordant pairs, probands from MZ discordant pairs, probands from DZ discordant pairs, healthy MZ co-twins from discordant pairs, healthy DZ co-twins from discordant pairs, and healthy twin pairs) as a fixed-effect predictor while covarying for age at imaging, history of substance disorder, sex, total cortical gray matter volume,[37] and the interactions of group with a history of substance disorder and sex (model 1). To test for possible differences in laterality, hemisphere entered the model as a within-subject repeated-measures factor, and a group×hemisphere interaction entered the model to test for possible differences in laterality among the groups. Whenever one of these terms contributed statistically significantly to the prediction of hippocampal volume, contrast analyses were per-

Table 2. Hippocampal Raw Volumes by Twin Type*

	MZ Probands		DC MZ Co-twins (n = 18)	CC MZ Controls (n = 56)	DZ Probands		DC DZ Co-twins (n = 28)	CC DZ Controls (n = 53)
	CC (n = 14)	DC (n = 16)			CC (n = 2)	DC (n = 32)		
Left hippocampus	4.10 (0.14)	4.01 (0.15)	4.18 (0.14)	4.46 (0.08)	3.82 (0.03)	4.12 (0.08)	4.26 (0.09)	4.62 (0.07)
Right hippocampus	4.17 (0.14)	4.20 (0.14)	4.38 (0.13)	4.58 (0.08)	3.98 (0.26)	4.26 (0.09)	4.34 (0.08)	4.68 (0.06)
Total hippocampus	8.27 (0.27)	8.21 (0.25)	8.55 (0.26)	9.04 (0.15)	7.81 (0.23)	8.37 (0.16)	8.60 (0.17)	9.29 (0.13)
Cortical gray matter	512 (14)	521 (17)	522 (18)	536 (8)	494 (4)	513 (13)	512 (12)	519 (7)
Intracranial volume	1248 (47)	1242 (35)	1248 (37)	1258 (17)	1164 (24)	1227 (28)	1205 (24)	1228 (14)

Abbreviations: CC, concordant; DC, discordant; DZ, dizygotic; MZ, monozygotic.
*Data are given as mean (SE) milliliters.

formed comparing conditions within the term collapsing over nonsignificant terms in the model. This approach maintains the hypothesis-wise type I error rate at 0.05 because a predictor's contribution to particular dependent measures is evaluated only if its effect is found to vary at the multivariate level. The significance of each predictor was tested while accounting for all other model terms simultaneously, and where mean differences were hypothesized, 1-tailed tests were used. These analyses were also performed for cortical gray matter while covarying for intracranial volume as a point of comparison to the hippocampus.

Intraclass correlations and their confidence intervals for index (concordant MZ and discordant MZ and DZ) and healthy (MZ and DZ) pairs were calculated using the analysis of variance method in SAS (version 6.12) for hippocampal, intracranial, cortical gray matter, and hippocampal corrected for cortical gray matter volumes. Predicted differences in ICCs between pairs were compared using 1-tailed *t* tests.

Finally, variance component analyses were performed using version 1.50d of MX[38] to determine the proportion of the variance in these volumes explained by additive genetic factors and shared and unique environmental factors in healthy and index twin pairs while covarying for age and sex.

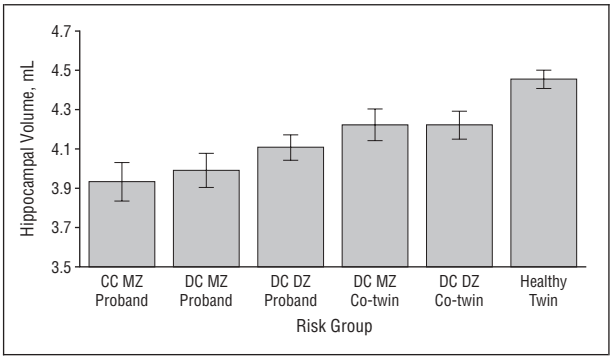
RESULTS

HIPPOCAMPAL VOLUME

Group Differences

There were significant effects for risk group ($F_{5,372}$=9.23; P<.001), hemisphere ($F_{1,372}$=3.95; P=.048), substance abuse ($F_{1,372}$=7.27; P=.007), and intracranial gray matter volume ($F_{1,372}$=45.43; P<.001) in predicting hippocampal volumes.

Given that the risk group effect did not vary by hemisphere, contrast analyses were performed on hippocampal volumes collapsed across hemisphere. Probands from MZ concordant pairs, MZ discordant pairs, and DZ discordant pairs had smaller mean hippocampal volumes than healthy individuals (t_{372}=4.0, t_{372}=4.9, and t_{372}=4.4, respectively; P<.001 for all). As predicted (hypothesis 1), none of the mean hippocampal volumes of the 3 proband groups differed statistically significantly from each other (**Figure**). Although the hippocampal volumes of the non-ill MZ and DZ co-twins from pairs discordant for schizophrenia were smaller than those of healthy control twins (t_{372}=2.6; P=.005 and t_{372}=3.2; P<.001, respectively), contrary to hypothesis 2, the non-ill MZ co-twins did not differ from the non-ill DZ co-twins (t_{372}=0.14; P=.45). Consistent with hypothesis 3, the probands from MZ discordant twin pairs had smaller hippocampal volumes than their non-ill MZ co-twins (t_{372}=2.0; P=.02). Probands from DZ discordant twin pairs did not have significantly smaller hippocampal volumes than their non-ill DZ co-twins (t_{372}=1.0; P=.15). However, hippocampal volumes from MZ and DZ probands combined were smaller than those of MZ (t_{372}=1.77; P=.04) and DZ (t_{372}=1.84; P=.03) co-twins, and these effects were even stronger when hippocampal volumes from MZ concordant probands were added (t_{372}=1.98 and t_{372}=2.1, respectively; P=.02 for both).



Least squares mean hippocampal volumes across risk groups. CC indicates concordant; MZ, monozygotic; DC, discordant; DZ, dizygotic. Error bars represent SEM.

Covariates

We replicated our previous findings of larger right than left hippocampal volumes (t_{372}=2.0; P=.02). The hippocampal volumes of individuals diagnosed as having a substance disorder were smaller than those without such a diagnosis (t_{372}=–2.7; P<.001).

Relative Risk

Percentage-wise, 11 (69%) of the 16 MZ and 19 (68%) of the 28 DZ probands had smaller hippocampal volumes than their healthy co-twins, and 12 (75%) of the 16 MZ and 25 (89%) of the 28 DZ co-twins had smaller hippocampal volumes than the average of the healthy twins.

Table 3. Intraclass Correlations and Comparison *t* Values for Twin Pairs

	CMZ*	CDZ*	CMZ vs CDZ*	CIMZ*	DIMZ*	DIDZ*	CIMZ vs DIDZ†	DIMZ vs DIDZ†
	(n = 28)	(n = 26)	(*df* = 53)	(n = 7)	(n = 16)	(n = 29)	(*df* = 35)	(*df* = 44)
THIP	0.79 (0.60 to 0.90)	0.43 (0.07 to 0.70)	10.8‡	0.65 (–0.04 to 0.93)	0.46 (–0.01 to 0.77)	0.46 (0.12 to 0.70)	2.9§	0.1
	(n = 24)	(n = 24)	(*df* = 47)	(n = 6)	(n = 14)	(n = 23)	(*df* = 28)	(*df* = 36)
IV	0.84 (0.67 to 0.93)	0.57 (0.23 to 0.79)	8.8‡	0.95 (0.76 to 0.99)	0.94 (0.83 to 0.98)	0.61 (0.28 to 0.81)	6.3‡	9.2‡
CGM	0.92 (0.83 to 0.97)	0.70 (0.42 to 0.85)	10.2‡	0.88 (0.44 to 0.98)	0.82 (0.55 to 0.94)	0.69 (0.40 to 0.85)	3.9‡	3.9‡
LS THIP (CGM)	0.74 (0.49 to 0.88)	0.42 (0.04 to 0.70)	8.1‡	0.70 (–0.03 to 0.95)	0.48 (–0.03 to 0.80)	0.26 (–0.16 to 0.60)	4.9‡	3.3§

Abbreviations: CDZ, control dizygotic; CGM, cortical gray matter volume; CIMZ, concordant index MZ; CMZ, control monozygotic; DIDZ, discordant index DZ; DIMZ, discordant index MZ; IV, intracranial volume; LS THIP (CGM), least squares THIP (corrected for CGM); n, number of pairs; THIP, total hippocampus.
*Intraclass correlations (95% confidence intervals).
†Comparison *t* values.
‡$P<.001$, 1-tailed.
§$P<.01$, 1-tailed.

CORTICAL GRAY MATTER VOLUME

Group Differences

There were significant effects for risk group ($F_{5,181}=3.0$; $P=.01$), risk group × sex ($F_{5,181}=3.14$; $P=.01$), substance abuse ($F_{1,181}=4.6$; $P=.03$), age ($F_{1,181}=33.8$; $P<.001$), and intracranial volume ($F_{1,402}=51.32$; $P<.001$) in predicting cortical gray matter volumes.

Contrast analyses showed that probands from pairs concordant for schizophrenia had less cortical gray matter than probands from discordant MZ ($t_{181}=2.2$; $P=.03$) and DZ ($t_{181}=2.5$; $P=.01$) pairs, non-ill MZ ($t_{181}=2.7$; $P=.009$) and DZ ($t_{181}=3.2$; $P=.002$) co-twins, and healthy twins ($t_{181}=3.7$; $P<.001$). None of the other groups differed from each other.

Covariates

Cortical gray matter volumes of individuals diagnosed as having a substance disorder were smaller than those of individuals without such a diagnosis ($t_{181}=-2.2$; $P=.03$). Cortical gray matter volumes in the overall sample seemed to decline with age (slope = –2.4 [SE = 0.41358462], $t_{181}=-5.8$; $P<.001$, 2-tailed). Female concordant patients had larger cortical gray matter volumes than male concordant patients ($t_{181}=2.4$; $P=.02$, 2-tailed), whereas female MZ co-twins and healthy twins had smaller cortical gray matter volumes than male MZ co-twins ($t_{181}=-2.3$; $P=.02$, 2-tailed) and male healthy twins ($t_{181}=-2.7$; $P=.009$, 2-tailed), respectively; none of the other groups showed sex differences in cortical gray matter volumes.

INTRACLASS CORRELATIONS

The ICCs for healthy MZ pairs were larger than those for healthy DZ pairs and the ICCs for concordant index MZ pairs were larger than those for discordant index MZ and DZ pairs on all measures (**Table 3**). Although the ICCs for hippocampal volumes in discordant MZ and DZ pairs were similar, those for hippocampal volumes corrected for cortical gray matter, and those for intracranial and cortical gray matter volume, were larger in discordant MZ compared with DZ pairs (Table 3). Finally, the ICC for hippocampal volumes in healthy MZ pairs was larger than that in discordant MZ pairs ($t_{43}=8.1$; $P<.001$).

VARIANCE COMPONENTS

In index and healthy pairs, unique environment/error–only models had significantly worse fit than additive genes, common environment, unique environment/error (ACE) models for hippocampal and total gray matter and intracranial volume (**Table 4**). Although not significantly different from the ACE model, based on parsimony and fit statistics, the AE model is the best-fitting model for all the regions in the healthy twins, whereas the CE model provides the best fit for hippocampal and total gray matter volume in the index twins and the AE model provides the best fit for intracranial volume and total hippocampal volume corrected for total cortical gray matter volume (Table 4). Based on this analysis, the variance component for the effect of additive genes on hippocampal volume corrected for cortical gray matter volume is 71% in the healthy twins and 42% in the discordant twins.

COMMENT

The principal finding of this study is that although hippocampal volumes in healthy twins are highly heritable, those in twins discordant for schizophrenia are subject to substantially great modulation by environmental factors.

The higher ICC for healthy MZ vs DZ pairs and the best fit for the AE model in the variance component analysis corroborate findings by other researchers[16,39,40] that hippocampal volume in healthy individuals is highly heritable. A combination of genetic[8,16,41,42] and unique environmental[12-15] effects on hippocampal volume in schizophrenia is indicated by (1) smaller hippocampal volumes in probands compared with their non-ill MZ co-twins, (2) larger ICCs for hippocampal volume in healthy compared with discordant MZ pairs, (3) higher variance components for additive genes in healthy compared with discordant twins, and (4) statistically significantly higher ICCs for discordant MZ compared with DZ twin hippocampal volume.

Interpretation of the data is based on 3 assumptions underlying the classic twin design: (1) MZ twins

Table 4. Univariate ACE Models With Linear Regression of Age and Sex on the Observed Total Hippocampal, Total Cortical Gray Matter, Intracranial, and Total Hippocampal Corrected for Total Cortical Gray Matter Volumes Fitted to the Raw Data*

Healthy Pairs									Index Pairs							
Model	A	C	E	−2LL	*df*	AIC	χ^2	*P* Value	Model	A	C	E	−2LL	*df*	AIC	χ^2	*P* Value
Total Hippocampal Volume																	
ACE	54	14	32	686.60	211	. . .	. . .	. . .	ACE	0	39	61	568.83	169	. . .	. . .	. . .
AE	***69 (48-82)***	. . .	***31 (18-52)***	686.77	212	−1.83	0.17	.68	AE	39	. . .	61	570.81	170	−0.02	1.98	.16
CE	. . .	57	43	689.29	212	0.70	2.70	.10	***CE***	. . .	***39 (10-62)***	***61 (38-90)***	568.83	170	−2.00	0.00	NS
E	. . .	. . .	100	710.89	213	20.30	24.30	<.001	E	. . .	. . .	100	575.32	171	2.49	6.49	.04
Total Cortical Gray Matter Volume																	
ACE	57	28	14	1414.71	202	. . .	. . .	. . .	ACE	23	45	32	1180.96	160	. . .	. . .	. . .
AE	***86 (74-92)***	. . .	***14 (8-26)***	1415.44	203	−1.27	0.74	.39	AE	72	. . .	28	1182.93	161	−0.03	1.97	.16
CE	. . .	74	26	1420.56	203	3.85	5.85	.02	***CE***	. . .	***61 (37-77)***	***39 (23-63)***	1181.38	161	−1.58	0.42	.52
E	. . .	. . .	100	3268.07	204	1857.37	1853.37	<.001	E	. . .	. . .	100	. . .	162	. . .	. . .	<.001
Intracranial Volume																	
ACE	65	7	28	1595.33	202	. . .	. . .	. . .	ACE	89	0	11	1282.31	160	. . .	. . .	. . .
AE	***72 (47-85)***	. . .	***28 (15-53)***	1595.36	203	−1.97	0.03	.87	***AE***	***89 (70-95)***	. . .	***11 (5-30)***	1282.31	161	−2.00	0.00	NS
CE	. . .	59	41	1598.12	203	0.79	2.79	.10	CE	. . .	53	47	1292.02	161	7.71	9.71	<.001
E	. . .	. . .	100	1608.26	204	8.92	12.92	<.001	E	. . .	. . .	100	1302.45	162	16.41	20.14	<.001
Total Hippocampal Volume Corrected for Total Cortical Gray Matter Volume																	
ACE	71	0	29	594.92	193	. . .	. . .	. . .	ACE	27	13	60	535.36	160	. . .	. . .	. . .
AE	***71 (41-84)***	. . .	***29 (16-53)***	594.92	194	−2.00	0.053	NA	***AE***	***42 (4-69)***	. . .	***58 (31-96)***	535.27	161	−1.91	0.09	.76
CE	. . .	53	47	599.53	194	2.61	4.61	.03	CE	. . .	33	67	535.42	161	−1.76	0.24	.63
E	. . .	. . .	100	615.19	195	16.28	20.28	<.001	E	. . .	. . .	100	539.90	162	0.72	4.7	.09

Abbreviations: ACE, standardized percentages of variance attributed to additive genes, common environment, and unique environment/error, respectively; −2LL, double-negative log-likelihood fit statistic (larger value indicates worse fit); AIC, Akaike Information Criterium (smaller or more negative value means better fit of reduced model compared with saturated model); χ^2, based on the hierarchical difference in fit, or Δ(−2LL), of the nested compared with the ACE model (a high χ^2 against a low gain in the *df* indicates a worse fit of the nested [AE/CE] models compared with the ACE model); NS, not significant; ellipses, not applicable.

*Boldface and italicized items indicate the values of the best-fitting model.

share 100% and DZ twins share, on average, 50% of their polymorphic genetic material, and current methods can adequately identify zygosity; (2) the environment shared among MZ and DZ twins is similar; and (3) twins are similar to singletons such that findings in twins can be generalized to nontwin populations.

Although it has been hypothesized that MZ twins discordant for schizophrenia share less of their polymorphic genetic material than concordant and healthy twins,[43-47] the empirical evidence for this claim remains controversial.[44,46,48] In contrast, obstetric complications seem to increase the risk for schizophrenia,[49-51] are associated with illness discordance,[52] and have been related to hippocampal volume reduction in schizophrenia.[9,10,14] It could still be argued that the smaller hippocampal volumes in probands compared with non-ill MZ co-twins are due to factors associated with disease status. However, in contrast to this hypothesis, and consistent with the results of Suddath and colleagues,[12] hippocampal volumes of probands were not associated with illness duration after covarying for age (SE=.04, t_{50}=1.6; *P*=.11) or with years receiving neuroleptic medication.

The findings are also interpreted assuming that MZ and DZ twins share environmental exposures to similar degrees. A competing interpretation of the similarity in mean hippocampal volume between the MZ and DZ co-twins and the higher ICC for MZ compared with DZ discordant twin pairs could be that DZ co-twins experienced more pregnancy complications than MZ co-twins from discordant pairs. In this sample, the frequency of prenatal and perinatal complications coded blindly from the original obstetric records on approximately half the studied twin pairs (T.D.C., unpublished observations, 2000) did not differ between discordant MZ and DZ pairs.[22] A full interpretation of the obstetric data is not possible because obstetric data were recorded by pair and not by individual twin.

The twin method is often criticized for nongeneralizability owing to differences in the intrauterine and family environment of twins compared with singletons. However, recent studies show that any differences in cognitive abilities between twins and their siblings no longer exist at age 5 years[53] and that although second-born twins have lower intracranial volumes than first-born twins, all other volumes are comparable when controlling for intracranial volume, suggesting that twin studies can provide reliable estimates of heritabilities in brain volume measures and that these can be generalized to singleton populations.[54]

The differences in overall cortical gray matter volume were different from those observed for the hippo-

campus, suggesting that the observed pattern of hippocampal volume reduction is not due to global effects on gray matter. Although intracranial gray matter volume was only reduced in concordant MZ probands relative to the other groups, a previous study[55] on regional cortical gray matter deficits showed that particular heteromodal cortical regions are affected by genetic liability and disease-related environmental factors.

The finding that the hippocampal volumes of probands from concordant pairs do not differ from those of discordant pairs is consistent with that of Weinberger and colleagues[18] in suggesting that the etiologies underlying the hippocampal volume reductions in these 2 types of probands are similar. However, the higher ICCs for concordant MZ twin pairs compared with discordant MZ and DZ twin pairs suggest that the environmental factor contributing to discordance may affect dissimilarity of hippocampal volume within twin pairs also.

Previously reported data on verbal episodic memory in the same sample,[22] thought to rely on the hippocampus[56] and previously shown to correlate with hippocampal volume,[8,23] match the pattern of hippocampal volume reduction and similarities observed in this study.

Strengths of this study are as follows: a random representative population sample was used such that the results can be generalized to the total population of twins, probands from concordant and discordant pairs were available such that their volumes could be compared directly, concordant and discordant pairs were available such that ICCs could be compared directly, high-resolution images were used to make the measurements, high reliabilities were achieved on the measurements, and the rater was blind to the presentation of the images (neurologic/radiologic) during data collection such that potential rater or other orientation biases were eliminated.

Several weaknesses of this study must also be noted. The measurements only reflect hippocampal volumes, and it is possible that there are also regional shape changes, in particular in the Sommer sector. Although the sample size is relatively large, the effects under examination are relatively small. Data for prenatal and perinatal complications were not available for the entire sample, making it impossible to directly examine the effects of specific environmental factors on hippocampal volume.

Finally, although hippocampal volume reduction in schizophrenia seems to be affected by genetic factors and unique environmental factors, it is yet to be determined in which parts of the hippocampal microstructure, and when during development, these factors act or interact.

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