

## **Hippocampal subfields and visuospatial associative memory across stages of schizophrenia-spectrum disorder**

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## Abstract

*Background:* While previous studies have identified relationships between hippocampal volumes and memory performance in schizophrenia, these relationships are not apparent in healthy individuals. Further, few studies have examined the role of hippocampal subfields in illness-related memory deficits, and no study has examined potential differences across varying illness stages. The current study aimed to investigate whether individuals with early and established psychosis exhibited differential relationships between visuospatial associative memory and hippocampal subfield volumes.

*Methods:* Measurements of visuospatial associative memory performance and grey matter volume were obtained from 52 individuals with a chronic schizophrenia-spectrum disorder, 28 youth with recent-onset psychosis, 52 older healthy controls, and 28 younger healthy controls.

*Results:* Both chronic and recent-onset patients had impaired visuospatial associative memory performance, however only chronic patients showed hippocampal subfield volume loss. Both chronic and recent-onset patients demonstrated relationships between visuospatial associative memory performance and hippocampal subfield volumes in the CA4/dentate gyrus and the stratum that were not observed in older healthy controls. There were no group by volume interactions when chronic and recent-onset patients were compared.

*Conclusions:* The current study extends the findings of previous studies by identifying particular hippocampal subfields, including the hippocampal stratum layers and the dentate gyrus, that appear to be related to visuospatial associative memory ability in individuals with both chronic and first-episode psychosis.

## Introduction

Memory impairments are considered to be a core cognitive dysfunction in schizophrenia, with patients exhibiting deficits across a wide range of memory tasks relative to healthy controls. While it has previously been suggested that memory ability is stably impaired in individuals with schizophrenia (Bora & Murray, 2013), recent longitudinal evidence indicates that one particular type of memory, visuospatial associative memory, is preserved in individuals with first-episode psychosis, with deterioration occurring only as the illness progresses (Haring *et al.* 2017; Wannan *et al.* 2017). It is therefore possible that deficits in visuospatial associative memory observed in chronic patients (Wood *et al.* 2002; Stip *et al.* 2005; Donohoe *et al.* 2008) are related to stage-specific brain abnormalities in regions associated with this ability (Bartholomeusz *et al.* 2016). Given that deficits in visuospatial associative memory have previously been associated with both concurrent and prospective functional impairment in individuals with first-episode and established psychotic illness (Barnett *et al.* 2005; Prouteau *et al.* 2005; Wannan *et al.* 2017), identifying the specific brain regions involved in this ability may be an important first step toward identifying potential underlying mechanisms or treatment targets for deficits in episodic memory that lead to impaired functioning in psychosis.

Visuospatial associative memory ability is thought to be heavily reliant on medial temporal lobe structures, particularly the hippocampus (Barnett *et al.* 2016). In healthy individuals and those in the early stages of Alzheimer's disease, visuospatial associative memory performance has been associated with bilateral hippocampal activation, with increased activation occurring alongside increasing memory load in healthy individuals (Gould *et al.* 2005; De Rover *et al.* 2011). Lesion studies also show that neurosurgical patients who have undergone amygdalo-hippocampectomy demonstrate significant impairments on tasks of visuospatial associative memory (Owen *et al.* 1995). Thus, there is evidence for the involvement of the hippocampus in visuospatial associative memory, although it is unclear whether this extends to measures of hippocampal volume as well as activation.

In individuals with schizophrenia, structural brain abnormalities have consistently been observed in brain regions that are associated with visuospatial associative memory. Bilateral hippocampal volume loss is well established in individuals with chronic schizophrenia (Velakoulis *et al.* 1999; Wright *et al.* 2000; Zakzanis *et al.* 2000; Wood *et al.* 2001; Shepherd *et al.* 2012; van Erp *et al.* 2016). While these findings are less consistent in recent-onset patients, several studies have shown left (Velakoulis *et al.* 1999), right (Fornito *et al.* 2009),

and bilateral (Copolov *et al.* 2000; Steen *et al.* 2006; Vita *et al.* 2006) hippocampal volume loss in early illness. Additionally, the hippocampus consists of several functionally distinct subfields, including the Cornu Ammonis regions (CA1-4), the dentate gyrus, the subiculum, and the stratum layers, (Duvernoy 1998). High-resolution magnetic resonance imaging (MRI) has revealed a pattern of progressive involvement of hippocampal subfields with increasing illness chronicity in schizophrenia (Mathew *et al.* 2014; Haukvik *et al.* 2015; Ho *et al.* 2016; Baglivo *et al.* 2018). Namely, in first-episode patients volume loss appears to be restricted to hippocampal subfields in the left hemisphere (Baglivo *et al.* 2018), whereas in chronic patients volume loss occurs bilaterally, across most or all investigated hippocampal subfields (Mathew *et al.* 2014; Haukvik *et al.* 2015; Ho *et al.* 2016).

Given that abnormalities of the hippocampus are considered to be among the most robust findings in schizophrenia, it is unsurprising that a number of studies have attempted to elucidate potential relationships between hippocampal volume and memory ability in this population. In individuals with first-episode psychosis (Hasan *et al.* 2011; Lappin *et al.* 2013) and chronic schizophrenia (Gur *et al.* 2000; Nestor *et al.* 2007), larger hippocampal volumes have been associated with superior memory performance. In healthy individuals however, relationships between hippocampal volumes and memory performance appear to be absent or attenuated (Van Petten 2004; Den Heijer *et al.* 2012; Lappin *et al.* 2013), suggesting that these relationships may be moderated by hippocampal pathology unique to patient groups. Of the two known studies correlating hippocampal subfield volumes with memory in schizophrenia, one found that smaller subiculum volumes were associated with poorer verbal memory performance (Haukvik *et al.* 2015), and the other found that left CA1, CA2/CA3, subiculum, and CA4/dentate gyrus volumes were positively correlated with list-learning (Mathew *et al.* 2014). However, while these findings provide initial evidence for the involvement of particular hippocampal subfields for memory performance in schizophrenia, it remains unclear whether these relationships are present at earlier illness stages, and whether similar results may be seen with measures of visuospatial associative rather than verbal memory.

The current study aimed to investigate relationships between visuospatial associative memory and hippocampal subfield volumes in early and established psychosis. The specific aims of the study were to (i) evaluate whether chronic schizophrenia patients and youth with recent-onset psychosis differed in visuospatial associative memory performance, and hippocampal subfield volumes, in comparison to healthy controls and each other, and (ii) to determine whether these patient groups exhibited differential relationships between hippocampal subfield volumes and visuospatial associative memory, compared to healthy controls and to

each other. We predicted that chronic patients would exhibit severe impairments in visuospatial associative memory relative to both recent-onset patients and older healthy controls, whereas recent-onset patients would perform at a similar level to younger healthy controls. We also expected to find widespread hippocampal subfield volume loss in chronic patients, relative to both recent-onset patients and healthy controls. Finally, we expected to find stronger relationships between visuospatial associative memory and hippocampal subfield volumes in patient groups relative to healthy controls, and stronger relationships in chronic patients relative to recent-onset patients.

## **Method**

### *Participants*

Fifty-two individuals with a chronic schizophrenia-spectrum disorder (illness duration > 5 years) and 28 youth with recent-onset psychosis (< 2.5 years since first presentation to a mental health service for treatment of psychosis, number of psychotic episodes  $\geq$  1) were recruited as part of two separate studies. Recent-onset patients were recruited from the Early Psychosis Prevention and Intervention Centre (EPPIC), Melbourne, Australia. EPPIC is a specialised public mental health service for people aged 15–25 years living in the north-western suburbs of metropolitan Melbourne who have experienced a first episode of psychosis. EPPIC intake criteria include: first presentation of suspected psychotic illness, or less than six-months previous antipsychotic treatment for a first psychotic episode. Chronic patients were recruited from inpatient (20%) and outpatient (80%) clinics in Melbourne, Australia. Study measures were identical across both studies, except for diagnostic and symptom measures. All patients were diagnosed with a schizophrenia-spectrum disorder according to the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) (American Psychiatric Association 1994). Diagnoses were confirmed using either The Mini International Neuropsychiatric Interview (MINI; Sheehan *et al.*, 1998) or the Structured Clinical Interview for DSM-IV Axis I Disorders (SCID; First *et al.* 2002).

Two groups of age-matched healthy control participants with similar socioeconomic and educational backgrounds were recruited from the general community using online advertising: young controls ( $n = 28$ ; mean age of 21.96), and older controls ( $n = 52$ ; mean age of 38.62). The MINI or the SCID were used to rule out current or past history of psychopathology in healthy controls.

Exclusion criteria for all participants were (1) a history of head injury or seizures, (2) diagnosis of a neurological disorder, (3) pregnancy, and (4) contraindication to MRI

scanning. Additional exclusion criteria specific to healthy controls included personal and first-degree relatives with a history of psychotic illness. All participants were either native English speakers or were fluent in English as determined by their self-report of numbers of years learnt English or schooled in English. Five older healthy controls, 4 younger healthy controls, and 3 recent-onset patients came from non-English speaking backgrounds. When analyses were re-run following removal of four older healthy controls who did not receive any formal education in English results remained unchanged.

The studies were approved by the Melbourne Health (2012.066, 2012.069) and Austin Health (H2012/04525) Human Research Ethics Committees and all participants provided written informed consent prior to participation.

#### *Clinical measures*

Negative symptoms in both patient cohorts were measured using the Scale for the Assessment of Negative Symptoms (SANS; Andreasen, 1983). Positive symptoms were measured using the Brief Psychiatric Rating Scale (BPRS; Overall & Gorham, 1962) or the Positive and Negative Syndrome Scale (PANSS; Kay, Fiszbein, & Opler, 1987). PANSS scores were converted to BPRS scores (Leucht *et al.* 2013), in order to provide a measure of general psychopathology and positive symptoms. Each participants' functioning was evaluated using the Social and Occupational Functioning Assessment Scale (SOFAS; Goldman, Skodol, & Lave, 1992). Current IQ was measured using the 2-subtest short form (Vocabulary and Matrix Reasoning) of the WAIS-III (Wechsler 1997). The Wechsler Test of Adult Reading (Wechsler 2001) was used to estimate premorbid IQ.

#### *Neuropsychological assessment*

Visuospatial associative memory was measured using the Visuospatial Paired-Associate Learning (PAL) Task from the CANTAB (Cambridge Cognition 2016). Details of this task have been described previously (Wannan *et al.* 2017). The total number of trials required to locate all of the patterns correctly in all stages of the task was the variable of interest, and therefore lower scores are better. For participants who did not complete all stages (due to failing at an earlier stage), the maximum score of 10 trials was added to their score for each stage that was not completed (Cambridge Cognition 2012).

#### *Imaging data acquisition*

Magnetic resonance images were acquired on a Siemens Avanto 3T Magnetom TIM Trio scanner. T1-weighted images were acquired using an optimized Magnetization-Prepared Rapid Acquisition Gradient Echo (MPRAGE) sequence. The sequence parameters were: 176

sagittal slices of 1 mm thickness without gap, field of view (FOV) =  $250 \times 250 \text{ mm}^2$ , repetition time (TR)=1980 ms, echo time (TE) = 4.3 ms, flip angle =  $15^\circ$ , using an acquisition matrix of  $256 \times 256$  resulting in a final reconstructed voxel resolution of  $0.98 \times 0.98 \times 1.0 \text{ mm}^3$ .

### *Image processing*

Intracranial volume (ICV) was estimated using FreeSurfer version 5.3 (<http://surfer.nmr.mgh.harvard.edu>)(Fischl *et al.* 2004). FreeSurfer determines estimated ICV using an atlas scaling factor (i.e. the determinant of an affine transformation matrix) derived from registering images to an average template using a full (12-parameter) affine transformation (Malone *et al.* 2015). For detailed information, see Buckner *et al.*, 2004. Fully-automated segmentation of the hippocampus was carried out using the MAGeT Brain algorithm (Chakravarty *et al.*, 2013: [//github.com/CobraLab/MAGeTbrain](https://github.com/CobraLab/MAGeTbrain)). This modified multi-atlas segmentation technique is designed to use a limited number of high-quality manually segmented atlases of the hippocampal subfields as input (Pipitone *et al.* 2014). These atlases include definitions for the right and left CA1, CA2CA3, CA4/dentate gyrus (CA4DG), strata radiatum/lacunosum/moleculare (stratum), and subiculum (see Figure 1). Atlas segmentations are propagated to a template library and then to subjects using nonlinear registration (Avants *et al.* 2008), and a subset of the population under study is used as the template library through which the final segmentation is bootstrapped. Each subject in the template library is segmented through nonlinear atlas-to-template registration followed by label propagation, yielding 5 unique definitions of the hippocampus for each of the templates. In the current study, 21 templates, consisting of ten controls and eleven patients, were selected based on age and gender to ensure a representative template set. This resulted in 105 candidate labels for each subject, and labels were then fused using a majority vote to complete the segmentation process. As the dentate gyrus and subiculum are included within the MAGeT Brain algorithm, the term “hippocampus” used throughout this manuscript encompasses the hippocampal formation. Quality assurance for each scan was carried out at three time points. First, unprocessed minc files were visually inspected for abnormalities and to ensure image quality and orientation. Second, following data pre-processing, scans were inspected to ensure successful registration to MNI space and inhomogeneity correction. Finally, following processing with MAGeT Brain, each participant’s hippocampal subfield segmentation was visually inspected to ensure segmentation quality using a three point system: 0 = fail; 0.5 = good pass; 1 = excellent pass (for more information on our detailed quality control procedure see Amaral *et al.*, 2018).

### **Data Analysis**

Data were analysed using SPSS version 24.

#### *Analysis of demographic data*

Group differences on demographic measures were examined using chi-square tests for categorical data, and t-tests for continuous variables.

#### *Analysis of PAL data*

The number of trials required to complete the PAL task was compared between groups using robust generalized linear models, with age and IQ included as covariates. Three separate analyses were conducted: recent-onset vs younger controls, chronic vs older controls, recent-onset vs chronic. Chi square tests were also used to compare the number of individuals who passed or failed the PAL task in each group.

#### *Between-group differences in hippocampal volumes*

ANCOVAs were used to compare total hippocampal and hippocampal subfield volumes between groups, with age, sex and ICV included as covariates. Three separate analyses were conducted: recent-onset vs younger controls, chronic vs older controls, recent-onset vs chronic. Results were corrected for multiple comparisons across each hemisphere using a False Discovery Rate (FDR) of  $p < .05$  (Benjamini & Hochberg 1995).

#### *Interactions between hippocampal subfield volumes and group*

In order to determine whether differential relationships existed between visuospatial memory ability and hippocampal subfield volumes between groups, a series of robust generalized linear models were conducted. Each model included main effects of group and volume and any group  $\times$  volume interactions, with age and ICV included as covariates. Main effects of volume and group  $\times$  volume interactions were the outcomes of interest. Results were corrected for multiple comparisons across each hemisphere using a False Discovery Rate (FDR) of  $p < .05$  (Benjamini & Hochberg 1995). For regions where there was a significant group  $\times$  volume interaction, post-hoc linear models were conducted separately for each group.

Sex was not included as a covariate in the analysis of PAL data as there were no between-group differences in PAL performance for males and females, and all groups were well matched for sex. Medication and illness duration were also considered as covariates when comparing the two patient groups. However as neither current antipsychotic dosage nor illness duration was correlated with either hippocampal subfield volumes or performance on the PAL task in either recent-onset or chronic patients, these were not included in further analyses.

## Results

### *Demographic results*

Demographic data are displayed in Table 1. There were no group differences for age or sex between the two patient groups and their respective healthy control groups. Chronic patients were significantly older than recent-onset patients. Both patient groups had significantly lower current IQ, premorbid IQ, years of education, and SOFAS scores compared to their respective healthy control groups. Chronic patients also had significantly lower current IQ and SOFAS scores than recent-onset patients, however there were no differences in premorbid IQ or years of education between patient groups. Chronic patients also had higher positive and negative symptom scores and antipsychotic doses than recent-onset patients. Although there were group differences in IQ, it is difficult to disentangle these effects from the disorder itself due to the intrinsic nature of IQ deficits to schizophrenia-spectrum illnesses (Kahn & Keefe 2013). When a particular variable is an attribute of a disorder, or intrinsic to the condition, it is suggested to be meaningless to include this variable as a covariate (Tupper & Rosenblood 1984; Miller & Chapman J 2001; Dennis *et al.* 2009). Therefore, IQ was not included as a covariate in any of the regression models.

### *Neuropsychological results*

As seen in Figure 2, Chronic patients required significantly more trials than older healthy controls on the PAL task, Wald  $\chi^2 = 30.83$ ,  $p < .0001$ , ( $\exp(\beta) = 730.25$  (95% CI: 71.22-7487.35)). Chronic patients were also significantly more likely to fail the PAL task compared to older controls,  $\chi^2 = 30.31$ ,  $p < .0001$ . recent-onset patients required significantly more trials than younger healthy controls on the PAL task, Wald  $\chi^2 = 4.59$ ,  $p = .032$ , ( $\exp(\beta) = 13.21$  (95% CI: 1.25-140.02)), however, they were no more likely than healthy controls to fail the PAL task  $\chi^2 = 1.98$ ,  $p = .16$ . There was no difference between chronic and recent-onset patients for number of trials required on the PAL task, Wald  $\chi^2 = 1.73$ ,  $p = .188$ , ( $\exp(B) = 13.56$  (95% CI: 0.28-657.85)), however chronic patients were significantly more likely to fail to complete the PAL task than were recent-onset patients,  $\chi^2 = 11.20$ ,  $p = <.001$ . Number of trials required to complete the PAL task for each group are displayed in Figure 2.

### *Between-group differences in hippocampal volumes*

Chronic patients had reduced volumes in the CA4DG, CA2CA3, and stratum bilaterally, and in the right subiculum relative to older healthy controls (see Table 2). There were no differences between recent-onset patients and younger healthy controls for any subfield

volume in either hemisphere. There were also no differences between recent-onset and chronic patients for any subfield volumes except for the right CA1, where recent-onset patients had volume reductions compared to chronic patients.

#### *Interactions between hippocampal subfield volumes and group*

##### *Recent-onset vs. younger controls*

As seen in Figure 3A, significant group  $\times$  volume interactions were observed in the left hemisphere for stratum, CA4DG, and CA2CA3 volume. Significant group  $\times$  volume interactions were also found in the right hemisphere for CA4DG and stratum volumes. Post-hoc analyses revealed that recent-onset patients had significant negative correlations between hippocampal subfield volumes and number of trials required to complete the PAL task in the left CA4DG and stratum, that were not observed in healthy control subjects. For the left CA2CA3 and the right CA4DG and stratum, recent-onset patients also demonstrated negative correlations with PAL trials that were not observed in healthy individuals, although these did not reach significance. Detailed results are provided in supplementary materials (Table S1).

##### *Chronic vs. older controls*

As seen in Figure 3B, significant group  $\times$  volume interactions were observed in the right hemisphere for CA1, CA4DG, CA2CA3, and stratum volumes. There was also a significant group  $\times$  volume interaction for the left stratum. Post-hoc analyses revealed that chronic patients had significant negative correlations between hippocampal subfield volumes and number of trials required to complete the PAL task in the right CA4DG, and in the stratum bilaterally, that were not observed in healthy control subjects. For the right CA1, chronic patients also demonstrated a negative correlation with PAL trials that were not observed in healthy individuals, although this did not reach significance. Older healthy controls also demonstrated a significant positive relationship between right CA2CA3 volume and PAL trials that was not observed in chronic patients. Detailed results are provided in supplementary materials (Table S2).

##### *Chronic vs. recent-onset*

There were no significant group  $\times$  volume interactions for chronic and recent-onset patients in any subfield region. However, there were significant main effects of volume for the left CA4DG and stratum, with larger subfield volumes associated with better performance on the PAL task. Detailed results are provided in supplementary materials (Table S3).

## **Discussion**

In this study, we examined visuospatial associative memory ability and hippocampal subfield volumes in individuals with recent-onset and chronic schizophrenia as well as their relationships. Both chronic patients and youth with recent-onset psychosis had impaired visuospatial associative memory performance, however, only chronic patients showed hippocampal subfield volume loss relative to healthy controls. recent-onset and chronic patients also demonstrated negative relationships between the number of trials required to complete the PAL task and hippocampal subfield volumes that were not observed in their respective healthy control groups, suggesting that smaller hippocampal subfields were associated with poorer visuospatial associative memory in both early and late illness stages. There were no group  $\times$  volume interactions for any hippocampal subfield when recent-onset and chronic patients were compared with each other.

#### *Visuospatial associative memory performance*

Contrary to our hypothesis, both chronic and recent-onset patients required significantly more trials than their respective healthy control groups to complete the PAL task. There was also no group difference in performance between chronic and recent-onset patients once age was taken into account. However, chronic patients were significantly more likely to fail to complete the PAL task than recent-onset patients, and there were no differences in pass rates between recent-onset patients and younger controls. Thus, although the recent-onset patients demonstrate visuospatial associative memory impairments, these appear to be milder than those observed in chronic patients. Although previous studies have also identified visuospatial associative memory deficits in individuals with chronic schizophrenia (Wood *et al.* 2002; Stip *et al.* 2005; Donohoe *et al.* 2008), prior studies of recent-onset patients found preserved functioning in this domain (Wood *et al.* 2002; Barnett *et al.* 2005; Wannan *et al.* 2017), with deterioration occurring only after illness onset (Haring *et al.* 2017; Wannan *et al.* 2017). One potential explanation for the visuospatial associative memory deficit in our recent-onset group is their longer duration of illness compared to previous studies (Wood *et al.* 2002; Wannan *et al.* 2017). Given that the most pronounced brain changes in schizophrenia-spectrum illness have been shown to occur in the first 6-12 months following illness onset (Bartholomeusz *et al.* 2016), we might expect that cognitive decline would occur in a similarly short period. With an average illness duration of over 1.5 years (including prodromal illness), it is possible that some deterioration of visuospatial associative memory had already occurred in this patient group, resulting in the observed deficits.

#### *Hippocampal subfield volumes in recent-onset and chronic schizophrenia*

Chronic patients had significant volume reductions in several hippocampal subfields, including the bilateral CA4DG, CA2CA3, and stratum, and the right subiculum, relative to older healthy controls. This is consistent with previous studies that identified volume reductions across most hippocampal subfields in chronic schizophrenia (Mathew *et al.* 2014; Haukvik *et al.* 2015; Ho *et al.* 2016). Recent-onset patients on the other hand, did not differ from younger controls in any hippocampal subfield volume. This is contrary to recent findings in which recent-onset patients were shown to have volume reductions across several hippocampal subfields, including the bilateral CA1 and CA4, and the left CA3 (Baglivo *et al.* 2018). Volumes of our recent-onset patients also largely did not differ from chronic patients: the right CA1 was the only subfield in which patient groups differed significantly, with the recent-onset patients demonstrating smaller volumes in this region. The smaller CA1 volume observed in recent-onset patients in the current sample may reflect continuing maturation of this region in the younger patient group: examination of the relationship between age and CA1 subfield volumes in recent-onset and chronic patients revealed that age was positively correlated with CA1 volumes in recent-onset patients, whereas chronic patients exhibited a negative relationship between age and CA1 volumes.

#### *Differential relationships between hippocampal subfield volumes and visuospatial associative memory*

Significant group  $\times$  volume interactions were observed in virtually identical subfields in the recent-onset and chronic groups relative to controls. Namely, the stratum layers and CA4DG were primarily affected in both groups, implicating these particular subfields as important for visuospatial associative memory performance across illness stages. However the two groups displayed noticeable differences in the laterality of their relationships, with post hoc relationships largely in the left hemisphere in recent-onset patients, and in the right hemisphere for chronic patients. These findings are of particular note given hippocampal volume loss, including in a recent study of hippocampal subfield volumes, has been restricted to the left hemisphere in recent-onset patients (Velakoulis *et al.* 1999, 2006; Baglivo *et al.* 2018) with bilateral volume loss observed only in chronic schizophrenia patients (Velakoulis *et al.* 1999, 2006). These findings suggest that initial deterioration in visuospatial associative memory ability may be related to left hippocampus atrophy in early psychosis, resulting in compensatory use of the right rather than bilateral hippocampus for performance on memory tasks in early illness stages. Ongoing memory deterioration in chronic patients may therefore be related to the spread of pathology to the previously unaffected right hippocampus. It should be noted that despite differences in laterality when compared to their respective healthy control groups, there were no group  $\times$  volume interactions in either hemisphere when the two patient groups were analysed together. Thus, recent-onset patients appear to have

similar relationships between visuospatial associative memory performance and hippocampal subfield volumes in the right hemisphere to chronic patients, though they may not be quite as strong.

The current study provides evidence for the critical role of the hippocampal stratum layers for visuospatial associative memory in schizophrenia. Differential relationships between stratum volumes and the number of trials required to complete the PAL task were observed in both chronic and recent-onset patients relative to their respective healthy control groups; poorer performance on the PAL task was associated with decreased stratum volumes bilaterally, in both patient groups. However, these relationships were not observed in either healthy control group. The stratum radiatum and stratum lacunar-moleculare layers of the hippocampus contain the apical and terminal dendrites of the CA pyramidal cells respectively, as well as some of the axons of the perforant pathway, which connects the entorhinal cortex to the hippocampus (Witter 2007). Chronic exposure to stress hormones has been shown to cause apical dendritic retraction in the hippocampus (Woolley *et al.* 1990; McEwen 2000), including the stratum radiatum (Chen *et al.* 2010), which in turn leads to impaired spatial memory ability (Conrad 2006). Indeed, a number of studies have identified relationships between impaired cognitive functioning and abnormalities in the Hypothalamic-Pituitary-Adrenal (HPA) axis, including increased cortisol levels, in individuals with schizophrenia (Lupien 2000; Walder *et al.* 2000; Aas *et al.* 2011). In this context, it is possible that our results support ongoing illness-related stress as having a potential role in both hippocampal and visuospatial associative memory abnormalities in schizophrenia, although further longitudinal studies are needed to fully explore these complex relationships over time.

An additional hippocampal subfield that showed differential relationships with visuospatial associative memory between patient groups and healthy controls was the CA4DG. The dentate gyrus forms part of the perforant pathway that receives inputs from the entorhinal cortex and projects via mossy fibres into CA3 (Amaral *et al.* 2007). This pathway is critical for associative memory formation (Carr *et al.* 2017), and is dependent on glutamate-mediated signalling via N-methyl-D-aspartate (NMDA) receptors (Zola-Morgan & Squire 1993; Lavenex & Amaral 2000) which comprise different subunits, including GluN1 and GluN2s. Post-mortem studies of schizophrenia patients have revealed decreased GluN1 messenger RNA and protein in the dentate gyrus (Gao *et al.* 2000; Law & Deakin 2001; Stan *et al.* 2015) but not in other hippocampal subfields (Stan *et al.*, 2015), which may suggest a unique pathology of dentate gyrus glutamate function in schizophrenia. This specific dentate gyrus pathology may exert downstream effects on the CA3 (Tamminga *et al.* 2012), which is itself an important region for the encoding of spatial information and the acquisition of relational

associations (Kesner 2007), which are both key aspects of visuospatial associative memory. Additionally, the granule cell layer of the dentate gyrus is a site of adult neurogenesis in humans; these adult-born neurons are thought to play a crucial role in episodic memory formation (Benarroch 2013; Drew *et al.* 2013; Gonçalves *et al.* 2016), particularly for spatial memories (Snyder *et al.* 2005). In individuals with schizophrenia however, there is evidence for reduced proliferation of adult-born dentate gyrus granule cells (Allen, Fung, & Weickert, 2016; Reif *et al.*, 2006), which may contribute to both hippocampal volume reductions and memory impairments in this population.

Our findings should be considered in the context of several limitations. Cumulative exposure to antipsychotic medication was not measured in the current study, and therefore it was not possible to control for the effects of ongoing exposure to medication in our analyses. Additionally, for recent-onset patients, duration of illness included both duration of prodromal and full-threshold symptoms. Furthermore, the lack of association between hippocampal measures and PAL performance in healthy control subjects may be due, in part, to ceiling effects rather than a lack of involvement of these regions for visuospatial associative memory performance in healthy people; Almost all of the healthy controls successfully completed the PAL task. The use of volumetric data in the current study also means that it is not possible to disentangle the potentially unique effects of factors such as hippocampal shape and grey matter surface area and thickness on PAL performance. Previous studies also suggest that the reliability and validity of hippocampal volume estimates may be influenced by the size of each individuals' hippocampi, resulting in over- or under-estimation of volumes for those with larger or smaller hippocampi (Sánchez-Benavides *et al.* 2010; Wenger *et al.* 2014; Schmidt *et al.* 2018). However, this issue was tested in a previous validation of the MAGeT Brain segmentation technique, and was not found to be true, with good estimation observed in individuals with small hippocampi (Pipitone *et al.* 2014). Finally, the current study was cross-sectional in nature and therefore unable to identify longitudinal relationships between PAL performance and hippocampal volume changes. Future studies that track changes in visuospatial associative memory ability and brain structure over time would therefore be beneficial.

In conclusion, the current study found that both chronic schizophrenia-spectrum patients and youth with recent-onset psychosis had impaired visuospatial associative memory performance, however only chronic patients showed hippocampal subfield volume loss relative to controls. Further, we identified relationships between visuospatial associative memory performance and hippocampal subfield volumes in recent-onset and chronic schizophrenia patients that were not present in healthy controls. In patients, poorer

performance on the PAL task was associated with reductions in hippocampal subfield volumes, primarily in the stratum layers and the CA4DG. In recent-onset patients, these relationships were observed in the left hemisphere, whereas in chronic patients findings were bilateral. Furthermore, when patient groups were compared, differential relationships between hippocampal subfield volumes and PAL performance were not observed, suggesting that similar hippocampal-memory relationships are present in both early and established illness. The current study extends the findings of previous studies that have examined relationships between hippocampal volume reductions and memory impairment in schizophrenia (Sanfilippo *et al.* 2002; Touloupoulou *et al.* 2004; Exner *et al.* 2008; Thoma *et al.* 2009) by identifying particular hippocampal subfields that appear to be related to visuospatial associative memory ability in individuals at early and late stages of a schizophrenia-spectrum illness. Further longitudinal studies that map progression of visuospatial associative memory ability and hippocampal subfield volumes from the prodromal illness stage would help to tease out how these two variables interact over time.

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Table 1. Demographics

	Group				t-statistic/ $\chi^2$		
	FEP	Younger HC	Chronic	Older HC	FEP vs. Younger HC	Chronic vs. Older HC	FEP vs. Chronic
Age	21.37 (2.00)	21.96 (1.96)	38.55 (9.21)	39.15 (10.43)	t = 1.19	t = -0.32	t = 12.92**
Gender (M/F)	21/7	18/10	38/14	33/18	$\chi^2 = 0.76$	$\chi^2 = 0.84$	$\chi^2 = 0.04$
Current IQ	95.50 (17.29)	118.89 (9.56)	86.20 (19.52)	115.32 (11.13)	t = 6.27**	t = 8.95**	t = 2.10*
Premorbid IQ	99.25 (15.41)	112.36 (11.87)	93.72 (14.41)	107.89 (8.82)	t = 3.57**	t = 5.74**	t = 1.56
Years of education	12.59 (2.45)	15.11 (1.92)	12.14 (3.05)	16.29 (2.79)	t = 4.25**	t = 6.82**	t = 0.66
SOFAS	56.14 (10.04)	85.79 (7.03)	46.69 (14.00)	79.29 (10.98)	t = 12.80**	t = 11.17**	t = 3.13**
Illness duration (years)	1.78 (0.84) <sup>#</sup>	-	16.69 (7.88)	-	-	-	-
Illness duration quartiles (25/75%)	1.10/2.33 <sup>#</sup>	-	10.50/21.50	-	-	-	-
CPZ equivalent	415.16 (444.61)	-	1045.76 (656.83)	-	-	-	t = 4.40**
Positive symptoms	12.93 (4.85)	-	16.96 (5.96)	-	-	-	t = 3.06**
Negative symptoms	23.42 (13.03)	-	40.04 (17.77)	-	-	-	t = 4.30**

\*  $p < .05$  \*\*  $p < .01$ <sup>#</sup> For FEP patients, illness duration includes both duration of full-threshold and prodromal symptoms

Abbreviations: FEP, first-episode psychosis; HC, healthy control; SOFAS, Social and Occupational Functioning Assessment Scale; CPZ, chlorpromazine

Table 2. Between-group comparisons of hippocampal subfield volumes

Hemisphere	Subfield	FEP		Younger HC		Chronic		Older HC		F statistic			Partial Eta Squared (90% CI)		
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	FEP vs. Younger HC	Chronic vs. Older HC	FEP vs. Chronic	FEP vs. Younger HC	Chronic vs. Older HC	FEP vs. Chronic
Left	CA1	730.89	65.80	740.14	78.91	754.41	93.37	767.31	103.91	0.03	2.76	0.988	0.001 (0-0.04)	0.03 (0-0.10)	0.01 (0-0.08)
	Subiculum	332.13	30.32	348.21	47.82	342.42	41.05	344.56	38.48	0.58	1.19	0.004	0.01 (0-0.10)	0.01 (0-0.07)	0.00 (0-0.00)
	CA4/DG	647.16	72.43	695.19	75.39	618.00	73.63	684.07	92.48	3.77	36.65*	1.124	0.07 (0-0.20)	0.27 (0.15-0.38)	0.02 (0-0.09)
	CA2/CA3	147.20	25.10	151.59	25.82	138.44	27.07	150.92	27.66	0.06	8.77*	2.797	0.001 (0-0.05)	0.08 (0.02-0.18)	0.04 (0-0.13)
	Stratum	603.42	63.19	618.64	68.16	569.70	70.77	608.68	89.79	0.16	11.80*	2.114	0.003 (0-0.07)	0.11 (0.03-0.21)	0.03 (0-0.12)
Right	CA1	740.93	66.89	772.71	95.04	832.87	101.07	842.47	111.04	1.10	2.64	9.29*	0.02 (0-0.12)	0.03 (0-0.10)	0.11 (0.02-0.23)
	Subiculum	322.27	39.50	329.31	45.55	322.55	50.29	330.78	41.45	0.04	3.04	1.536	0.001 (0-0.043)	0.03 (0-0.10)	0.02 (0-0.10)
	CA4/DG	646.31	77.36	695.96	83.84	639.55	72.91	689.19	84.30	2.68	28.47*	0.008	0.05 (0-0.17)	0.22 (0.11-0.33)	0.00 (0-0.01)
	CA2/CA3	161.82	29.33	168.51	22.16	173.49	30.40	186.51	25.80	0.14	12.2*	2.373	0.003 (0-0.07)	0.11 (0.03-0.21)	0.03 (0-0.12)

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Stratum	574.00	63.61	613.29	81.91	548.86	77.51	582.35	84.35	2.25	10.84*	2.103	0.04 (0-0.16)	0.10 (0.02-0.20)	0.03 (0-0.11)
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\* p < .05 , FDR corrected

Abbreviations: FEP, first-episode psychosis; HC, healthy control

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