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OPEN

Neuropsychopharmacology (2017) 42, 1361–1370
Official journal of the American College of Neuropsychopharmacology

www.neuropsychopharmacology.org

Progressive Decline in Hippocampal CA1 Volume in Individuals at Ultra-High-Risk for Psychosis Who Do Not Remit: Findings from the Longitudinal Youth at Risk Study

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Most individuals identified as ultra-high-risk (UHR) for psychosis do not develop frank psychosis. They continue to exhibit subthreshold symptoms, or go on to fully remit. Prior work has shown that the volume of CA1, a subfield of the hippocampus, is selectively reduced in the early stages of schizophrenia. Here we aimed to determine whether patterns of volume change of CA1 are different in UHR individuals who do or do not achieve symptomatic remission. Structural MRI scans were acquired at baseline and at 1–2 follow-up time points (at 12-month intervals) from 147 UHR and healthy control subjects. An automated method (based on an *ex vivo* atlas of ultra-high-resolution hippocampal tissue) was used to delineate the hippocampal subfields. Over time, a greater decline in bilateral CA1 subfield volumes was found in the subgroup of UHR subjects whose subthreshold symptoms persisted ($n=40$) and also those who developed clinical psychosis ($n=12$), compared with UHR subjects who remitted ($n=41$) and healthy controls ($n=54$). No baseline differences in volumes of the overall hippocampus or its subfields were found among the groups. Moreover, the rate of volume decline of CA1, but not of other hippocampal subfields, in the non-remitters was associated with increasing symptom severity over time. Thus, these findings indicate that there is deterioration of CA1 volume in persistently symptomatic UHR individuals in proportion to symptomatic progression.
Neuropsychopharmacology (2017) **42,** 1361–1370; doi:10.1038/npp.2017.5; published online 1 February 2017

INTRODUCTION

Individuals at risk for psychosis are generally identified by the presence of subthreshold symptoms. Many studies of this population largely focus on examining characteristics of at-risk subjects who subsequently develop full-blown psychotic symptoms. Yet, the majority of the at-risk individuals do not convert to frank psychosis (Simon *et al*, 2011). The subthreshold symptoms observed at baseline typically either persist or resolve over time. It has been argued that clinical care for individuals with persistent subthreshold psychotic symptoms is indicated, given the functional impairment seen in this population (Carpenter, 2014). To stratify treatment for at-risk individuals with varying outcomes (Kapur *et al*, 2012), attention needs to be directed towards understanding the biological predictors of these potential outcomes, as it has become increasingly clear that in psychiatric illnesses, biological changes precede the appearance of symptoms in a dimensional manner (Cuthbert and Insel, 2010).

Several lines of evidence suggest that abnormalities of the hippocampus may represent candidate biological markers of psychosis (Heckers and Konradi, 2010). Two recent independent large-scale consortium studies have separately found the hippocampus to exhibit the most extensive volume reductions among all of the subcortical brain regions examined in schizophrenia (Okada *et al*, 2016; van Erp *et al*, 2015). Also, several meta-analyses have consistently found hippocampal volume reductions not just in chronic schizophrenia but also within first-episode populations (Adriano *et al*, 2012; Haijma *et al*, 2013). However, the timing of the emergence of hippocampal abnormalities during the course of the illness remains unclear.

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Received 29 September 2016; revised 5 December 2016; accepted 4 January 2017; accepted article preview online 12 January 2017

Findings from cross-sectional studies of hippocampal volume in at-risk subjects, which have included familial (first-degree relatives) and/or clinical (those with subsyndromal symptoms) at-risk groups, have been mixed thus far—with findings of volume reduction, or no changes (Ganzola *et al*, 2014; Hurlemann *et al*, 2008; McDonald *et al*, 2006; O'Driscoll *et al*, 2001; Ongur *et al*, 2006; Seidman *et al*, 2002; Witthaus, 2010; Witthaus *et al*, 2009; Wood *et al*, 2005, 2010). Longitudinal studies of hippocampal volumes in at-risk subjects could help to resolve the discrepancies among prior findings by determining whether volume deficits of the hippocampus are already present during the prodromal phase of psychosis, and map the potential differences in the trajectories of hippocampal volumes for subjects with different outcomes.

Also, studies must now account for evidence that the cytoarchitecturally distinct subfields of the hippocampus (ie, CA1, CA2/3, CA4, dentate gyrus, and subiculum) are differentially affected by the disease process associated with psychosis (Talati *et al*, 2014), particularly early on in the illness (Ho *et al*, 2016; Schobel *et al*, 2013). Metabolic abnormalities selectively in the CA1 subfield of the hippocampus have been found to precede the development of psychosis and a subsequent change in the shape of a hippocampal region likely corresponding to CA1 (Schobel *et al*, 2013). Consistent with these findings, we recently identified, using a validated automated method of segmenting the hippocampus (Iglesias *et al*, 2015), a selective volume deficit in the CA1 subfield in early-course schizophrenia patients (Ho *et al*, 2016). Therefore, in the current investigation, we sought to extend this finding by determining whether CA1 volume deficits are present during the prodrome and are predictive of persistence or worsening of symptoms.

To test this hypothesis, we prospectively followed a group of ultra-high-risk (UHR) individuals, who met the consensus criteria for the UHR state (Yung *et al*, 2005), and healthy control (HC) subjects, who reside in a region of the world with one of the lowest rates of illicit substance use worldwide (Lee *et al*, 2013) and thus are likely minimally affected by the potentially confounding effects of substance use on hippocampal volume (Solowij *et al*, 2013; Thompson *et al*, 2004). We collected MRI scans and clinical measures at the time of recruitment (baseline), and at the 12- and 24-month follow-up time points, or until the point of transition to clinical psychosis. Based on prior work (Ho *et al*, 2016; Schobel *et al*, 2013), we predicted that we would observe a progressive decline in volume of the CA1 subfield in the subgroup of UHR subjects who exhibited persistent or worsening symptoms (the 'non-remitters') but this decline would be absent in the UHR subjects who remitted.

MATERIALS AND METHODS

Participants

We studied 93 UHR individuals and 54 HCs. These participants were part of the Longitudinal Youth At-Risk Study conducted from 2008 to the fall of 2015 in Singapore (Lee *et al*, 2013; Wang *et al*, 2016). The UHR participants met the criteria for the at-risk mental state defined by the Comprehensive Assessment of At-Risk Mental States (CAARMS) version 12/2006 (Yung *et al*, 2006), which includes the positive subscale of the CAARMS and the Social and Occupational Functioning Assessment Scale (SOFAS) (Goldman *et al*, 1992; Yung *et al*, 2005); the youths were of genetic risk for psychosis and/or showed subthreshold symptoms, and deterioration in functioning in the past 12 months. See Supplementary Methods S1 for details of the recruitment and screening process.

Exclusion criteria for the UHR participants included (i) past or current history of psychosis, or mental retardation, (ii) any treatment with mood stabilizers or antipsychotic medications, (iii) medical conditions that could account for their prodromal psychotic symptoms, (iv) current substance use, abuse, or dependence, and (v) current alcohol abuse or dependence, assessed using the Structured Clinical Interview for DSM-IV Axis I Disorders—Patient version (SCID-I/P) (First, 2002b).

Exclusion criteria for the HC included (i) a history of major psychiatric conditions, (ii) first-degree relatives with any axis I disorder, and (iii) any history of substance or alcohol abuse/dependence, assessed using the Structured Clinical Interview for DSM-IV Axis I Disorders—Non-Patient version (SCID-I/NP) (First MB, 2002a). In addition, the HC did not meet the CAARMS criteria for UHR status.

General exclusion criteria for all of the subjects included contraindications for MRI scans, and major medical conditions. Ethics approval for this study was provided by the Singapore National Healthcare Group's Domain Specific Review Board. Written informed consent was obtained from all participants aged 21 years and above, or from a legally acceptable representative for participants under 21 years of age with their assent.

Clinical Measures

The CAARMS and SOFAS, as part of the CAARMS version 12/2006, were administered to the UHR subjects at 6-month intervals, or until the point of transition to clinical psychosis. The SCID-I/P was administered every 12 months to the UHR subjects. The SCID-I/NP was administered to the HC subjects during the baseline visit and at the end of follow-up, which was 24 months later.

The UHR subjects were assigned to three different groups, based on their combined CAARMS and SOFAS status at their last MRI scan. UHR subjects were deemed to have remitted if they no longer met the CAARMS/SOFAS criteria for UHR status (UHR remitters, UHR-R). UHR subjects who transitioned to psychosis during the course of study, and UHR subjects who remained at risk because of persistent prodromal symptoms were combined into one group to afford greater power for subsequent analyses (UHR-non-remitted, UHR-NR). Two of the UHR subjects with unstable symptomatology, that is, who were symptom free for 12 months, and then experienced a second onset of subsyndromal symptoms, were excluded from the primary analysis.

MRI Acquisition and Image Processing

Parameters of the structural scans, which were performed at baseline, 12 months, and 24 months, are detailed in Supplementary Methods S2. The MRI scans were performed

within 2 weeks after the clinical and neuropsychological assessments were conducted.

Standard preprocessing of the structural images, followed by longitudinal preprocessing steps that further reduced within-subject variability, were performed using FreeSurfer version 5.3 (http://surfer.nmr.mgh.harvard.edu) (Fischl *et al*, 2002, 2004; Reuter *et al*, 2012). The segmentation of the hippocampus into its subfields was performed using a recently validated segmentation algorithm that was based on an atlas constructed from ultra-high-resolution *ex vivo* hippocampal tissue (Iglesias *et al*, 2015). Details of these methods can be found in Ho *et al* (2016). Visual quality control of the image processing that included checking for excessive motion was performed (Supplementary Methods S3). The proportion of UHR subjects excluded because of motion was not different from healthy subjects. Further, data set from an UHR-NR subject was removed from the analysis because of poor longitudinal image reconstruction. Hippocampal segmentation results were visually checked for each subject; there were no instances of incomplete labeling of hippocampal subregions or mislabeling of extrahippocampal regions in the remaining 147 subjects.

Volumes of the seven subfields of the hippocampus (Burwell and Agster, 2009): the GCL, CA4, CA2/3, CA1, ML, the hippocampal tail, and the subiculum were extracted. The volumes of the seven subfields for each hemisphere were summed to obtain a measure of the volume of the global left and global right hippocampus. To facilitate comparisons with other studies, the volumes of the hippocampus (from the standard output of the FreeSurfer automated labeling of major subcortical structures) were also extracted for a supplementary analysis.

Statistical Analyses

All analyses were performed using open-source R software (version 3.2) (The R Core Team, 2015).

Participant characteristics. We examined three groups of participants: HC, UHR-R, and UHR-NR. Baseline between-group demographic, clinical, and cognitive differences were tested using χ^2 tests and analysis of variance (ANOVA).

Primary analyses. We tested whether there was a main effect of group on baseline volumes, as well as an interaction effect between group and time, by fitting a linear mixed-effects model for each volume measure. Before model fitting, Shapiro–Wilks test for normality and the Bartletts test of homogeneity of variance were first performed to test for normal distributions and equal variances of all the volume measures of the hippocampus and subfields at each time point among the groups.

In our model, the fixed-effects included group, time (in months), interaction between group and time, ICV, baseline age, and gender. Random effects included individual intercept and individual slope of time. An autoregressive structure was used to model the variance–covariance matrix, which recognizes that proximal observations are more correlated than distant observations. An ANOVA was performed on the linear mixed-effects model to test whether there was statistical evidence of group differences in intercept (effect of group at baseline) and slope (interaction effect between group and time). If so, this was followed by *post hoc* pair-wise group comparisons using Wald tests to compute asymptotic χ^2 statistics, with *p*-values adjusted for between-group multiple comparisons using the Holm–Bonferroni method (Fox and Weisberg, 2011; Hothorn *et al*, 2008).

Secondary analyses. (1) We first tested whether the presence of psychiatric comorbidity (ie, SCID-assessed lifetime history of depressive spectrum disorders, anxiety spectrum disorders, alcohol abuse/dependence, and/or substance abuse/dependence) influenced our primary hypotheses by adjusting for comorbidity in the statistical model. (2) We also tested whether treatment with antidepressants or benzodiazepines at any time-point affected the findings by adding these as time-varying predictors in the model. (3) We then tested whether the inclusion of two subjects with fluctuating symptoms over time impacted the primary findings. (4) Last, we tested whether CA1 volume decline was different in individuals who developed clinical psychosis, by further subtyping the non-remitted UHR subjects into those who remained symptomatic at a subthreshold level (UHR-NR-non-psychosis or 'non-converters') and those who transitioned to full psychosis (UHR-NR-psychosis or 'converters').

Post hoc correlations between symptoms and hippocampal subfields found to exhibit group differences. We examined the within-group relationship between rates of volume change in the affected hippocampal subfields and symptoms. First, we fitted linear mixed-effect models for symptom measures, with CAARMS scores as dependent variables; time, age, and gender as fixed-effect variables; and individual intercept and slope of time as random-effect variables. The slope estimates of the subjects' measures of symptoms were then regressed onto the slope estimates of the affected hippocampal subfield volumes (extracted from the linear mixed-effect models constructed for the above-mentioned primary hypothesis).

RESULTS

Subject Cohort Characteristics at Baseline and Follow-Up

At baseline, the three groups of subjects, the HC ($n=54$), UHR-R ($n=41$), and UHR-NR ($n=52$, including 12 whose symptoms worsened to exceed subthreshold), were well matched in terms of age, gender, ethnicity, handedness, and ICV (which was used as an estimate of head size) (Table 1). A further breakdown of the baseline clinical characteristics of the UHR subpopulations, that is, a comparison of the UHR-R and UHR-NR, found no group differences in CAARMS scores, as well as the proportion of individuals with (1) lifetime and/or current history of depressive or anxiety disorder, (2) past history of alcohol abuse or dependence, and/or (3) past history of substance abuse or dependence (Supplementary Table S1).

Table 1 Baseline Sociodemographic, Imaging, and Clinical Characteristics of Three Groups Healthy Controls, UHR for Psychosis subjects who Subsequently Remitted (UHR-R), and the Combined Group of UHR Subjects who Remained at Risk/Converted to Psychosis (UHR-NR)

	Controls	UHR-R	UHR-NR	χ^2, F, or *t* (d.f.)	*p*-Value
N (baseline scan)	54	41	52 (40 remained at risk, 12 converted)	—	—
Gender	27M, 27F	27M, 14F	36M, 16F	4.64 (2)	0.10
Age (years)	21.22 ± 4.28, range 14–29	20.76 ± 3.33, range 14–29	21.96 ± 3.91 range 14–29	1.14 (2,90)	0.32
Ethnicity	28C, 14M, 10I, 2O	25C, 11M, 3I, 2O	40C, 8M, 4I	10.36 (6)	0.11
Handedness	47R, 2L, 5A	37R, 2L, 2A	41R, 6L, 5A	3.85 (4)	0.43
UHR subtype	—	34 APS, 7 GRD	45 APS, 7 GRD	0.23 (1)	0.63
N with depressive and/or anxiety disorder (%)	—	34 (82.9%)	39 (75%)	0.86 (1)	0.36
N with history of alcohol abuse/ dependence (%)	—	3 (7.3%)	4 (7.7%)	0.005 (1)	0.95
N with history of drug abuse/dependence (%)	—	4 (9.8%)	1 (1.9%)	2.77 (1)	0.10
CAARMS composite scores	—	22.56 ± 12.60	26.38 ± 16.74	1.22 (91)	0.23
Intracranial volume (mm^3)	155 3021 ± 137362.2	1 594 989 ± 171744.8	1 572 357 ± 168665.9	0.79 (2, 90)	0.46
N (first/second follow-up)	31/20	38/9	25[a]/8[b]	—	—
N (antidepressants at baseline/first/second follow-up)	—	13/8/3	30/5/5	—	—
N (benzodiazepines at baseline/first/second follow-up)	—	6/3/3	4/0/0	—	—
N (antipsychotics at baseline/first/second follow-up)	—	—	0/0/2[c]		

Abbreviations: A, ambidextrous; APS, attenuated psychotic syndrome; C, Chinese; CAARMS, Comprehensive Assessment of At-Risk Mental States; d.f., degrees of freedom; F, females; I, Indian; L, left; M, males; M, Malay; MRI, magnetic resonance imaging; O, others; R, right; UHR, ultra-high-risk; V, vulnerability/genetic risk group. Measures of continuous variables are indicated by their means ± standard deviation. χ^2, analysis of variance, or independent *t*-tests were used to examine possible group differences in the variables.

[a]We conducted the first follow-up MRI scan for 5 of the UHR subjects who had developed psychosis, but did not conduct the second follow-up scan for these five subjects.

[b]Among the subjects with second follow-up scans, one subject had converted to psychosis between the first and second follow-up, whereas one subject had converted to psychosis 10 months after the second follow-up scan.

[c]At the point of the second follow-up, two participants were newly diagnosed with clinical psychosis and were prescribed antipsychotics (risperidone and quetiapine). The others were scanned before receiving medication.

No Group Differences in Hippocampal Volumes at Baseline

At baseline, no between-group differences in global measures of bilateral hippocampal volumes were found; also, similar results were found using the standard subcortical segmentation method (see Supplementary Table S2). However, a significant group by time effect was found for the volume of the right (but not the left) global hippocampus ($F_{2,\ 148} = 9.69$, $p = 0.0001$, Table 2A). *Post hoc* analyses found that UHR-NR, compared with UHR-R and HC subjects, showed greater decline in right hippocampal volume over time (HC and UHR-NR groups: $\beta = 3.7$, $\chi^2 = 14.5$, $p < 0.0003$; UHR-R and UHR-NR group: $\beta = 3.93$, $\chi^2 = 17.8$, $p < 0.0001$) (Figure 1a).

Progressive Volume Decline of the CA1 Subfield in Non-Remitting UHR Subjects

We then tested our primary hypothesis that a volume decline in CA1 would be observed over time in UHR-NR, compared with UHR-R subjects. While no initial group differences were found in the volume of CA1 in both the left and right hemispheres, a significant group by time interaction effect was observed (right: $F_{2,\ 148} = 9.115$, $p = 0.0002$; left: $F_{2,\ 148} = 3.077$, $p = 0.049$; Table 2B). *Post hoc* analysis (adjusted for multiple comparisons between groups) revealed that the CA1 volume decline was more pronounced in the UHR-NR compared with HC (right: $\beta = 1.05$, $\chi^2 = 7.33$, $p < 0.013$; left: $\beta = 0.9$, $\chi^2 = 5.74$, $p = 0.049$) and UHR-R subjects (right: $\beta = 1.59$, $\chi^2 = 18.15$, $p < 0.0001$; left: $\beta = 0.77$, $\chi^2 = 4.42$, $p = 0.07$), but there was no such difference between the UHR-NR and HC subjects (Figures 1b and c). These findings remained unchanged after adjusting for comorbidity (which had an effect on several other subfields but not CA1; see Supplementary Table S3) and treatment with benzodiazepine and antidepressant medication (Supplementary Table S4A). Also, inclusion of two subjects with fluctuating symptomatology (in a secondary analysis) did not substantively alter the results (Supplementary Table S5).

No CA1 Volume Trajectory Differences Between Non-Remitting Converters and Non-Converters

Secondary analyses of additional subgroups (UHR-R, UHR-NR-non-psychosis, and UHR-NR-psychosis) and HC

Table 2 Summary Statistics of Changes in Volumes of The Hippocampus and its Subfields Over Time in HC Subjects, UHR-R Subjects, and UHR-NR Subjects (Those who had Persistent Subthreshold Symptoms and Those who Subsequently Developed Clinical Psychosis)

	ANOVA of group by time	***Post hoc* statistics of group by time effect**		
		UHR-R and HC	**UHR-NR and HC**	**UHR-NR and UHR-R**
(A) Global hippocampus				
Left	$F_{2,\ 148} = 0497$, $p = 0.61$			
Right	$F_{2,\ 148} = 8.18$, $p = 0.0004$[a]	$\beta = 0.2$, $\chi^2 = 0.09$, $p = 0.76$, CI = − 1.60, 0.48	$\beta = -2.94$, $\chi^2 = 12.09$, $p = 0.0007$, CI = − 4.45, − 1.17	$\beta = -3.14$, $\chi^2 = 15.06$, $p = 0.0002$, CI = − 1.60, 0.49
(B) CA1				
Left CA1	$F_{2,\ 148} = 3.10$, $p = 0.048$[a]	$\beta = -0.14$, $\chi^2 = 0.23$, $p = 0.63$, CI = − 0.74, 0.45	$\beta = -0.9$, $\chi^2 = 5.56$, $p = 0.05$, CI = − 0.16, − 0.15	$\beta = -0.76$, $\chi^2 = 4.26$, $p = 0.07$, CI = − 1.46, − 0.02
Right CA1	$F_{2,\ 148} = 9.08$, $p = 0.0002$[a]	$\beta = 0.54$, $\chi^2 = 2.93$, $p = 0.09$, CI = − 0.08, 1.16	$\beta = -1.05$, $\chi^2 = 7.32$, $p = 0.007$, CI = − 1.8, − 0.28	$\beta = -1.6$, $\chi^2 = 18.15$, $p < 0.0001$, CI = − 2.33, − 0.85
(C) Other hippocampal subfields				
Left GCL	$F_{2,\ 148} = 0.72$, $p = 0.49$			
Right GCL	$F_{2,\ 148} = 1.40$, $p = 0.25$			
Left CA4	$F_{2,\ 148} = 0.62$, $p = 0.54$			
Right CA4	$F_{2,\ 148} = 1.24$, $p = 0.30$			
Left CA3	$F_{2,\ 148} = 0.83$, $p = 0.43$			
Right CA3	$F_{2,\ 148} = 4.97$, $p = 0.008$[a]	$\beta = 0.09$, $\chi^2 = 0.56$, $p = 0.46$, CI = − 0.15, 0.33	$\beta = -0.37$, $\chi^2 = 5.84$, $p = 0.031$, CI = − 0.68, − 0.068	$\beta = -0.46$, $\chi^2 = 9.78$, $p = 0.005$, CI = − 0.76, − 0.17
Left molecular layer	$F_{2,\ 148} = 0.76$, $p = 0.47$			
Right molecular layer	$F_{2,\ 148} = 2.65$, $p = 0.07$			
Left subiculum	$F_{2,\ 148} = 0.91$, $p = 0.40$			
Right subiculum	$F_{2,\ 148} = 0.60$, $p = 0.55$			
Left tail	$F_{2,\ 148} = 0.55$, $p = 0.58$			
Right tail	$F_{2,\ 148} = 1.38$, $p = 0.26$			

Abbreviations: ANOVA, analysis of variance; CI, confidence interval; HC, healthy control; UHR, ultra-high-risk; UHR-NR, non-remitted UHR; UHR-R, remitted UHR. A linear mixed-effects model was used to test whether there were group differences in volume measures over time. As there were three groups, an ANOVA was first performed to test whether there was statistical evidence of a group by time effect on the following measures: (A) the global hippocampus, that is, the sum of the seven subfields of the hippocampus in the left and right hemisphere of the brain, (B) *a priori* CA1 hippocampal subfields; and (C) the other subfields of the hippocampus. [a]Significance at a threshold of $p < 0.05$. *Post hoc* pair-wise group comparisons were then performed using Wald tests (based on sampling covariance matrix of the parameter estimates) to compute an asymptotic χ^2, with *p*-values were adjusted for multiple comparisons between the groups using the Holm–Bonferroni method. The βs indicate the differences in slopes between the two groups. For example, in the case of right CA1, the slope of UHR-NR relative to HC is − 1.05 per month. The βs can be considered as unstandardized effect sizes. The 95% CIs are also shown; a CI covering the value of zero means the null hypothesis cannot be rejected.

revealed significant group differences in the right CA1 volume trajectory (right: $F_{3,\ 147} = 6.9$, $p = 0.0002$), while group differences in the left CA1 were subtle ($F_{3,\ 147} = 2.36$, $p = 0.074$). Consistent with the findings of the primary analysis, *post hoc* tests showed a steeper right CA1 volume decline in the UHR-NR-non-psychosis group compared with the UHR-R ($\beta = 1.4$, $\chi^2 = 11.9$, $p = 0.003$), and also in the UHR-NR-psychosis group compared with the HC group ($\beta = 1.93$, $\chi^2 = 7.1$, $p = 0.03$) and UHR-R group ($\beta = 2.46$, $\chi^2 = 11.9$, $p = 0.003$). The volume trajectories did not differ between the UHR-NR-psychosis and UHR-NR-non-psychosis groups.

Volume Trajectories of Other Hippocampal Subfields

With the exception of the right CA3 ($F_{2,\ 148} = 4.97$, $p = 0.0082$), the group by time effect was not observed in the other subfields (Table 2C). UHR-NR subjects displayed a steeper volume decline compared with HC ($\beta = -0.37$, $\chi^2 = 5.84$, $p = 0.03$) and UHR-R subjects ($\beta = -0.46$, $\chi^2 = 9.8$, $p = 0.005$) for the right CA3. However, when we accounted for treatment with antidepressant and benzodiazepine medications, there was no longer a significant group by time effect for the right CA3 (Supplementary Table S4B). Also, the secondary pair-wise comparisons among the further subtyped UHR subjects and HC groups did not reveal any significant group differences in CA3 volumes.

Rate of CA1 Volume Decline Correlates With Rate of Worsening CAARMS Scores

We then examined whether there was a longitudinal relationship between decline in volume of the affected subfields, that is, right CA1, left CA1, and right CA3, and

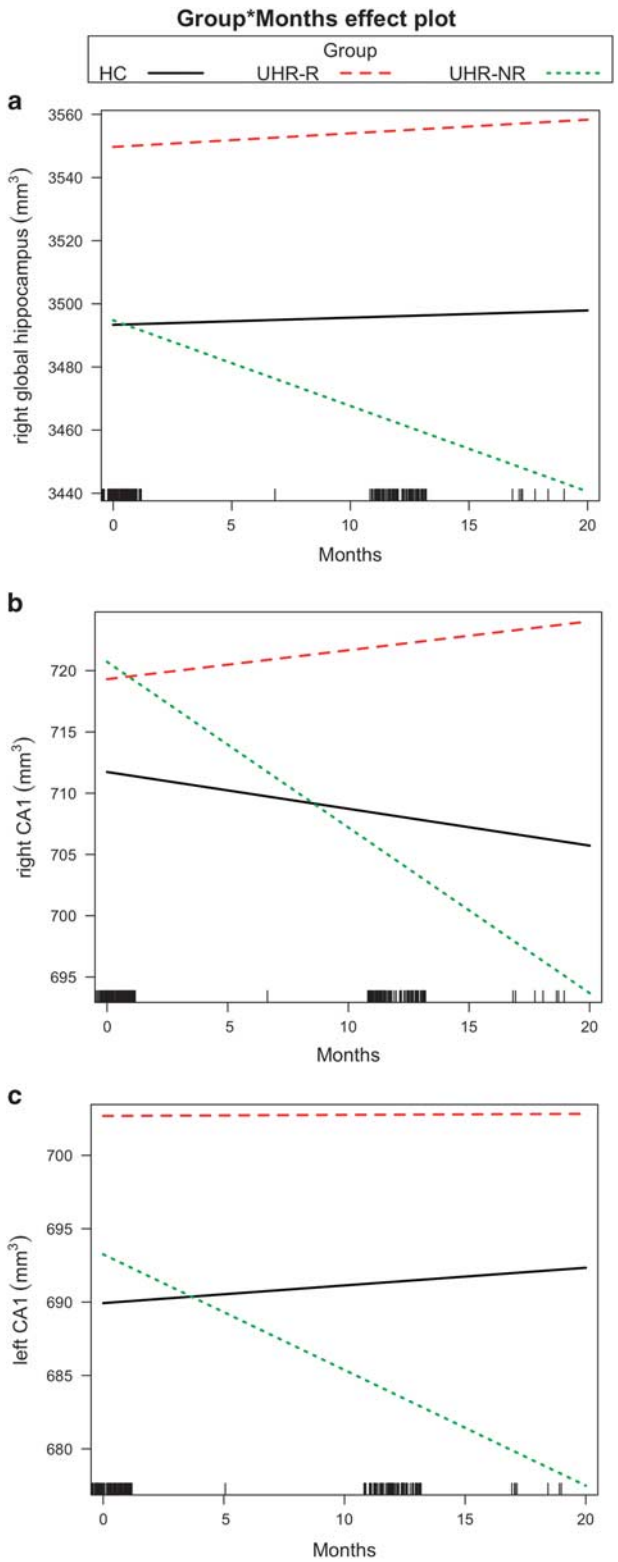

Figure 1 CA1 volume declines over time in ultra-high-risk (UHR) subjects showing persistent or worsening symptoms. Compared with healthy controls (HCs) and UHR subjects who remitted (UHR-R), the UHR 'non-remitters', whose symptoms persisted or worsened over time (UHR-NR), showed steeper progressive decline in volume in measures of the (a) right hippocampus, that is, sum of hippocampal subfields in the right hemisphere, (b) right CA1, and (c) left CA1. The bars at the bottom of the x axis represent the number of observations over time.

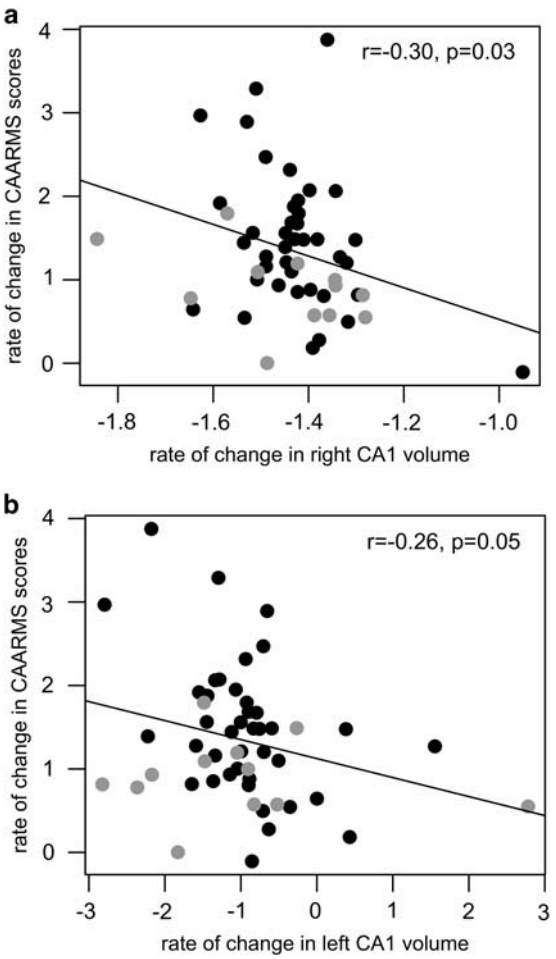

Figure 2 CA1 volume decline is associated with worsening of symptoms over time. In the ultra-high-risk non-remitters (UHR-NR), an inverse correlation was found between the rate of change in Comprehensive Assessment of At-Risk Mental States (CAARMS) scores and the (a) rate of change in right CA1 volume and (b) rate of change in left CA1 volume. Black circles indicate UHR-NR subjects who have persistent but subthreshold symptoms (UHR-NR-non-psychosis); open circles indicate UHR subjects who developed clinical psychosis (UHR-NR-psychosis). Secondary analyses revealed no differences in these CA1 volume–symptom relationships between the UHR-NR-non-psychosis and UHR-R-psychosis groups.

change in symptom scores in the UHR-NR group. We found an association between the rates of volume decline of CA1 and rates of increasing severity in CAARMS composite scores (right CA1: $r=-0.30$, $p=0.03$; Figure 2a; left CA1: $r=-0.26$, $p=0.05$; Figure 2b). This relationship was not found for the right CA3 volumes. Also see Supplementary Figure S1.

DISCUSSION

Summary of Main Findings

At baseline, no volume deficits of the hippocampus or of any of its subfields were observed in a cohort of UHR young adults. Over time, however, progressive decline of the volume of the CA1 subfield was found in UHR subjects who remained persistently symptomatic or developed full-blown psychosis. Moreover, the decline in CA1 volume in this non-remitting group was associated with increasing

symptom severity. In contrast, CA1 volume remained unchanged in UHR individuals who remitted.

No Initial Hippocampus Volume Deficit in At-Risk Individuals

The absence of baseline global hippocampal volume deficits in UHR subjects observed here agrees with findings of some prior studies (Buehlmann *et al*, 2010; Cannon *et al*, 2015; Klauser *et al*, 2015; Schobel *et al*, 2013; Velakoulis *et al*, 2006) but not others (Dean *et al*, 2015; Francis *et al*, 2013; O'Driscoll *et al*, 2001; Seidman *et al*, 2002, 2014; Wood *et al*, 2010). The inconsistency of results of prior studies may be related to variability across studies in the phases at which the UHR subjects were studied, for example, some of the studies may have captured subjects during the late prodrome (when hippocampal changes may have begun or at least become detectable at a macroscopic level), while others may have assessed subjects in early prodromal stages (before measurable changes in hippocampal volumes have occurred) (Addington and Heinssen, 2012; Fusar-Poli *et al*, 2014). The discrepancies in this literature may also be related to methodologic differences across studies and/or heterogeneity within and across cohorts, that is, variability in MRI acquisition parameters (Bois *et al*, 2014; O'Driscoll *et al*, 2001; Seidman *et al*, 2002) and hippocampal analyses methods used (Bois *et al*, 2014; Dean *et al*, 2015; Fusar-Poli *et al*, 2011), as well as the potentially confounding effects of comorbid mood, anxiety, and substance abuse disorders that have been linked to alterations in hippocampal structure and function (Cha *et al*, 2016; Smith *et al*, 2015; Whittle *et al*, 2014). Of note, the UHR subjects in the present study cohort were never treated with antipsychotic and mood-stabilizing medications, and predominantly lacked histories of substance abuse.

Emergence of CA1 Volume Decline in At-Risk Individuals Differentiates Non-Remitters From Remitters

Gradual volume decline in bilateral CA1 and right CA3—but not in other hippocampal subfields—was found in subjects who remained persistently symptomatic at a subthreshold level, or whose symptoms worsened to the point of meeting criteria for an episode of clinical psychosis. The trajectory of CA1 volume changes observed here in non-remitting UHR subjects is similar to the pattern of hypermetabolism, which preceded shape abnormalities, of CA1 that was previously found in clinical high-risk subjects who transitioned to psychosis (Schobel *et al*, 2013). In a recent study, we reported a selective CA1 volume deficit in subjects in the early stages of schizophrenia (Ho *et al*, 2016). The current observation of a similar pattern of findings in UHR subjects further indicates that changes in the CA1 subfield of the hippocampus occur early on in the illness process and are not attributable to secondary effects of having the illness, such as treatment or chronicity. In addition, the finding of an association between progressive CA1 volume decline in the UHR-NR subjects and increasing symptom severity replicates a similar association observed in patients with schizophrenia (Ho *et al*, 2016). Taken together, these findings provide converging evidence that CA1 atrophy over time, regardless of illness stage, is closely linked to clinical deterioration.

As the transition to frank psychosis is clinically defined, it is unclear whether criteria for a psychosis diagnosis can be precisely demarcated by any biomarker threshold (Yung *et al*, 2010). Recently, converging clinical, cognitive, biological, and genetic evidence across psychotic spectrum disorders, and at both early and chronic stages, has supported a dimensional model of psychosis (Craddock *et al*, 2009; Hill *et al*, 2013; Kuswanto *et al*, 2016; O'Donovan *et al*, 2009; Reininghaus *et al*, 2013; Tamminga *et al*, 2013). Consistent with this model, the findings of minimal CA1 volume trajectory differences between the non-remitters who remained persistently symptomatic at a subthreshold level and those who progressed to full-blown psychosis suggest that the CA1 volume changes may be most closely linked, in a dimensional manner, to the emergence of psychotic symptoms, rather than to the diagnosis of schizophrenia *per se*.

Possible Underlying Cellular Mechanisms

The progressive reduction of CA1 volume in individuals with persistent subthreshold symptoms could be related to changes in neuronal, glial, synaptic or vascular cytoarchitecture, or density over time (Ho *et al*, 2013). Post-mortem studies attempting to identify cytoarchitectural changes in the hippocampus in schizophrenia have generally found no reductions in the number of pyramidal neurons (Heckers *et al*, 1991; Konradi *et al*, 2011; Walker *et al*, 2002) but some evidence for decreased neuronal size and dendritic density throughout the hippocampus (Arnold *et al*, 1995; Benes *et al*, 1991, 2001; Rosoklija *et al*, 2000; Zaidel *et al*, 1997). Among the hippocampal subfields, CA1 has the largest number of γ-aminobutyric acid interneurons, which are decreased in density in patients with schizophrenia (Konradi *et al*, 2011). Based on this finding and related work, it has been proposed that the balance between excitation and inhibition is disrupted in CA1, as a result of either blockage of the *N*-methyl-D-aspartate receptors on interneurons or a reduction in the number of interneurons, leading to disinhibition of the CA1 pyramidal cells (Heckers and Konradi, 2015; Lisman *et al*, 2010). Extending this hypothesis, we suggest that this excitatory–inhibitory imbalance leads to alterations in synaptic architecture and density in CA1, and an overall thinning of the CA1 apical neuropil.

Limitations and Future Directions

These findings should be viewed in light of several limitations of the study. First, the sample size of the converters is modest. The rates of conversion in the present sample cohort (14%) are lower than the initial UHR cohorts (~30–40%) (Cannon *et al*, 2008; Yung *et al*, 2003), although they are similar to recent at-risk cohorts across multiple worldwide sites (Cannon *et al*, 2016; Carrion *et al*, 2016; Ruhrmann *et al*, 2010); indeed, rates of conversion have been steadily declining over the past decade to ~7–15% (Fusar-Poli *et al*, 2015; Hartmann *et al*, 2016).

Second, the present findings of progressive CA1 volume decline in the converters was significant only in the right hemisphere; this appears inconsistent with prior work in prodromal subjects, which detected metabolic and shape changes primarily in the left CA1 (Schobel *et al*, 2013). However, it is notable that both the prior and current study

observed similar trends in the contralateral hemisphere. In light of this and the modest sample sizes of these studies, differences in image acquisition and analysis methods, as well as the lack of evidence for any strong laterality of hippocampal abnormalities in schizophrenia (Okada *et al*, 2016), we suggest that the changes in CA1 volume over time associated with psychotic symptoms are not limited to one hemisphere.

Third, the internal boundaries of the hippocampal subfields are not visible at 1 mm resolution, thus the automated segmentation method relies heavily on prior knowledge (ie, the probabilistic *ex vivo* atlas). While segmentations on 1 mm data cannot delineate the subfields as accurately as higher resolution scans, it has been shown, on several large publicly available data sets of different age groups and disorders, that the segmentations on 1 mm scans are highly reliable (Whelan *et al*, 2016), even across different MRI scanner platforms and field strengths (Whelan *et al*, 2016). Also, on large data sets with the same resolution as our data, it has been demonstrated that the segmentations based on local changes within the hippocampal subfield(s) can discriminate between diseased and healthy states with higher accuracy than the whole hippocampus (Iglesias *et al*, 2015).

Future larger-scale, prospective studies of UHR subjects could assess the value of including a CA1 volume measure in a multidomain index, which may include proinflammatory cytokines in the plasma (Cannon *et al*, 2015), negative symptoms (Piskulic *et al*, 2012), cognitive abilities (Lin *et al*, 2013), and white matter markers (Bloemen *et al*, 2010), designed to predict risk and transition to full psychosis.

FUNDING AND DISCLOSURE

The authors declare no conflict of interest.

ACKNOWLEDGMENTS

This work was supported by the National Medical Research Council Translational and Clinical Research Flagship Programme (grant number NMRC/TCR/003/2008), Biomedical Research Council, Singapore (grant number BMRC 04/1/36/19/372 to JZ), National Medical Research Council, Singapore (grant number CBRG/0088/2015 to JZ), and National Medical Research Council Centre Grant Programme (grant number NMRC/CG/004/2013 to NFH). JL is further supported by the National Healthcare Group Clinician Scientist Career Scheme. JEI is supported by a Marie Sklodowska-Curie fellowship (Project No. 654911 'THALAMODEL') and by the Spanish Ministry of Economy and Competitiveness (reference TEC2014-51882-P). DJH is supported by the National Institute of Mental Health (grant number RO1MH095904). The funding bodies had no roles in the design of the study, collection and analysis of data, and decision to publish.

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Supplementary Information accompanies the paper on the Neuropsychopharmacology website (http://www.nature.com/npp)