ORIGINAL ARTICLE

Left Hippocampal Volume as a Vulnerability Indicator for Schizophrenia

A Magnetic Resonance Imaging Morphometric Study of Nonpsychotic First-Degree Relatives

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Background: Clues to the causes of schizophrenia can be derived from studying first-degree relatives because they are genetically related to an ill family member. Abnormalities observed in nonpsychotic relatives are indicators of possible genetic vulnerability to illness, independent of psychosis. We tested 4 hypotheses: (1) that hippocampal volume is smaller in nonpsychotic relatives than in controls, particularly in the left hemisphere; (2) that hippocampi will be smaller in multiplex relatives as compared with simplex relatives, and both will be smaller than in controls; (3) that hippocampal volumes and verbal declarative memory function will be positively correlated; and (4) that hippocampi will be smaller in patients with schizophrenia than in their nonpsychotic relatives or in controls.

Methods: Subjects were 45 nonpsychotic adult first-degree relatives from families with either 2 people ("multiplex," n=17) or 1 person ("simplex," n=28) diagnosed with schizophrenia, 18 schizophrenic relatives, and 48 normal controls. Sixty contiguous 3-mm coronal, T1-weighted 3-dimensional magnetic resonance images of the brain were acquired on a 1.5-T magnet. Volumes of the total cerebrum and the hippocampus were measured.

Results: Compared with controls, relatives, particularly from multiplex families, had significantly smaller left hippocampi. Verbal memory and left hippocampal volumes were significantly and positively correlated. Within families, hippocampal volumes did not differ between schizophrenic patients and their nonpsychotic relatives.

Conclusions: Results support the hypothesis that the vulnerability to schizophrenia includes smaller left hippocampi and verbal memory deficits. Findings suggest that smaller left hippocampi and verbal memory deficits are an expression of early neurodevelopmental compromise, reflecting the degree of genetic liability to schizophrenia.

Arch Gen Psychiatry. 2002;59:839-849

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CURRENT perspectives on the cause of schizophrenia have focused attention on neurobiological vulnerability to the illness.[1-3] Children at risk for schizophrenia, and nonpsychotic adult relatives manifest electrophysiological, neurocognitive, symptomatic, and behavioral abnormalities, usually to a milder degree than patients with frank psychosis.[4,5] A few magnetic resonance imaging (MRI) studies of the brain in relatives have demonstrated abnormalities in structures relevant to schizophrenia.[6-8] Both younger[9-11] and older[12-16] nonpsychotic relatives manifest volumetric abnormalities, especially in the hippocampus-amygdala region and in the thalamus, suggesting that these abnormalities, at least in part, reflect vulnerability to the illness. Advances in understanding the biological vulnerability to schizophrenia will be facilitated by increasing the precision of measurement of the abnormalities, by evaluating whether putatively linked risk factors are related to each other (ie, left hippocampus and verbal declarative memory), and by determining whether these deficits are associated with genetic factors.

Of the myriad manifestations of schizophrenia, structural brain abnormalities and neurocognitive deficits are among the most replicated findings. The most consistent MRI abnormalities are enlarged ventricles and smaller temporal lobe and limbic system volumes, particularly in the hippocampus.[17-19] Postmortem studies have also demonstrated subtle anomalies in limbic structures, most consistently in the hippocampus,[20] including reduced neuronal size and reduced levels of synaptic proteins.[21,22] The hippocampus is considered to

be important,[20-22] especially because of its role in learning and memory.[23-25] Some lateralized temporal lobe abnormalities have been observed in schizophrenia (more often left-sided),[26] and some have proposed that this pattern reflects a genetic, neurodevelopmental vulnerability.[27]

Verbal declarative or "explicit" memory (the conscious recollection of words, stories, or events) is one of the most robustly impaired neurocognitive functions in schizophrenia.[28,29] It is commonly impaired in diseases affecting the "medial temporal lobe memory system," particularly the left hippocampus.[23,30] These findings suggest that hippocampal abnormalities, especially left-sided ones, and verbal memory deficits are associated candidates for vulnerability indicators.

The study of biological relatives is a valuable strategy used to investigate vulnerabilities to schizophrenia.[5-7] Abnormalities in relatives may provide clues to the cause of the illness, suggesting potential genetic effects.[31,32] Unlike patients, nonpsychotic relatives are not affected by antipsychotic medications, hospitalization, and putative neurotoxic effects of psychosis. Adult relatives, who have passed through the peak age of risk for psychosis (20-35 years) are unlikely to develop schizophrenia, and thus may manifest abnormal traits associated with vulnerability and not with psychosis.[33]

Our prior work suggests that verbal declarative memory[34,35] and hippocampal volume[13] might represent genetic markers of vulnerability to schizophrenia. Most researchers agree that a single gene theory is untenable, even if that theory allows for many different single gene variants.[33,36-39] The multifactorial model of schizophrenia has found some, although not complete, support.[33,36-39] In accordance with the multifactorial model, the amount of impairment in relatives should increase with their genetic loading for schizophrenia. Supporting this hypothesis, verbal memory was significantly worse in nonpsychotic persons from "multiplex" families (containing 2 first-degree relatives with schizophrenia) compared with persons from "simplex" families (containing 1 first-degree relative with schizophrenia).[40] Other investigators have found similar results assessing "integrative" neurological signs.[41]

Based on this model, we tested 4 hypotheses. First, hippocampal volume will be smaller in relatives than in normal controls, and the abnormalities will be primarily left-sided. Second, hippocampal volume will be smaller in multiplex, as compared with simplex relatives, and both will be smaller than in controls. Third, hippocampal volume and verbal memory will be significantly and positively correlated. Fourth, hippocampal volumes will be smaller in patients with schizophrenia than in their nonpsychotic relatives or in controls.

SUBJECTS AND METHODS

SUBJECTS

Subjects comprised an extended sample from a previous study.[13,34] Subjects (45 nonpsychotic, first-degree relatives of schizophrenic patients, 48 controls, and 18 patients with schizophrenia) were 20 to 68 years of age, had at least an eighth-grade education, with English as their first language, and an estimated IQ of at least 70. Exclusion criteria were (1) substance abuse within the past 6 months; (2) head injury with documented cognitive sequelae or loss of consciousness greater than 5 minutes; (3) neurologic disease; and (4) medical illnesses that impair neurocognitive function.

After describing the study, written informed consent was obtained, including permission by the schizophrenic patients ("probands") for us to contact their relatives. *DSM-III-R*[42] diagnoses in patients were established using the Schedule for Affective Disorders and Schizophrenia[43] or Diagnostic Interview for Genetic Studies,[44] and a systematic review of the medical record. Substance use was assessed by a semi-structured interview to determine quantity, frequency, and duration of use.[34] Blindness of assessments was maintained among psychiatric, neuropsychological, and MRI data.

Relatives

Relatives were free of psychosis during their lifetime. There were 28 simplex (16 siblings, 7 offspring, 5 parents) and 17 multiplex (16 siblings, 1 offspring) relatives from 34 unique families. Twenty-six families provided a single relative, 3 families had 3 relatives, and 5 families had 2 relatives. All available relatives were interviewed to determine if the family was simplex or multiplex. Relatives were interviewed with the Structured Clinical Interview for *DSM-III-R*[45] or Diagnostic Interview for Genetic Studies for Axis I disorders, and the Structured Interview for *DSM-III* Personality Disorders.[46] Fifty-six percent had nonpsychotic diagnoses—mainly Axis I disorders such as past major depressive disorder or substance abuse. One relative had schizotypal personality disorder. Three relatives had received a psychotropic medication (1 antianxiety and 2 antidepressant medications).

We also analyzed a subset of 18 (of 45) relatives (8 males, 10 females) from the 13 families who had a proband with schizophrenia who had an MRI scan. This sample included 1 father, 2 mothers, 13 siblings (6 sisters, 7 brothers), and 2 daughters of patients. Nine families had 1, three families had 2, and 1 family had 3 nonpsychotic relatives. Thirteen were from multiplex families, and 5 were from simplex families.

Patients With Schizophrenia

Eighteen patients participated. They are a subset of 90 patients (40 simplex and 50 multiplex) with schizophrenia who had received an MRI scan, and have been described elsewhere.[47,48] There were 10 males and 8 females from 13 families (4 simplex, 9 multiplex). Of the 9 multiplex families, 5 had 2 ill members, and 4 had 1 member.

Healthy Controls

Forty-eight controls came from unrelated families acquired through advertisements in the catchment areas of the hospitals, from which the patients had been ascertained. Our goal was to acquire demographically similar controls as patients and relatives. Controls underwent a similar screening process, as did other subjects, except, as in our previously published studies with this control sample,[12,13,34,35,40] they were screened for current psychopathological disorders using a short form of the Minnesota Multiphasic Personality Inventory (MMPI-168)[49] rather than interviewed. We excluded potential controls if they had a personal or family history of psychosis or psychiatric hospitalization, or had MMPI elevations above 70 on the clinical scales. Controls were also administered the substance use section of the Schedule for Affective Disorders and Schizophrenia. We did not screen for a lifetime history of psychopathological or neuropsychological dysfunction. In

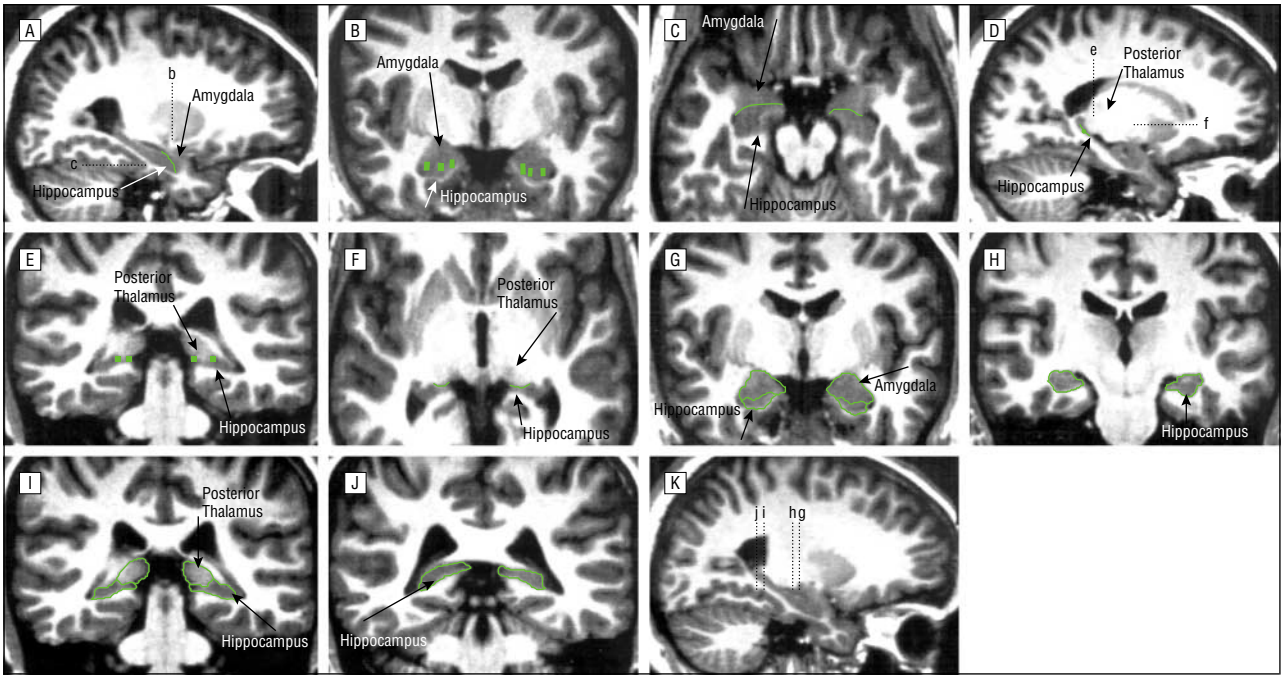

Figure 1. Method of segmentation of the hippocampus (observed in radiological convention) as performed in our study (under the supervision of Nikos Makris, MD, PhD). Identification of the hippocampus is achieved using a cross-referencing tool,[66] which allows visualization of the structure in 3 coregistered cardinal views (coronal, axial, and sagittal). A-F, Dotted lines (b, c, e, f) on sagittal slices (A) and (D) indicate where coronal slices (B and E) and axial slices (C and F) lie. D presents a more medial view compared with A, while the image in F is more superior to that in C, and the image in E is more posterior than that of B. Colored outlines separating the hippocampus from its neighboring structures are shown. Colored lines distinguish the structures. Specifically, 2 lines were drawn to demarcate the boundary between the anterior hippocampus and amygdala (A, B, and C) and the boundary between the hippocampus and posterior thalamus (D, E, and F). G-J, Segmentation of the hippocampus is shown in 4 representative coronal sections, which is determined by outlines previously drawn in sagittal (A and D) and axial (C and F) planes. G, Coronal slice in the anterior tip of the hippocampus (where it borders amygdala). H, Coronal slice is in the middle of the hippocampus. I, Coronal slice is in the posterior hippocampus (where it borders posterior thalamus). J, Coronal slice in the tail of the hippocampus. K, Dotted vertical lines g, h, i, and j indicate the corresponding coronal planes.

choosing a control group, we attempted to balance 2 competing sources of bias. Unscreened controls frequently have rates of psychopathology and neuropsychological dysfunction above the population expectation.[50-52] Thus, unscreened controls can obscure the effects of interest. However, excessive screening of controls can exaggerate the effects of interest.[53,54] The data we collected from tests having extensive normative data provide some indication of the "normalcy" of our controls. The mean (SD) score for controls on the Wide Range Achievement Test—Revised (WRAT-R)[55] reading subtest was 105.6 (11.1), well within the normal range. Two controls received antianxiety medications.

NEUROPSYCHOLOGICAL MEASURES

The vocabulary and block design tests of the Wechsler Adult Intelligence Scale—Revised[56] estimated current intelligence,[57] and the reading test of the WRAT-R estimated intellectual potential.[58] Handedness was determined by questionnaire.[59] Verbal declarative memory was assessed with the Logical Memory Stories test of the Wechsler Memory Scale—Revised.[60] Data consisted of raw scores at immediate and 30-minute delayed recall and the percentage retained[61] (delayed recall/immediate recall × 100).

MRI PROCEDURES

MRI Image Acquisition and Morphometric Analysis

Subjects received a brain MRI scan usually after neuropsychological testing (median, 36 days). The MRI scans were obtained at the Massachusetts General Hospital (MGH) on a General Electric 1.5-T Signa Scanner (Milwaukee, Wis). Image acquisitions included conventional sagittal scout, a coronal T2-weighted sequence to rule out gross pathology and a coronal volumetric T1-weighted spoiled gradient echo imaging sequence for morphometric analysis, using the following parameters: pulse sequence, 3D-SPGR (spoiled GRASS-gradient refocused acquisition in the steady-state); TR (time to repeat), 40 ms; TE (echo time), 8 ms; flip angle, 50°; field of view, 30 cm; slice thickness, 3.0 mm; number of slices, 60 contiguous coronal images of the entire brain; matrix, 256 × 256; number of excitations, 1.

Images were positionally normalized to overcome variations in head position by imposing a standard 3-dimensional brain coordinate system on each scan using the midpoints of the decussations of the anterior and posterior commissures and the midsagittal plane at the level of posterior commissure as points of reference for rotation and translation.[62,63]

Gray matter–white matter segmentation was performed on each T1-weighted, normalized, 3-dimensional coronal scan using a semiautomated intensity contour algorithm for external border definition, and signal intensity histogram distributions for demarcation of gray-white borders.[64] Regions of interest for this study included total cerebrum and the hippocampus. Prior to measurement of the hippocampus, volumetric morphometry was undertaken by methods employed previously for a series of adult controls,[62,63] and in our previous study, which included 54 of the 111 subjects whose cases are reported here.[13] To measure the hippocampus, we applied a new, entirely manual, anatomically guided, segmentation boundary that was adapted from a procedure described previously[65] (**Figure 1**).

Anatomical Definition

In our MRI system, the hippocampus is based on an anatomical definition of hippocampal formation[65(p40),66] that excludes the parahippocampal gyrus.

Segmentation Procedure

The amygdala and hippocampus are first defined as a continuous gray matter mass in the primary segmentation.[63] They are then manually partitioned from each other at the rostral coronal plane where the hippocampus appears (Figure 1). This includes clearly defined segments of hippocampus in ventromedial relation to the anterior tip of the ventral horn of the lateral ventricle. The caudal pole of the amygdala is present in medial and superior relation to the hippocampus in the coronal plane.[67] The anterior tip of the hippocampus is separated from the ventral and posterior border of the amygdala. Using lateral and sagittal views, one can distinguish and trace this border of the amygdala, which is usually enhanced by the anterior end of the temporal horn of the inferior lateral ventricle. In cross-reference, corresponding axial views help identify this border. In the coronal view, the saw-tooth pattern of the hippocampus is identified and traced.

INTERRATER RELIABILITY

In 16 blindly segmented brains, intraclass correlation coefficients (r) were 0.93 for total cerebral volume, 0.91 for the left hippocampus, and 0.92 for the right hippocampus.

VOLUMETRIC ANALYSIS

The volume of each structure was calculated by multiplying the number of voxels assigned to that structure on each slice by the slice thickness, and summing across all slices in which the structure appeared.

DATA ANALYSIS

Primary comparisons were made between controls, relatives from simplex families, and relatives from multiplex families. Analyses included tests of overall group effects, linear trends (control-simplex-multiplex), and pairwise comparisons between each group of relatives with each other and with controls. Additional analyses compared schizophrenia probands with a subgroup of their relatives, and with normal controls. In testing for hippocampal differences, total cerebral volume and potential confounds (age, sex, handedness, ethnicity, and parental education) were used as covariates in all analyses. Some analyses also included psychiatric diagnosis and IQ as covariates, or were based on subsets of these subject groups (eg, subjects 36 years and older were studied to evaluate effects in relatives who are very unlikely to experience the onset of schizophrenia). Statistical significance was $P<.05$ (2-tailed).

Some families yielded more than 1 subject. Generalized estimating equations regression models account for the potential error in the estimation of standard errors of parameter estimates resulting from correlations between family members.[68,69] The generalized estimating equations approach provides consistent estimates of means and SEs under weak assumptions about the population distribution of the data. We used a working independence correlation structure implemented in SAS PROC GENMOD (SAS Institute Inc, Cary, NC; Version 6.12). Heuristically, the observations are assumed to be independent, and an empirical variance estimator is used to account for clustering within families. Previous research has shown this to be a good choice for small sample sizes.[70,71]

RESULTS

DEMOGRAPHIC AND CLINICAL CHARACTERISTICS IN RELATIVES AND CONTROLS

Groups did not differ significantly on age, parental socioeconomic status,[72] parental education, ethnicity, handedness, or use of alcohol and other drugs (**Table 1**). There were significant differences by sex, education, and estimated IQ, and a nonsignificant trend in reading ($P<.10$).

Our primary regression models included main effects for group, sex, age, handedness, ethnicity, parental education, and total cerebral volume. We found no significant evidence for pairwise interactions between sex, age, or total cerebral volume.

EFFECTS OF RELATIVE AND CONTROL STATUS ON HIPPOCAMPAL VOLUMES

There were no significant differences for total cerebral volume, but group differences were significant for left hippocampal volume (**Table 2**). A test of the linear trend of hippocampal volumes (control>simplex>multiplex) was significant (**Table 3**). Multiplex relatives had significantly smaller left hippocampi than simplex relatives (Table 3). Multiplex subjects had significantly smaller left hippocampi compared with controls (Table 3 and **Figure 2**A). As a percentage of the control volume, the left multiplex hippocampus was 9.3% smaller. The effect size, taking into account the confounders, was 1.0. Simplex relatives showed a trend ($P<.10$) toward significantly smaller volumes as compared with controls. The left simplex hippocampus was 4.8% smaller than the control volume. The effect size was 0.44. Results were similar when controlling for IQ. There were no significant differences or nonsignificant trends for any comparison of the right hippocampus; thus, in Table 3, we report only the statistical comparison of left hippocampi.

EFFECTS OF RELATIVE AND CONTROL STATUS ON HIPPOCAMPAL VOLUMES IN SUBGROUPS OF RELATIVES

Although sample sizes were smaller, results for relatives who would be highly unlikely to develop schizophrenia (those 36 years or older, beyond the peak age of risk) were comparable to the overall sample (Table 3 presents the size of effects as measured by β weights). Analyses for siblings only (multiplex, n=16; simplex, n=16) and males and females produced similar results to the overall sample and were comparable on most analyses. Eliminating 2 subjects with mild hypertension did not change the results (Tables 2 and 3; Figure 2).

EFFECTS OF PSYCHIATRIC DIAGNOSIS ON HIPPOCAMPAL VOLUMES IN RELATIVES AND CONTROLS

To assess the potential confound of psychopathology, we examined the effect of the presence vs the absence of any

Table 1. Demographic Variables for Controls and Relatives of Patients With Schizophrenia*

Variable	Control Subjects (n = 48)	Simplex Relatives (n = 28)	Multiplex Relatives (n = 17)	F Score	*P* Value
Mean (SD) age at MRI, y	40.1 (10.8)	41.9 (12.7)	38.9 (10.6)	0.76	.68
Mean (SD) parental SES	2.6 (1.0)	3.1 (1.2)	2.9 (1.0)	2.90	.24
Mean (SD) years of parental education	12.1 (2.3)	11.3 (3.4)	11.7 (2.1)	1.05	.59
Mean (SD) years of education	15.1 (2.2)	14.0 (2.5)	12.2 (2.2)	12.13	.002
Mean (SD) WAIS-R vocabulary	13.2 (3.5)	10.5 (2.6)	11.5 (3.8)	9.53	.009
Mean (SD) WAIS-R block design	11.4 (2.4)	11.0 (2.0)	11.3 (3.5)	0.60	.74
Mean (SD) IQ estimate†	112.9 (13.0)	104.3 (9.9)	107.9 (18.6)	7.09	.03
Mean (SD) WRAT-R reading	105.6 (11.1)	99.8 (12.1)	99.2 (12.9)	5.32	.07
Sex (% male)	56.3	35.7	41.2	. . .	.05
Ethnicity (% white)	93.8	92.9	94.1	. . .	.99
Handedness (% right)	91.7	89.3	88.2	. . .	.89
Present drug use (% of subjects)‡					
0	91.7	92.9	100.0	. . .	
1	8.3	3.6	0.0	. . .	
2	0.0	3.6	0.0	. . .	.52
3	0.0	0.0	0.0	. . .	
4	0.0	0.0	0.0	. . .	
Past drug use (% of subjects)‡					
0	77.1	92.9	64.7	. . .	
1	8.3	3.6	23.5	. . .	
2	12.5	0.0	5.9	. . .	.08
3	2.1	3.6	5.9	. . .	
4	0.0	0.0	0.0	. . .	
Present alcohol use (% of subjects)‡					
0	39.6	60.7	41.2	. . .	
1	47.9	32.1	41.2	. . .	
2	12.5	7.1	11.8	. . .	.33
3	0.0	0.0	5.9	. . .	
4	0.0	0.0	0.0	. . .	
Past alcohol use (% of subjects)‡					
0	29.2	50.0	29.4	. . .	
1	50.0	35.7	35.3	. . .	
2	16.7	7.1	17.7	. . .	.26
3	4.2	7.1	11.8	. . .	
4	0.0	0.0	5.9	. . .	

*MRI indicates magnetic resonance imaging; SES, socioeconomic status (for which a lower score indicates a higher status[72]); WAIS-R, Wechsler Adult Intelligence Scale–Revised[56]; WRAT-R, Wide Range Achievement Test–Revised; and ellipses, not applicable.[55] Tests for sex, ethnicity, and handedness were performed using χ^2 analysis (5.95, 0.02, and 0.23, respectively). Frequency of substance abuse, both past and present, was tested by Fisher exact test.

†IQ estimate was derived from vocabulary and block design age scale scores.[57]

‡For substance abuse ratings, 0 indicates never/occasional use; 1, recreational (episodic) use; 2, regular use; 3, abuse (for a period of 6 months to 5 years); and 4, sustained abuse (for longer than 5 years).

Table 2. Brain Volumes and Verbal Memory Performance in Controls and Relatives of Patients With Shizophrenia*

Variable	Control Subjects (n = 48)	Simplex Relatives (n = 28)	Multiplex Relatives (n = 17)	χ^2 Analysis	*P* Value
MRI measures, cm^3					
Total cerebral exterior	1073 (101.10)	1067 (99.40)	1104 (93.40)	3.78	.15
Left hippocampus	4.14 (0.44)	3.94 (0.47)	3.75 (0.41)	10.89	.004
Right hippocampus	3.99 (0.44)	4.12 (0.41)	4.00 (0.52)	3.37	.19
Neuropsychological measures†					
Logical memory–immediate recall	28.4 (5.7)	27.8 (6.8)	22.1 (6.5)	9.46	.009
Logical memory–delayed recall	25.0 (6.4)	24.9 (7.4)	19.4 (7.4)	7.18	.03
Percent retention‡	87.2 (11.2)	89.1 (12.1)	85.6 (19.5)	0.61	.74

*Data are presented as means (SDs). MRI indicates magnetic resonance imaging. For all neuropsychological measures, higher numbers indicate better scores for each of the 3 logical memory variables.

†These 93 subjects are a subset of 177 subjects (100 controls, 36 multiplex relatives, and 41 simplex relatives) whose cases were previously reported in a study of verbal memory and the effect of genetic loading.[40] In that study, relatives from multiplex families performed significantly worse than controls and simplex relatives on immediate and delayed logical memories. Relatives from simplex families performed significantly worse than controls only on immediate logical memories. Group × gender interactions were observed. The worse performance of the multiplex group was seen in females. In the table above, statistics are reported for overall group effects for display purposes only.

‡Percent retention = (logical memory delayed recall/logical memory immediate recall)×100.

Table 3. Regression Models for Left Hippocampus Analyzing Different Subsets of Relatives vs Controls*

Subjects Groups	No. of Subjects	Multiplex vs Controls β Weight	Multiplex vs Controls z Score	Multiplex vs Controls P Value	Simplex vs Controls β Weight	Simplex vs Controls z Score	Simplex vs Controls P Value	Multiplex vs Simplex χ^2 Analysis	Multiplex vs Simplex P Value	Linear Trend χ^2 Analysis	Linear Trend P Value	Overall Group χ^2 Analysis	Overall Group P Value
Full sample	90	−.4673	−4.15	<.001	−.1899	−1.79	.07	4.94	.03	11.31	.001	10.89	.004
Age >35 y	53	−.4734	−2.88	.004	−.3207	−2.59	.009	0.92	.34	6.37	.01	7.79	.02
Men	40	−.5640	−2.94	.003	−.3031	−1.59	.11	1.25	.26	4.71	.03	4.80	.09
Women	50	−.4533	−3.76	<.001	−.1450	−1.17	.244	4.72	.03	7.51	.006	7.52	.02
Siblings	77	−.4781	−4.12	<.001	−.2125	−1.43	.153	2.70	.10	9.71	.002	9.87	.007
Adjusting for IQ estimate	90	−.4577	−4.22	<.001	−.1693	−1.53	.13	4.92	.03	11.31	<.001	10.78	.005
Deleting subjects with alcohol dependence†	85	−.4996	−3.95	<.001	−.2333	−2.25	.02	3.01	.08	10.74	.001	11.31	.004
Deleting subjects with SPD†	89	−.4360	−3.90	<.001	−.1774	−1.57	.12	3.60	.06	10.23	.001	9.72	.008
Deleting subjects with MDD†	79	−.4482	−3.61	<.001	−.2649	−2.29	.02	1.47	.23	9.19	.002	10.71	.006
Deleting subjects with hypertension†	88	−.4601	4.24	<.001	−.1765	−1.55	.12	4.69	.03	11.42	<.001	10.92	.004

*All analyses are adjusted for age, sex, handedness, ethnicity, parental education, and total cerebral volume (3 subjects did not have parental education values, so the largest sample size was 90 of 93 subjects). No analyses were significant for the right hippocampus. Multiplex and simplex groups each had 16 sublings. SPD indicates schizotypal personality disorder; and MDD, major depressive disorder.

†These analyses were all adjusted for IQ in addition to age, sex, handedness, ethnicity, parental education, and total cerebral volume.

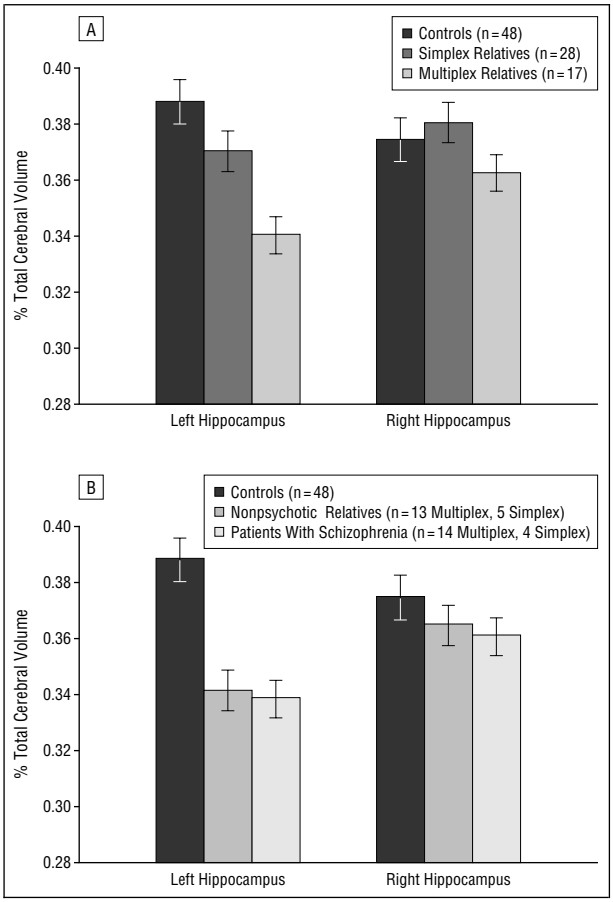

Figure 2. Left and right hippocampal volumes proportionally adjusted for total cerebral volume in control subjects, relatives of schizophrenics, and patients with schizophrenia. A, Comparison of controls and nonpsychotic relatives. B, Comparison of controls, nonpsychotic relatives, and patients with schizophrenia. Percent of total cerebral volume indicates hippocampal volume/total cerebral volume × 100; SEs are corrected for intrafamilial correlation.

psychiatric diagnosis and the number of psychiatric diagnoses per individual on hippocampal volumes. After correcting for the sum of diagnoses, group differences remained significant for the left hippocampus (χ^2_2=7.7, P<.02). A test of linear trend was also significant (χ^2_1=7.7, P<.006). Multiplex relatives (z=−3.86, P<.001) and simplex relatives (z=−2.10, P<.04) had significantly smaller volumes than controls. Multiplex relatives had significantly smaller left hippocampal volumes than simplex relatives (χ^2_1=5.03, P<.03). For the right hippocampus, the results remained nonsignificant.

When we tested whether the presence (vs absence) of any psychiatric diagnosis would attenuate the results, the results did not change. The results did not change when excluding subjects who had other disorders that could affect hippocampal volume (past alcohol dependence [n=5], schizotypal personality disorder [n=1], major depressive disorder [n=11]), nor while controlling for IQ in addition to the other confounders (Table 3).

RELATIONSHIP OF VERBAL DECLARATIVE MEMORY AND HIPPOCAMPAL VOLUMES

We used regression models for verbal memory variables with and without left and right hippocampal volumes included as predictors. Other covariates included sex, group (relatives, controls), the interaction between sex and group, handedness, ethnicity, parental education, and IQ. The left hippocampus was significantly associated with immediate verbal memory (z=3.66, P<.001); the right hippocampus was not (z=−1.86, P=.06). The left hippocampus was significantly associated with delayed verbal memory (z=3.15, P=.001). Group (relatives, controls), the sex × group interaction, and ethnicity remained significant in these models for immediate and delayed verbal memory. The right

hippocampus remained nonsignificant. Hippocampal volumes did not significantly predict percent retention.

To help in understanding the magnitude of the relationship between hippocampal volumes and the residual verbal memory scores, we calculated Pearson correlations, partialling out the confounders described above. For the entire sample, immediate verbal memory was more strongly associated with left (r=0.32) than right (r=0.14) hippocampal volumes. Similarly, delayed verbal memory was more strongly associated with left (r=0.27) than right (r=0.12) hippocampus. Within-group analyses showed that the relationship between left hippocampus and memory seemed to be strongest in the multiplex relatives (**Table 4**).

Table 4. Correlations Between Hippocampal Volumes and Verbal Memory Performance in Controls and Relatives of Patients With Schizophrenia*

	Controls (n = 47)		Simplex Relatives (n = 28)		Multiplex Relatives (n = 15)	
Variables	**Left**	**Right**	**Left**	**Right**	**Left**	**Right**
Logical memory						
Immediate recall	0.28	0.26	0.35	0.01	0.49	0.00
Delayed recall	0.27	0.25	0.20	–0.06	0.51	0.00

*Pearson correlations are of residualized memory scores (adjusted for sex, group, group by sex, handedness, ethnicity, parental education, and IQ) with left and right hippocampal (absolute) volumes. One control subject was not included in the analysis because he or she did not have parental education values. Two multiplex relatives were not included in the analysis because they did not have parental education values.

EFFECTS OF PATIENTS AND THEIR RELATIVES ON HIPPOCAMPAL VOLUMES COMPARED WITH CONTROLS

Groups did not differ significantly on age, sex, parental socioeconomic status, parents' education, ethnicity, handedness, and drug or alcohol use (**Table 5**). There were expected significant differences in education and estimated IQ involving the patients.

There were no significant differences in total cerebral volume among groups (**Table 6**) or between patients vs

Table 5. Demographic Variables for Controls, Patients With Schizophrenia, and Their Nonpsychotic Relatives*

Variable	Controls (n = 48)	Patients (n = 18)	Relatives (n = 18)	F Score	*P* Value
Mean (SD) age at MRI, y	40.1 (10.8)	43.2 (8.3)	44.6 (13.0)	1.09	.34
Mean (SD) parental SES	2.6 (1.0)	3.1 (1.1)	2.7 (1.0)	2.14	.13
Mean (SD) years of parental education	12.1 (2.3)	12.0 (2.6)	11.7 (2.3)	0.23	.80
Mean (SD) years of education	15.1 (2.2)	12.2 (2.4)	13.1 (2.1)	13.37	<.001
Mean (SD) WAIS-R vocabulary	13.2 (3.5)	9.5 (2.5)	11.6 (3.6)	7.73	<.001
Mean (SD) WAIS-R block design	11.4 (2.4)	8.4 (3.1)	12.1 (3.2)	10.03	<.001
Mean (SD) IQ estimate†	112.9 (13.0)	94.1 (13.5)	110.7 (17.5)	11.27	<.001
Mean (SD) WRAT-R reading	105.6 (11.1)	97.0 (17.8)	99.8 (12.5)	3.24	.04
Gender (% male)	56.3	55.6	44.5	. . .	.68
Ethnicity (% white)	93.8	88.9	94.5	. . .	.76
Handedness (% right)	91.7	88.9	83.3	. . .	.64
Present drug use (% of patients)‡					
0	91.7	88.2	100.0	. . .	
1	8.3	11.8	0.0	. . .	
2	0.0	0.0	0.0	. . .	.30
3	0.0	0.0	0.0	. . .	
4	0.0	0.0	0.0	. . .	
Past drug use (% of patients)‡					
0	77.1	70.6	66.7	. . .	
1	8.3	11.8	22.2	. . .	
2	12.5	11.8	5.6	. . .	.45
3	2.1	5.9	5.6	. . .	
4	0.0	0.0	0.0	. . .	
Present alcohol use (% of patients)‡					
0	39.6	70.6	50.0	. . .	
1	47.9	17.7	38.9	. . .	
2	12.5	11.8	11.1	. . .	.41
3	0.0	0.0	0.0	. . .	
4	0.0	0.0	0.0	. . .	
Past alcohol use (% of patients)‡					
0	29.2	47.1	38.9	. . .	
1	50.0	23.5	33.3	. . .	
2	16.7	11.8	16.7	. . .	.37
3	4.2	17.7	11.1	. . .	
4	0.0	0.0	0.0	. . .	

*MRI indicates magnetic resonance imaging; SES, socioeconomic status (for which a lower score indicates a higher status[72]); WAIS-R, Wechsler Adult Intelligence Scale–Revised[56]; WRAT-R, Wide Range Achievement Test-Revised; and ellipses, not applicable.[55] Tests for gender, ethnicity, and handedness were performed using χ^2 analysis (0.77, 0.55, and 0.90, respectively). Frequency of substance use, both past and present, was tested by Fisher exact test.

†IQ estimate was derived from vocabulary and block design age scale scores.

‡For substance abuse ratings, 0 indicates never/occasional use; 1, recreational (episodic) use; 2, regular use; 3, abuse (for a period of 6 months to 5 years); and 4, sustained abuse (for longer than 5 years).

Table 6. Brain Volumes in Controls, Patients With Schizophrenia, and Their Nonpsychotic Relatives*

Variable	Control Subjects (n = 48)	Patients (n = 18)	Relatives (n = 18)	χ^2 Analysis	*P* Value
Total cerebral exterior	1073.0 (101.10)	1096.0 (140.60)	1122.8 (88.60)	2.83	.24
Left hippocampus	4.14 (0.44)	3.68 (0.52)	3.63 (0.40)	13.09	.001
Right hippocampus	3.99 (0.44)	3.94 (0.68)	4.08 (0.46)	0.79	.67

*Data are presented as mean (SD) unadjusted absolute brain volumes, in cubic centimeters^3, as determined by magnetic resonance imaging.

relatives (χ^2_1=2.97, *P*=.10). There was a significant overall group effect for the left, but not the right hippocampus. Patients (β [SE]=–.4819 [.1141], *P*<.001) and relatives (β [SE]=–.4046 [.1010], *P*<.001) had significantly smaller left hippocampi than controls, whereas there were no significant differences for the right hippocampus (Figure 2B). There were no significant differences between patients and relatives for the right hippocampus (χ^2_1=0.27, *P*=.61) or the left hippocampus (χ^2_1=0.64, *P*=.43).

COMMENT

We found strong support for our hypotheses regarding hippocampal volumes and verbal declarative memory deficits as manifestations of vulnerability to schizophrenia. First, nonpsychotic relatives, primarily those from multiplex families, had significantly smaller left hippocampi than controls. Second, there was a linear trend indicating smaller left hippocampi in multiplex as compared with simplex relatives and controls, and significantly smaller left hippocampi in multiplex as compared with simplex relatives. Third, the positive association between verbal memory function and left hippocampal volumes suggests that smaller hippocampi are related to important cognitive dysfunctions. Fourth, patients with schizophrenia had smaller left but not right hippocampal volumes than controls, which is identical to the pattern seen in relatives. While the effect in probands was marginally larger than in nonpsychotic relatives from the same families, there were no significant differences in left hippocampal volumes between them. These results are consistent with the hypothesis that increased genetic liability to schizophrenia affects brain structure[73] and verbal memory,[40] supporting the hypothesis that a smaller left hippocampus and verbal memory deficits are associated vulnerability indicators for schizophrenia.

Our statistical analysis allowed us to adjust for the evaluation of more than one relative per family. Results were equally robust when controlling for psychiatric diagnosis. These findings are virtually identical to our previous reports of no significant effect of psychiatric diagnosis on brain volumes in a smaller sample of mainly simplex relatives[13] and on neuropsychological dysfunction in relatives.[34] Because there were no differences between the controls, relatives, or patients in substance use, these factors cannot account for volumetric differences between relatives and controls. Thus, psychopathology in relatives does not explain their smaller left hippocampi. Because we controlled for demographic features and IQ, these cannot account for the results.

We found comparable effects in relatives who have passed through the peak age of risk for schizophrenia. This suggests that our findings cannot be accounted for by people who will develop schizophrenia. Others have demonstrated significant reductions in *N*-acetyl-aspartate in the hippocampus of unaffected adult siblings of patients with schizophrenia.[74] Smaller hippocampal volumes, especially in the left hemisphere, have been reported in adolescents and young adults (aged 15-25 years) at risk for schizophrenia,[11] suggesting that hippocampal abnormalities are already present by midadolescence.

The absence of a significant difference in left hippocampal volume between nonpsychotic relatives (mainly siblings) and patients with schizophrenia is striking. This argues against the idea that secondary effects of psychosis or its treatment cause smaller hippocampi. Longitudinal studies of first-episode patients with schizophrenia do not demonstrate changes in hippocampal volumes over time.[75-77] These data together point to processes affecting hippocampal volume preceding the onset of illness.

Our study demonstrates an association between left hippocampal volumes and verbal declarative memory in relatives and controls. The association was strongest in multiplex relatives. However, because the sample sizes were small, we have to interpret these correlations cautiously. Nevertheless, the convergence of 2 deficits, theoretically and empirically linked in the broader literature on brain-behavior relationships, provides strong support for the construct validity of our findings.

The cause of these abnormalities is unknown, but our finding of smaller hippocampi in multiplex compared with simplex relatives implicates genes. The distribution of impairment among families is consistent with multifactorial models of familial transmission. Presumably, multiplex families harbor more schizophrenia genes than simplex families, putting relatives at greater risk for both schizophrenia and genetically related deficits. Our results, however, do not address the genetic vs environmental causes of hippocampal deficits, given the inferential limitations of family studies that do not include twins or adoptive relatives.[78] Nor do our data rule out other causes affecting left hippocampi in nonpsychotic relatives, such as acquired brain injury[79] or effects of psychosocial stress on the hippocampus,[80] which could interact with genetic vulnerability. Currently, there are no published studies demonstrating either association in relatives of patients with schizophrenia. It is possible that the hippocampal abnormalities originate from subtle brain injuries similar to those occurring in schizophrenia[81] that are caused by obstetric complications[82] or viruses.[83] There is some support for slightly elevated rates of obstetric com-

plications in nonpsychotic relatives of patients with schizophrenia.[84-86] We also cannot rule out the possibility of later-occurring alterations in developmental processes such as abnormal synaptic pruning or myelination, which could account for the abnormal hippocampus. However, consistent with the occurrence of earlier abnormal brain development, children at risk for schizophrenia show signs of neurological, cognitive, and socioaffective maladjustment as early as the preschool years.[4]

The nature of the subtle memory problems observed in relatives suggests several points worthy of follow-up research. Unlike patients with schizophrenia[61] or patients with amnestic disorders,[23] the relatives do not have abnormal rates of forgetting as compared with controls.[34,35,40] Thus, their memory deficits suggest defects in the acquisition or retrieval, rather than storage, of information. Such difficulties have been linked to posterior hippocampus and other associated structures such as the parahippocampal gyrus,[87] as well as the prefrontal cortex.[88] Further research can determine whether defects in related processes of working memory, encoding, or attention explain the memory impairments, and whether associated brain regions important for memory are impaired in relatives.

Our results must be interpreted in light of some limitations. It would have been optimal to have diagnosed controls in the same way as relatives. Nevertheless, the groups were comparable on demographic factors and did not differ in substance or psychotropic medication use, Moreover, psychiatric diagnoses were not associated with hippocampal abnormalities in relatives. In addition, the smaller left hippocampi in patients with schizophrenia are comparable to those reported by others.[18] Although we did not administer an extensive family history diagnostic interview to the controls, the absence of this information would not bias our findings. This mitigates against the idea that our control group is "super normal." Nevertheless our screening method using elevations on the MMPI could have resulted in a psychiatrically clean control group.

In summary, these results provide support for the hypothesis that expressions of the liability to schizophrenia include a smaller left hippocampus and inefficient verbal declarative memory. Because both genetic factors and obstetric complications have been suggested as risk factors for schizophrenia and for hippocampal dysfunction,[89-91] it is important to investigate the possibility that independent or interactive aspects of these causes may result in left hippocampal abnormalities in relatives. This work also helps to differentiate between vulnerability factors and factors associated with schizophrenic psychosis per se, which is an important distinction for improved treatment and prevention of schizophrenia.[92]

Submitted for publication May 21, 2001; final revision received September 7, 2001; accepted October 1, 2001.

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We thank the following people for their contributions to this project: Mimi Braude, MSW, Deborah Catt, Joanne Donatelli, Elizabeth Hoge, MD, Lynda Jacobs, Jennifer Koch, Genichi Matsuda, MD, Camille McPherson, Catherine Monaco, PhD, James Myers, John Pepple, PhD, Anne Shore, Jason Tourville, Michael Ward, PhD, Heidi Wencel, PhD, Judith Wides, and Andrew Worth, PhD.

This article was supported in part by grants from the Theodore and Vada Stanley Foundation, Bethesda, Md, and the National Association for Research in Schizophrenia and Affective Disorders (NARSAD), Great Neck, NY (Dr Seidman); grants SDA K21 MH 00976 and MH 56956 from the National Institute of Mental Health, Bethesda (Dr Goldstein); a grant from NARSAD (Dr Makris); a grant from the Fairway Trust, Kingston Upon Thames, England (Dr Kennedy); and the NARSAD Distinguished Investigator Award and grants MH 43518 and 46318 from the National Institute of Mental Health (Dr Tsuang).

This work was presented, in part, at the Annual Meeting of the American College of Neuropsychopharmacology, Acapulco, Mexico, December 13, 1999, and the Annual Meeting of the Society of Biological Psychiatry, Chicago, Ill, May 11, 2000.

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