

ORIGINAL ARTICLE

Progression from selective to general involvement of hippocampal subfields in schizophrenia

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Volume deficits of the hippocampus in schizophrenia have been consistently reported. However, the hippocampus is anatomically heterogeneous; it remains unclear whether certain portions of the hippocampus are affected more than others in schizophrenia. In this study, we aimed to determine whether volume deficits in schizophrenia are confined to specific subfields of the hippocampus and to measure the subfield volume trajectories over the course of the illness. Magnetic resonance imaging scans were obtained from Data set 1: 155 patients with schizophrenia (mean duration of illness of 7 years) and 79 healthy controls, and Data set 2: an independent cohort of 46 schizophrenia patients (mean duration of illness of 18 years) and 46 healthy controls. In addition, follow-up scans were collected for a subset of Data set 1. A novel, automated method based on an atlas constructed from ultra-high resolution, post-mortem hippocampal tissue was used to label seven hippocampal subfields. Significant cross-sectional volume deficits in the CA1, but not of the other subfields, were found in the schizophrenia patients of Data set 1. However, diffuse cross-sectional volume deficits across all subfields were found in the more chronic and ill schizophrenia patients of Data set 2. Consistent with this pattern, the longitudinal analysis of Data set 1 revealed progressive illness-related volume loss (~2–6% per year) that extended beyond CA1 to all of the other subfields. This decline in volume correlated with symptomatic worsening. Overall, these findings provide converging evidence for early atrophy of CA1 in schizophrenia, with extension to other hippocampal subfields and accompanying clinical sequelae over time.

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INTRODUCTION

Abnormalities of the hippocampus are among the most consistently reported findings in studies of schizophrenia, and have been hypothesized to underlie the neuropsychological deficits and symptoms observed in the disorder.^{1–3} Meta-analyses of numerous structural magnetic resonance imaging (MRI) studies show reductions of the hippocampus in patients in both the early and chronic stages of illness.^{4–6} A recent large-scale multisite consortium study found that among the subcortical regions examined in schizophrenia, the largest magnitude of volume deficits was in the hippocampus.⁷ However, it is less clear whether the volume deficits of the hippocampus worsen during the course of illness, with some studies finding no atrophy over time^{8–11} and other studies suggesting progressive volume loss that begins at later stages.^{12–14}

It is also unclear whether certain portions of the hippocampus are affected more than others.¹⁵ The hippocampus comprises the dentate gyrus (DG), Cornu Ammonis (CA) regions CA4, CA3, CA2 and CA1 of the hippocampus proper, and the subiculum (Sub).^{16,17} The study of these cellularly demarcated, inter-connected hippocampal subfields, which have distinct functions,^{18–22} could offer insights into the underlying pathogenic mechanisms of hippocampal abnormalities in schizophrenia.³ With new advances in MRI data acquisition and analysis methods,

many studies have shown that it is now possible to examine the subfields of the hippocampus separately.^{23–28}

Previous structural MRI studies of the hippocampal subfields in schizophrenia have produced mixed results. In cross-sectional studies of schizophrenia, semiautomated shape analyses—which involve manually tracing the perimeters of each individual subject's hippocampus and high-dimensional mapping with a hippocampal anatomical template—have found deformity in regions corresponding to the CA1 in first-episode²⁹ and chronic patients.³⁰ Also, using an automated approach of labeling the subfields, one study reported volume reductions in CA1 and CA2/3 in chronic patients.³¹ However, two subsequent, larger-scale cross-sectional studies of subjects with chronic schizophrenia reported the greatest degree of volume reductions in the CA2/3, CA4/DG and Sub instead.^{32,33} The discrepancies among these findings could be due to differences in the stages of illness of the schizophrenia patients examined, the image acquisition sequences or the methods used to delineate the hippocampal subfields. Surface-based shape analyses cannot adequately model the subfields that are embedded deep in the hippocampal formation, such as the DG and CA4.³⁴ Also, the initial iteration of the automated method of labeling the subfields (used in the above-mentioned studies)^{32,33} relied on an atlas constructed from *in vivo* hippocampal scans of limited MRI contrast.^{23,35,36} A newly

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developed approach, used in this study, relies on the much greater spatial resolution obtainable in ultra-high field scans of *ex vivo* hippocampal tissue.³⁷ The higher level of segmentation accuracy associated with this approach should help clarify remaining questions about the distribution and time course of hippocampal volume loss in schizophrenia.

Hence in this study, we sought to determine the extent and trajectory of volume deficits of hippocampal subfields in schizophrenia, using this novel, automated method to label the subfields. We measured hippocampal subfield volume in two independent cohorts of schizophrenia patients and controls cross-sectionally; one cohort consisted of patients who were primarily in the early stages of illness, whereas the other cohort included a greater number of patients with chronic schizophrenia. Finally, a longitudinal analysis was performed in one cohort, to assess any changes in hippocampal subfield volumes that occur over the course of the illness.

MATERIALS AND METHODS

Participants

Written informed consent was obtained from all subjects in accordance with the guidelines of the National Healthcare Group (Singapore), National Neuroscience Institute (NNI, Singapore), Partners Healthcare and Harvard University (Boston, MA, USA) institutional review boards.

Clinically stable outpatients with schizophrenia were recruited at two sites: the Institute of Mental Health (IMH), Singapore, from 2006 to 2013 (Data set 1), and the Massachusetts General Hospital (MGH) in Boston, MA, USA, from 2008 to 2013 (Data set 2). Diagnosis of schizophrenia for the patients was confirmed by the Structured Clinical Interview for DSM-IV disorders (SCID)-Patient version.³⁸ Healthy controls were recruited from the community at the same time through advertisements by the study team of KS at IMH, and DJH and JLR at MGH, and the Cognitive Neuroscience Laboratory based at both Harvard University and MGH. Healthy controls were screened using the SCID-Non-Patient (SCID-NP) interview; none had

any history of Axis I disorders. Also, none of the participants had a history of neurological or neurodevelopmental disorders, or a diagnosis of substance or alcohol abuse 3 months preceding the study, claustrophobia or any other contraindications for having an MRI.

Demographic, clinical and imaging information for Data set 1 (155 patients and 79 controls) and Data set 2 (46 patients and 46 controls) is described in Supplementary Tables 1 and 2. In addition, we conducted a secondary analysis of a subset of 53 patients from Data set 1 in early stages of schizophrenia, who at the time of baseline data collection, had an onset of illness at an age < 35 years, zero or just one hospitalization and < 5 years of psychosis, and 61 demographically matched controls (Supplementary Table 3). Also, demographic information about the 34 patients and 41 controls (from Data set 1) that were followed up naturally for 2–7 years are described in Supplementary Table 4.

Symptom severity of all the patients was assessed using the Positive and Negative Symptom Scale (PANSS).³⁹ Also, estimates of antipsychotic dosages were calculated using daily chlorpromazine (CPZ) equivalent dosages.

MRI acquisition and image processing

All MRI scans were performed within 2 weeks after the clinical and neuropsychological assessments. All participants of Data set 1, including those scanned a second time 2–7 years later, were scanned on the same 3-Tesla whole-body scanner MRI (Philips Achieva, Best, The Netherlands) with an 8-channel SENSE (Sensitivity Encoding) head coil at the NNI, Singapore. Participants of Data set 2 were scanned on one of two identical 3-Tesla Tim Trio Siemens MRI scanners located at either the Athinoula A Martinos Center for Biomedical Imaging or at the Harvard University Center for Brain Science. There were no major scanner hardware or software upgrades during the MRI data collection period at either site. Scan parameters are detailed in Supplementary Methods 1.

Preprocessing of the structural images collected at both sites was performed using the open-source FreeSurfer pipeline (version 5.3, <http://surfer.nmr.mgh.harvard.edu>).^{40–43} The longitudinal data were additionally processed using a specialized longitudinal processing stream where unbiased subject-specific templates were created, and then within-subject images for each time-point re-processed with common

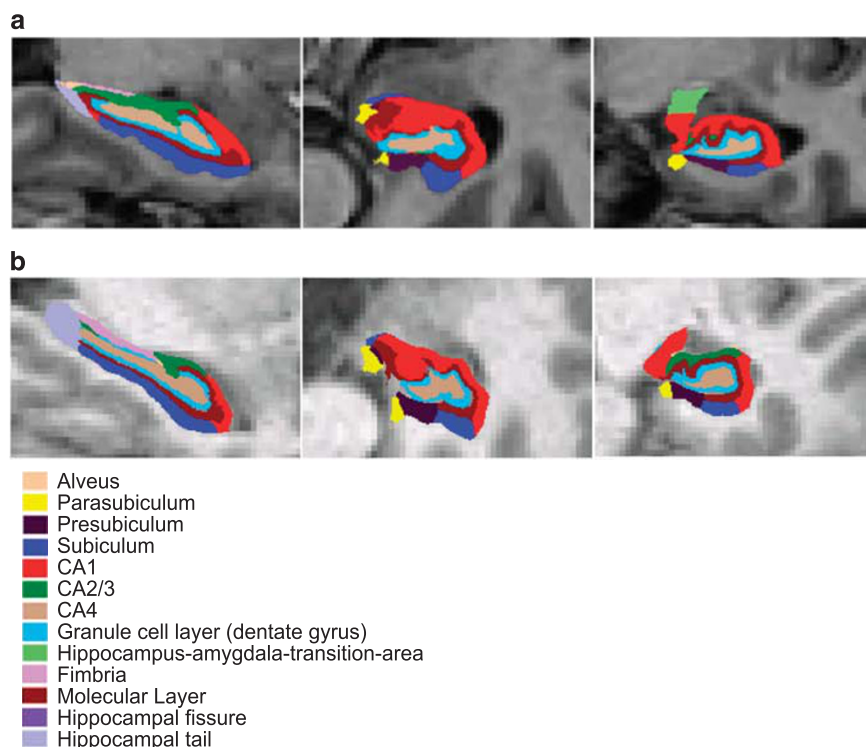


Figure 1. Representative subfield labels of the hippocampus generated by an automated *ex vivo* hippocampal segmentation approach. Subfield labels of the left anterior hippocampus of a representative healthy control subject from (a) Data set 1 and (b) Data set 2, in the sagittal, axial and coronal planes, are shown.

information from the template.⁴⁴ This has been demonstrated to reduce the variability in within-subject morphological measures, and hence result in greater statistical power and the ability to detect small changes.⁴⁴

To label the subfields, a new automated algorithm from FreeSurfer was used (Figure 1). This algorithm was based on a computational atlas built from *ex vivo* MRI data of post-mortem medial temporal tissue from 15 subjects, acquired at an average of 0.13 mm isotropic resolution on a 7-T scanner, and an *in vivo* atlas that provided information about adjacent extrahippocampal structures.³⁷ Compared with the previous algorithm developed by FreeSurfer,⁴⁵ the volumes generated by this new algorithm are more comparable with histologically based measurements of the subfields.³⁷ It also provides a more comprehensive, fine-grained segmentation of the structures of the hippocampus, including areas such as the granule cell layer (GCL) of the DG, and the molecular layer (ML) of the CA fields and Sub. See Supplementary Methods 2 for comparisons of volume measures derived from both the older and new segmentation methods.

We measured volumes of the (i) overall hippocampus, generated by the widely used automated FreeSurfer subcortical segmentation script (based on the *in vivo* atlas)⁴² and (ii) seven structures considered to be subfields of the hippocampus:^{16,17} the GCL, CA4, CA2/3 (CA2 and CA3 were combined in the atlas because of the lack of distinguishing MRI contrast), CA1, ML, the hippocampal tail (the posterior end of the hippocampus, which includes portions of the CA fields and DG undistinguishable with the MRI contrast) and the Sub.

Statistical analysis

All analyses were performed using open-source R software (version 3.1.3).⁴⁶ Cross-sectional demographic differences between the patients and controls were tested using χ^2 tests for categorical variables (gender, handedness and ethnicity) and F tests or independent t-tests for continuous variables (age, intracranial volume (ICV), CPZ and time between scans). Longitudinal change in clinical variables was assessed using paired t-tests (duration of illness, PANSS scores and CPZ). Differences in age, ICV, CPZ, duration of illnesses between the data sets were assessed using the Welch two-sample t-test of unequal variances.

Cross-sectional analyses (Data sets 1 and 2). We first determined whether there were any group-based differences in the overall mean hippocampus volume of Data set 1 and Data set 2, as well as the early course patients of Data set 1 and matched controls. A multiple linear regression, with volume as the dependent variable, diagnosis as the main predictor, and ICV, age and gender as covariates, was conducted. We then investigated whether schizophrenia differentially affects the volume measures of the inter-related hippocampal subfields. The Shapiro–Wilk test and Bartlett's test of homoscedasticity was first performed to ensure multivariate normality of the subfield volumes and equal variances of in the healthy control and patient groups of each data set, respectively. The subfield values were then log transformed. A multivariate analysis of covariance, with the 14 subfields as dependent (and correlated) variables, diagnosis as the main predictor, and ICV, age, gender and duration of illness as covariates, was conducted. The alpha was set at $P < 0.05$. This was followed by a *post-hoc* univariate analysis of covariance to determine which subfield (dependent variable) contributed to the significant overall effect of illness.

Longitudinal analysis (Data set 1). We sought to determine whether there was an interaction effect between diagnosis and time (between baseline and follow-up scans) for each hippocampal subfield. A separate multi-level model was constructed here, which accounted for the unevenly spaced time-points among subjects and the intra-individual variability in initial subfield volumes and their trajectories (Supplementary Methods 3). After model fitting, fixed effects included diagnosis, time, interaction between diagnosis and time, ICV, CPZ, age, and gender. Random effects included individual intercept and slope of time. The change in volume was modeled

linearly, as volume trajectories in studies of gray matter in schizophrenia⁴⁷ and the hippocampus in childhood-onset schizophrenia (which followed subjects until their late twenties)⁴⁸ have been shown to be linear. In addition, the annualized rate of change in subfield volume measures was calculated: $(\text{Volume}_{\text{follow-up}} - \text{Volume}_{\text{baseline}})/(\text{Volume}_{\text{baseline}} \times \text{time})$.

Secondary analyses. As some studies have reported effects of treatment with antipsychotics,¹¹ antidepressants⁴⁹ and mood stabilizers⁵⁰ on hippocampal structure, we repeated our primary analyses with the dosage or use of medication classes of antipsychotics, antidepressants and mood stabilizers as covariates. To minimize the potential confounding effect of ethnicity, we also repeated our analyses with ethnicity included as a covariate.

Post-hoc correlations between illness severity and hippocampal subfield volumes in subjects with schizophrenia

Cross-sectional analyses: We examined the relationship between clinical measures (PANSS subscales) and absolute volume measures of the subfields that were significantly different in the patients. A linear regression model was used, with the clinical measures as primary variables of interests, and age and sex among the covariates. We also tested the hypothesis that duration of illness is correlated with subfield volumes. In addition, we tested for correlations between antipsychotic dosages and subfield volumes.

Longitudinal analysis: We examined whether there was an intra-individual relationship between the rate of change in clinical symptoms—calculated by $(\text{Scores}_{\text{follow-up}} - \text{Scores}_{\text{baseline}})/(\text{Scores}_{\text{baseline}} \times \text{time})$ —and the annualized rate of change in the subfield volume measures using similar regression modeling, controlling for age, sex, CPZ and baseline duration of illness.

To address the multiple testing for the various hypotheses, the Holm–Bonferroni method controlling for family-wise errors at alpha level (0.05) was applied.⁵¹

RESULTS

Subject and cohort characteristics

Within each data set, the schizophrenia patients and controls were well-matched in terms of age, gender, ethnicity, and handedness. Between the cross-sectional Data sets 1 and 2, no cohort differences were found in the MRI estimates of head sizes, or in handedness or sex, for either the controls ($t_{106,7} = -0.14$, $P = 0.89$; $\chi^2 = 0.34$, $P = 0.56$; $\chi^2 = 1.7$, $P = 0.20$, respectively) or the patients ($t_{81,7} = 0.23$, $P = 0.82$; $\chi^2 = 1.24$, $P = 0.54$; $\chi^2 = 1.39$, $P = 0.25$, respectively). Also, there was no difference in mean age of illness onset between the two patient cohorts ($t_{79,33} = 1.11$, $P = 0.28$). However, as expected, the mean age of the subjects was higher in Data set 2 (patients: 42.9 ± 10.2 years; controls: 41.9 ± 9.1) compared with Data set 1 (patients: 32.5 ± 8.8 years; controls: 31.2 ± 9.9 years) (controls: $t_{100,6} = 6.2$, $P = 1.4 \times 10^{-8}$; patients: $t_{67,1} = 6.05$, $P = 7 \times 10^{-8}$). Also, compared with the patients of Data set 1, the patients of Data set 2 had a significantly longer mean duration of illness (Data set 1: 6.6 ± 7.0 years; Data set 2: 18.2 ± 11.0 years) ($t_{57,8} = 46.78$, $P = 7 \times 10^{-9}$), received higher mean daily doses of antipsychotic medication (Data set 1: 212.3 ± 191.3 mg; Data set 2: 525.4 ± 444.9 mg) ($t_{51,3} = 4.7$, $P = 2 \times 10^{-5}$) and were more symptomatic (see Supplementary Tables 1; positive: $t_{61,7} = 7.45$, $P = 3.58 \times 10^{-10}$; negative: $t_{58,2} = 13.67$, $P = 2.2 \times 10^{-16}$; general psychopathology: $t_{47,1} = 8.44$, $P = 5.53 \times 10^{-11}$).

Figure 2. Cross-sectional hippocampal subfield volume deficits in schizophrenia. Group-based comparisons of the hippocampal subfield volumes in (a) Data set 1 and (b) Data set 2. In each data set, the subfield volumes of individual controls (blue) and schizophrenia subjects (red) are shown after co-adjusting for cohort-averaged head size. Multivariate analysis of covariance of the combined volumes of the 14 hippocampal subfields, followed by *post-hoc* univariate analysis of covariance and a Holm–Bonferroni correction for multiple comparisons across the 14 subfields showed that the volume deficit is limited to the CA1 in the schizophrenia patients who are at an early-to-mid stage of illness (Data set 1), whereas the volume deficits involve multiple subfields in chronic patients (Data set 2). *Indicates significance in corrected P-values controlling for family-wise error rate of alpha level of < 0.05 . GCL, granule cell layer; ML, molecular layer; Sub, subiculum.

Cross-sectional findings of Data set 1

Comparison of overall hippocampal volumes indicated that, compared with the controls, the schizophrenia patients had a

smaller left hippocampus ($\beta = -150.7$, $s.e. = 52.1$, $t_{230} = -2.89$, $P = 0.0042$) and right hippocampus ($\beta = -169.9$, $s.e. = 47.7$, $t_{230} = -3.56$, $P = 0.00046$). Following measurement of the volumes of the

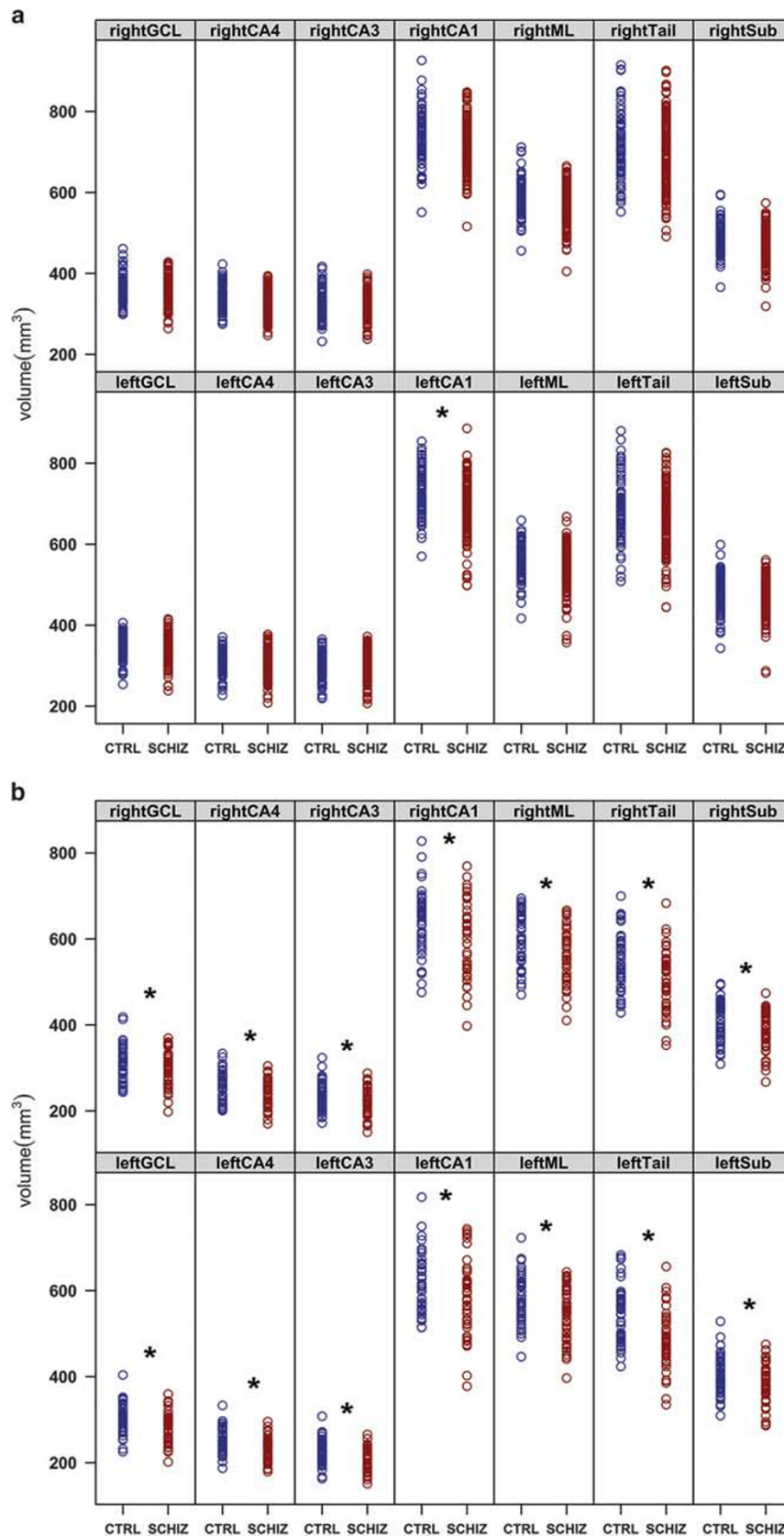


Table 1a. Group-wise comparisons of hippocampal subfields in (A) Data set 1

| (A) | Data set 1 | | | | |
|------------|------------------|----------------|---------|---------------------|-----------|
| Subfields | Healthy controls | Schizophrenia | F-value | P-value | Cohen's d |
| Left GCL | 343.65 (29.14) | 337.53 (30.75) | 1.32 | 0.2512 | 0.14 |
| Right GCL | 366.48 (32.66) | 354.94 (30.42) | 5.40 | 0.0210 | 0.26 |
| Left CA4 | 310.28 (28.67) | 304.65 (29.78) | 1.19 | 0.2769 | 0.14 |
| Right CA4 | 336.49 (30.47) | 324.84 (29.55) | 6.27 | 0.0130 | 0.29 |
| Left CA3 | 302.35 (31.99) | 298.34 (32.53) | 0.41 | 0.5204 | 0.08 |
| Right CA3 | 327.37 (36.31) | 318.30 (30.41) | 2.67 | 0.1036 | 0.21 |
| Left CA1 | 726.95 (57.22) | 695.45 (63.97) | 11.10 | 0.0010 ^a | 0.49 |
| Right CA1 | 735.72 (63.87) | 716.69 (60.54) | 6.99 | 0.0088 | 0.39 |
| Left ML | 559.18 (44.30) | 543.14 (51.57) | 4.25 | 0.0404 | 0.23 |
| Right ML | 586.99 (46.00) | 569.67 (45.57) | 5.51 | 0.0197 | 0.25 |
| Left tail | 687.80 (72.98) | 666.28 (75.06) | 5.12 | 0.0246 | 0.27 |
| Right tail | 714.18 (80.66) | 697.02 (80.87) | 3.67 | 0.0566 | 0.26 |
| Left Sub | 482.38 (45.26) | 462.56 (46.08) | 3.01 | 0.0844 | 0.22 |
| Right Sub | 485.71 (38.54) | 471.28 (40.83) | 1.67 | 0.1975 | 0.14 |

Abbreviations: CA, Cornu Ammonis; GCL, granule cell layer; ML, molecular layer; Sub, subiculum. The mean volumes (\pm s.d.) in cubic millimeters of the hippocampal subfields of the healthy control and patient group are indicated. The F-values and P-values represent the results of *post-hoc* univariate analysis of covariance (with intracranial volume, age, sex and duration of illness in years as covariates) testing the effect of diagnosis on group means and variances of the subfield volumes. ^aIndicates significance ($P < 0.05$) after Holm–Bonferroni correction for multiple comparisons across the 14 subfields. Cohen's *d* provides approximates of the effect sizes, or magnitude of group differences in the mean volume measures of each subfield.

subfields, a significant effect of group on the combined volumes of the subfields GCL, CA4, CA2/3, CA1, ML, tail and Sub (Pillai's trace=0.11, $F_{14, 215}=1.83$, $P=0.036$) was observed (Figure 2a). *Post-hoc* testing, after correction for multiple comparisons, revealed a significant volume deficit in the left CA1 (but not in the other subfields) in the schizophrenia patients, relative to the controls ($P=0.0010$; Table 1a).

The first 5 years of illness

To determine whether a change in CA1 is present during the earliest stages of schizophrenia, we conducted a secondary analysis in 53 patients in the first 5 years of illness and 61 healthy controls of Data set 1. This analysis showed reduced volumes of the left hippocampus ($\beta=-154.8$, s.e.=63.29, $t_{110}=-2.45$, $P=0.016$) and right hippocampus ($\beta=-179.6$, s.e.=63.43, $t_{110}=-2.83$, $P=0.0055$) in the patients (mean age 27.6 ± 4.9 years), compared with the controls (mean age 27.2 ± 4.7 years). There was a marginal effect of group on the combined subfield volumes (Pillai's trace=0.12, $F_{14, 151}=1.58$, $P=0.09$). *Post-hoc* testing revealed that the subtle hippocampal volume deficit found in the schizophrenia patients of this subgroup was due to a smaller left and right CA1, as well as right GCL (Table 1b).

Cross-sectional findings of Data set 2

Comparisons of the overall hippocampal volumes showed a significant effect of group (that is, smaller in the patients) for both the left ($\beta=-260.1$, s.e.=86.2, $t_{88}=-3.02$, $P=0.003$) and right ($\beta=-219.4$, s.e.=86.6, $t_{88}=-2.53$, $P=0.013$) hippocampus. Also, there was a significant effect of group on the combined subfield volumes (Pillai's trace=0.27, $F_{14, 73}=1.91$, $P=0.037$) (Figure 2b). *Post-hoc* testing revealed that all of the subfields contributed to this overall main effect (Table 1c).

Longitudinal findings of Data set 1

In 41 controls (mean age 31.6 ± 9.3 years) and 34 patients (mean age 30.9 ± 9.1 years, of which 13 were in the first 5 years of illness), we tested whether there was an interaction effect between group and time for each subfield. In this subsample, only a subtle group

difference in baseline volumes was seen in CA1 ($\beta=-32.5$, s.e.=14.54, $P=0.043$; uncorrected). Over time, the volumes of all subfields, except the left tail, decreased at a significantly greater rate in the schizophrenia cohort than in the healthy control cohort (Figure 3a). Specifically, subfields showing significant volume loss in the schizophrenia group (as indicated by the beta coefficients, as well as derived *P*-values that survived multiple comparisons) included the left CA1, right CA1, right ML, right CA2/3, left GCL and right GCL (Table 2a).

Secondary analyses

After adjusting for different medication classes, the CA1 volume deficit in Data set 1 remained significant (Supplementary Table 5A). Similarly, the extensive volume deficits across the hippocampal subfields of the more chronic patients of Data set 2 remained present, as well as the progressive volume decline across multiple subfields in the patients of the longitudinal cohort (Supplementary Table 5B). Also, the results of our primary analyses remained unchanged after adjusting for ethnicity.

Relationships of the findings to symptoms and illness duration

Across the two cross-sectional data sets, no significant correlations were found between symptom levels and absolute volume measures of all of the subfields, after correction for multiple comparisons. Also, no relationships between medication dosages and subfield volumes were found (Supplementary Table 6). However, negative associations between duration of illness and CA1 volumes were observed in both cohorts (Data set 1, left CA1: $r=-0.22$, $P=0.006$, right CA1: $r=-0.21$, $P=0.01$; Data set 2, left CA1: $r=-0.27$, $P=0.065$, right CA1: $r=-0.32$, $P=0.03$) (Supplementary Table 7).

Within the patient cohort of the longitudinal analysis, there were—on average—mild improvements in positive symptoms ($P < 0.012$) and general psychopathology symptoms ($P < 0.06$), but no significant changes in negative symptoms, over time (between baseline and follow-up). However, when we examined intra-individual relationships between symptoms and subfield volumes over time (adjusting for age, sex, antipsychotic dosages and baseline duration of illness), associations between rate of worsening of symptoms across all symptom domains and the rate

Table 1b. Group-wise comparisons of hippocampal subfields in (B) a subset of Data set 1 in the first 5 years of illness

| (B) <i>Data set 1 subset</i> | | | | | |
|------------------------------|------------------|----------------------------|---------|---------------------|------------------|
| Subfields | Healthy controls | Early course schizophrenia | F-value | P-value | Cohen's <i>d</i> |
| Left GCL | 348.39 (29.04) | 333.98 (35.39) | 1.84 | 0.1764 | 0.18 |
| Right GCL | 366.95 (35.53) | 349.40 (37.71) | 8.69 | 0.0037 ^a | 0.37 |
| Left CA4 | 315.10 (27.65) | 301.36 (33.31) | 1.45 | 0.2310 | 0.17 |
| Right CA4 | 337.08 (33.45) | 320.19 (36.36) | 5.65 | 0.0186 | 0.39 |
| Left CA3 | 305.87 (30.83) | 292.87 (35.71) | 1.06 | 0.3047 | 0.14 |
| Right CA3 | 327.16 (38.52) | 310.89 (36.86) | 6.67 | 0.0107 | 0.31 |
| Left CA1 | 737.79 (67.38) | 691.32 (80.88) | 11.22 | 0.0010 ^a | 0.41 |
| Right CA1 | 740.07 (71.62) | 705.42 (72.60) | 9.30 | 0.0027 ^a | 0.36 |
| Left ML | 568.18 (45.89) | 538.11 (60.42) | 4.97 | 0.0272 | 0.29 |
| Right ML | 589.62 (53.99) | 562.94 (55.19) | 7.95 | 0.0054 | 0.34 |
| Left tail | 690.49 (84.22) | 655.75 (77.86) | 3.49 | 0.0635 | 0.25 |
| Right tail | 713.75 (86.07) | 680.19 (95.87) | 3.53 | 0.0621 | 0.23 |
| Left Sub | 487.22 (47.74) | 460.02 (53.08) | 5.60 | 0.0191 | 0.32 |
| Right Sub | 488.09 (44.71) | 465.28 (47.35) | 6.15 | 0.0141 | 0.31 |

Abbreviations: CA, Cornu Ammonis; GCL, granule cell layer; ML, molecular layer; Sub, subiculum. The mean volumes (\pm s.d.) in cubic millimeters of the hippocampal subfields of the healthy control and patient group are indicated. The F-values and P-values represent the results of *post-hoc* univariate analysis of covariance (with intracranial volume, age, sex and duration of illness in years as covariates) testing the effect of diagnosis on group means and variances of the subfield volumes. ^aIndicates significance ($P < 0.05$) after Holm-Bonferroni correction for multiple comparisons across the 14 subfields. Cohen's *d* provides approximates of the effect sizes, or magnitude of group differences in the mean volume measures of each subfield.

Table 1c. Group-wise comparisons of hippocampal subfields in (C) Data set 2

| (C) <i>Data set 2</i> | | | | | |
|-----------------------|------------------|----------------|---------|---------------------|------------------|
| Subfields | Healthy controls | Schizophrenia | F-value | P-value | Cohen's <i>d</i> |
| Left GCL | 304.66 (34.86) | 280.71 (35.04) | 11.12 | 0.0013 ^a | 0.69 |
| Right GCL | 318.23 (40.22) | 295.62 (41.05) | 7.20 | 0.0088 ^a | 0.56 |
| Left CA4 | 250.77 (28.67) | 232.86 (27.48) | 9.68 | 0.0025 ^a | 0.64 |
| Right CA4 | 261.05 (32.63) | 243.51 (32.70) | 6.65 | 0.0116 ^a | 0.54 |
| Left CA3 | 225.34 (30.23) | 203.98 (26.91) | 12.94 | 0.0005 ^a | 0.75 |
| Right CA3 | 236.61 (32.51) | 219.33 (33.45) | 6.46 | 0.0128 ^a | 0.52 |
| Left CA1 | 620.96 (64.39) | 580.20 (86.01) | 7.63 | 0.0070 ^a | 0.54 |
| Right CA1 | 640.91 (72.54) | 596.85 (84.26) | 7.95 | 0.0062 ^a | 0.56 |
| Left ML | 579.85 (56.53) | 541.76 (62.67) | 9.63 | 0.0026 ^a | 0.64 |
| Right ML | 597.67 (61.57) | 561.76 (62.13) | 7.63 | 0.0070 ^a | 0.58 |
| Left tail | 545.27 (66.98) | 482.53 (65.86) | 20.56 | 0.0000 ^a | 0.94 |
| Right tail | 550.78 (63.32) | 500.90 (68.96) | 13.23 | 0.0005 ^a | 0.75 |
| Left Sub | 407.23 (46.08) | 384.50 (45.22) | 5.94 | 0.0168 ^a | 0.50 |
| Right Sub | 406.30 (45.59) | 382.42 (45.33) | 6.31 | 0.0139 ^a | 0.53 |

Abbreviations: CA, Cornu Ammonis; GCL, granule cell layer; ML, molecular layer; Sub, subiculum. The mean volumes (\pm s.d.) in cubic millimeters of the hippocampal subfields of the healthy control and patient group are indicated. The F-values and P-values represent the results of *post-hoc* univariate analysis of covariance (with intracranial volume, age, sex and duration of illness in years as covariates) testing the effect of diagnosis on group means and variances of the subfield volumes. ^aIndicates significance ($P < 0.05$) after Holm-Bonferroni correction for multiple comparisons across the 14 subfields. Cohen's *d* provides approximates of the effect sizes, or magnitude of group differences in the mean volume measures of each subfield.

of change in subfield volume were observed (Table 2b). Figure 3b displays a plot of the correlation between the rate of left CA1 atrophy and rate of increase in negative symptom severity ($r = -0.54$, $P = 0.0023$).

DISCUSSION

Here we demonstrated that MRI volume estimates of the CA1 of the hippocampus were selectively reduced in patients in the early (including those in the first 5 years of illness) to mid-course of schizophrenia, when compared with healthy controls. In contrast, in a cohort of chronic schizophrenia patients, the volume deficits were widespread across the subfields. Consistent with this pattern, the results of our longitudinal analysis indicated that over time,

the focal atrophy associated with early illness extends beyond CA1, involving other subfields such as the CA2/3 and GCL. Finally, correlational analyses revealed that subfield volumes were (1) cross-sectionally smaller with greater illness duration and (2) declined with illness progression (that is, worsening symptoms) over time.

Early changes in CA1 in schizophrenia, with later involvement of other subfields

The pattern of disproportionate reductions in CA1 volume in schizophrenia patients of Data set 1 agrees with convergent evidence from shape morphometric studies of first-episode schizophrenia patients.²⁹ The diffuse subfield volume deficits in the chronic patients of Data set 2 are consistent with prior

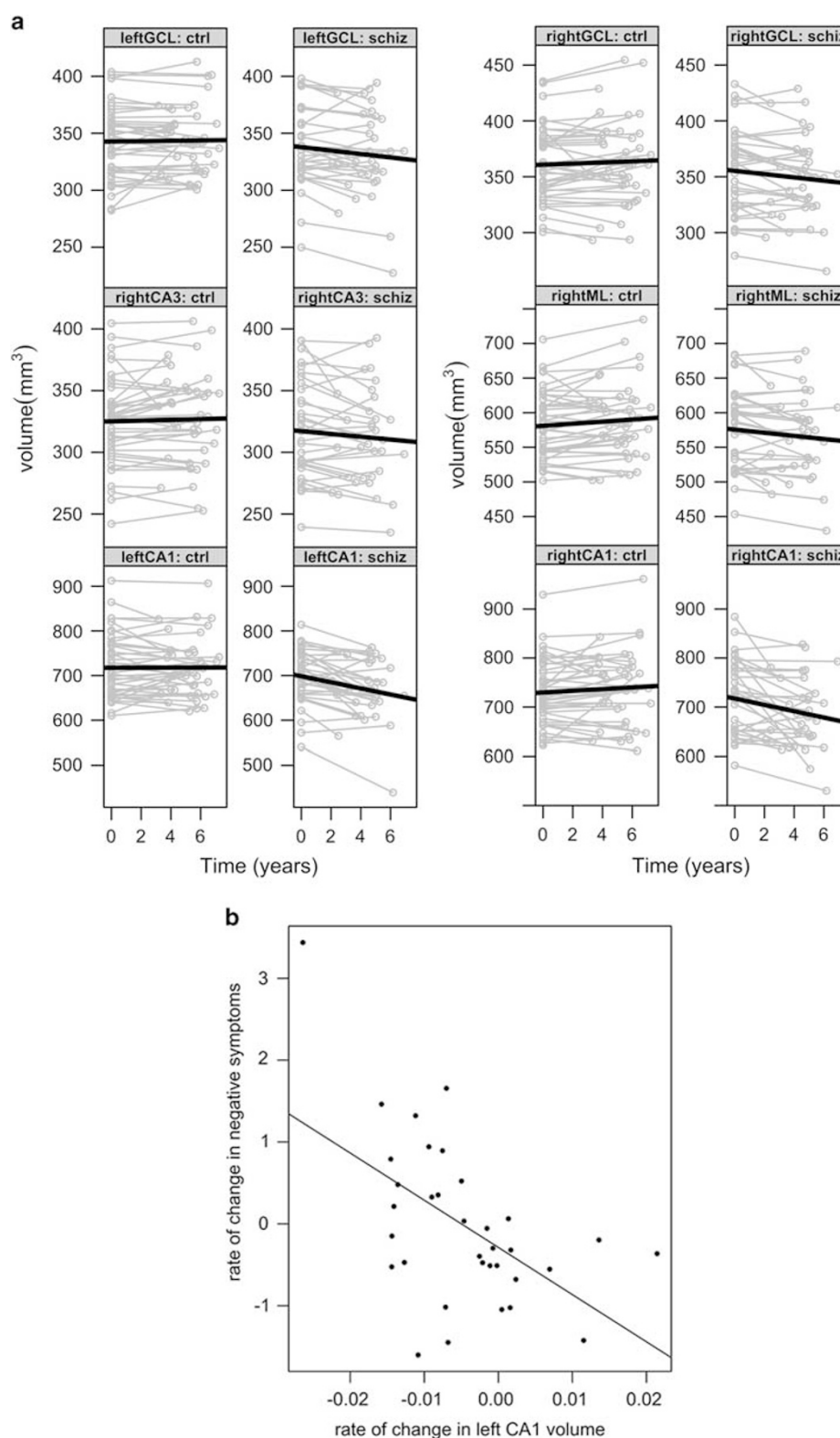


Figure 3. Longitudinal change in hippocampal subfield volumes in schizophrenia over time. **(a)** Spaghetti plots are shown indicating the trajectories of volumes of the hippocampal subfields, which showed a steeper rate of loss in patients compared with controls (in a subset of Data set 1). Bold lines indicate the group mean linear regression line. GCL, granule cell layer; ML, molecular layer; Sub, subiculum. **(b)** Also, in this cohort, the rate of atrophy of left CA1 was correlated with the rate of increasing negative symptom severity in the patients. The scatter plot showing the standardized rate of change of negative symptoms versus the rate of change of left CA1 volume across all schizophrenia patients ($r = -0.54$, $P = 0.0023$) is displayed.

Table 2a. Longitudinal group-based comparisons between schizophrenia patients and healthy controls of slopes of hippocampal subfield volumes

| Hemisphere | Subfield | Results after model fitting | | | | Controls | Patients |
|------------|----------|---|------|---------|---------------------|--------------------------|--------------------------|
| | | Fixed effects parameters for interaction between diagnosis and time | | | | Mean annualized % change | Mean annualized % change |
| | | Beta | s.e. | t-value | P-value | | |
| Left | GCL | -2.04 | 0.65 | -3.17 | 0.0023 ^a | 0.34 (2.75) | -2.0 (3.05) |
| | CA4 | -0.87 | 0.63 | -1.38 | 0.17 | 0.24 (3.10) | -1.5 (3.20) |
| | CA2/3 | -1.26 | 0.81 | -1.55 | 0.12 | 0.31 (3.60) | -1.5 (3.94) |
| | CA1 | -5.96 | 1.66 | -3.60 | 0.0006 ^a | -0.2 (6.43) | -5.9 (9.40) |
| | ML | -2.42 | 1.02 | -2.37 | 0.02 | 0.46 (4.34) | -3.1 (4.47) |
| | Tail | 1.38 | 1.80 | 0.77 | 0.45 | 1.24 (7.67) | 2.30 (8.23) |
| Right | Sub | -1.15 | 0.85 | -1.35 | 0.18 | 0.23 (3.44) | -1.9 (4.94) |
| | GCL | -2.08 | 0.63 | -3.29 | 0.0016 ^a | 0.24 (3.16) | -2.2 (4.04) |
| | CA4 | -1.77 | 0.68 | -2.62 | 0.01 | 0.25 (3.04) | -2.2 (4.29) |
| | CA2/3 | -2.15 | 0.64 | -3.35 | 0.0013 ^a | 0.50 (3.15) | -2.2 (4.61) |
| | CA1 | -8.87 | 2.15 | -4.13 | 0.0001 ^a | 1.57 (7.34) | -6.1 (10.0) |
| | ML | -3.73 | 0.99 | -3.78 | 0.0003 ^a | 1.05 (4.39) | -3.4 (6.36) |
| | Tail | -0.99 | 2.05 | 0.48 | 0.63 | 0.0 (6.81) | -0.89 (11.1) |
| | Sub | -1.81 | 0.79 | -2.29 | 0.03 | 0.31 (3.51) | -2.3 (5.71) |

Abbreviations: CA, Cornu Ammonis; GCL, granule cell layer; ML, molecular layer; Sub, subiculum. The mean annualized percentage change of each hippocampal subfield in each group is also indicated. ^aIndicates significance ($P < 0.05$) after Holm-Bonferroni correction for multiple comparisons across the 14 subfields.

Table 2b. Correlations between rate of symptom change and rate of volume change in the hippocampal subfields showing significant atrophy in schizophrenia over time

| Subfield | Positive and Negative Symptom Scale, subscales | | | | | | | | | | | |
|-------------|--|------|---------|----------------|----------|------|----------------------|----------------|-------------------------|------|---------------------|----------------|
| | Positive | | | | Negative | | | | General psychopathology | | | |
| | Beta | s.e. | P-value | r ² | Beta | s.e. | P-value | r ² | Beta | s.e. | P-value | r ² |
| Left CA1 | -1.60 | 1.95 | 0.419 | 0.04 | -7.83 | 7.83 | 0.00018 ^a | 0.41 | -3.80 | 1.14 | 0.0024 ^a | 0.42 |
| Right CA1 | -3.04 | 1.22 | 0.019 | 0.19 | -1.32 | 1.57 | 0.410 | 0.060 | -2.39 | 0.70 | 0.0018 ^a | 0.30 |
| Left GCL | -4.79 | 2.01 | 0.024 | 0.18 | -6.49 | 2.30 | 0.0085 | 0.244 | -3.66 | 1.16 | 0.0038 ^a | 0.26 |
| Right GCL | -4.01 | 2.06 | 0.134 | 0.06 | -3.20 | 2.52 | 0.22 | 0.086 | -3.44 | 1.18 | 0.0068 | 0.23 |
| Right CA2/3 | -2.59 | 1.41 | 0.123 | 0.08 | -1.34 | 1.75 | 0.449 | 0.055 | -2.56 | 0.78 | 0.0028 ^a | 0.28 |
| Right ML | -3.69 | 1.61 | 0.029 | 0.17 | -4.02 | 1.93 | 0.046 | 0.162 | -3.15 | 0.90 | 0.0015 ^a | 0.30 |

Abbreviations: CA, Cornu Ammonis; GCL, granule cell layer; ML, molecular layer. The negative betas across all subfields investigated suggested a pattern of association between rate of symptom worsening and rate of volume decline. ^aIndicates significant association ($P < 0.05$) after Holm-Bonferroni correction for multiple comparisons across the 6 subfields and 3 symptom subscales.

evidence of widespread volume deficits along the anterior-posterior extent of the hippocampus in patients with long-term schizophrenia.¹⁵ Negative correlations between illness duration and subfield volumes were also found in both data sets, similar to prior reports of an association between hippocampal volume reduction and illness duration in chronic schizophrenia.^{12,52,53} Taken together, these cross-sectional findings raise the possibility that a progressive extension of atrophy across the hippocampus occurs during the illness. We tested this hypothesis by conducting a longitudinal analysis in a subset of subjects of Data set 1. Here we found subtle volume reductions at baseline in the left CA1—but not in the other subfields—in the schizophrenia patients when compared with the controls. Over time, significant effects of the illness were observed not only in the CA1 but also in other subfields that are part of the trisynaptic circuit, that is, CA2/3, ML and GCL.^{16,54} The extent of atrophy averaged about 2–6% loss per year in the most affected subfields, with the greatest amount of volume change in the CA1 subregion (~6%). We found that the

rate of atrophy across the subfields in schizophrenia correlated with the rate of symptom worsening over time. Thus, our findings suggest that the greatest decline in subfield volume over time may occur in patients who have a poor course of illness.

The present finding of an initial, selective loss of CA1 volume during the early stages of illness is consistent with a finding of increased CA1 regional cerebral blood volume in prodromal subjects who subsequently became ill, compared with those that did not.⁵⁵ In this prior study, CA1 hypermetabolism predated changes in shape measures of CA1 and subicular atrophy in these subjects.⁵⁵ The present finding is also consistent with prior findings of selective CA1 hypermetabolism or shape deformity in patients in the early-to-mid stages of illness, including patients with mean illness duration of ≤ 10 years.^{29,30,56,57}

Our findings, however, are not in line with two recent studies, which found pronounced volume deficits in the CA2/3, CA4/DG and Sub subfields of the hippocampus in schizophrenia patients, with little or no differences in CA1.^{32,33} The discrepancy between

these prior findings and ours is likely due to differences in hippocampal subfield labeling methods. It has recently been shown that portions of CA1 are misattributed as CA2/3, CA4-DG and Sub by the older automated algorithm used in these prior studies.^{23,36,58} Indeed, our own calculations indicate that volume estimates⁴⁵ of the CA1 using the prior algorithm are approximately 40% smaller than the current, *ex vivo* algorithm's CA1 volume estimates, and that the previous volume estimates of the CA2/3 are four times larger than the *ex vivo* algorithm's CA2/3 volume estimates.

Possible mechanisms underlying the selective-to-diffuse changes in the hippocampus in schizophrenia

Small *et al.*³ and Schoebel *et al.*⁵⁵ have proposed a framework to explain an early involvement of CA1 in the pathophysiological process underlying psychosis. They suggest a sequence of events involving an increase in synaptic glutamate levels, subsequent increase in metabolic demand and blood flow, and down-regulation of GABAergic interneurons in the hippocampus.⁵⁵ Owing to the greater density of glutamate receptors (N-Methyl-D-aspartate receptor and α -Amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid receptor) in CA1 compared with the other hippocampal subfields,⁵⁹ CA1 may be particularly vulnerable to dysregulation of glutamatergic neurotransmission and excitotoxic injury.³ Moreover, as Konradi *et al.*⁶⁰ have shown, CA1 has the largest numbers of primary hippocampal interneuron subtypes among the subfields, the loss of function of these interneurons, which control information flow arriving from the entorhinal cortex and excitatory hippocampal pyramidal cell activity,⁶¹ may produce excessive, feedforward excitation of the trisynaptic hippocampal circuit,^{54,62} driving an extension of these abnormalities in excitation-inhibition balance to other portions of the hippocampus and beyond.^{61,63–66} Empirical support for this model has been produced using ketamine-treated mice, which showed hypermetabolism followed by atrophy selectively in CA1 and the Sub; these effects were subsequently blocked by reducing synaptic glutamate levels.⁵⁵

The progression of atrophy from CA1 to other hippocampal subfields could result from an extension of the pathophysiological process underlying psychosis, such as the one hypothesized above. Consistent with this possibility are the findings of extensive reductions in interneuron subtypes throughout the hippocampus (in the CA1, CA2/3 and CA4 fields) in post-mortem studies of schizophrenia.⁶⁰ This possibility is also supported by the association between progressive atrophy and symptomatic worsening observed in this study. Alternatively, this progression could result from an interaction between (1) a fundamental cellular abnormality and (2) environmental factors,⁶¹ including stress, substance use and/or treatment with antipsychotic medications, all of which have been associated with decreases in brain tissue volume.^{67–69} However, when we controlled for antipsychotic medication dosages (as well as the use of mood stabilizers and antidepressants) in the analyses, both our cross-sectional and longitudinal findings in both data sets remained significant, suggesting that progression of atrophy across the hippocampus over time in schizophrenia is not a consequence of medication treatment. Also, it is notable that our longitudinal data were collected in a country (Singapore) with extremely strict prohibitions against (and hence very little) illicit substance use. Thus, although substance use, cannabis in particular, has been linked to reductions in hippocampal volume in both healthy and schizophrenia groups,^{70–72} it is unlikely to have had a role in our longitudinal findings.

However, we speculate that elevated stress levels,⁶⁸ as well as unhealthy lifestyle practices common in patients with chronic schizophrenia, such as poor nutrition, cigarette smoking^{73,74} and lack of exercise,^{75,76} exacerbate dysfunction and structural

changes of the hippocampus in the illness. Future studies can quantitatively measure these environmental factors longitudinally to determine which influence progression of hippocampal atrophy in schizophrenia.

Intriguingly, a recent multisite structural MRI study of major depressive disorder show a pattern of results that is similar to those of this study, with hippocampal volume deficits in chronic patients, but no such deficit in first-episode depression subjects.⁷⁷ Taken together with evidence for hippocampal hypermetabolism in depression,^{78,79} these findings suggest that related, overlapping mechanisms may be responsible for hippocampal abnormalities in schizophrenia and depression, as recently proposed.³ The possibility that identical mechanisms, affecting overlapping but partially distinct circuits, underlie psychosis and depression, could account both for the overlap in symptoms (for example, anhedonia) as well as the phenomenological differences across the two disorders.

Potential limitations and summary

There are several potential limitations of our study. First, although the hippocampal segmentations of all subjects studied here were visually inspected individually, it is possible that schizophrenia disrupts the folding of the multi-layered hippocampus, influencing the accuracy of the hippocampal segmentation process. However, the current segmentation algorithm has been used, with a high level of accuracy, to delineate the subfields of the hippocampus in patients with Alzheimer's disease—who exhibit greater cellular degradation and atrophy of the hippocampus than patients with schizophrenia,³⁷ suggesting that this method is likely fairly robust to disease-induced alterations.

Second, it is possible that there may be differences in hippocampal structure across ethnic groups that may limit the validity of examining hippocampal subfields across cohorts with different ethnic compositions. However, prior studies, including one that directly compared the brain structures of 140 cognitively matched young and old Chinese Singaporeans and non-Asian Americans, have found no evidence of effects of ethnicity on hippocampal volume measures.^{80,81} Hippocampal volume deficits are also consistently reported in studies of schizophrenia across patients of varying ethnicities;^{4,7,82–86} thus it is unlikely that the differences in ethnicity across the two sites of our study impacts the generalizability of these results.

In conclusion, this study reports selective volume deficits of the CA1 in the early-to-mid phases of schizophrenia, with evidence for an extension of this atrophy to the remaining hippocampal subfields over the course of the illness. Correlations between these findings and measures of illness progression suggest that these anatomical changes may have direct clinical consequences, which could be treated, in future trials, by interventions aimed at restoring or preserving the hippocampus.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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