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ORIGINAL ARTICLE

Hippocampal subregion volume changes associated with antipsychotic treatment in first-episode psychosis

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Background. Hippocampal dysfunction is considered central to many neurobiological models of schizophrenia, yet there are few longitudinal *in vivo* neuroimaging studies that have investigated the relationship between antipsychotic treatment and morphologic changes within specific hippocampal subregions among patients with psychosis.

Method. A total of 29 patients experiencing a first episode of psychosis with little or no prior antipsychotic exposure received structural neuroimaging examinations at illness onset and then following 12 weeks of treatment with either risperidone or aripiprazole in a double-blind randomized clinical trial. In addition, 29 healthy volunteers received structural neuroimaging examinations at baseline and 12-week time points. We manually delineated six hippocampal subregions [i.e. anterior cornu ammonis (CA) 1–3, posterior CA1–3, subiculum, dentate gyrus/CA4, entorhinal cortex, and fimbria] from 3T magnetic resonance images using an established method with high inter- and intra-rater reliability.

Results. Following antipsychotic treatment patients demonstrated significant reductions in dentate gyrus/CA4 volume and increases in subiculum volume. Healthy volunteers demonstrated non-significant volumetric changes in these subregions across the two time points. We observed a significant quadratic (i.e. inverted U) association between changes in dentate gyrus/CA4 volume and cumulative antipsychotic dosage between the scans.

Conclusions. This study provides the first evidence to our knowledge regarding longitudinal *in vivo* volumetric changes within specific hippocampal subregions in patients with psychosis following antipsychotic treatment. The finding of a non-linear relationship between changes in dentate gyrus/CA4 subregion volume and antipsychotic exposure may provide new avenues into understanding dosing strategies for therapeutic interventions relevant to neurobiological models of hippocampal dysfunction in psychosis.

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Key words: Antipsychotic treatment, dendate gyrus, hippocampus, psychosis, subiculum.

Introduction

Neurobiological models of schizophrenia have emphasized a critical role for hippocampal dysfunction (Tamminga *et al.* 2010). This was recently supported by a large prospective meta-analysis from the ENIGMA consortium that revealed smaller hippocampus volume in 2028 patients with schizophrenia compared with 2540 healthy controls (van Erp *et al.* 2016). Moreover, hippocampal abnormalities have also been identified among individuals with psychotic bipolar disorder (Frey *et al.* 2007) and those vulnerable to psychosis (Fusar-Poli *et al.* 2007), suggesting they play a critical role in psychotic phenomenology and do not respect traditional diagnostic boundaries.

Although antipsychotic treatment has been typically linked to subcortical dopaminergic changes, medication effects have also been hypothesized to be secondary to a loss of inhibitory signaling (i.e. hyperactivity) within dopamine systems regulated by the hippocampus (Lodge & Grace, 2011; Perez & Lodge, 2014). Additionally, this theory has been used to guide animal models of schizophrenia (Peleg-Raibstein *et al.*

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2012). Better understanding the relationship between antipsychotic treatment and hippocampal structure/function is critical as this work has important implications for both patients' ability to flexibly generalize past experiences within new environments (Shohamy *et al.* 2010) and declarative memory deficits that have been linked to memories with psychotic content (Tamminga *et al.* 2010).

Considerable evidence indicates that antipsychotic treatment may be associated with changes in hippocampal structure and function. Cross-sectional studies reported that antipsychotic dosage was associated with abnormal hippocampal shape (Zierhut *et al.* 2013) and less volume (Yang *et al.* 2015) in patients with schizophrenia. Although the use of longitudinal neuroimaging could more directly facilitate our understanding of how antipsychotics affect hippocampal morphology, findings have been highly inconsistent. Rizos *et al.* (2014) reported total hippocampal volume reductions in patients following monotherapy with second-generation antipsychotics and Ebdrup *et al.* (2011) reported hippocampal volume loss among patients treated with quetiapine. This work has been supported by animal studies linking hippocampal volume reduction with olanzapine treatment (Barr *et al.* 2013). In contrast, some studies reported that atypical antipsychotics are associated with less longitudinal change in hippocampal volume (Koolschijn *et al.* 2010), especially when compared with typical antipsychotics such as haloperidol (Mamah *et al.* 2012). Other studies, however, reported that longitudinal changes in hippocampal volume did not differ significantly in patients treated with olanzapine *v.* haloperidol (Panenka *et al.* 2007; McClure *et al.* 2013).

The hippocampus is a highly heterogeneous structure comprised of multiple subregions that have different afferent and efferent projections relevant to the neurobiology of psychosis and its pharmacological treatment (Tamminga *et al.* 2010; Benes, 2015). Distinguishing different parts of the hippocampus from cellular features has been difficult to accomplish with the majority of *in vivo* magnetic resonance (MR) imaging sequences widely used today. Although some protocols have been developed to resolve the internal architecture of the hippocampus, they require higher (e.g. 7 T) field strengths (e.g. Wieshmann *et al.* 1999; Thomas *et al.* 2008; Kerchner *et al.* 2010) not typically available outside of academic centers. In addition, several prior investigations of hippocampal subregions are limited to the body of the hippocampus where they can be most clearly identified (Mueller & Weiner, 2009; Mueller *et al.* 2009, 2010; Wang *et al.* 2010). Other strategies incorporate surface shape analysis by projecting post-mortem atlases onto three-dimensional (3D) renderings of the entire hippocampus (Frisoni *et al.* 2008; Xie *et al.* 2009; Boccardi *et al.* 2010; Cole *et al.* 2010), and are therefore limited to subregions located on the external surface of the hippocampus. Some studies have 'unfolded' the hippocampus to translate its 3D subregions into two-dimensional space (Ekstrom *et al.* 2009; Donix *et al.* 2010). Hippocampal subregion delineation has also incorporated manual techniques (Mueller *et al.* 2007; La Joie *et al.* 2010; Malykhin *et al.* 2010), automated techniques (Van Leemput *et al.* 2009; Bonnici *et al.* 2012), or a combination of these two approaches (Yushkevich *et al.* 2010). Such approaches are highly valuable given their potential to accommodate high throughput, but the majority of these techniques did not assess inter-rater and/or intra-rater reliability.

Manual delineation remains the 'gold standard' for quantifying the hippocampus and its subregions from MR images, but few studies have successfully implemented such an approach with high reliability using a T1-weighted contrast. We previously reported a reliable set of manual delineation criteria to measure the anterior cornu ammonis (CA) 1–3, posterior CA1–3, subiculum, dentate gyrus (DG)/CA4, entorhinal cortex and fimbria at 3 T with high inter- and intra-rater reliability assessed using both intra-class correlation (ICC) coefficients and Dice indices (Rhindress *et al.* 2015). In this approach we relied on anatomical rather than cytoarchitectural features where they would be more evident. The goal of the current study was to examine volumetric changes in hippocampal subregion morphology following antipsychotic treatment in first-episode psychosis. Based on prior published work (Barr *et al.* 2013; Haukvik *et al.* 2015; Kawano *et al.* 2015) and neurobiological models of hippocampal dysfunction (Tamminga *et al.* 2010) we hypothesized that antipsychotic treatment would be associated with volume reductions within CA4 and the DG.

Method

All procedures contributing to this work comply with the ethical standards of the relevant national and institutional committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008.

Subjects

A total of 29 patients experiencing a first-episode of psychosis were recruited from admissions to the in-patient service at The Zucker Hillside Hospital in Glen Oaks, NY and were enrolled in a National Institute of Mental Health-funded double-blind randomized controlled trial comparing aripiprazole, a dopamine and serotonin 5HT1A partial agonist, *v.*

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risperidone, a dopamine and serotonin antagonist (R01MH060004). All patients were required to have 2 weeks or fewer of cumulative lifetime exposure to antipsychotics to enter the clinical trial and received a physical examination and laboratory screening to rule out medical causes for their initial psychotic episode. All patient (lifetime) diagnoses were based on the Structured Clinical Interview for DSM-IV Axis I disorders (SCID; First *et al.* 1998) supplemented by information from clinicians and, when available, family members. First-episode patients met Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) criteria for schizophrenia ($n = 14$ for undifferentiated and $n = 7$ for paranoid), schizophreniform disorder ($n = 4$), schizo-affective disorder ($n = 1$) or psychosis not otherwise specified ($n = 3$).

Patients had, on average, a total lifetime exposure of 6.7 days (S.D. = 8.1 days) of antipsychotic treatment prior to the baseline scan; data were unavailable for one patient. Patients were scanned, on average, within 2.9 days (S.D. = 8.3 days) of entry into the clinical trial. Of the patients, 10 were antipsychotic drug-naïve at the time of the baseline scan. On the date of the baseline scan, nine of the 29 patients in this study received medications other than antipsychotics. Six patients received lorazepam, with dosages ranging from 0.5 to 6 mg. One patient received both lorazepam (1 mg) and benztropine (4 mg). One patient was given loratadine (10 mg), a multivitamin, and a nicotine patch. One patient was given a 15% gel solution of azelaic acid. Mean age at first psychotic symptoms was 19.4 years (S.D. = 2.9 years); data were unavailable for two patients.

Healthy volunteers ($n = 29$) were recruited to participate in imaging studies being conducted at the North Shore – Long Island Jewish Hospital System (NSLIJHS, now Northwell Health), using advertisements in local newspapers and through word of mouth. All healthy controls were administered the SCID-non-patient edition (SCID-NP; First *et al.* 2001) to ensure the absence of an Axis I psychiatric disorder.

In addition, all individuals met the following exclusion criteria: (1) fulfilling DSM-IV criteria for a current substance-induced psychotic disorder, psychotic disorder due to a general medical condition, or mental retardation; (2) having a serious medical condition known to have an impact on the brain; (3) having any medical condition that required treatment with a medication with psychotropic effects; (4) having a risk of suicidal or homicidal behavior; (5) MR imaging contraindications; and (6) pregnancy. All procedures were approved by the NSLIJHS Institutional Review Board and written informed consent was obtained either from study participants or a legal guardian in the case of minors. All minors provided written assent to participate in the study.

Treatment trial antipsychotic titration schedule

Research psychiatrists followed a flexible dosing titration schedule. The initial daily dose for patients in the treatment trial was 5 mg for aripiprazole and 1 mg for risperidone. The dose was initially increased after 3 days of treatment and then further adjustments were made every 1 to 3 weeks until the patient improved, developed side effects that precluded a dose increase, or reached a maximum daily dose of 30 mg of aripiprazole or 6 mg of risperidone. All patients were receiving either aripiprazole or risperidone at the time of the second scan except one patient who discontinued treatment 2 weeks prior. Lorazepam was prescribed for anxiety or agitation. Patients were not allowed to receive antidepressants or mood stabilizers. Cogentin and/or propranolol was prescribed for extrapyramidal symptoms.

Clinical assessments

Patients completed the 18-item Brief Psychiatric Rating Scale – anchored version (BPRS-A; Overall & Gorham, 1962) and we derived a total score by summing all items. The average BPRS score was 44.2 (S.D. = 9.4) at the time of baseline scanning and 27.7 (S.D. = 7.3) at follow-up.

Chlorpromazine and olanzapine equivalents

To examine the relationship between volumetric changes and antipsychotic exposure over the course of the study we converted each patient's total antipsychotic exposure between the scans into chlorpromazine equivalents using techniques described by Woods (2003). To confirm our results, these values were also converted into olanzapine equivalents using the method recommended by Leucht *et al.* (2015).

MR imaging and image processing

All study participants underwent MR imaging examinations at North Shore University Hospital at the baseline and 12-week time points. Images were acquired in the coronal plane using a 3D spoiled gradient sequence (repetition time = 7.5 ms, echo time = 3 ms, matrix = 256 × 256, field of view = 240 mm) on a single 3 T scanner (GE Signa HDx; General Electric, USA), producing 216 contiguous images (slice thickness = 1 mm) through the whole head. All scans were reviewed by a neuroradiologist for gross neuroanatomic abnormalities (e.g. tumors) that would preclude participation in this study. Scans were also reviewed by a member of the research team for quality control. All scans were aligned along the anterior and posterior commissures using previously published software (Ardekani & Bachman, 2009) and resampled into a 512 × 512 matrix

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yielding voxel dimensions of 0.47 × 0.47 × 1 mm. Measurements were conducted using ITK-SNAP (Yushkevich *et al.* 2006) and completed by a single operator (K.R.) blind to time point and group membership.

Intracranial volume

Intracranial volumes were computed using the FreeSurfer image analysis suite (version 5.1), which is documented and freely available for download online (http://surfer.nmr.mgh.harvard.edu). The technical details for procedures are described in prior publications (e.g. Fischl & Dale, 2000; Fischl *et al.* 2004; Reuter *et al.* 2012). Mean intracranial volume was 1529 cm^3 (S.D. = 257) for patients and 1562 cm^3 (S.D. = 166) for healthy volunteers.

Hippocampal delineation criteria

The approach for manual delineation of hippocampal subregions and its reliability analyses have been described in detail previously (Rhindress *et al.* 2015). Briefly, this approach uses anatomical landmarks and differences in the images' intensities to identify the location of six subregions throughout the hippocampal formation (i.e. anterior CA1–3, posterior CA1–3, subiculum, DG/CA4, entorhinal cortex and fimbria) as illustrated in Figs 1 and 2. Tracing of the subregions was conducted in the coronal plane while simultaneously viewing the images in the axial and sagittal planes. Tracings began on the most posterior slice of the hippocampus and progressed anteriorly. It took approximately 2.5 h for an operator to manually delineate all subregions within a single hippocampus.

Two operators manually traced the hippocampal regions of interest in the right and left hemispheres blind to the other operator's work to establish inter-rater reliability. One operator (K.R.) manually traced these regions of interest again approximately 1 week later to determine intra-rater reliability. Reliability was assessed using the Dice index (Dice, 1945), which is defined as: $2(A \cap B)/(A + B)$, that is, the size of intersection (agreement) between two labels ($A \cap B$) divided by their average size $(A + B)/2$. Dice indices range from 0 (no overlap) to 1 (perfect agreement) and were computed for hippocampal subregions using both inter-rater and intra-rater reliability. In addition, ICC coefficients were computed using the Statistical Package for the Social Sciences (SPSS) using a two-way mixed-effects model based on the ICC value for a single operator. Thus, each subject is assessed by both operators, but the operators are the only ones of interest. ICC coefficients were computed based on the premise that a single operator would conduct the tracings and not that the average of the two operators would be used for the measurements. Inter-rater reliability performed using this approach yielded an average ICC of 0.85 (range 0.71–0.98, median 0.86) and an average Dice index of 0.75 (range 0.70–0.81, median 0.75), whereas the intra-rater reliability metrics yielded an average ICC of 0.92 (range 0.66–0.99, median 0.97) and an average Dice index of 0.85 (range 0.82–0.90, median 0.85).

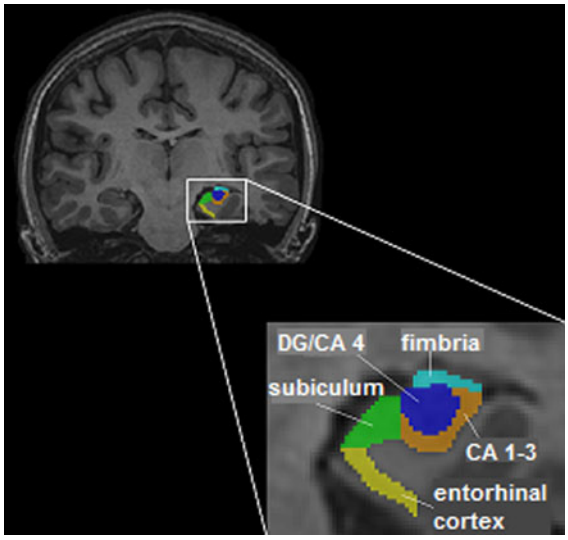

Fig. 1. Hippocampal subregions delineated on a coronal image in the left hemisphere. DG, Dentate gyrus; CA, cornu ammonis.

Handedness

All individuals completed a modified version of the Edinburgh Inventory. The total number of right- and left-hand items was scored and the laterality quotient was computed according to the following formula: (total R − total L)/(total R + total L). This yielded a range from +1.00 (totally dextral) to −1.00 (totally non-dextral). Mean laterality quotient was 0.74 (S.D. = 0.39) for healthy volunteers and 0.71 (S.D. = 0.40) for patients.

Statistical analyses

Analyses were conducted using the Statistical Package for the Social Sciences (SPSS; v. 11.5, USA). We used analysis of covariance to compare the hippocampal subregions with group as a between-subjects factor. As none of the group × hemisphere × time point interactions were statistically significant for any of the individual hippocampal subregions, right and left hemisphere volumes were combined for analysis. The within-subjects factors included six levels of subregion (anterior CA1–3, posterior CA1–3, subiculum, DG/CA4, entorhinal cortex and fimbria) as well as time

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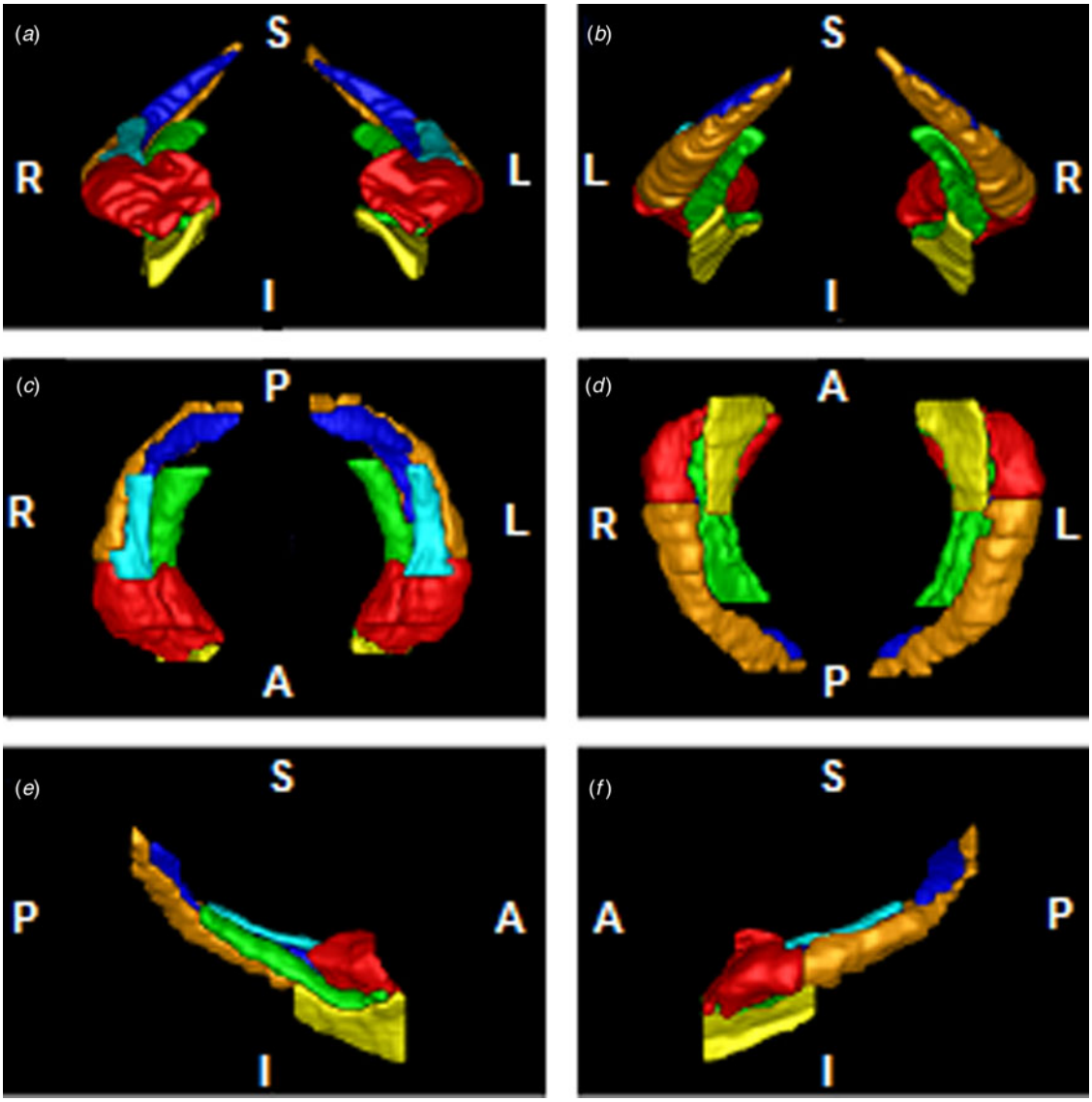

Fig. 2. Three-dimensional mesh of an individual hippocampal formation produced by ITK-SNAP and viewed: (*a*) anteriorly (A); (*b*) posteriorly (P); (*c*) superiorly (S); (*d*) inferiorly (I); (*e*) medially (left hemisphere only); and (*f*) laterally (left hemisphere only). The color scheme for the subregions is as follows: anterior cornu ammonis (CA) 1–3, red; posterior CA1–3, orange; subiculum, green; dentate gyrus/CA4, blue; entorhinal cortex, yellow; and fimbria, cyan. L, Left; R, right.

point (baseline and follow-up). We included age, sex, handedness and intracranial volume as covariates. Due to the sphericity assumption being violated (epsilon = 0.75) we used the Greenhouse–Geisser correction to amend degrees of freedom (df) in the analyses (Mauchly's W = 0.315 for the region × time point interaction with corresponding $\chi^2 = 58$, $p < 0.001$).

We used curve estimation procedures employing both linear and quadratic models to assess the relationship between subregion volume changes and total antipsychotic exposure between the scans. We set α to 0.05 (two-tailed) for all analyses.

Results

Sample demographics are described in Table 1. The two groups did not differ significantly in distributions of age, sex, handedness or intra-cranial volume. As expected, there was a significant difference in education level between the groups ($F_{1,56} = 5.64$, $p = 0.02$). There was no significant difference in patients being treated with risperidone *v.* aripiprazole in either chlorpromazine or olanzapine equivalents between the two time points. Descriptive statistics for each of the hippocampal subregions at the time of the baseline and

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Table 1. *Sample demographics*

	Patients ($n = 29$)	Healthy volunteers ($n = 29$)
Mean age, years (S.D.)	21.9 (4.8)	22.2 (4.9)
Sex, *n*		
Male	22	22
Female	7	7
Handedness, mean (S.D.)	0.71 (0.40)	0.74 (0.39)
Mean duration of education, years (S.D.)	12.6 (1.8)	13.1 (2.3)
Antipsychotic, *n*		–
Aripiprazole	13	
Risperidone	16	
Ethnicity, *n*		
African American	11	4
Hispanic	4	5
Caucasian	10	16
Asian/Pacific islander	4	2
Other	0	2
Brief Psychiatric Rating Scale, mean (S.D.)		
Baseline	44.2 (9.4)	–
Follow-up	27.7 (7.3)	–

S.D., Standard deviation.

follow-up scans are provided in Table 2 for descriptive purposes only, with individual change scores regarding the subiculum and DG/CA4 illustrated in Fig. 3.

The main finding that distinguished the groups was a significant group × time × subregion interaction ($F_{3.73,194} = 3.29$, $p = 0.01$). *Post-hoc* analyses revealed significant group × time interactions for the subiculum ($F_{1,52} = 4.79$, $p = 0.03$) and DG/CA4 ($F_{1,52} = 7.10$, $p = 0.01$). Paired-samples *t* tests were used to compare each hippocampal subregion volume at baseline with its respective volume at 12 weeks. Patients demonstrated a significant increase in subiculum volume (mean paired difference = 32 mm^3) as well as a significant volumetric decrease in the DG/CA4 subregion (mean paired difference = −68 mm^3). Changes in subiculum and DG/CA4 volume did not correlate significantly with symptom changes over the course of the trial. No significant ($p > 0.05$) changes in any of the hippocampal subregion volumes were evident between the baseline and 12-week follow-up time points in healthy volunteers.

The percentage difference in volume between time points was calculated for each hippocampal subregion using the following equation: [(12-week volume – baseline volume)/(baseline volume)] × 100. Independent-samples *t* tests were computed for the volumetric percentage differences of each hippocampal subregion to determine if patients had changes that were significantly different from healthy volunteers between the two time points. Consistent with the paired-samples *t* tests, patients displayed significantly larger increases in subiculum volumes ($t = 2.34$, df = 56, $p = 0.02$) and significantly larger decreases in DG/CA4 volumes ($t = -2.49$, df = 56, $p = 0.02$) compared with healthy volunteers.

Mean dosage for patients between the two scans was 16 177 mg (S.D. = 4800 mg) in chlorpromazine and 835 mg (S.D. = 256 mg) in olanzapine equivalents. One patient was excluded from analysis due to being an outlier (>3 S.D.s from the mean) on both chlorpromazine and olanzapine equivalents. Curve estimation did not reveal any significant association between chlorpromazine/olanzapine equivalents and changes in subiculum volume. There was, however, a significant association ($R^2 = 0.34$) between chlorpromazine equivalents and change in DG/CA4 volume when data were analysed using a quadratic model ($F_{2,25} = 6.29$, $p = 0.006$; Fig. 4); this effect was not evident with a linear model. These results were replicated when olanzapine equivalents were used in the analysis ($R^2 = 0.32$, $F_{2,25} = 5.81$, $p = 0.008$). When the individual with the largest reduction in DG/CA4 volume was removed from the analysis both quadratic models remained statistically significant for the analyses with chlorpromazine and olanzapine equivalents.

Discussion

Antipsychotics are widely used in the treatment of schizophrenia and other psychotic disorders. Post-mortem animal data have indicated that antipsychotic treatment is associated with brain volume reductions compared with vehicle-treated control subjects through stereology and the use of *ex vivo* MR imaging (Vernon *et al.* 2011). There are, however, little *in vivo* data regarding their potential impact on hippocampal subregion morphology. Moreover, these subregions may be differentially affected by antipsychotic medications as they are known to be heterogeneous in cytoarchitecture and maintain different afferent and efferent connections. In this regard our study provides the first evidence, to our knowledge, of longitudinal *in vivo* changes within specific hippocampal subregions associated with antipsychotic treatment. Strengths of our study include the use of a longitudinal design, patients studied early in the course of illness prior to extensive antipsychotic treatment, and manual methods for mensuration of hippocampal subregions with high inter-rater/intra-rater reliability assessed using both Dice indices and ICC coefficients. Specifically, while patients demonstrated significant volumetric changes in the DG/CA4 and subiculum over the course

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Table 2. *Baseline and follow-up volumes (mm^3) for patients and healthy volunteers*[a]

	Healthy volunteers			Patients			Statistics		
Subregion	Baseline mean (S.D.)	12 Weeks mean (S.D.)	Mean % change	Baseline mean (S.D.)	12 Weeks mean (S.D.)	Mean % change	*t*	df	*p*
A CA1–3	2343 (453)	2337 (447)	−0.13	2316 (381)	2315 (393)	−0.05	−0.07	56	0.95
P CA1–3	1150 (238)	1164 (244)	1.24	1181 (203)	1171 (200)	−0.54	0.93	56	0.36
Subiculum	1438 (180)	1430 (183)	−0.53	1369 (248)	1400 (245)	2.55	−2.34	56	0.024
DG/CA4	1014 (234)	1017 (216)	0.91	1150 (254)	1082 (241)	−5.28	2.49	56	0.016
EC	842 (161)	862 (150)	3.06	835 (187)	839 (168)	1.51	−0.53	56	0.59
Fimbria	333 (59)	336 (57)	1.58	341 (74)	347 (70)	2.54	−0.41	56	0.68

S.D., Standard deviation; df, degrees of freedom; A CA1–3, anterior cornu ammonis 1–3; P CA1–3, posterior cornu ammonis 1–3; DG/CA4, dentate gyrus/cornu ammonis 4; EC, entorhinal cortex.

[a] Subregion volumes were computed by summing right and left hemisphere volumes.

of the treatment trial, healthy volunteers demonstrated only minor (non-significant) volumetric changes (i.e. about 0.3%) in these subregions over a comparable 12-week time period, providing strong support for the reliability of our manual mensuration approach.

Few studies have investigated changes in hippocampal subregion morphology in relationship to antipsychotic treatment and, thus, it is difficult to compare our results with prior work. Our findings do converge, however, with cross-sectional *in vivo* work (Haukvik *et al.* 2015) that used FreeSurfer to identify smaller CA4/DG volumes in a large cohort of 402 previously treated patients with schizophrenia or bipolar spectrum disorders compared with 300 healthy volunteers. Moreover, Kawano *et al.* (2015) reported that CA4/DG volume was significantly smaller in first-episode, subchronic and chronic patients compared with healthy volunteers. Regarding that study, it may be noteworthy that there was a small, but statistically significant, reduction in left CA4/DG volume that was evident over the first 6 months of illness and that may be consistent with antipsychotic treatment effects. Conversely, both Haukvik *et al.* (2015) and Kawano *et al.* (2015) utilized automatic mensuration techniques in these protocols, raising the issue of compatibility of their methods with our own manual approach.

In one neurobiological model of hippocampal pathology in schizophrenia, lower excitatory signaling within the DG has been linked to altered plasticity within CA3 – thereby increasing neuronal activity and concomitant encoding mistakes leading to false memories consisting of psychotic content (Tamminga *et al.* 2010). Moreover, simulations (Faghihi & Moustafa, 2015) and *in vivo* data (Das *et al.* 2014) suggest that abnormalities within the DG are associated with deficits in separation efficiency in schizophrenia, thus further highlighting their functional sequelae and relevance for phenomenology. Post-mortem studies reported molecular changes within the DG in patients with schizophrenia compared with other parts of the hippocampus (Gao *et al.* 2000; Knable *et al.* 2004; Allen *et al.* 2016), which may be consistent with reduced glutamate transmission in this region and the efferent mossy fiber pathway (Tamminga *et al.* 2010). Animal studies indicate that chronic olanzapine exposure predicted abnormal hippocampal connectivity, particularly within inhibitory terminals of the DG (Ramos-Miguel *et al.* 2015), and was associated with DG volume reductions in sedentary rats with exercise reversing the majority of this loss (Barr *et al.* 2013). Thus, the restoration of hippocampal neurogenesis within the DG and associated CA4 subregion could be an important therapeutic target for treating hippocampal dysfunction in psychosis (Newton & Duman, 2007).

Our finding of an inverted U function between chlorpromazine/olanzapine equivalents and changes in DG volume provides the first evidence to our knowledge regarding a non-linear relationship between antipsychotic administration and changes in hippocampal morphology. Our findings are consistent, however, with cognitive studies reporting a non-linear relationship between antipsychotic dosage and functions subserved by the hippocampus, including measures of vigilance (Sakurai *et al.* 2013). For example, D_2 occupancy above approximately 80% was demonstrated to increase the risk for extrapyramidal side effects and cognitive impairment. In this regard it may be noteworthy that dopamine receptor stimulation and working memory performance follow an inverted U function such that either too little or too much dopamine negatively has an impact on cognitive functioning (Vijayraghavan *et al.* 2007). Moreover,

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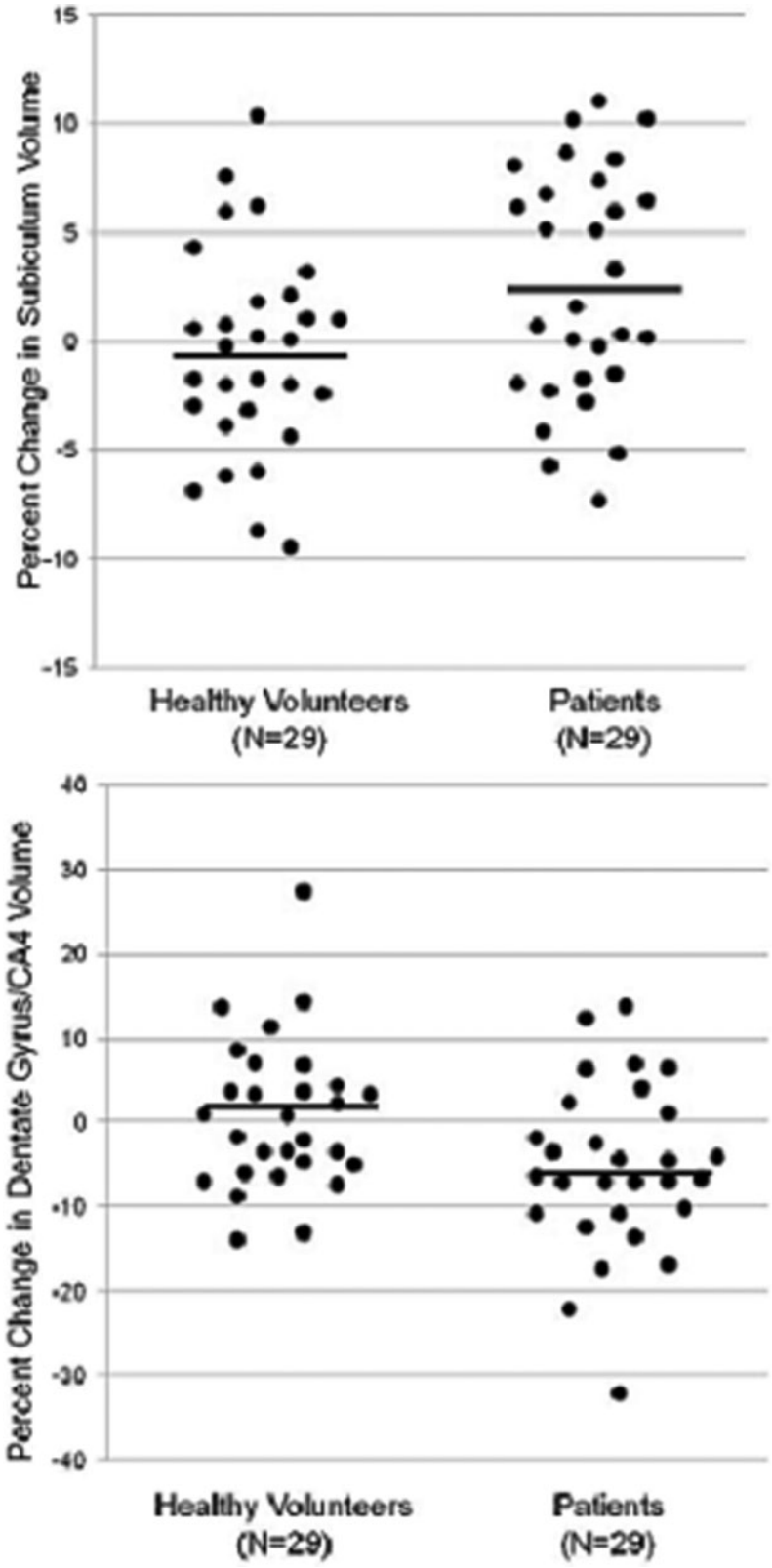

Fig. 3. Scatterplots of change scores for patients and healthy volunteers in the subiculum and dentate gyrus/cornu ammonis (CA) 4. Horizontal lines represent mean values.

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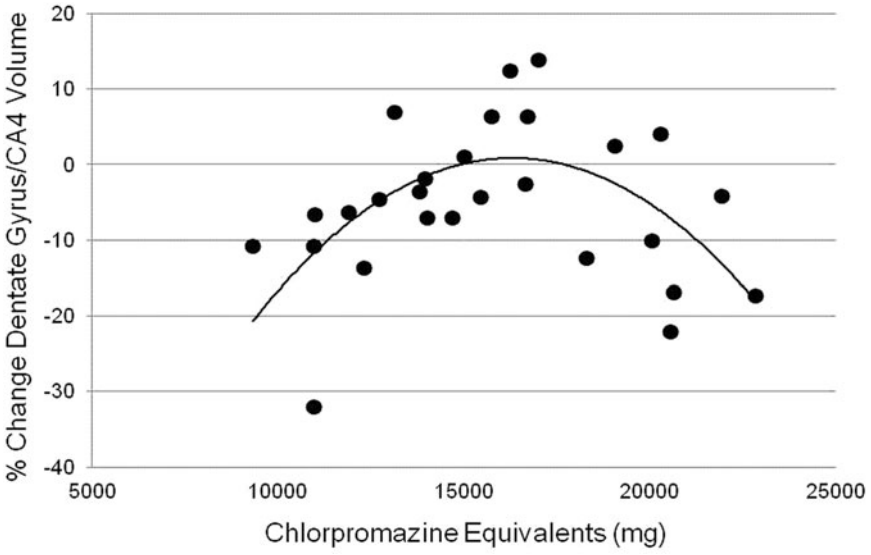

Fig. 4. Change in dentate gyrus/cornu ammonis (CA) 4 volume and chlorpromazine equivalents.

several studies indicated that D_2-like (Monte-Silva *et al.* 2009) and D_1 receptor activation (Fresnoza *et al.* 2014) was associated with a dose-dependent inverted U-shaped impact on neuroplasticity in healthy subjects.

We also identified changes in subiculum volume in patients with psychosis following antipsychotic treatment. It should first be acknowledged that estimates of the hippocampus proper have sometimes included the region of the subicular complex (La Joie *et al.* 2010; Malykhin *et al.* 2010; Winterburn *et al.* 2013), and have at other times excluded it (Szeszko *et al.* 2003; Wellington *et al.* 2013; Rhindress *et al.* 2015). This inconsistency occurs because the cellular structure of the subicular complex varies considerably. Namely, some areas of the subicular complex (i.e. the subiculum) possess cytoarchitectural features that resemble those of traditional hippocampus proper subregions such as CA1. Other areas of the subicular complex (e.g. the presubiculum), however, demonstrate cytoarchitecture that is more similar to the entorhinal cortex – an area that is included in the hippocampal formation but is not often considered to be a part of the hippocampus proper (Amaral & Lavenex, 2006).

The subiculum, a major output region of the hippocampus, is a potential site of action for antipsychotics and animal data demonstrate an interaction of subicular and dopaminergic inputs to the nucleus accumbens (Greene, 1996). Moreover, there are animal data relevant to the phenomenology of schizophrenia including latent inhibition (Meyer & Louilot, 2011) and pre-pulse inhibition of the acoustic startle response (Caine *et al.* 1992) that have implicated dysfunction of this region. Using structural MR imaging, Bearden *et al.* (2009) reported that previous treatment with atypical antipsychotics in patients with major depression was associated with larger hippocampal volume (including subicular regions) compared with patients not treated with atypicals – suggesting potential neuroprotective effects in this region. Moreover, moderate but significant increases in [^{3}H]AFDX-384 binding sites within the subiculum were associated with 3 months of exposure to olanzapine (Terry *et al.* 2006).

There were no significant changes in regions comprising the CA of the hippocampus; however, prior research has implicated structural changes in these regions in relation to antipsychotic usage in both human and animal studies. Possible differences between the current study and prior work include defining the regions of interest, especially along the medial aspects of the CA subfields using shape analysis. For example, Zierhut *et al.* (2013) found that patients with schizophrenia demonstrated shape deformations corresponding neuroanatomically to the CA1 subfield, which was related to positive symptom severity and antipsychotic dosage. Similarly, Schobel *et al.* (2009) reported *in vivo* abnormal cerebral blood flow increases in the CA1 subfield, which predicted onset of psychosis from the prodrome and correlated with psychotic symptoms. Furthermore, animal studies implicate GABAergic system hypofunctioning in the CA1 subfield (Steiner *et al.* 2016) and suggest that antipsychotics reduced the number of *N*-methyl-D-aspartate (NMDA) receptors and associated glutamatergic signaling within the CA1/CA2 brain regions (Krzystanek *et al.* 2015). In this regard it is noteworthy that treatment with lorazepam could be a mediator/source of bias in examining the relationship between

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antipsychotic usage and hippocampal volume. Specifically, a key feature of the model proposed by Lodge & Grace (2011) involves γ-aminobutyric acid (GABA) signaling. In addition, it has been reported that within the hippocampus a compensatory increase in glutamate develops in association with chronic lorazepam treatment (Bonavita *et al.* 2003).

There were a number of limitations with our study that should be acknowledged. Although the use of manual volumetry remains the 'gold standard' for computerized mensuration of the hippocampus, this process is labor-intensive and therefore not practical for large sample sizes. On the other hand, direct comparisons of manual volumetry of the hippocampus with automated approaches have reported inconsistent findings for case–control comparisons (Clerx *et al.* 2013; Arnold *et al.* 2015). Moreover, automated approaches for hippocampal delineation such as FreeSurfer may overestimate volumes, especially among younger individuals, and produce less pronounced right greater than left volume asymmetry (Wenger *et al.* 2014) that has typically been observed in studies employing manual mensuration (Wellington *et al.* 2013). It should also be acknowledged that the current neurobiological evidence for imaging findings in schizophrenia and associated psychotic disorders has been inconclusive (Weinberger & Radulescu, 2016) and factors such as smoking, substance use, and medical co-morbidities could potentially influence findings. However, the presence of an inverted U response demonstrates that the simple 'less is worse', or 'more is better' dichotomy is not supported by the current investigation. Furthermore, limitations of MR imaging must also be acknowledged. For example, there is a possibility that chronic drug use could influence the MR imaging signal (e.g. Cousins *et al.* 2013). In addition, we acknowledge that chlorpromazine (and olanzapine) equivalence is determined based on clinical dosing and not biological factors (e.g. receptor occupancy). Larger sample sizes would also be useful to investigate the differential effects of antipsychotics on volumetric changes.

In sum, we report *in vivo* volumetric changes of hippocampal subregions using manual volumetry in patients with first-episode psychosis following 12 weeks of antipsychotic treatment. Changes in DG/CA4 volume demonstrated an inverted U pattern in association with cumulate antipsychotic exposure over the course of the treatment trial. Significant volumetric changes among patients were observed alongside negligible volumetric changes among healthy volunteers, thus providing evidence for the excellent reliability of our mensuration approach. This is especially notable given the presence of well-known limitations with automated measurements of these subregions.

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Declaration of Interest

None.

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