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Hippocampal Volume Is Reduced in Schizophrenia and Schizoaffective Disorder But Not in Psychotic Bipolar I Disorder Demonstrated by Both Manual Tracing and Automated Parcellation (FreeSurfer)

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This study examined hippocampal volume as a putative biomarker for psychotic illness in the *Bipolar-Schizophrenia Network on Intermediate Phenotypes* (B-SNIP) psychosis sample, contrasting manual tracing and semiautomated (FreeSurfer) region-of-interest outcomes. The study sample (n = 596) included probands with schizophrenia (SZ, n = 71), schizoaffective disorder (SAD, n = 70), and psychotic bipolar I disorder (BDP, n = 86); their first-degree relatives (SZ-Rel, n = 74; SAD-Rel, n = 62; BDP-Rel, n = 88); and healthy controls (HC, n = 145). Hippocampal volumes were derived from 3Tesla T1-weighted MPRAGE images using manual tracing/3DSlicer3.6.3 and semiautomated parcellation/FreeSurfer5.1,64bit. Volumetric outcomes from both methodologies were contrasted in HC and probands and relatives across the 3 diagnoses, using mixed-effect regression models (SAS9.3 Proc MIXED); Pearson correlations between manual tracing and FreeSurfer outcomes were computed. SZ (P = .0007–.02) and SAD (P = .003–.14) had lower hippocampal volumes compared with HC, whereas BDP showed normal volumes bilaterally (P = .18–.55). All relative groups had hippocampal volumes not different from controls (P = .12–.97) and higher than those observed in probands (P = .003–.09), except for FreeSurfer measures in bipolar probands vs relatives (P = .64–.99). Outcomes from manual tracing and FreeSurfer showed direct, moderate to strong, correlations (r = .51–.73, P < .05). These findings from a large psychosis sample support decreased hippocampal volume as a putative biomarker for schizophrenia and schizoaffective disorder, but not for psychotic bipolar I disorder, and may reflect a cumulative effect of divergent primary disease processes and/or lifetime medication use. Manual tracing and semiautomated parcellation regional volumetric approaches may provide useful outcomes for defining measurable biomarkers underlying severe mental illness.

Key words: schizophrenia/psychotic bipolar disorder/hippocampus/manual tracing/FreeSurfer

Introduction

Alterations in medial temporal lobe anatomy and function are consistently reported in schizophrenia and include (1) hippocampal volume reduction,[1–5] (2) elevated hippocampal regional blood flow,[6] (3) reduced task-associated activation as probed by novelty and memory tasks,[7–10] (4) associations between hippocampal alterations and severity of psychosis,[4,6] and (5) attenuation of hippocampal-dependent relational memory dysfunction by antipsychotic medication.[11] Furthermore, structural and functional hippocampal abnormalities are found in other psychotic, mood, and anxiety disorders, as well as neurodegenerative conditions[12–15] suggesting that hippocampal vulnerability may be a common biomarker underlying a broad array of psychiatric and neurologic phenotypes. Although molecular mechanisms of these hippocampal alterations remain unknown, several putative determinants have been proposed, including glutamate/NMDA-[16,17], GABA-[18,19], and cortisol-mediated[20] metaplasticity changes resulting in hippocampal subfield-specific disease vulnerability and, possibly, psychosis formation.[16,21] Given this broad link between hippocampal alterations and psychosis, we examined whether regional hippocampal volumetric characteristics show common and/or distinctive features across the schizophrenia–psychotic bipolar I disorder diagnoses in

a large sample of probands and their first-degree relatives from the *Bipolar-Schizophrenia Network on Intermediate Phenotypes* (B-SNIP),[22] with the aim of understanding the biological determinants of lifetime psychosis.

Hippocampal volumetric characteristics have been previously examined in psychotic disorders, mostly in small samples, using a "gold standard" manual tracing region-of-interest (ROI) approach[1,13,23–26] and, more recently, semiautomated methods utilizing edge-detection and tissue intensity differences, such as FreeSurfer.[2,27,28] In schizophrenia, reduced hippocampal volumes compared with healthy controls (HC) are well documented,[1–5] especially for the left hippocampus,[27,29,30] though some studies report no volume differences.[13,26] In bipolar disorder, data are inconsistent, ranging from normal,[1,24,26,31] to decreased,[12,13] to increased[32] hippocampal volumes compared with controls, with some reports of asymmetric alterations, eg, smaller volume in the right but not the left hippocampus.[12,13] A recent meta-analysis[33] reported smaller bilateral hippocampal volumes in probands with schizophrenia (SZ) vs bipolar probands. A few small studies have specifically focused on hippocampal volumes in psychotic vs nonpsychotic bipolar phenotypes: Strasser *et al*[24] found no differences in hippocampal volumes between SZ and nonpsychotic and psychotic bipolar disorder probands (BDP), whereas McDonald *et al*[31] reported smaller bilateral hippocampi in SZ than in BDP; both found no differences in BDP vs HC. Furthermore, analyses that included the amygdala-hippocampus complex into a single ROI have shown similar volumes across schizophrenia, psychotic bipolar disorder,[15,34] and psychotic depression,[15] suggesting that the ROI definition may introduce further variability in the volumetric outcomes. No study has examined hippocampal volume in schizoaffective disorder as a unique group, but manual tracing reports from mixed schizophrenia and schizoaffective proband (SAD) samples have shown smaller volumes of hippocampus[25,35] and amygdala-hippocampus complex[34] compared with HC.

Hippocampal volume alterations have also been reported in biological relatives of psychosis probands, albeit with substantial variability in findings. Studies in relatives of SZ (SZ-Rel)[25,36,37] have reported hippocampal volumes intermediate between those found in SZ and HC, with characteristic hippocampal volume reductions observed in unaffected relatives.[36,38–40] Seidman *et al*[39] reported more substantial left hippocampal volume reductions in individuals with 2 or more first-degree relatives with schizophrenia, suggesting familial cosegregation of this biomarker in psychosis. In contrast, other studies found normal hippocampal volumes in SZ-Rel.[2,27,41] A few small reports in first-degree relatives of BDP (BDP-Rel) showed hippocampal volumes similar to those in HC,[31,42] but larger than those found in probands.[43] To date, no study has examined hippocampal characteristics unique to relatives of SAD (SAD-Rel) although the data from mixed schizoaffective disorder/schizophrenia relatives samples have produced outcomes consistent with those in SZ-Rel alone.[25,35] No studies have contrasted hippocampal characteristics in relatives based on psychosis-relevant clinical manifestations, ie, relatives with psychosis spectrum disorders vs unaffected relatives.

Overall, hippocampal volume outcomes from the "gold standard" manual and semiautomated studies, all of limited sample size, support diminished volumes in SZ and SAD, with more variable observations in BDP and in biological relatives of psychosis probands. The hippocampal volume abnormalities associated with psychosis are subtle, averaging 2%–4% decrease in SZ (see meta-analyses);[3,5] thus, larger samples are necessary to support reliable characterization of these putative brain structure biomarkers.

This study examined hippocampal volume using manual tracing and semiautomated parcellation, FreeSurfer, contrasting these outcomes across the schizophrenia-bipolar I disorder psychosis dimension in a large sample of probands and their first-degree relatives. We tested whether hippocampal volume measures would show common or divergent characteristics across the 3 psychoses diagnoses—schizophrenia, schizoaffective disorder, and psychotic bipolar I disorder—contrasting (1) probands and HC, (2) relatives and HC, and (3) probands and relatives. We hypothesized that (1) probands will show decreased hippocampal volumes compared with HC, consistent across schizophrenia, schizoaffective disorder, and psychotic bipolar I disorder diagnoses, and (2) relatives will show hippocampal volumes similar across the 3 diagnostic groups and intermediate in magnitude between volumes observed in their respective probands and controls.

Methods

Study Sample

Five hundred ninety-six subjects were included in this analysis, including 227 psychosis probands (71 SZ, 70 SAD, and 86 BDP), 224 first-degree relatives (74 SZ-Rel, 62 SAD-Rel, and 88 BDP-Rel), and 145 HC from 2 B-SNIP sites (University of Chicago, J.A.S.; UT Southwestern Medical Center at Dallas, C.A.T.). Detailed characteristics of the B-SNIP clinical population are described elsewhere.[22] The study was approved by the Institutional Review Boards at each site and was consistent with standards for the ethical conduct of human research. All subjects provided written informed consent after the study procedures had been fully explained. Axis I diagnoses in probands and affected relatives were established based on the Structured Clinical Interview for DSM-IV Diagnosis (SCID-I/P),[44] and Axis II diagnoses in relatives were established based on the Structured Interview for DSM-IV Personality Disorders (SIDP-IV).[45] Probands were clinically stable, mostly medicated outpatients. Relatives

with lifetime psychiatric diagnoses were asymptomatic/mildly symptomatic at the time of the imaging acquisition. Given potential confounders related to lifetime disease burden and treatments, relatives with lifetime Axis I psychotic disorders (n = 26 with "proband-like" psychosis diagnoses, ie, schizophrenia, schizoaffective disorder, and psychotic bipolar disorder, and n = 2 with other Axis I psychoses) were excluded. Relatives with psychosis spectrum disorders (cluster A: schizoid, paranoid, and schizotypal personality disorders) (n = 24), those with nonpsychotic Axis I and/or II diagnoses (eg, mood and anxiety disorders, cluster B, C personality disorders) (n = 72), and those completely unaffected (n = 126) were included, providing basis for an exploratory analysis contrasting hippocampal volumes in affected vs unaffected relatives. Rates of DSM-IV Axis I-II diagnoses in relatives are presented in supplementary table 1.

Demographic, clinical, and cognitive characteristics of the study sample are detailed in table 1. There were no between-group differences in age or handedness. The groups differed in sex due to a higher proportion of males among SZ and SAD compared with other groups. While no differences in ethnicity were observed, the groups differed in race due to a higher proportion of African Americans among SZ and SAD. There were differences in years of education, where SZ had lower education attainment than BDP, BDP-Rel, and HC. The proband groups did not differ in age of illness onset, age at first psychiatric hospitalization, or lifetime number of hospitalizations. SZ and SAD had higher Positive and Negative Syndrome Scale (PANSS)[46] total, positive, negative, and general subscales scores compared with BDP. SAD showed the highest scores on Montgomery-Asberg Depression Rating Scale (MADRS)[47] and Young Mania Research Scale (YMRS).[48] All proband and relative groups scored lower than controls on the Global Assessment of Functioning, with the lowest ratings observed in SZ and SAD. The Reading Subtest scores from Wide Range Achievement Test (WRAT), used as an estimate of premorbid intellectual functioning, differed across groups, accounted for by lower scores in SZ compared with that in BDP, all relative groups, and HC, as well as lower scores in SZ-Rel compared with BDP and BDP-Rel. Additionally, probands had lower composite and subscale scores on the Brief Assessment of Cognition in Schizophrenia (BACS) neuropsychological test battery,[49] compared to relatives and controls, consistent with our previous report.[50] Most probands were actively treated with various psychotropic medications including antipsychotics (81.9%), mood stabilizers (48.0%), and antidepressants (45.8%); 73.6% of probands were treated with psychotropic agents of more than 1 class. Among relatives, the majority was unmedicated (82.4% SZ-Rel, 69.4% SAD-Rel, and 64.8% BDP-Rel); antidepressants were the most common treatment in all relative groups.

Structural Magnetic Resonance Imaging Acquisition and the Hippocampal ROI Definitions

Whole brain structural magnetic resonance imaging (sMRI) 3D images were acquired on 3Tesla scanners (University of Chicago: GE Signa, UT Southwestern Medical Center: Philips Achieva). All subjects at each site were scanned on the same magnet. High-resolution isotropic T1-weighted Magnetization Prepared RApid Gradient Echo (MPRAGE) sequences were obtained following the Alzheimer's Disease Neuroimaging Initiative protocol (http://adni.loni.usc.edu). The MPRAGE sequence parameters were comparable across sites (detailed in supplementary methods).

All images were processed by experienced analysts (manual ROI tracing: S.J.M.A., T.A.G., A.P.R.; FreeSurfer: A.N.F., N.T.) blind to subjects' clinical characteristics. Manual hippocampal ROI tracing was performed using 3DSlicer/v.3.6.3 (http://www.slicer.org). Within- and between-rater reliability was established at >90% agreement and checked every 4 weeks throughout tracing period. Tracing was performed on the coronal view, with all views simultaneously visible in 3DSlicer.[51] The area defined as hippocampal ROI is bounded laterally and medially by the lateral ventricle, anteriorly by the hippocampal-amygdala transitional zone, posteriorly by the crus of the fornix, inferiorly by the subiculum, and superiorly by the alveus, similar to Keshavan *et al*[23] (detailed in supplementary methods and supplementary figure 1A). Hippocampal mask labeling relied on a tissue-based definition of the hippocampus proper and included cornu ammonis1 (CA1), CA2/3, dentate gyrus/CA4, and fimbria, avoiding subiculum, entorhinal cortex, and hippocampus-amygdala transitional zone. All ROI definitions were checked against a standardized anatomical brain atlas.[52]

Hippocampal volume outputs were extracted from FreeSurfer/v.5.1-64bit (http://surfer.nmr.mgh.harvard.edu), following standardized steps of preprocessing, subcortical and cortical parcellation, and automated labeling algorythm[53–55] (detailed in supplementary methods). The hippocampal mask included CA1, CA2/3, CA4/dentate gyrus, subiculum/presubiculum, and fimbria (http://surfer.nmr.mgh.harvard.edu), yielding a more inclusive ROI than our manual tracing definition (supplementary figure 1B). Left and right hippocampal volumes (as primary outcomes) as well as total intracranial volume (as a covariate in mixed model analyses) from FreeSurfer were used. Intracranial volumes (group mean ± standard deviation, mm^3: SZ, 1488796.49 ± 217813.15; SAD, 1389376.66 ± 186550.83; BDP, 1444642.19 ± 175834.12; SZ-Rel, 1425362.36 ± 197966.93; SAD-Rel, 1417176.02 ± 158748.94; BDP-Rel, 1495038.38 ± 158848.76; HC, 1451021.05 ± 177621.46) were comparable to previously reported in similar clinical populations.[28,56] There was an effect of diagnostic group on intracranial volume ($F(6,505) = 2.78$, $P = .01$), accounted for by lower volume

Table 1. Sociodemographic, Clinical, and Cognitive Characteristics of the Study Sample

	SZ (n = 71)	SAD (n = 70)	BDP (n = 86)	SZ-Rel (n = 74)	SAD-Rel (n = 62)	BDP-Rel (n = 88)	HC (n = 145)	Test Statistic	P Value
Sociodemographic characteristics									
Age, years; Mean (SD)	35.9 (12.4)	37.6 (12.7)	35.8 (13.2)	40.7 (16.1)	39.3 (15.4)	38.9 (16.2)	38.4 (12.4)	$F(6,589) = 1.24$	.29
Sex/Male; n (%)	44 (62.0)	32 (45.7)	25 (29.1)	18 (24.3)	18 (29.0)	28 (31.8)	67 (46.2)	$\chi^2(6) = 34.49$	<.001[a]
Handedness; n (%)[b]								$\chi^2(12) = 20.11$	.07
Right handed	59 (83.1)	64 (91.4)	72 (83.7)	66 (89.2)	59 (95.2)	74 (84.1)	129 (89.0)		
Left handed	9 (12.7)	3 (4.3)	14 (16.3)	6 (8.1)	2 (3.2)	14 (15.9)	13 (9.0)		
Ambidextrous	1 (1.4)	3 (4.3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.7)		
Ethnicity/Hispanic; n (%)	10 (14.2)	6 (8.6)	6 (7.0)	11 (14.9)	3 (4.8)	8 (9.1)	22 (15.2)	$\chi^2(6) = 8.22$	.22
Race; n (%)								$\chi^2(12) = 51.24$	<.001[c]
Caucasian	32 (45.1)	32 (45.7)	61 (70.9)	39 (52.7)	41 (66.1)	75 (85.2)	96 (66.2)		
African American	34 (47.9)	35 (50.0)	19 (22.1)	28 (37.8)	19 (30.6)	11 (12.5)	36 (24.8)		
Other	5 (7.0)	3 (4.3)	6 (7.0)	7 (9.5)	2 (3.2)	2 (2.3)	13 (9.0)		
Education, years; Mean (SD)	12.8 (2.5)	13.3 (2.5)	14.2 (2.5)	14.0 (2.3)	13.8 (2.9)	14.6 (2.8)	14.5 (2.3)	$F(6,581) = 5.59$	<.001[d]
Clinical characteristics; Mean (SD)									
Age of illness onset, years	20.4 (8.3)	19.0 (9.4)	19.8 (9.2)	—	—	—	—	$F(2,222) = 0.46$	.63
Age of first hospitalization, years	22.6 (7.5)	24.2 (9.4)	24.7 (10.1)	—	—	—	—	$F(2,190) = 0.92$	.4
Number of lifetime hospitalizations	5.6 (6.5)	6.6 (7.0)	4.6 (6.0)	—	—	—	—	$F(2,185) = 1.34$	.26
PANSS									
Total	75.9 (15.7)	74.6 (14.0)	58.8 (13.6)	—	—	—	—	$F(2,220) = 34.33$	<.001[e]
Positive subscale	19.4 (4.9)	20.3 (4.1)	13.8 (4.2)	—	—	—	—	$F(2,220) = 50.66$	<.001
Negative subscale	19.2 (6.3)	16.1 (4.6)	13.4 (4.3)	—	—	—	—	$F(2,220) = 24.39$	<.001
General symptoms subscale	37.3 (7.8)	38.2 (7.8)	31.6 (7.9)	—	—	—	—	$F(2,220) = 16.24$	<.001
YMRS	7.2 (5.8)	8.4 (5.5)	5.8 (6.2)	—	—	—	—	$F(2,220) = 4.81$	.009[f]
MADRS	10.1 (8.1)	14.5 (9.6)	10.9 (9.3)	—	—	—	—	$F(2,220) = 3.79$	.02[g]
GAF	43.1 (9.7)	44.5 (9.7)	58.9 (12.0)	76.0 (12.3)	76.1 (12.9)	75.2 (12.1)	86.2 (5.0)	$F(6, 586) = 234.32$	<.001[h]
Cognitive characteristics									
WRAT IQ, Mean (SD)	92.5 (16.5)	96.2 (13.7)	102.2 (14.2)	94.9 (15.0)	101.3 (17.0)	102.5 (13.3)	100.9 (14.2)	$F(6,580) = 5.76$	<.001[i]
BACS, z-score, Mean (SD)									
Composite	−1.7 (1.2)	−1.4 (.2)	−0.9 (1.3)	−0.5 (1.1)	−0.3 (1.3)	−0.08 (1.2)	−0.09 (1.2)	$F(6,570) = 21.8$	<.001[j]
Verbal memory	−1.1 (1.3)	−0.8 (1.3)	−0.5 (1.3)	−0.4 (1.3)	−0.2 (1.1)	−0.2 (1.2)	−0.1 (1.1)	$F(2,533) = 5.82$	<.001
Digit sequencing	−1.2 (1.2)	−0.9 (1.2)	−0.7 (1.2)	−0.4 (1.1)	−0.2 (1.1)	−0.04 (1.1)	−0.2 (1.2)	$F(2,533) = 9.9$	<.001
Token motor	−1.3 (1.2)	−1.5 (1.0)	−0.9 (1.3)	−0.4 (1.0)	−0.2 (0.9)	−0.2 (1.0)	−0.02 (1.1)	$F(2,527) = 20.9$	<.001
Verbal fluency	−0.8 (1.2)	−0.3 (1.1)	−0.2 (1.1)	−0.1 (1.1)	0.2 (1.3)	0.2 (1.2)	0.1 (1.1)	$F(2, 33) = 6.34$	<.001
Symbol coding	−1.3 (1.1)	−1.2 (1.2)	−0.9 (1.0)	−0.5 (1.1)	−0.2 (1.0)	−0.2 (1.0)	0.02 (1.0)	$F(2,533) = 19.6$	<.001
Tower of London	−0.8 (1.4)	−0.6 (1.3)	−0.2 (1.2)	−0.2 (1.0)	−0.2 (1.2)	0.2 (0.8)	−0.002 (1.1)	$F(2,532) = 6.6$	<.001
Concomitant medications; n (%)[b]									
Off-psychotropic medications	7 (9.9)	6 (8.6)	5 (5.9)	61 (82.4)	43 (69.4)	57 (64.8)	140 (96.6)	—	—
Antipsychotics (any)	62 (87.3)	59 (84.3)	65 (75.6)	1 (1.4)	2 (3.2)	5 (5.7)	0 (0.0)	—	—
Typical	9 (12.7)	5 (7.1)	8 (9.3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	—	—
Atypical	53 (74.7)	54 (77.1)	57 (66.3)	1 (1.4)	2 (3.2)	5 (5.7)	0 (0.0)	—	—
Mood stabilizers (any)	14 (19.7)	38 (54.3)	57 (66.3)	0 (0.0)	6 (9.7)	8 (9.1)	0 (0.0)	—	—
Lithium	3 (4.2)	7 (10.0)	20 (23.3)	0 (0.0)	2 (3.2)	2 (2.3)	0 (0.0)	—	—
Other	11 (15.5)	31 (44.3)	37 (43.0)	0 (0.0)	4 (6.5)	6 (6.8)	0 (0.0)	—	—

Table 1. Continued

	SZ (n = 71)	SAD (n = 70)	BDP (n = 86)	SZ-Rel (n = 74)	SAD-Rel (n = 62)	BDP-Rel (n = 88)	HC (n = 145)	Test Statistic	P Value
Antidepressants (any)	26 (36.6)	36 (51.4)	42 (48.8)	10 (13.5)	15 (24.2)	19 (21.6)	1 (0.7)	—	—
Tricyclic	1 (1.4)	0 (0.0)	2 (2.3)	1 (1.4)	1 (1.6)	0 (0.0)	0 (0.0)	—	—
Other (SSRI, SNRI, etc.)	25 (35.2)	36 (51.4)	40 (46.5)	9 (12.2)	14 (22.6)	19 (21.6)	1 (0.7)	—	—
Antiparkinsonian	11 (15.5)	7 (10.0)	8 (9.3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	—	—
Stimulants	6 (8.5)	4 (5.7)	7 (8.1)	0 (0.0)	3 (4.8)	6 (6.8)	0 (0.0)	—	—
Combined medications	43 (60.6)	57 (81.4)	67 (77.9)	3 (4.1)	7 (11.3)	10 (11.4)	0 (0.0)	—	—

Note: SZ, probands with schizophrenia; SAD, probands with schizoaffective disorder; BDP, probands with psychotic bipolar I disorder; SZ-Rel, relatives of probands with schizophrenia; SAD-Rel, relatives of probands with schizoaffective disorder; BDP-Rel, relatives of probands with psychotic bipolar I disorder; HC, healthy controls; PANSS, the Positive and Negative Syndrome Scale; YMRS, the Young Mania Rating Scale; MADRS, the Montgomery-Asberg Depression Rating Scale; GAF, the Global Assessment of Functioning; WRAT, Wide Range Achievement Test IQ estimate; BACS, the Brief Assessment of Cognition in Schizophrenia.

[a]Sex: Higher proportion of males in (1) SZ vs BDP ($\chi^2(1) = 15.78$, $P < .001$), all relative groups (SZ-Rel [$\chi^2(1) = 19.47$, $P < .001$], SAD-Rel [$\chi^2(1) = 13.14$, $P < .001$], BDP-Rel [$\chi^2(1) = 13.23$, $P < .001$]), and HC ($\chi^2(1) = 4.13$, $P = .04$); (2) SAD vs BDP ($\chi^2(1) = 3.92$, $P < .047$) and SZ-Rel ($\chi^2(1) = 6.35$, $P = .01$); and (3) HC vs BDP ($\chi^2(1) = 6.35$, $P = .01$) and all relative groups (SZ-Rel [$\chi^2(1) = 8.98$, $P = .003$], SAD-Rel [$\chi^2(1) = 4.95$, $P = .03$], BDP-Rel [$\chi^2(1) = 4.48$, $P = .03$]).

[b]Missing data: (1) Handedness: 2/71 SZ, 2/74 SZ-Rel, 1/62 SAD-Rel, 2/145 HC; (2) Concomitant medications: 2/71 SZ, 2/74 SZ-Rel, 1/62 SAD-Rel, 1/88 BDP-Rel, 1/145 HC. Each psychotropic medication class in each subject is reported separately. The number of subjects who were treated with more than 1 psychotropic medication is indicated under "Combined medications."

[c]Race: Higher proportion of African Americans in (1) SZ vs BDP ($\chi^2(1) = 10.89$, $P = .001$), HC ($\chi^2(1) = 10.28$, $P = .001$), and relatives (SAD-Rel [$\chi^2(1) = 4.3$, $P = .04$], BDP-Rel [$\chi^2(1) = 25.04$, $P < .001$]; and (2) SAD vs BDP [$\chi^2(1) = 11.54$, $P < .001$], HC [$\chi^2(1) = 11.01$, $P < .001$], and relatives (SAD-Rel [$\chi^2(1) = 4.67$, $P = .03$], BDP-Rel [$\chi^2(1) = 26.03$, $P < .001$]). Lower proportion of African Americans in BDP vs SZ-Rel [$\chi^2(1) = 4.66$, $P = .03$].

[d]Education: Lower years of education in (1) SZ vs BDP ($P = .009$), BDP-Rel ($P < .001$), and HC ($P < .001$); (2) SAD vs BDP-Rel ($P = .02$) and HC ($P = .02$).

[e]PANSS/total: Lower scores in BDP vs SZ ($P < .001$) and SAD ($P < .001$). PANSS/positive: Lower scores in BDP vs SZ ($P < .001$) and SAD ($P < .001$). PANSS/negative: Lower scores in BDP vs SZ ($P < .001$) and SAD ($P = .002$). PANSS/general: Lower scores in BDP vs SZ ($P < .001$) and SAD ($P < .001$).

[f]YMRS: Higher scores in SAD vs BDP ($P = .02$).

[g]MADRS: Higher scores in SAD vs BDP ($P = .03$).

[h]GAF: Lower scores in (1) all proband and relative groups vs HC (all $P < .001$); (2) SZ and SAD vs BDP, all relative groups, and HC (all $P < .001$).

[i]WRAT: (1) Lower scores in SZ vs BDP ($P < .001$), SAD-Rel ($P < .01$), BDP-Rel ($P < .001$), and HC ($P < .002$); (2) SZ-Rel vs BDP ($P = .03$) and BDP-Rel ($P = .02$).

[j]BACS composite: (1) Lower scores in SZ vs BDP ($P = .002$), all relative groups ($P < .001$), and HC ($P < .001$); (2) SAD vs BDP, all relatives and HC (all $P < .001$); (3) BDP vs SAD-Rel ($P = .045$), BDP-Rel and HC (all $P < .001$). Verbal memory: (1) Lower scores in SZ vs SZ-Rel ($P = .02$), SAD-Rel ($P = .006$), BDP-Rel and HC (all $P < .001$); (2) SAD vs HC ($P = .01$). Digit sequencing: (1) Lower scores in SZ vs BDP ($P = .048$), all relatives and HC (all $P < .001$); (2) SAD vs SAD-Rel ($P = .03$), BDP-Rel and HC (all $P < .001$); BDP vs BDP-Rel ($P = .006$). Token motor: (1) Lower scores in SZ vs all relatives and HC (all $P < .001$); (2) SAD vs BDP ($P = .01$), all relatives and HC (all $P < .001$). Verbal fluency: (1) Lower scores in SZ vs BDP ($P = .04$); SZ-Rel ($P = .02$); SAD-Rel, BDP-Rel, and HC (all $P < .001$). Symbol coding: (1) Lower scores in SZ and SAD vs all relatives and HC (all $P < .001$, except for SAD vs SZ-Rel, $P = .006$); (2) BDP vs SAD-Rel ($P = .009$), BDP-Rel and HC ($P < .001$). Tower of London: (1) Lower scores in SZ and SAD vs BDP-Rel and HC (all $P < .001$, except for SAD vs HC, $P = .02$).

in SZ vs BDP-Rel (adjusted $P = .02$); the rest of pairwise group comparisons were nonsignificant.

Statistical Analyses

A one-way analysis of variance with a subsequent post hoc Tukey Honest Significant Difference test and Yates corrected chi-square test were used, as appropriate, for demographic, clinical, and cognitive variables. To test a priori hypotheses, the left and right hippocampal volumes from manual tracing and semiautomated parcellation/FreSurfer were contrasted in (1) probands (SZ, SAD, and BDP) and controls, (2) relatives (SZ-Rel, SAD-Rel, and BDP-Rel) and controls, and (3) probands and their respective relatives (SZ vs SZ-Rel, SAD vs SAD-Rel, and BDP vs BDP-Rel). A maximum likelihood approach was used to fit mixed-effect regression models for the left and right hippocampal volume from manual or semiautomated tracing variables (SAS9.3 Proc MIXED procedure) controlling for the following covariates: age, sex, handedness (left-handed indicator), and FreeSurfer-derived total intracranial volume. The family cluster (ie, a random code assigned to all probands and relatives from the same pedigree) and site variables were incorporated as random effects in the model. The model specified the covariance structure within subjects using an unstructured model to account for the within-subject correlation across family clusters and sites. A family cluster effect was included in the mixed-effect model only for analyses comparing probands and their respective relatives. F test was used to test a diagnostic group variance in mean estimates, and t test was used to test pairwise between-group differences in mean estimates of the left and right hippocampal volumes from manual tracing and FreeSurfer.

Fourteen percent of the study sample (including 9 SZ, 6 SAD, 7 BDP, 17 SZ-Rel, 7 SAD-Rel, 21 BDP-Rel, and 17 HC) had missing FreeSurfer-derived hippocampal volume and total intracranial volume data. The missing intracranial volume variables were imputed using multiple imputation (MI) method[57] and subsequently used as a covariate in both manual- and FreeSurfer-based hippocampal volume analyses. MI is a simulation-based inferential tool operating on multiple completed data sets, where the missing values are replaced by random draws from their respective predictive distributions following Monte Carlo Markov Chain (SAS9.3 Proc MI procedure). This study used 50 sets of completed data, then analyzed by standard complete data methods, and the results were combined into a single inferential statement using rules to yield estimates, standard errors, and P values that formally incorporate the missing-data uncertainty into the modeling process (SAS9.3 Proc MIANALYZE).

In addition, a series of exploratory analyses was conducted to examine potential disease and medication effects on hippocampal volume using maximum likelihood mixed-effect regression models (SAS9.3 Proc MIXED). (1) To explore whether hippocampal volume outcomes cosegregate in probands and relatives within the same pedigree, relatives were stratified by the hippocampal volumes in their respective probands using a "median split" approach.[8] Subsequently, relatives of probands with hippocampal volumes above vs below group median were compared. (2) To examine effect of lifetime mild psychosis manifestations on hippocampal volume, relatives with psychosis spectrum/cluster A personality disorders ($n = 24$) were contrasted with relatives without lifetime psychosis ($n = 198$), including those completely unaffected ($n = 126$) and those with nonpsychotic Axis I/II disorders ($n = 72$). (3) Pearson correlations were computed between hippocampal volumes and symptom severity scores from PANSS, YMRS, and MADRS (in probands) and between hippocampal volumes and BACS composite and declarative/verbal memory scores (in proband, relative, and control groups). (4) To investigate effect of active medication use, hippocampal volume outcomes were contrasted in all probands combined who were actively treated with antipsychotic medications vs those off-antipsychotics and those on- vs off-lithium. In addition, Pearson correlations between antipsychotic dose chlorpromazine equivalents[58] and hippocampal volumes from manual tracing and FreeSurfer were calculated.

Pearson correlations between hippocampal volume outcomes from the 2 sMRI methodologies were also computed.

Results

Hippocampal Volume in Probands

Hippocampal volume outcomes in the psychosis probands and HC from manual tracing and FreeSurfer are presented in table 2A and figure 1A. Effect of the diagnostic group was observed for the left manual ($P = .088$, trend) and bilateral FreeSurfer (left, $P = .005$; right, $P = .001$) measures. A priori planned pairwise comparisons showed lower hippocampal volumes in SZ and SAD compared with HC based on manual tracing ($P = .02$–$.09$, except for the right hippocampus in SAD $P = .14$) and FreeSurfer ($P = .0007$–$.009$). In contrast, all outcomes in BDP were not different from controls ($P = .18$–$.55$). Furthermore, FreeSurfer measurements showed reduced hippocampal volumes in SZ and SAD compared with BDP ($P = .02$–$.07$).

Hippocampal Volume in Relatives

Hippocampal volume outcomes in relatives of the psychosis probands and HC based on manual tracing and FreeSurfer are reported in table 2B and figure 1B. No overall effect of the diagnostic group ($P = .39$–$.99$) was observed for any of the hippocampal measures, using

Table 2. Hippocampal Volume Outcomes in Probands, Relatives, and Healthy Controls Based on Manual Tracing and FreeSurfer

Hippocampal Volume Measure	Hippocampal Volume, Mean ± SD, mm^3	Diagnostic Group Effect	Planned Pairwise Comparisons	df	*t*	*P*
A. Probands						
Manual[a]						
Left (*n* = 368)	SZ, 2691.73 ± 447.45	**$F(3,8.7\times10^5) = 2.18$, $P = .09$[b]**	**SZ vs HC**	**353.00**	**2.33**	**.02**
	SAD, 2638.75 ± 344.41		**SAD vs HC**	**353.03**	**1.67**	**.09**
	BDP, 2689.55 ± 351.71		BDP vs HC	354.68	1.34	.18
	HC, 2752.59 ± 357.48		SZ vs SAD	351.07	0.56	.57
			SZ vs BDP	353.28	0.93	.35
			SAD vs BDP	354.09	0.35	.72
Right (*n* = 368)	SZ, 2621.78 ± 392.91	$F(3,1.64\times10^5) = 1.52$, $P = .21$[b]	**SZ vs HC**	**354.65**	**1.92**	**.06**
	SAD, 2579.01 ± 333.83		SAD vs HC	353.86	1.50	.14
	BDP, 2612.02 ± 391.66		BDP vs HC	355.57	0.99	.32
	HC, 2671.77 ± 339.26		SZ vs SAD	353.25	0.36	.72
			SZ vs BDP	354.42	0.86	.39
			SAD vs BDP	354.32	0.49	.63
FreeSurfer[a]						
Left (*n* = 328)	SZ, 3952.95 ± 453.58	**$F(3,319) = 4.39$, $P = .005$**	**SZ vs HC**	**319**	**2.64**	**.009**
	SAD, 3821.80 ± 415.74		**SAD vs HC**	**319**	**3.01**	**.003**
	BDP, 3979.70 ± 371.57		BDP vs HC	319	0.60	.55
	HC, 4030.24 ± 388.65		SZ vs SAD	319	0.29	.77
			SZ vs BDP	**319**	**1.85**	**.07**
			SAD vs BDP	**319**	**2.16**	**.03**
Right (*n* = 332)	SZ, 4000.92 ± 491.60	**$F(3,323) = 5.46$, $P = .001$**	**SZ vs HC**	**323**	**3.41**	**.0007**
	SAD, 3886.05 ± 412.06		**SAD vs HC**	**323**	**2.98**	**.003**
	BDP, 4064.61 ± 451.76		BDP vs HC	323	0.86	.39
	HC, 4112.88 ± 405.26		SZ vs SAD	323	0.39	.70
			SZ vs BDP	**323**	**2.33**	**.02**
			SAD vs BDP	**323**	**1.92**	**.06**
B. Relatives						
Manual[a]						
Left (*n* = 367)	SZ-Rel, 2746.96 ± 395.51	$F(3,1.7\times10^5) = 0.05$, $P = .99$[b]	SZ-Rel vs HC	341.89	0.36	.72
	SAD-Rel, 2727.64 ± 335.09		SAD-Rel vs HC	349.82	0.11	.91
	BDP-Rel, 2802.42 ± 379.17		BDP-Rel vs HC	339.66	0.08	.93
	HC, 2752.59 ± 357.48		SZ-Rel vs SAD-Rel	346.28	0.21	.83
			SZ-Rel vs BDP-Rel	338.78	0.26	.79
			SAD-Rel vs BDP-Rel	342.75	0.03	.97
Right (*n* = 365)	SZ-Rel, 2656.15 ± 414.76	$F(3,2.0\times10^5) = 0.23$, $P = .87$[b]	SZ-Rel vs HC	340.04	0.48	.63
	SAD-Rel, 2658.91 ± 385.94		SAD-Rel vs HC	346.49	0.13	.89
	BDP-Rel, 2718.95 ± 367.04		BDP-Rel vs HC	341.00	0.79	.43
	HC, 2671.77 ± 339.26		SZ-Rel vs SAD-Rel	343.19	0.29	.77
			SZ-Rel vs BDP-Rel	336.05	0.28	.78
			SAD-Rel vs BDP-Rel	345.15	0.55	.58

Table 2. Continued

Hippocampal Volume Measure	Hippocampal Volume, Mean ± SD, mm^3	Diagnostic Group Effect	Planned Pairwise Comparisons	df	*t*	*P*
FreeSurfer[a]						
Left (n = 303)	SZ-Rel, 3998.0 ± 399.33	$F(3,294) = 0.39$, $P = .76$	SZ-Rel vs HC	294	.43	.67
	SAD-Rel, 3951.89 ± 381.76		SAD-Rel vs HC	294	.68	.49
	BDP-Rel, 4060.88 ± 459.32		BDP-Rel vs HC	294	.48	.63
	HC, 4030.24 ± 388.65		SZ-Rel vs SAD-Rel	294	.96	.34
			SZ-Rel vs BDP-Rel	294	.79	.43
			SAD-Rel vs BDP-Rel	294	.20	.84
Right (n = 306)	SZ-Rel, 4102.44 ± 449.27	$F(3,297) = 1.00$, $P = .39$	SZ-Rel vs HC	297	0.65	.52
	SAD-Rel, 4023.24 ± 389.15		SAD-Rel vs HC	297	0.72	.47
	BDP-Rel, 4117.12 ± 457.78		BDP-Rel vs HC	297	1.16	.25
	HC, 4112.88 ± 405.26		SZ-Rel vs SAD-Rel	297	1.19	.23
			SZ-Rel vs BDP-Rel	297	1.57	.12
			SAD-Rel vs BDP-Rel	297	0.34	.74
C. Probands vs Relatives						
Manual						
Left	—	—	**SZ vs SZ-Rel**	**57.56**	**2.72**	**.009**
			SAD vs SAD-Rel	**47.07**	**1.78**	**.08**
			BDP vs BDP-Rel	**63.34**	**2.18**	**.03**
Right	—	—	**SZ vs SZ-Rel**	**57.76**	**2.95**	**.005**
			SAD vs SAD-Rel	**47.05**	**1.84**	**.07**
			BDP vs BDP-Rel	**66.34**	**1.72**	**.09**
FreeSurfer						
Left	—	—	**SZ vs SZ-Rel**	**37**	**3.25**	**.003**
			SAD vs SAD-Rel	**40**	**1.98**	**.05**
			BDP vs BDP-Rel	51	0.46	.64
Right	—	—	**SZ vs SZ-Rel**	**38**	**3.37**	**.002**
			SAD vs SAD-Rel	**40**	**2.43**	**.02**
			BDP vs BDP-Rel	52	0.00	.997

Note: SZ, probands with schizophrenia; SAD, probands with schizoaffective disorder; BDP, probands with psychotic bipolar I disorder; SZ-Rel, relatives of probands with schizophrenia; SAD-Rel, relatives of probands with schizoaffective disorder; BDP-Rel, relatives of probands with psychotic bipolar I disorder; HC, healthy controls.

[a]Sample sizes are indicated per hippocampal volume measure.

[b]Adjusted degrees of freedom (df) for pairwise group comparisons for manual hippocampal volumes were calculated similar to Barnard and Rubin [87] due to a large df following original MI method suggested by Rubin.[55]

Statistically significant and trend level outcomes are indicated in Bold.

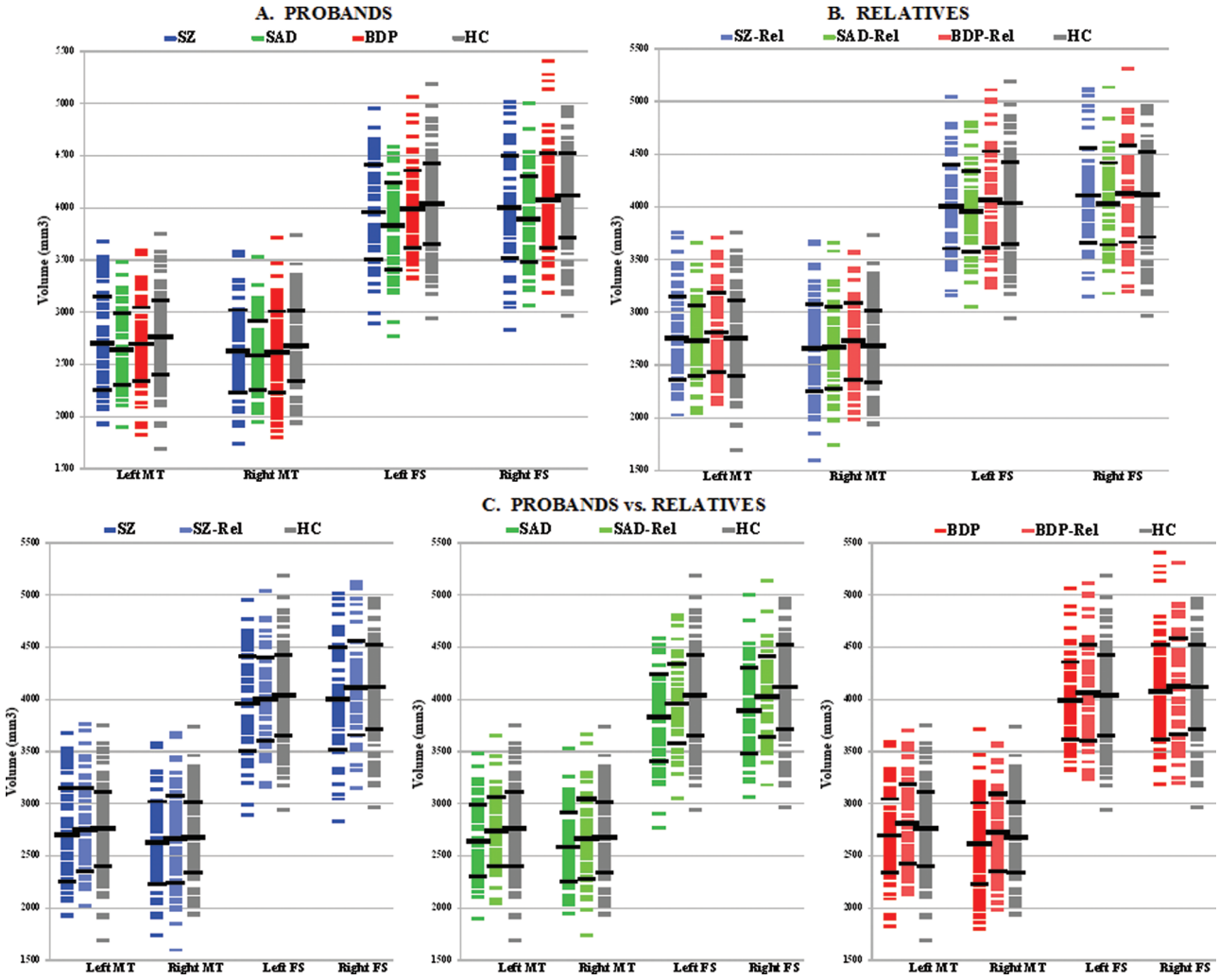


Fig. 1. Hippocampal volume outcomes from manual tracing and FreeSurfer in probands, relatives, and healthy controls. The scatter plots show individual hippocampal volumes derived from manual tracing and FreeSurfer in each proband, relative, or HC subject. The horizontal bars indicate group means and standard deviations. SZP, probands with schizophrenia; SADP, probands with schizoaffective disorder; BDP, probands with psychotic bipolar I disorder; SZR, relatives of probands with schizophrenia; SADR, relatives of probands with schizoaffective disorder; BDR, relatives of probands with psychotic bipolar I disorder; HC, healthy controls; L Hipp, the left hippocampus; R Hipp, the right hippocampus; MT, manual tracing; FS, FreeSurfer.

either methodology. Likewise, planned pairwise comparisons showed no between-group differences in any of the relative groups vs controls (P = .25–.93) or across the 3 relative diagnostic groups (P = .12–.97).

Hippocampal Volume Outcomes in Probands vs Relatives

Hippocampal volume outcomes in the psychosis probands contrasted with their relatives are presented in table 2C and figure 1C. SZ and SAD had lower hippocampal volumes than their respective relatives based on manual tracing (P = .005–.08) and semiautomated parcellation (P = .002–.05). BDP showed decreased hippocampal volumes compared with their relatives based on manual tracing (P = .03–.09), but not FreeSurfer (P = .64–.997).

Effect of Illness and Medication on Hippocampal Volume

Relatives' subgroups stratified by hippocampal volumes in their respective probands based on "median split" showed numerically lower volumes in the relatives of probands whose hippocampal volumes fell below group median compared with relatives of probands with volumes above group median (supplementary table 2). These differences were statistically significant in BDP-Rel for the left manual tracing volume (P = .001) and at a trend

level in SZ-Rel (left manual, $P = .051$; right FreeSurfer, $P = .052$) and SAD-Rel (left manual, $P = .068$) relative subgroups.

No between-group differences in hippocampal volume were found in relatives with lifetime psychosis spectrum disorders vs relatives unaffected by psychosis for either manual ($P = .64$–$.73$) or semiautomated ($P = .58$–$.71$) hippocampal ROIs (supplementary table 3).

PANSS total and positive subscale scores correlated inversely with bilateral hippocampal volumes from manual tracing and FreeSurfer ($r = -.14$ to $-.3$, $P < .05$) (supplementary table 4), whereas no correlations were found with PANSS negative and general subscale scores, YMRS, or MADRS scores in any of the proband groups. BACS composite and verbal memory scores showed direct correlations with manual tracing and FreeSurfer hippocampal volume outcomes across proband, relative, and control groups (supplementary table 4).

No differences in hippocampal volumes were found in probands actively treated with antipsychotic medication(s) vs those off-antipsychotic(s) ($P = .65$–$.94$) (table 3A). Probands treated with lithium had numerically higher hippocampal volumes compared with those off-lithium across all measures. However, this difference was statistically significant only for the right hippocampal volume from FreeSurfer ($P = .047$). All correlations between antipsychotic dose chlorpromazine equivalents and manual and FreeSurfer hippocampal volume outcomes were nonsignificant (table 3B).

Correlations Between Manual Tracing and Semiautomated Hippocampal Outcomes

Direct, moderate to strong correlations were found between hippocampal volume outcomes from manual tracing and semiautomated parcellation ($r = .51$–$.73$, $P < .05$) across all proband, relative, and HC groups (figure 2). Manual tracing yielded lower bilateral hippocampal volumes than FreeSurfer: 31.46% and 34.58% volume differences for the left and right hippocampal ROIs, respectively, averaged across all study groups.

Discussion

This study examined hippocampal volume characteristics in probands and their first-degree relatives across the schizophrenia-bipolar psychosis dimension from a large multisite sample (B-SNIP) using 2 ROI methodologies: The "gold standard" manual tracing and FreeSurfer. The outcomes showed bilateral hippocampal volume reductions in SZ and SAD compared with controls, but normal bilateral hippocampal volumes in BDP. Hippocampal volumes in relatives contrasted by their probands' diagnoses were normal, with no differences found between relatives with and without lifetime psychosis spectrum disorders. However, relatives' subgroup analysis, where hippocampal outcomes in relatives were stratified by their respective probands' volumes (ie, above and below group median), suggested a cosegregation of hippocampal volume outcomes in probands and relatives from the same pedigree: Relatives whose probands had "high" volumes also had "high" hippocampal volumes, whereas relatives whose probands had "low" volumes also showed "low" hippocampal volumes. These differences were strongest in BDP-Rel (left manual measure, $P = .001$) but were also observed in SZ-Rel (left manual, $P = .051$; right FreeSurfer, $P = .052$) and SAD-Rel (left manual, $P = .068$) at a trend level. Furthermore, hippocampal volume outcomes from the conventional manual tracing approach with rigorous reliability standards and semiautomated regional parcellation, FreeSurfer, correlated highly. Exploratory analyses revealed inverse correlations between hippocampal volume and PANSS psychosis and total scores, suggesting a relationship between the volume reduction and severity of psychosis. Direct correlations were obtained between hippocampal volume and BACS declarative memory and composite scores, suggesting a cognition cost to hippocampal volume reduction. Active treatment with antipsychotics had no effect on hippocampal volume outcomes, whereas lithium was associated with an increased hippocampal volume, albeit based on a single measure (right FreeSurfer, $P = .047$).

The divergent findings for hippocampal volume outcomes in schizophrenia/schizoaffective disorder vs psychotic bipolar I disorder are in agreement with several prior reports,[1,24,31,33] but not all.[13,24,26] The volume decreases found in schizophrenia/schizoaffective cases are subtle, consistent with previously observed range of 2%–4% decrease based on meta-analyses;[3,5] thus, larger samples may be necessary to detect these regional alterations. The mechanisms underlying these hippocampal volume differences across schizophrenia vs bipolar disorder phenotypes are unknown. Nevertheless, postmortem findings from schizophrenia and bipolar cases parallel these hippocampal characteristics captured with sMRI. Tissue from SZ cases show reductions in whole hippocampal volume compared with controls[59,60] (although see).[61,62] Decreased hippocampal volume may be due to reduced neuropil volume along with normal cell size and number, similar to Goldman-Rakic and Selemon's[63] observations in prefrontal cortex in schizophrenia. Furthermore, hippocampal subfield alterations have been reported in schizophrenia, suggesting subfield-specific disease vulnerability[16]: Cell size is decreased in the left cornu ammonis 1 (CA1) and CA2, and the right CA3[64]; nonpyramidal cell size and density in CA2 were found to be decreased in a mixed schizophrenia/bipolar disorder sample.[65] However, 2-dimensional cell counting studies carry methodological limitations due to tissue shrinkage by fixation and staining procedures.[62] Additionally, problems may be caused by irregular cell shape and size (ie, pyramidal neurons), nonrandom orientation, and cutting of cells during sectioning.[66] Stereological studies

Table 3. Associations Between Psychotropic Medications and Hippocampal Volume Outcomes

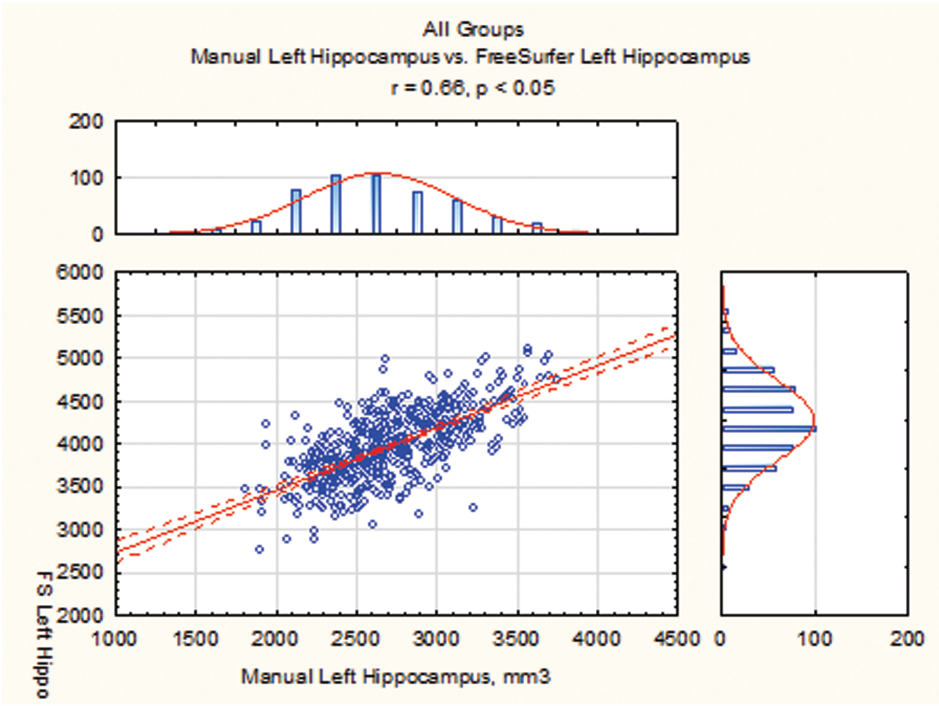
A. Hippocampal Volume Comparisons in Probands On- and Off-Antipsychotic Medications and Lithium					
Hippocampal Volume Measure	Proband Groups by Active Medication Use[a]	Hippocampal Volume, Mean ± SD, mm^3	df	*t*	*P*
Antipsychotics					
Manual left	On (*n* = 184)	2678.05 ± 372.76	220.25	0.46	.65
	Off (*n* = 39)	2631.19 ± 414.40			
Manual right	On (*n* = 185)	2613.52 ± 354.90	220.27	0.46	.65
	Off (*n* = 39)	2545.04 ± 448.60			
FS left	On (*n* = 168)	3935.34 ± 403.89	194	0.07	.94
	Off (*n* = 33)	3833.45 ± 461.79			
FS right	On (*n* = 169)	4016.38 ± 439.42	196	0.28	.78
	Off (*n* = 34)	3882.74 ± 519.12			
Lithium					
Manual left	On (*n* = 30)	2686.48 ± 362.69	220.25	0.43	.67
	Off (*n* = 193)	2667.27 ± 383.24			
Manual right	On (*n* = 30)	2640.55 ± 359.67	220.27	0.10	.92
	Off (*n* = 194)	2595.58 ± 375.20			
FS left	On (*n* = 25)	4019.88 ± 351.42	194	1.13	.26
	Off (*n* = 176)	3904.23 ± 421.53			
FS right	**On (*n* = 26)**	**4171.31 ± 453.90**	**196**	**2.00**	**.047**
	Off (*n* = 177)	**3967.95 ± 450.69**			

B. Pearson Correlations Between Antipsychotic Dose Chlorpromazine Equivalents and Hippocampal Volumes			
Antipsychotic Dose Chlorpromazine Equivalents[b]			
	SZ (*n* = 39)	SAD (*n* = 25)	BDP (*n* = 86)
Mean ± SD, mg	472.11 ± 365.93	619.72 ± 639.13	306.75 ± 450.26
Pearson Correlations in All Probands Combined			
Hippocampal Volume Measure	*n*	*r*	*P*
Manual left	109	.02	.86
Manual right	104	.08	.39
FS left	110	−.07	.47
FS right	104	.02	.82

Note: Manual right, right hippocampal volume from manual tracing; Manual left, left hippocampal volume from manual tracing; FS right, right hippocampal volume from FreeSurfer; FS left, left hippocampal volume from FreeSurfer; SZ, probands with schizophrenia; SAD, probands with schizoaffective disorder; BDP, probands with psychotic bipolar I disorder.
[a]Sample sizes are indicated per hippocampal volume measure.
[b]Chlorpromazine equivalents were calculated for concomitant (taken during the study) antipsychotic medications according to Andreasen *et al*.[58]
Statistically significant outcomes are indicated in Bold.

failed to detect alterations in the total cell number in CA1, CA2/3, CA4, or subiculum in schizophrenia cases vs matched controls.[61,67] Nevertheless, a decreased oligodendrocyte number in the bilateral deep polymorph layer of the dentate gyrus/CA4 has been detected and linked to hippocampal volume decreases and white matter tracts alterations captured with in vivo imaging techniques.[62] Postmortem findings in bipolar probands are even less consistent, with some studies reporting subtle hippocampal volume reductions compared with controls.[68] Although the total number of hippocampal neurons is normal,[69] size of pyramidal neurons in CA1[70] and nonpyramidal cell layer volume in CA2/3[69] were found to be decreased in bipolar cases vs HC. In contrast, some studies report no differences in neuronal densities in hippocampus, superior temporal cortex, and dorsolateral prefrontal cortex in bipolar vs controls tissue,[71] consistent with the finding of overall normal cortical thickness in bipolar cases.[72] These distinct postmortem findings in schizophrenia and bipolar disorder, with volumetric/cellular reductions found in schizophrenia and less severe, if any, tissue volume changes in bipolar disorder,[73] suggest at least partially unique anatomical underpinnings for the 2 disorders and provide plausible cellular correlates for

(A) The left hippocampal volume: Pearson correlations between manual tracing and FreeSurfer in all groups combined ($p < .05$)



(B) The right hippocampal volume: Pearson correlations between manual tracing and FreeSurfer in all groups combined ($p < .05$)

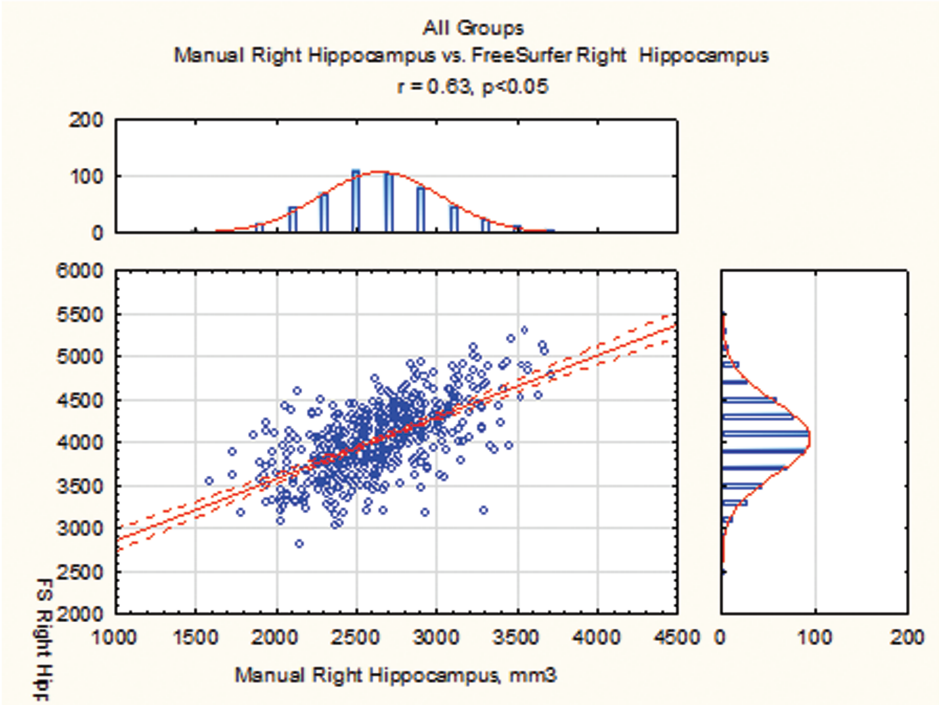


Fig. 2. Pearson correlations between the hippocampal volume outcomes from the 2 ROI methodologies–manual tracing and FreeSurfer–in all study groups combined. Manual tracing yielded lower bilateral hippocampal volumes than FreeSurfer: left, manual tracing volume = 2721.38 mm^3, FreeSurfer = 3970.78 mm^3, 31.46% difference; right, manual tracing volume = 2645.51 mm^3,

the divergent hippocampal volume outcomes in SZ/SAD and BDP observed here.

Alternatively, it is possible that hippocampal volume preservation in BDP could be secondary to a medication effect (ie, chronic treatment with lithium) and that, even if a "primary" disease-associated loss of hippocampal volume exists in these probands, it could be obscured by a volume-enhancing effect of chronic exposure to lithium.[32,33] Our findings of higher hippocampal volumes in probands actively treated with lithium vs those who were not support this notion, albeit the exploratory nature of these analyses merits cautious interpretation. Moreover, active treatment effects tested here could be obscured by high frequency of combined medication use (61%–81%) across all proband groups, as well as by longitudinal effects of both disease and treatments on hippocampal structure.[74–78] Disambiguating disease and medication effects is difficult, especially in a cross-sectional study such as ours.

The finding of overall normal hippocampal volumes in biological relatives, when contrasted based on their probands' diagnoses, is in agreement with some[2,27,31,41,42] but not all[25,35–39] reports. Hippocampal abnormalities in relatives may be even more subtle than in probands, possibly manifesting on functional rather than structural level, supported by well-established deficits in declarative memory in SZ-Rel, SAD-Rel, and BDP-Rel[79–81]; and correlations with BACS observed here. Our findings argue for disease effects that accompany psychosis in probands (eg, characteristic cellular abnormalities in SZ), which may be not present in relatives. Longitudinal MRI studies find hippocampal volume decreased in first-episode and chronic psychosis probands alike but not in ultrahigh-risk individuals,[20] suggesting this biomarker's specificity to frank, fully manifested psychosis within psychosis dimension. Subsequent exploratory analyses stratifying relatives' hippocampal outcomes by volumes in their related probands suggest a consistent pattern of hippocampal outcomes within the same pedigrees: Relatives of probands with "low" hippocampal volumes had "low" volumes as well, whereas relatives of probands with "high" volumes had consistently "high" hippocampal outcomes. These findings indicate substantial heterogeneity within the relatives' sample, with a range of heritable[82] "hippocampal biomarker load," clustering in probands and relatives within the same pedigree. These hippocampal volume characteristics do not map on either the DSM "schizophrenia/bipolar" or "psychosis spectrum disorders/unaffected" relatives diagnostic groups, and advocate for future studies testing heritability and genetic underpinnings for brain structure biomarkers.

Hippocampal outcomes from manual and semiautomated volumetric approaches showed direct, moderate to strong correlations (r = .51–.73, P < .05), consistent with previous studies.[12,83] Nevertheless, these methodologies utilize entirely different volume-defining approaches, reflected in observed here differences in volumes (ie, lower volumes with manual tracing than FreeSurfer), similar to prior reports.[12,83] Manual tracing remains a "gold standard" in ROI-focused investigations, providing precise regional definitions and is highly sensitive to within- and between-subject anatomical variabilities. It is especially useful in investigation of brain structures known to have significantly heterogeneous tissue intensity properties, eg, subcortical structures, as well as in hippocampal subfield-level analyses.[84,85] However, this methodology requires rigorous reliability standards, high scan resolution, and ROI definition specificity, particularly in the hippocampus.[51] FreeSurfer is semiautomated and therefore highly reproducible and more efficient in person hours, making it useful for large samples. However, region-specific inconsistencies in volume estimates have been reported with this technique, eg, less precision in anterior hippocampus,[83] and overall larger/more inclusive hippocampal ROIs compared with manual tracing,[12,83] accounted for by inclusion of areas avoidable during manual tracing (eg, subiculum/presubiculum, hippocampus-amygdala transitional zone). Nevertheless, the 2 methodologies show high correlations in volume estimates, and both are reliable and valid for volumetric analysis.

These findings from a large psychosis family sample (B-SNIP) support structural alterations in hippocampus as a putative biomarker for the 2 major psychosis phenotypes, schizophrenia and schizoaffective disorder, but not for psychotic bipolar I disorder. Both the "primary" disease effects, as demonstrated by postmortem schizophrenia vs bipolar tissue findings,[63–65,68–70,72] and chronic treatments (eg, a volume-enhancing effect of lithium)[32,33] likely contribute to these outcomes. Structural and functional hippocampal alterations, which are long known to play a crucial role in learning and memory processes,[86] have been recently linked to psychosis formation,[16] providing a framework for future testing of the biological mechanisms of psychosis. The strengths of our study are

FreeSurfer = 4043.89 mm^3, 34.58% difference (all volumes are averaged across all study groups). Correlation coefficients (r) in: (1) All groups combined: left, r = .66; right, r = .63; (2) probands: SZ, left, r = .73; right, r = .63; SAD, left, r = .66; right, r = .74; BDP, left, r = .65; right, r = .64; (3) relatives: SZ-Rel, left, r = .68; right, r = .69; SZD-Rel, left, r = .6; right, r = .55; BDP-Rel, left, r = .72; right, r = .71; and (4) HC: left, r = .57; right, r = .51. All correlations are statistically significant at P < .05. SZ, probands with schizophrenia; SAD, probands with schizoaffective disorder; BDP, probands with psychotic bipolar I disorder; SZ-Rel, relatives of probands with schizophrenia; SAD-Rel, relatives of probands with schizoaffective disorder; BDP-Rel, relatives of probands with psychotic bipolar I disorder.

the relatively large sample, study of psychosis probands and their relatives across DSM psychosis diagnoses, and comprehensive hippocampal ROI characterization via 2 complimentary volumetric methodologies: The "gold standard" manual tracing and semiautomated parcellation by FreeSurfer. Limitations include the cross-sectional nature of the study, potential confounds related to chronic and active medication use (ie, only a small subset of probands were off-antipsychotics, mood stabilizers, and/or other psychotropic medications at the time of imaging acquisition), as well as state of illness. In addition, psychosis diagnoses are limited to schizophrenia, schizoaffective disorder, and psychotic bipolar I disorder, and do not cover full psychosis spectrum or relevant nonpsychotic phenotypes (eg, nonpsychotic bipolar disorder). Future studies investigating brain structure characteristics in individuals with severe mental illness, using multimodal imaging techniques coupled with genetic and molecular testing, focusing on disease dimensions (eg, psychosis, affect, and cognition) and underlying circuitries may help to elucidate disease mechanisms and define disease biomarkers.

Supplementary Material

Supplementary material is available at http://schizophreniabulletin.oxfordjournals.org.

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