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Dentate Gyrus Volume Deficit in Schizophrenia

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Author contributions

Drs. Nakahara and Van Erp had full access to all of the data in the study, conducted the statistical analysis, and take responsibility for the integrity of the data and the accuracy of the data analysis. Drs. Nakahara and Van Erp drafted the manuscript. All authors critically reviewed the manuscript, provided comments, and approved the manuscript for publication.

Conflict of interest

Dr. Soichiro Nakahara’s effort was supported by Astellas Pharma Inc. while he was a visiting scholar in University of California, Irvine. Dr. Bustillo consulted with Novartis and Otsuka Pharmaceuticals. Dr. Mathalon consulted for Boehringer Ingelheim and Takeda. Dr. Preda consulted for Boehringer-Ingelheim. Dr. Potkin has financial interests in Bristol-Myers Squibb, Eisai, Inc., Eli Lilly, Forest Laboratories, Genentech, Janssen Pharmaceutical, Lundbeck, Merck, Novartis, Organon, Pfizer, Roche, Sunovion, Takeda Pharmaceutical, Vanda Pharmaceutical, Novartis, Lundbeck, Merck, Sunovion and has received grant funding from Amgen, Baxter, Bristol-Myers Squibb, Cephalon, Inc., Eli Lilly, Forest Laboratories, Genentech, Janssen Pharmaceutical, Merck, Otsuka, Pfizer, Roche, Sunovion, Takeda Pharmaceutical, Vanda Pharmaceutical, NIAAA, NIBIB, NIH/NCRR, University of Southern California, UCSF, UCSD, Baylor College of Medicine. Dr. Van Erp has had a contract with Otsuka Pharmaceuticals. The remaining authors declare no potential conflict of interest.

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Abstract

Background—Schizophrenia is associated with robust hippocampal volume deficits but subregion volume deficits, their associations with cognition, and contributing genes remain to be determined.

Methods—Hippocampal formation subregion volumes were obtained using FreeSurfer 6.0 from individuals with schizophrenia ($n=176$, mean age \pm SD=39.0 \pm 11.5, 132 males) and healthy volunteers ($n=173$, mean age \pm SD=37.6 \pm 11.3, 123 males) with similar mean age, gender, handedness, and race distributions. Relationships between the hippocampal formation subregion volume with the largest between group difference, neuropsychological performance, and single-nucleotide polymorphisms were assessed.

Results—This study found a significant group by region interaction on hippocampal subregion volumes. Compared to healthy volunteers, individuals with schizophrenia had significantly smaller dentate gyrus (Cohen's $d = -0.57$), Cornu Ammonis 4, molecular layer of the hippocampus, hippocampal tail, and Cornu Ammonis 1 volumes, when statistically controlling for intracranial volume; dentate gyrus ($d = -0.43$) and Cornu Ammonis 4 volumes remained significantly smaller when statistically controlling for mean hippocampal volume. Dentate gyrus volume showed the largest between group difference and significant positive associations with visual memory and speed of processing in the overall sample. Genome-wide association analysis with dentate gyrus volume as the quantitative phenotype identified rs56055643 (Beta=10.8, $p < 5 \times 10^{-8}$, 95% CI: 7.0-14.5) on chromosome 3 in high linkage disequilibrium with *MOBP*. Gene-based analyses identified associations between *SLC25A38* and *RPSA* and dentate gyrus volume.

Conclusions—This study suggests that dentate gyrus dysfunction is fundamentally involved in schizophrenia pathophysiology, that it may contribute to cognitive abnormalities in schizophrenia, and that underlying biological mechanisms may involve contributions from *MOBP*, *SLC25A38*, and *RPSA*.

Keywords

imaging; genetics; hippocampus; subfield; genome-wide association analysis

Introduction

The hippocampal formation (HF) is among the brain structures that show the most robust volume deficits in schizophrenia (Haijma et al. 2013; Okada et al. 2016; van Erp et al. 2016) though subregion abnormalities, relationships with cognitive performance, and contributing genetic loci remain to be determined. Regional HF abnormalities have been hypothesized and observed (Schobel et al. 2009; Tamminga et al. 2010, 2012; Small et al. 2011). Tamminga and colleagues hypothesized that a primary deficit in the hippocampal dentate gyrus (DG) disrupts memory presentations in schizophrenia (Tamminga et al. 2010), while Small and colleagues hypothesized that a primary deficit in Cornu Ammonis (CA) 1 disrupts memory integration in schizophrenia (Small et al. 2011). The HF's trisynaptic circuit, includes subregions that subservise different functions and differ in cellular make-up -e.g., the principal cell type in the DG is the granule cell and in the CA the pyramidal cell (Amaral & Witter 1989; Amaral et al. 2007; Small et al. 2011; Wheeler et al. 2015; Nakahara et al. 2018). Hence, different biological mechanisms may contribute to HF subregion volume abnormalities observed in schizophrenia.

Several automated HF segmentation methods exist (Van Leemput et al. 2008, 2009; Yushkevich et al. 2009; Iglesias et al. 2013; Adler et al. 2014). Two of these methods (Van Leemput et al. 2008, 2009; Iglesias et al. 2013) are implemented in FreeSurfer (Fischl et al. 2002; Fischl 2012) and have been most widely used in schizophrenia studies. Subregions shown to have lower volume in schizophrenia compared to controls include the CA1 (Hýža et al. 2016; Ho et al. 2017a, 2017b; Ota et al. 2017; Sauras et al. 2017), CA2/3 (Ho et al. 2017a, 2017b), DG (Ho et al. 2017b; Ota et al. 2017) and the subiculum (Francis et al. 2013; Mathew et al. 2014; Haukvik et al. 2015). These findings suggest that regional deficits in DG-CA3-CA1-subiculum circuitry may contribute to the emergence of psychosis (Yushkevich et al. 2008, 2009; Van Leemput et al. 2009).

Most schizophrenia HF subregion studies employed FreeSurfer 5.1-5.3; (Haukvik et al. 2018; Nakahara et al. 2018), for which limitations have been reported (Wisse et al. 2014). Hitherto, only two schizophrenia studies assessed HF subregion volumes using FreeSurfer 6.0's improved ex-vivo hippocampal subregion atlas segmentations (Iglesias et al. 2013) run on FreeSurfer 5.3 whole hippocampus segmentations (Ho et al. 2017a, 2017b). Ho and colleagues found progressive decline in CA1 volume in individuals at risk for psychosis (Ho et al. 2017a) and more widespread volume deficits and progressive decline in DG and CA3 after illness onset (Ho et al. 2017b). To our knowledge, only one schizophrenia study has reported HF subregion volumes obtained using only FreeSurfer 6.0 (Baglivo et al. 2017). A major advantage of FreeSurfer 6.0 is that its ex-vivo atlas provides separate DG and CA4 volume estimates (Iglesias et al. 2013). This distinction is important for testing Tamminga and colleague's hypothesis (Tamminga et al. 2010) because it focuses on the DG and CA3 but not CA4. Moreover, given that no study to date has tested for a group by region interaction on HF subregion volumes, the possibility of differential regional HF volume abnormalities remains untested.

Schizophrenia is a heritable and HF subregion volume heritabilities are estimated at 40-70% (Greenspan et al. 2016; Whelan et al. 2016), suggesting that genetic variation may contribute

to psychosis related HF abnormalities. However genetic mechanisms contributing to HF subregion volume deficits remain undetermined.

In this study, we compared HF subregion volumes obtained using Freesurfer 6.0 between 176 individuals with schizophrenia and 173 healthy volunteers recruited into the Function Biomedical Informatics Research Network (FBIRN) Phase 3 study. We tested for a group by region interaction on regional volumes and computed group contrast effect sizes. Based on our review of the literature (Nakahara et al. 2018), a meta-analysis of predominantly FreeSurfer 5.3 studies (Haukvik et al. 2018) and studies that employed FreeSurfer 6.0 (Baglivo et al. 2017; Ho et al. 2017a, 2017b), we hypothesized a significant group by region interaction with the largest schizophrenia-control effect sizes for the DG/CA4 and CA1 and smallest effect sizes for the subicular regions. We also explored relationships between regions with the largest group difference effect sizes and cognitive performance. To identify possible novel contributing genetic variants to schizophrenia related HF subregion volume deficits, we performed, to our knowledge the first, schizophrenia genome-wide association (GWA) analysis with DG volume (largest group difference effect size) as the quantitative phenotype. Finally, we explored possible relationships between DG volume and age at onset, duration of illness, symptom severity, smoking, and medication dose.

Methods

Participants

Individuals with schizophrenia (n=176, mean age±SD=38.9±11.5, 132 males) and healthy volunteers (n=173, mean age±SD=37.6±11.3, 123 males) with similar mean age, gender handedness, and ethnicity distributions, recruited from seven sites, participated in this multi-center cross sectional case-control study (Table 1) (Friedman & Glover 2006; Friedman et al. 2006; Greve et al. 2011; Glover et al. 2012). Each participant was assessed with high-resolution T1-weighted scans and clinical assessments (Oldfield 1971; de Belmont Hollingshead 1975; Kay et al. 1989; Uttil 2002a, 2002b). Inclusion criteria were clinically stable -no antipsychotic medication or dose changes within the last two months- schizophrenia diagnosis based on DSM-IV-TR (First 2002). Schizophrenia and healthy volunteers with a history of major medical illness, drug dependence in the last five years (except for nicotine), current substance abuse disorder, or MRI contraindications, were excluded. Excluded were individuals with schizophrenia with significant tardive dyskinesia and healthy volunteers with current or past history of major neurological or psychiatric illness or a first-degree relative with an Axis-I psychotic disorder diagnosis (Supplementary methods). Written informed consent, including permission to share de-identified data between the centers, approved by the University of California (UC) Irvine, UC Los Angeles, and UC San Francisco, Duke University, University of North Carolina, New Mexico, Iowa, and Minnesota Institutional Review Boards, was obtained from all study participants.

Image analysis

Hippocampal tail, subiculum, CA1, hippocampal-fissure, presubiculum, parasubiculum, molecular layer of the hippocampus (molecular_layer_HP), granule cell and molecular cell layer of the DG (GC-ML-DG), CA3, CA4, fimbria, and hippocampal-amygdaloid transition

region (HATA) (Figure. 1A) as well as intracranial volume (ICV) and overall hippocampal volumes (Supplementary Table 1) were extracted from high-resolution T1-weighted images of the brain, that had previously been determined of good quality (little motion or other artifacts based on visual inspection) (van Erp et al. 2014; Esteban et al. 2017), using Freesurfer 6.0 (<https://surfer.nmr.mgh.harvard.edu>) (Fischl et al. 2002; Van Leemput et al. 2008, 2009; Fischl 2012; Iglesias et al. 2013).

Neuropsychological battery

Study participants performed the Computerized Multiphasic Interactive Neurocognitive DualDisplay System (CMINDS®) FBIRN task battery and the attention/vigilance, speed of processing, working memory, verbal learning, visual learning, and reasoning/problem cognitive domain scores as well as a total composite (global cognition) score (Supplementary Table 2) were computed as described in detail previously (van Erp et al. 2015).

Genetic imputation and quality control

DNA samples from unrelated and mixed ethnicity subjects (schizophrenia=130, healthy volunteers=145; Supplementary Methods & Table 3) were genotyped using the Illumina MEGA+Psych chip (Illumina, SD, USA) between January 2016 - May 2016 at Illumina Genomics Services (San Diego). Data were filtered to remove single-nucleotide polymorphisms (SNPs) with low minor allele frequency (MAF<0.01), deviations from Hardy–Weinberg equilibrium ($p < 1 \times 10^{-6}$), or poor genotyping call rate (<95%) using PLINK software (<http://pngu.mgh.harvard.edu/purcell/plink>) (Purcell et al. 2007). Filtered data were imputed to the 1000 Genomes Project reference panel (phase 1, version 3; <ftp://ftp.1000genomes.ebi.ac.uk/vol1/ftp/release/20130502>) using the Michigan imputation server (<https://imputationserver.sph.umich.edu/index.html>) (Das et al. 2016).

For individuals with genotyping (n=275; schizophrenia=130, healthy volunteers=145), imputed SNPs with estimated linkage disequilibrium $R^2 < 0.8$ or low MAF (<0.5%) were removed, resulting in a final dataset of 12,049,533 SNPs.

Statistical analyses

Prior to the main multivariate statistical analyses, we ran univariate mixed model regression analyses predicting ICV, left and right total hippocampal volumes (Supplementary results), and left and right HF subregions with group, site, gender, age, hemisphere (repeated measure), group \times site, group \times hemisphere, site \times hemisphere, and group \times site \times hemisphere interactions to visually examine the residuals for normality (Proc Univariate, SAS v9.4, SAS Institute Inc.); analysis of ICV did not include a hemisphere term. Residuals appeared normally distributed, no additional data points were excluded, and no data transforms were applied.

To enable proper testing of the diagnosis \times region interaction on hippocampal subregion volumes, data were equated for scalar differences between the subregions by normalization to the mean healthy volunteers subregion volumes [normalized volume = (participant volume - healthy volunteer mean volume) / healthy volunteers standard deviation]. The normalization

was performed with the healthy volunteer mean of left and right hemisphere volumes (mean across healthy volunteer subjects of $[\text{left}+\text{right volumes}/2]$) to allow for examination of hemisphere effects. The diagnosis \times region interaction on hippocampal subregion volumes was also tested in a model excluding fissure volumes which should be larger in individuals with lower hippocampal volumes such as individuals with schizophrenia.

Group differences for each region were examined using mixed model regression analyses (Proc Mixed, SAS v9.4, SAS Institute Inc.), predicting normalized hippocampal subregion volumes with group, region, and hemisphere and their 2 and 3-way interactions. Region and hemisphere entered the model as repeated measures factors, and gender, and site along with 2- and 3-way interactions between site, group, and region entered the model as categorical and age and ICV as continuous covariates (*Model 1*). Degrees of freedom were estimated using the Satterthwaite option. Possible confounding influences of smoking status on HF subregion volumes (Durazzo et al. 2013; Schneider et al. 2014) were assessed by including smoking status (current smoker, ex-smoker, never-smoker), current pack-year, or lifetime pack-year as covariates in *Model 1*, respectively. We also ran models in current non-smokers and never smokers only. Possible confounding effects of ethnicity were examined and ethnicity was not found to be a significant predictor of HF subregion volumes ($p>0.05$).

To assess the independence of the pattern of hippocampal subregion abnormalities from overall hippocampal volume deficits, a second analysis included *left and right hippocampal volumes*, instead of ICV, as covariates (*Model 2*). For the regions with a-priori defined directional hypotheses based on the literature (CA1, CA2-3, DG/CA4, and Subiculum) the significance threshold was set at $p<0.05$ (one-tailed). We also indicate which of the findings pass the more conservative Bonferroni multiple comparison corrected threshold for a test of subregion differences of $p<0.004$ ($0.05 \div 12$ hippocampal subregions, two tailed). Percent volume differences and Cohen's d weighted mean effect sizes, based on Model 1, were computed for each of the subregions.

We assessed the relationships between HF subregion volumes (with moderate between group effect sizes $d<-0.40$) and cognitive performance, age at onset, duration of illness, symptom severity (SANS and SAPS scores), and medication dose using regressions statistically controlling for sex, site (categorical), age, and ICV (two-tailed; Supplementary methods). Unequal slope analyses (comparing diagnoses) did not show significant diagnosis \times volume interactions on cognitive performance. Therefore, only overall sample associations with cognitive performance are reported and type I error was controlled using Bonferroni correction (see Supplementary Table 2). All analyses only included subjects with available anatomical, cognitive, or symptom data, no extrapolation or imputation of these data were performed to generate missing data.

Genome-wide association and gene-based analyses

The GWA analysis predicted mean bilateral DG volume with diagnosis, SNP, and the diagnosis \times SNP interaction, while statistically controlling for age, sex, whole hippocampus volume, site (scanner), and 4 multidimensional-scaling (MDS) components using PLINK. Whole hippocampal volume, rather than ICV was included in the GWA analysis, as it may more likely yield GWA findings unique to the DG volume. The standard genome-wide

significant threshold of $p < 5 \times 10^{-8}$ was applied to identify significant SNP (Risch & Merikangas 1996; Barsh et al. 2012) and diagnosis \times SNP interaction effects (Hancock et al. 2012). A fast and flexible gene- or set-based association test using genome-wide association study (GWAS) summary data of DG volume was performed using GCTA (see Supplementary Methods). The manhattan plot (Figure 2) was created using SNP2GENE software (<http://fuma.ctglab.nl/>) (Watanabe et al. 2017). Genome wide significance of gene was defined at $p = 0.05/24,765$ (the number of total genes) $= 2.0 \times 10^{-6}$.

Results

Main and interaction effects on hippocampal formation subregion volumes

Mixed model regression analyses, controlling for individual differences in ICV (Model 1), showed significant main effects of diagnosis, region, hemisphere, gender, site, and ICV as well as significant diagnosis \times region, hemisphere \times region, diagnosis \times site, and site \times region interactions on HF subregion volumes (Table 2). Mixed model regression analyses, controlling for individual differences in left and right hippocampal volume (Model 2), showed significant main effects of region, hemisphere, gender, age, site, and hippocampal volume, as well as significant diagnosis \times region, hemisphere \times region, and site \times region interactions, though the effects of diagnosis was no longer significant (Table 2). The diagnosis \times region interaction was also significant when fissure volumes were excluded from the analysis [$F(10,613) = 2.64$, $p < 0.004$ (Model 1); $F(10,635) = 2.83$, $p < 0.002$ (Model 2)].

Decomposition of the diagnosis \times region interaction in the model controlling for ICV (Model 1) showed that subjects with schizophrenia, compared to healthy subjects, had significantly smaller GC-ML-DG ($t_{644} = -4.82$, $p < 0.0001$; -4.1% , Cohen's $d = -0.57$), CA4 ($t_{640} = -4.64$, $p < 0.001$; -4.0% , $d = -0.55$), molecular_layer_HP ($t_{644} = -3.94$, $p < 0.0001$; -4.0% , $d = -0.47$), hippocampal tail ($t_{660} = -3.7$, $p = 0.0002$; -3.1% , $d = -0.44$), CA1 ($t_{645} = -3.26$, $p = 0.001$; -3.1% , $d = -0.39$), presubiculum ($t_{645} = -2.88$, $p = 0.004$; -2.9% , $d = -0.34$), fimbria ($t_{623} = -3.67$, $p = 0.008$; -5.4% , $d = -0.32$), subiculum ($t_{650} = -2.61$, $p = 0.009$; -2.3% , $d = -0.31$), HATA ($t_{641} = -2.41$, $p = 0.02$; -3.0% , $d = -0.29$), CA3 ($t_{635} = -2.03$, $p < 0.04$; -2.4% , $d = -0.24$), and parasubiculum ($t_{615} = -1.97$, $p < 0.05$; -3.4% , $d = -0.24$) volumes, and larger hippocampal fissure ($t_{632} = 3.18$, $p = 0.002$; $+5.0\%$, $d = 0.38$) volumes (all p-value reported are two-tailed). GC-ML-DG, CA4, molecular_layer_HP, hippocampal tail, CA1, and hippocampal fissure group differences were also significant at the Bonferroni multiple comparison correction threshold of $p < 0.004$ when controlling for ICV (Figure. 1B).

Decomposition of the diagnosis \times region interaction in the model controlling for hippocampal volume (Model 2) showed a similar profile to that in model 1. Subjects with schizophrenia, compared to healthy subjects, had significantly smaller GC-ML-DG ($t_{599} = -3.6$, $p = 0.0003$; Cohen's $d = -0.43$), CA4 ($t_{605} = -3.25$, $p = 0.001$; $d = -0.39$), molecular_layer_HP ($t_{490} = -2.8$, $p = 0.005$; $d = -0.33$), and hippocampal tail ($t_{645} = -2.14$, $p < 0.03$; $d = -0.26$), but not CA1 ($d = -0.20$), presubiculum ($d = -0.09$), fimbria ($d = -0.08$), subiculum ($d = -0.06$), HATA ($d = -0.03$), CA3 ($d = 0$), and parasubiculum ($d = 0$); in this secondary analysis, the hippocampal fissure remained significantly larger in schizophrenia compared with healthy volunteers ($t_{638} = 5.36$, $p < 0.0001$; $d = 0.65$) volumes. Only the GC-ML-DG, CA4, molecular_layer_HP, and hippocampal fissure group differences were

significant at the Bonferroni multiple comparison correction threshold of $p < 0.004$ when controlling for hippocampal volume (Figure. 1C). Given that DG volume showed the largest differences between individuals with schizophrenia and healthy volunteers, further association and GWA analysis focused on this region.

Decomposition of the region \times hemisphere interaction based on the Model 1 analysis showed that left hemisphere GC-ML-DG, CA1, CA3, CA4, molecular_layer_HP, hippocampal tail, and HATA region volumes were smaller than right hemisphere volumes (all p -values < 0.0001), while left hemisphere subiculum ($p < 0.05$), presubiculum ($p < 0.0001$), and parasubiculum volumes were larger than right hemisphere volumes. Left and right hemisphere volumes for the fimbria and hippocampal fissure were equivalent. Finally, there is a consistent pattern of lower GC-ML-DG volume in individuals with schizophrenia compared with controls across the seven sites (Supplementary Figure 2).

Associations with neuropsychological performance

DG (GC-ML-DG) and CA4 volumes showed significant associations with visual learning and speed of processing, molecular layer volumes showed significant associations with visual learning and CMINDS composite, while the hippocampal tail showed significant associations with attention/vigilance ($p < 0.0018$, $0.05/7/4 = 0.0018$, Bonferroni; see Supplementary Table 2).

Novel genome-wide markers associated with DG volume

No SNPs showed a genome-wide significant main effect on DG volume. The GWA analysis did identify a genome-wide significant diagnosis \times SNP interaction ($p < 5 \times 10^{-8}$) on DG volume for rs56055643 (Beta=10.75, 95% CI: 7.0-14.5, SE=1.91; T-statistic=5.63, $p = 4.8 \times 10^{-8}$) located on Chromosome 3 near *MOBP* (Figure 2 and Supplementary Table 4). Decomposition of the diagnosis \times SNP interaction showed a positive main effect of the rs56055643 A allele in schizophrenia (Beta=4.79, 95% CI: 1.8-7.82, SE=1.54; T-statistic=3.10, $p = 0.002$) and a negative main effect of the rs56055643 A allele in healthy volunteers (Beta=-5.14, 95% CI: -7.9--2.41, SE=1.40; T-statistic=-3.69, $p = 0.0003$; Fig. 2D). The effect of genotype remained significant within the schizophrenia group when either antipsychotic type (typical, atypical, both, or none) or chlorpromazine medication dose were included as covariates in the analysis. Gene-based analysis based on this GWA summary, found that several additional genes, including *MOBP*, *RPSA*, and *SLC25A38*, were significantly associated with DG volume (Table 3). The gene expression heatmap shows that each of these genes is expressed in brain, including in the hippocampus (Supplementary Figure 1).

Discussion

We found, to our knowledge for the first time, a significant group by HF subregion interaction, indicating regional variation in HF volume deficits in schizophrenia, significant associations between DG volumes and visual memory and speed of processing in the overall sample, and a genome-wide significant SNP associated with DG volume.

All HF subregion volumes were smaller and hippocampal fissure volume was larger (at $p < 0.05$) in individuals with schizophrenia compared to healthy controls when controlling for individual differences in ICV; GC-ML-DG, CA4, molecular_layer_HP, Hippocampal tail, CA1, and hippocampal fissure volume group differences survived multiple comparison correction.

GC-ML-DG, CA4, molecular_layer_HP, and hippocampal tail volumes were smaller and hippocampal fissure volume was larger (at $p < 0.05$) in individuals with schizophrenia compared to healthy controls when controlling for individual differences in hippocampal volumes; GC-ML-DG, CA4, and hippocampal fissure group differences survived multiple comparison correction.

We found that these regional volume abnormalities were independent from possible confounding effects of differences in smoking habits between schizophrenia and healthy volunteers, were not associated with ethnicity, and were not correlated with antipsychotic medication dose.

Our findings are largely consistent with prior studies that also found CA1, CA2/3, CA4/DG, molecular_layer_HP, subiculum, presubiculum, hippocampal tail volume deficits (Francis et al. 2013; Mathew et al. 2014; Haukvik et al. 2015; Kawano et al. 2015; Hýža et al. 2016; Ho et al. 2017a, 2017b; Ota et al. 2017; Rhindress et al. 2017) at $p < 0.05$. Moreover, our freesurfer 6.0 findings are also consistent with a recent meta-analysis, which reported the largest effect size for combined DG/CA4 volume deficits in schizophrenia based on predominantly FreeSurfer 5.3 studies (Haukvik et al. 2018). Lower bilateral CA4, DG, and CA1 volumes have also been observed in individuals with first-episode psychosis (Baglivo et al. 2017). We found fimbria ($p < 0.05$) and fissure volume abnormalities in schizophrenia compared to healthy volunteers, which were not reported previously. Possible reasons for the discrepancy are lower measurement accuracy of the smaller subregions, as well as between study differences in status and duration of illness (Ho et al. 2017a). Freesurfer 6.0 enables separate estimates for GC-ML-DG, CA4, and CA3 volumes, which was not possible in previous Freesurfer versions (5.1/5.3). Our finding that DG volume is significantly smaller in schizophrenia compared to control subjects based on Freesurfer 6.0, corroborates that from an independent study that assessed HF volumes with automatic segmentation of hippocampal subfields (ASHS) (Ota et al. 2017).

Furthermore, while some studies suggest that hippocampal volume deficits are largely similar across subregions (Haukvik et al. 2015), the present study found a significant group \times region interaction even when controlling for whole hippocampal volume. These findings indicate regional variability in HF subregion volume abnormalities in schizophrenia independent from overall hippocampal volume. Moreover, the interaction remained significant when the hippocampal fissure was excluded from the analysis. The present study thus provides robust evidence for regional variability in volume deficits within the HF in schizophrenia.

DG volume was significantly positively associated with visual memory and speed of processing performance in the overall sample, findings that are in line with the dysfunctional

DG hypothesis in schizophrenia (Tamminga et al. 2010, 2012). Interestingly, several mouse models of schizophrenia -including Ca^{2+} /calmodulin-dependent protein kinase II α heterozygous, Schnurri-2 knockout, mutant Synaptosomal-associated protein 25 knock-in, and forebrain-specific calcineurin knockout mice- show DG dysfunction due to an immature DG, which has also been observed in schizophrenia (Walton et al. 2012) and could underlie the observed volume deficit (Hagihara et al. 2013; Ohira et al. 2013). These mouse models of psychosis all show memory deficits, suggesting that DG dysfunction may be associated with memory deficits in schizophrenia. Though, the association between visual memory and volume was not unique to the DG, and was also observed for the CA4 and the molecular layer. Differential associations between HF subregion volumes and cognitive performance will require additional study and replication. We found no significant associations between DG volume and age at onset, duration of illness, or symptom severity, though associations between brain volumes and symptom measures are often weak and may require larger samples.

Although there have been several GWA studies on hippocampal volume, to our knowledge this is the first report to identify loci associated with DG volume in a schizophrenia sample. The top SNP, rs56055643, with a p-value less than the 5×10^{-8} standard genome-wide threshold, and several SNPs in high linkage disequilibrium with p-values less than 1×10^{-7} are located on chromosome 3 near the genes *RPSA* and *MOBP*. Gene-based analysis also suggests the association of these genes with DG volume.

RPSA, expressed in the hippocampus, encodes 40S ribosomal protein SA that is required for the assembly and/or stability of the 40S ribosomal subunit. This protein plays a role as a cell surface receptor for laminin, which plays an important role in cell differentiation, migration, and adhesion as well as synapse stabilization (Omar et al. 2017). Importantly, this protein interacts with ZNF804A, a well-known schizophrenia risk gene (Steinberg et al. 2011; Zhang et al. 2011), and rescues the migration and translational defects caused by *ZNF804A* knockdown. *RPSA* is enriched in mature compared to immature DG granule cells (Chatzi et al. 2016) consistent with models that have hypothesized an immature DG in schizophrenia (Walton et al. 2012)

MOBP, expressed in hippocampal oligodendrocytes, encodes myelin-associated oligodendrocyte basic protein. Recent studies suggest that oligodendrocyte function and myelination are disrupted in schizophrenia (Kubicki et al. 2005; McInnes & Lauriat 2006) and a study combining PGC schizophrenia risk loci, lifespan gene expression data, and schizophrenia methylome data has identified *MOBP* as a SNP-rich gene expression hub (Hegyí 2017). Lower numbers of oligodendrocytes have been observed in the hippocampal CA4 subfield in individuals with schizophrenia (Schmitt et al. 2009) and altered levels of myelin basic protein (MBP) have been reported in schizophrenia and bipolar disorder compared to controls (Chambers & Perrone-Bizzozero 2004) providing evidence for the myelin hypothesis in schizophrenia. Muted myelination could contribute to lower CA4 and DG volumes in schizophrenia. The rs56055643 SNP, located on near *MOBP*, was also associated with CA4 volume (Beta= 9.30, 95% CI: 5.77-12.82, SE=1.80; T-statistic=5.17, $p=4.7 \times 10^{-7}$) but not with whole hippocampus volume ($p=0.64$). Likewise, gene-based

analysis also showed that *MOBP* is associated with CA4 ($p=0.0002$) but not with whole hippocampus volume ($p=0.60$).

SLC25A38, or apoptosin, belongs to the SCL25 mitochondria solute carrier family, is expressed in hippocampal neurons, and has been linked to apoptosis (Zhang et al. 2012). Moreover, *MOBPSNPs* were strongly associated with apoptosin expression (Höglinger et al. 2011). These findings may suggest that *SLC25A39* and *MOBP* may be differentially involved in the regulation of survival or maturation of newly generated DG neurons in schizophrenia compared to controls (Chambers & Perrone-Bizzozero 2004).

It has been argued that imaging associations that are observed only in patients should be viewed with caution (Carter et al. 2017). We agree with this viewpoint but also note that genetic loci may have differential effects within the context of a disease. Therefore testing for diagnosis by SNP interaction effects may provide additional insights that may be missed when testing only for main effects of SNPs across diagnostic groups. Moreover, the observation that rs56055643 alleles show opposing effects in individuals with schizophrenia and healthy individuals could not have been observed in studies that perform genetic analyses using categorical (e.g. case-control) as opposed to quantitative traits.

This imaging study has several notable strengths. First, this study has a robust sample sizes for an imaging study including 176 schizophrenia and 173 healthy subjects recruited from multiple centers strengthening the generalizability of the findings. Second, this study demonstrated a significant group \times region interaction on hippocampal subregion volumes indicating, to our knowledge for the first time, that in schizophrenia not all HF subregions are affected equally. Third, the percent difference in volume between schizophrenia and healthy volunteers tracks the group contrast effect sizes and suggest that the effect sizes are not merely due to difference in measurement error between the regions. Fourth, this study tests for group by SNP interactions on a quantitative phenotype, an analysis strategy that is less frequently employed but may yield novel insights into gene-phenotype relationships. Finally, this study adheres to the best of its abilities to the “Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) and Strengthening the REporting of Genetic Association studies” (STREGA) guidelines (Supplementary Table 5).

Some limitations must also be noted. First, FreeSurfer’s hippocampal subregion method was developed using ultra-high resolution brain imaging data with a within-plane resolution of $380\mu\text{m}$ and 0.8mm slice thickness (Van Leemput et al. 2008) and may require additional validation for use with conventional resolution imaging data. It should be noted that our findings in part replicate previous reports based on ultra-high resolution scans as discussed above (Ota et al. 2017), corroborating prior reports that the method may also be applicable to high-quality conventional resolution structural imaging data (Iglesias et al. 2013). Furthermore, the fact that most subregion volumes are smaller while the fissure is larger in individuals with schizophrenia when compared to healthy controls indicates that the method makes local adjustments to the subregions. Second, we report to our knowledge a first GWAS with DG volume as a quantitative trait in schizophrenia and these findings warrant replication in an independent sample. Third, while no effects of ethnicity on HF subregion volumes were observed and while differences in ethnicity in the GWA analyses were

controlled for using MDS components, only analyses in ethnically homogeneous samples can fully exclude ethnic influences on GWA findings. Finally, while the use of multi-scanner imaging data may be viewed as a weakness, numerous studies have shown that larger samples based on multi-scanner data improves power to detect effects rather than increases noise over signal (Fennema-Notestine et al. 2007; Segall et al. 2009; van Erp et al. 2014; Boedhoe et al. 2017). Moreover, mean DG volume was smaller in schizophrenia than healthy subjects within each site.

In conclusion, we found volumetric abnormalities in some but not all hippocampal subregions in schizophrenia. In particular, DG was most severely affected and associated with visual memory and speed of processing deficits. Further, we identified novel genome-wide significant loci associated with DG volume. These findings support the DG dysfunction hypothesis of psychosis in schizophrenia (Tamminga et al. 2010, 2012). They also suggest novel biological insights in DG pathophysiology [e.g., oligodendrocyte (*MOBP*), cell differentiation, migration, adhesion (*RPSA*), or maturation / apoptosis (*SLC25A38*) aberrancies]. Overall, our finding that not all HF subregion volumes are equally affected in schizophrenia suggests involvement of different biological mechanisms between HF subregion, and that examining HF subregion abnormalities, including DG morphology and function as representative intermediate phenotypes, combined with genetics may allow for more accurate identification of the role of the HF in schizophrenia.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

- Adler DH, Pluta J, Kadivar S, Craige C, Gee JC, Avants BB, Yushkevich PA (2014). Histology-derived volumetric annotation of the human hippocampal subfields in postmortem MRI. *NeuroImage* 84, 505–523. [PubMed: 24036353]
- Amaral DG, Scharfman HE, Lavenex P (2007). The dentate gyrus: fundamental neuroanatomical organization (dentate gyrus for dummies). *The Dentate Gyrus: A Comprehensive Guide to Structure, Function, and Clinical Implications*, 3–790.
- Amaral DG, Witter MP (1989). The three-dimensional organization of the hippocampal formation: a review of anatomical data. *Neuroscience* 31, 571–591. [PubMed: 2687721]

- Baglivo V, Cao B, Mwangi B, Bellani M, Perlini C, Lasalvia A, Dusi N, Bonetto C, Cristofalo D, Alessandrini F, Zoccatelli G, Ciceri E, Dario L, Enrico C, Francesca P, Mazzi F, Paolo S, Balestrieri M, Soares JC, Ruggeri M, Brambilla P, GET UP Group (2017). Hippocampal Subfield Volumes in Patients With First-Episode Psychosis. *Schizophrenia bulletin* 44, 552–559.
- Barsh GS, Copenhaver GP, Gibson G, Williams SM (2012). Guidelines for Genome-Wide Association Studies. *PLoS genetics* 8, e1002812. [PubMed: 22792080]
- de Belmont Hollingshead A (1975). Four Factor Index of Social Status
- Boedhoe PSW, Schmaal L, Abe Y, Ameis SH, Arnold PD, Batistuzzo MC, Benedetti F, Beucke JC, Bollettini I, Bose A, Brem S, Calvo A, Cheng Y, Cho KIK, Dallaspezia S, Denys D, Fitzgerald KD, Fouche J-P, Giménez M, Gruner P, Hanna GL, Hibar DP, Hoexter MQ, Hu H, Huyser C, Ikari K, Jahanshad N, Kathmann N, Kaufmann C, Koch K, Kwon JS, Lazaro L, Liu Y, Lochner C, Marsh R, Martínez-Zalacain I, Mataix-Cols D, Menchón JM, Minuzzi L, Nakamae T, Nakao T, Narayanaswamy JC, Piras F, Piras F, Pittenger C, Reddy YCJ, Sato JR, Simpson HB, Soreni N, Soriano-Mas C, Spalletta G, Stevens MC, Szeszko PR, Tolin DF, Venkatasubramanian G, Walitza S, Wang Z, van Wingen GA, Xu J, Xu X, Yun J-Y, Zhao Q, ENIGMA OCD Working Group, Thompson PM, Stein DJ, van den Heuvel OA (2017). Distinct Subcortical Volume Alterations in Pediatric and Adult OCD: A Worldwide Meta- and Mega-Analysis. *The American journal of psychiatry* 174, 60–69. [PubMed: 27609241]
- Carter CS, Bearden CE, Bullmore ET, Geschwind DH, Glahn DC, Gur RE, Meyer-Lindenberg A, Weinberger DR (2017). Enhancing the Informativeness and Replicability of Imaging Genomics Studies. *Biological psychiatry* 82, 157–164. [PubMed: 27793332]
- Chambers JS, Perrone-Bizzozero NI (2004). Altered myelination of the hippocampal formation in subjects with schizophrenia and bipolar disorder. *Neurochemical research* 29, 2293–2302. [PubMed: 15672553]
- Das S, Forer L, Schönherr S, Sidore C, Locke AE, Kwong A, Vrieze SI, Chew EY, Levy S, McGue M, Schlessinger D, Stambolian D, Loh P-R, Iacono WG, Swaroop A, Scott LJ, Cucca F, Kronenberg F, Boehnke M, Abecasis GR, Fuchsberger C (2016). Next-generation genotype imputation service and methods. *Nature genetics* 48, 1284–1287. [PubMed: 27571263]
- Durazzo TC, Pennington DL, Schmidt TP, Mon A, Abé C, Meyerhoff DJ (2013). Neurocognition in 1-month-abstinent treatment-seeking alcohol-dependent individuals: interactive effects of age and chronic cigarette smoking. *Alcoholism, clinical and experimental research* 37, 1794–1803.
- van Erp TGM, Greve DN, Rasmussen J, Turner J, Calhoun VD, Young S, Mueller B, Brown GG, McCarthy G, Glover GH, Lim KO, Bustillo JR, Belger A, McEwen S, Voyvodic J, Mathalon DH, Keator D, Preda A, Nguyen D, Ford JM, Potkin SG, Fbirm (2014). A multi-scanner study of subcortical brain volume abnormalities in schizophrenia. *Psychiatry research* 222, 10–16. [PubMed: 24650452]
- van Erp TGM, Hibar DP, Rasmussen JM, Glahn DC, Pearson GD, Andreassen OA, Agartz I, Westlye LT, Haukvik UK, Dale AM, Melle I, Hartberg CB, Gruber O, Kraemer B, Zilles D, Donohoe G, Kelly S, McDonald C, Morris DW, Cannon DM, Corvin A, Machielsen MWJ, Koenders L, de Haan L, Veltman DJ, Satterthwaite TD, Wolf DH, Gur RC, Gur RE, Potkin SG, Mathalon DH, Mueller BA, Preda A, Macciardi F, Ehrlich S, Walton E, Hass J, Calhoun VD, Bockholt HJ, Sponheim SR, Shoemaker JM, van Haren NEM, Pol HEH, Ophoff RA, Kahn RS, Roiz-Santiañez R, Crespo-Facorro B, Wang L, Alpert KI, Jönsson EG, Dimitrova R, Bois C, Whalley HC, McIntosh AM, Lawrie SM, Hashimoto R, Thompson PM, Turner JA (2016). Subcortical brain volume abnormalities in 2028 individuals with schizophrenia and 2540 healthy controls via the ENIGMA consortium. *Molecular psychiatry* 21, 585. [PubMed: 26283641]
- van Erp TGM, Preda A, Turner JA, Callahan S, Calhoun VD, Bustillo JR, Lim KO, Mueller B, Brown GG, Vaidya JG, McEwen S, Belger A, Voyvodic J, Mathalon DH, Nguyen D, Ford JM, Potkin SG, FBIRN (2015). Neuropsychological profile in adult schizophrenia measured with the CMINDS. *Psychiatry research* 230, 826–834. [PubMed: 26586142]
- Esteban O, Birman D, Schaer M, Koyejo OO, Poldrack RA, Gorgolewski KJ (2017). MRIQC: Advancing the automatic prediction of image quality in MRI from unseen sites. *PLoS one* 12, e0184661. [PubMed: 28945803]
- Fennema-Notestine C, Gamst AC, Quinn BT, Pacheco J, Jernigan TL, Thal L, Buckner R, Killiany R, Blacker D, Dale AM, Fischl B, Dickerson B, Gollub RL (2007). Feasibility of multi-site clinical

structural neuroimaging studies of aging using legacy data. *Neuroinformatics* 5, 235–245. [PubMed: 17999200]

First MB (2002). User's Guide for the Structured Clinical Interview for DSM-IV-TR Axis I Disorders: SCID-I

Fischl B (2012). FreeSurfer. *NeuroImage* 62, 774–781. [PubMed: 22248573]

Fischl B, Salat DH, Busa E, Albert M, Dieterich M, Haselgrove C, van der Kouwe A, Killiany R, Kennedy D, Klaveness S, Montillo A, Makris N, Rosen B, Dale AM (2002). Whole brain segmentation: automated labeling of neuroanatomical structures in the human brain. *Neuron* 33, 341–355. [PubMed: 11832223]

Francis AN, Seidman LJ, Tandon N, Shenton ME, Thermenos HW, Mesholam-Gately RI, van Elst LT, Tuschen-Caffier B, DeLisi LE, Keshavan MS (2013). Reduced subicular subdivisions of the hippocampal formation and verbal declarative memory impairments in young relatives at risk for schizophrenia. *Schizophrenia research* 151, 154–157. [PubMed: 24144441]

Friedman L, Glover GH (2006). Report on a multicenter fMRI quality assurance protocol. *Journal of Magnetic Resonance Imaging* 23, 827–839. [PubMed: 16649196]

Friedman L, Glover GH, Fbirn Consortium (2006). Reducing interscanner variability of activation in a multi-center fMRI study: controlling for signal-to-fluctuation-noise-ratio (sfnr) differences. *NeuroImage* 33, 471–481. [PubMed: 16952468]

Glover GH, Mueller BA, Turner JA, van Erp TGM, Liu TT, Greve DN, Voyvodic JT, Rasmussen J, Brown GG, Keator DB, Calhoun VD, Lee HJ, Ford JM, Mathalon DH, Diaz M, O'Leary DS, Gadde S, Preda A, Lim KO, Wible CG, Stern HS, Belger A, McCarthy G, Ozyurt B, Potkin SG (2012). Function biomedical informatics research network recommendations for prospective multicenter functional MRI studies. *Journal of magnetic resonance imaging: JMIR* 36, 39–54. [PubMed: 22314879]

Greenspan KS, Arakelian CR, van Erp TGM (2016). Heritability of Hippocampal Formation Subregion Volumes. *Journal of neurology and neuroscience* 7

Greve DN, Mueller BA, Liu T, Turner JA, Voyvodic J, Yetter E, Diaz M, McCarthy G, Wallace S, Roach BJ, Ford JM, Mathalon DH, Calhoun VD, Wible CG, Brown GG, Potkin SG, Glover G (2011). A novel method for quantifying scanner instability in fMRI. *Magnetic Resonance in Medicine* 65, 1053–1061. [PubMed: 21413069]

Hagihara H, Takao K, Walton NM, Matsumoto M, Miyakawa T (2013). Immature dentate gyrus: an endophenotype of neuropsychiatric disorders. *Neural plasticity* 2013, 318596. [PubMed: 23840971]

Hajima SV, Van Haren N, Cahn W, Koolschijn PCMP, Hulshoff Pol HE, Kahn RS (2013). Brain volumes in schizophrenia: a meta-analysis in over 18 000 subjects. *Schizophrenia bulletin* 39, 1129–1138. [PubMed: 23042112]

Hancock DB, Soler Artigas M, Gharib SA, Henry A, Manichaikul A, Ramasamy A, Loth DW, Imboden M, Koch B, McArdle WL, Smith AV, Smolonska J, Sood A, Tang W, Wilk JB, Zhai G, Zhao JH, Aschard H, Burkart KM, Curjuric I, Eijgelsheim M, Elliott P, Gu X, Harris TB, Janson C, Homuth G, Hysi PG, Liu JZ, Loehr LR, Lohman K, Loos RJJ, Manning AK, Marciante KD, Obeidat M 'en, Postma DS, Aldrich MC, Brusselle GG, Chen T-H, Eiriksdottir G, Franceschini N, Heinrich J, Rotter JI, Wijmenga C, Williams OD, Bentley AR, Hofman A, Laurie CC, Lumley T, Morrison AC, Joubert BR, Rivadeneira F, Couper DJ, Kritchevsky SB, Liu Y, Wjst M, Wain LV, Vonk JM, Uitterlinden AG, Rochat T, Rich SS, Psaty BM, O'Connor GT, North KE, Mirel DB, Meibohm B, Launer LJ, Khaw K-T, Hartikainen A-L, Hammond CJ, Gläser S, Marchini J, Kraft P, Wareham NJ, Völzke H, Stricker BHC, Spector TD, Probst-Hensch NM, Jarvis D, Jarvelin M-R, Heckbert SR, Gudnason V, Boezen HM, Barr RG, Cassano PA, Strachan DP, Fornage M, Hall IP, Dupuis J, Tobin MD, London SJ (2012). Genome-wide joint meta-analysis of SNP and SNP-by-smoking interaction identifies novel loci for pulmonary function. *PLoS genetics* 8, e1003098. [PubMed: 23284291]

Haukvik UK, Tamnes CK, Söderman E, Agartz I (2018). Neuroimaging hippocampal subfields in schizophrenia and bipolar disorder: A systematic review and meta-analysis. *Journal of psychiatric research* 104, 217–226. [PubMed: 30107268]

- Haukvik UK, Westlye LT, Mørch-Johnsen L, Jørgensen KN, Lange EH, Dale AM, Melle I, Andreassen OA, Agartz I (2015). In vivo hippocampal subfield volumes in schizophrenia and bipolar disorder. *Biological psychiatry* 77, 581–588. [PubMed: 25127742]
- Hegy H (2017). Connecting myelin-related and synaptic dysfunction in schizophrenia with SNP-rich gene expression hubs. *Scientific reports* 7, 45494. [PubMed: 28382934]
- Höglinger GU, Melhem NM, Dickson DW, Sleiman PMA, Wang L-S, Klei L, Rademakers R, de Silva R, Litvan I, Riley DE, van Swieten JC, Heutink P, Wszolek ZK, Uitti RJ, Vandrovcova J, Hurtig HI, Gross RG, Maetzler W, Goldwurm S, Tolosa E, Borroni B, Pastor P, PSP Genetics Study Group, Cantwell LB, Han MR, Dillman A, van der Brug MP, Gibbs JR, Cookson MR, Hernandez DG, Singleton AB, Farrer MJ, Yu C-E, Golbe LI, Revesz T, Hardy J, Lees AJ, Devlin B, Hakonarson H, Müller U, Schellenberg GD (2011). Identification of common variants influencing risk of the tauopathy progressive supranuclear palsy. *Nature genetics* 43, 699–705. [PubMed: 21685912]
- Ho NF, Holt DJ, Cheung M, Iglesias JE, Goh A, Wang M, Lim JK, de Souza J, Poh JS, See YM, Adcock AR, Wood SJ, Chee MW, Lee J, Zhou J (2017a). 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. *Neuropsychopharmacology: official publication of the American College of Neuropsychopharmacology* 42, 1361–1370. [PubMed: 28079061]
- Ho NF, Iglesias JE, Sum MY, Kuswanto CN, Sitoh YY, De Souza J, Hong Z, Fischl B, Roffman JL, Zhou J, Sim K, Holt DJ (2017b). Progression from selective to general involvement of hippocampal subfields in schizophrenia. *Molecular psychiatry* 22, 142–152. [PubMed: 26903271]
- Hýža M, Kuhn M, ešková E, Ustohal L, Kašpárek T (2016). Hippocampal volume in first-episode schizophrenia and longitudinal course of the illness. *The world journal of biological psychiatry: the official journal of the World Federation of Societies of Biological Psychiatry* 17, 429–438.
- Iglesias JE, Sabuncu MR, Van Leemput K, Alzheimer's Disease Neuroimaging Initiative (2013). Improved inference in Bayesian segmentation using Monte Carlo sampling: application to hippocampal subfield volumetry. *Medical image analysis* 17, 766–778. [PubMed: 23773521]
- Kawano M, Sawada K, Shimodera S, Ogawa Y, Kariya S, Lang DJ, Inoue S, Honer WG (2015). Hippocampal subfield volumes in first episode and chronic schizophrenia. *PloS one* 10, e0117785. [PubMed: 25658118]
- Kay SR, Opler LA, Lindenmayer J-P (1989). The Positive and Negative Syndrome Scale (PANSS): Rationale and Standardisation. *British Journal of Psychiatry* 155, 59–65.
- Kubicki M, McCarley RW, Shenton ME (2005). Evidence for white matter abnormalities in schizophrenia. *Current opinion in psychiatry* 18, 121–134. [PubMed: 16639164]
- Mathew I, Gardin TM, Tandon N, Eack S, Francis AN, Seidman LJ, Clementz B, Pearlson GD, Sweeney JA, Tamminga CA, Keshavan MS 71, 769–777.
- McInnes LA, Lauriat TL (2006). RNA metabolism and dysmyelination in schizophrenia. *Neuroscience and biobehavioral reviews* 30, 551–561. [PubMed: 16445981]
- Nakahara S, Matsumoto M, van Erp TGM (2018). Hippocampal subregion abnormalities in schizophrenia: A systematic review of structural and physiological imaging studies. *Neuropsychopharmacology Reports*, 1–11.
- Ohira K, Kobayashi K, Toyama K, Nakamura HK, Shoji H, Takao K, Takeuchi R, Yamaguchi S, Kataoka M, Otsuka S, Takahashi M, Miyakawa T (2013). Synaptosomal-associated protein 25 mutation induces immaturity of the dentate granule cells of adult mice. *Molecular brain* 6, 12. [PubMed: 23497716]
- Okada N, Fukunaga M, Yamashita F, Koshiyama D, Yamamori H, Ohi K, Yasuda Y, Fujimoto M, Watanabe Y, Yahata N, Nemoto K, Hibar DP, van Erp TGM, Fujino H, Isobe M, Isomura S, Natsubori T, Narita H, Hashimoto N, Miyata J, Koike S, Takahashi T, Yamasue H, Matsuo K, Onitsuka T, Iidaka T, Kawasaki Y, Yoshimura R, Watanabe Y, Suzuki M, Turner JA, Takeda M, Thompson PM, Ozaki N, Kasai K, Hashimoto R (2016). Abnormal asymmetries in subcortical brain volume in schizophrenia. *Molecular psychiatry* 21, 1460–1466. [PubMed: 26782053]
- Oldfield RC (1971). The assessment and analysis of handedness: The Edinburgh inventory. *Neuropsychologia* 9, 97–113. [PubMed: 5146491]

- Omar MH, Campbell MK, Xiao X, Zhong Q, Brunken WJ, Miner JH, Greer CA, Koleske AJ (2017). CNS Neurons Deposit Laminin $\alpha 5$ to Stabilize Synapses. *Cell reports* 21, 1281–1292. [PubMed: 29091766]
- Ota M, Sato N, Hidese S, Teraishi T, Maikusa N, Matsuda H, Hattori K, Kunugi H (2017). Structural differences in hippocampal subfields among schizophrenia patients, major depressive disorder patients, and healthy subjects. *Psychiatry research* 259, 54–59. [PubMed: 27987389]
- Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MAR, Bender D, Maller J, Sklar P, de Bakker PIW, Daly MJ, Sham PC (2007). PLINK: A Tool Set for Whole-Genome Association and Population-Based Linkage Analyses. *American journal of human genetics* 81, 559–575. [PubMed: 17701901]
- Rhindress K, Robinson DG, Gallego JA, Wellington R, Malhotra AK, Szeszko PR (2017). Hippocampal subregion volume changes associated with antipsychotic treatment in first-episode psychosis. *Psychological medicine* 47, 1706–1718. [PubMed: 28193301]
- Risch N, Merikangas K (1996). The Future of Genetic Studies of Complex Human Diseases. *Science* 273, 1516–1517. [PubMed: 8801636]
- Sauras R, Keymer A, Alonso-Solis A, Díaz A, Molins C, Nuñez F, Rabella M, Roldán A, Grasa E, Alvarez E, Portella MJ, Corripio I (2017). Volumetric and morphological characteristics of the hippocampus are associated with progression to schizophrenia in patients with first-episode psychosis. *European psychiatry: the journal of the Association of European Psychiatrists* 45, 1–5. [PubMed: 28728089]
- Schmitt A, Steyskal C, Bernstein H-G, Schneider-Axmann T, Parlapani E, Schaeffer EL, Gattaz WF, Bogerts B, Schmitz C, Falkai P (2009). Stereologic investigation of the posterior part of the hippocampus in schizophrenia. *Acta neuropathologica* 117, 395–407. [PubMed: 18777029]
- Schneider CE, White T, Hass J, Geisler D, Wallace SR, Roessner V, Holt DJ, Calhoun VD, Gollub RL, Ehrlich S (2014). Smoking status as a potential confounder in the study of brain structure in schizophrenia. *Journal of psychiatric research* 50, 84–91. [PubMed: 24373929]
- Schobel SA, Lewandowski NM, Corcoran CM, Moore H, Brown T, Malaspina D, Small SA (2009). Differential targeting of the CA1 subfield of the hippocampal formation by schizophrenia and related psychotic disorders. *Archives of general psychiatry* 66, 938–946. [PubMed: 19736350]
- Segall JM, Turner JA, van Erp TGM, White T, Bockholt HJ, Gollub RL, Ho BC, Magnotta V, Jung RE, McCarley RW, Schulz SC, Lauriello J, Clark VP, Voyvodic JT, Diaz MT, Calhoun VD (2009). Voxel-based morphometric multisite collaborative study on schizophrenia. *Schizophrenia bulletin* 35, 82–95. [PubMed: 18997157]
- Small SA, Schobel SA, Buxton RB, Witter MP, Barnes CA (2011). A pathophysiological framework of hippocampal dysfunction in ageing and disease. *Nature reviews. Neuroscience* 12, 585–601. [PubMed: 21897434]
- Steinberg S, Mors O, Børglum AD, Gustafsson O, Werge T, Mortensen PB, Andreassen OA, Sigurdsson E, Thorgeirsson TE, Böttcher Y, Olason P, Ophoff RA, Cichon S, Gudjonsdottir IH, Pietiläinen OPH, Nyegaard M, Tuulio-Henriksson A, Ingason A, Hansen T, Athanasiu L, Suvisaari J, Lonnqvist J, Paunio T, Hartmann A, Jürgens G, Nordentoft M, Hougaard D, Norgaard-Pedersen B, Breuer R, Möller H-J, Giegling I, Glenthøj B, Rasmussen HB, Mattheisen M, Bitter I, Réthelyi JM, Sigmundsson T, Fossdal R, Thorsteinsdottir U, Ruggeri M, Tosato S, Strengman E, Genetic Risk and Outcome in Psychosis, Kremeney LA, Melle I, Djurovic S, Abramova L, Kaleda V, Walshe M, Bramon E, Vassos E, Li T, Fraser G, Walker N, Touloupoulou T, Yoon J, Freimer NB, Cantor RM, Murray R, Kong A, Golimbet V, Jönsson EG, Terenius L, Agartz I, Petursson H, Nöthen MM, Rietschel M, Peltonen L, Rujescu D, Collier DA, Stefansson H, St Clair D, Stefansson K (2011). Expanding the range of ZNF804A variants conferring risk of psychosis. *Molecular psychiatry* 16, 59–66. [PubMed: 20048749]
- Tamma CA, Southcott S, Sacco C, Wagner AD, Ghose S (2012). Glutamate dysfunction in hippocampus: relevance of dentate gyrus and CA3 signaling. *Schizophrenia bulletin* 38, 927–935. [PubMed: 22532703]
- Tamma CA, Stan AD, Wagner AD (2010). The hippocampal formation in schizophrenia. *The American journal of psychiatry* 167, 1178–1193. [PubMed: 20810471]
- Uttil B (2002a). North American Adult Reading Test: age norms, reliability, and validity. *Journal of clinical and experimental neuropsychology* 24, 1123–1137. [PubMed: 12650237]

- Uttl B (2002b). North American Adult Reading Test: age norms, reliability, and validity. *Journal of clinical and experimental neuropsychology* 24, 1123–1137. [PubMed: 12650237]
- Van Leemput K, Bakkour A, Benner T, Wiggins G, Wald LL, Augustinack J, Dickerson BC, Golland P, Fischl B (2008). Model-based segmentation of hippocampal subfields in ultra-high resolution in vivo MRI. *Medical image computing and computer-assisted intervention: MICCAI ... International Conference on Medical Image Computing and Computer-Assisted Intervention* 11, 235–243. [PubMed: 18979753]
- Van Leemput K, Bakkour A, Benner T, Wiggins G, Wald LL, Augustinack J, Dickerson BC, Golland P, Fischl B (2009). Automated segmentation of hippocampal subfields from ultra-high resolution in vivo MRI. *Hippocampus* 19, 549–557. [PubMed: 19405131]
- Walton NM, Zhou Y, Kogan JH, Shin R, Webster M, Gross AK, Heusner CL, Chen Q, Miyake S, Tajinda K, Tamura K, Miyakawa T, Matsumoto M (2012). Detection of an immature dentate gyrus feature in human schizophrenia/bipolar patients. *Translational psychiatry* 2, e135. [PubMed: 22781168]
- Watanabe K, Taskesen E, van Bochoven A, Posthuma D (2017). Functional mapping and annotation of genetic associations with FUMA. *Nature communications* 8
- Wheeler DW, White CM, Rees CL, Komendantov AO, Hamilton DJ, Ascoli GA (2015). Hippocampome.org: a knowledge base of neuron types in the rodent hippocampus. *eLife* 4
- Whelan CD, Hibar DP, van Velzen LS, Zannas AS, Carrillo-Roa T, McMahon K, Prasad G, Kelly S, Faskowitz J, deZubiracay G, Iglesias JE, van Erp TGM, Frodl T, Martin NG, Wright MJ, Jahanshad N, Schmaal L, Sämann PG, Thompson PM, Alzheimer's Disease Neuroimaging Initiative (2016). Heritability and reliability of automatically segmented human hippocampal formation subregions. *NeuroImage* 128, 125–137. [PubMed: 26747746]
- Wisse LEM, Biessels GJ, Geerlings MI (2014). A Critical Appraisal of the Hippocampal Subfield Segmentation Package in FreeSurfer. *Frontiers in aging neuroscience* 6, 261. [PubMed: 25309437]
- Yushkevich PA, Avants BB, Pluta J, Das S, Minkoff D, Mechanic-Hamilton D, Glynn S, Pickup S, Liu W, Gee JC, Grossman M, Detre JA (2009). A high-resolution computational atlas of the human hippocampus from postmortem magnetic resonance imaging at 9.4 T. *NeuroImage* 44, 385–398. [PubMed: 18840532]
- Yushkevich PA, Avants BB, Pluta J, Minkoff D, Detre JA, Grossman M, Gee JC (2008). Shape-based alignment of hippocampal subfields: evaluation in postmortem MRI. *Medical image computing and computer-assisted intervention: MICCAI ... International Conference on Medical Image Computing and Computer-Assisted Intervention* 11, 510–517. [PubMed: 18979785]
- Zhang H, Zhang Y-W, Chen Y, Huang X, Zhou F, Wang W, Xian B, Zhang X, Masliah E, Chen Q, Han J-DJ, Bu G, Reed JC, Liao F-F, Chen Y-G, Xu H (2012). Apoptosin is a novel pro-apoptotic protein and mediates cell death in neurodegeneration. *The Journal of neuroscience: the official journal of the Society for Neuroscience* 32, 15565–15576. [PubMed: 23115192]
- Zhang R, Valenzuela RK, Lu S, Meng L, Guo T, Du X, Kang W, Ma J (2011). Is the conserved mammalian region of ZNF804A locus associated with schizophrenia? A population-based genetics analysis. *Schizophrenia research* 133, 159–164. [PubMed: 21993378]

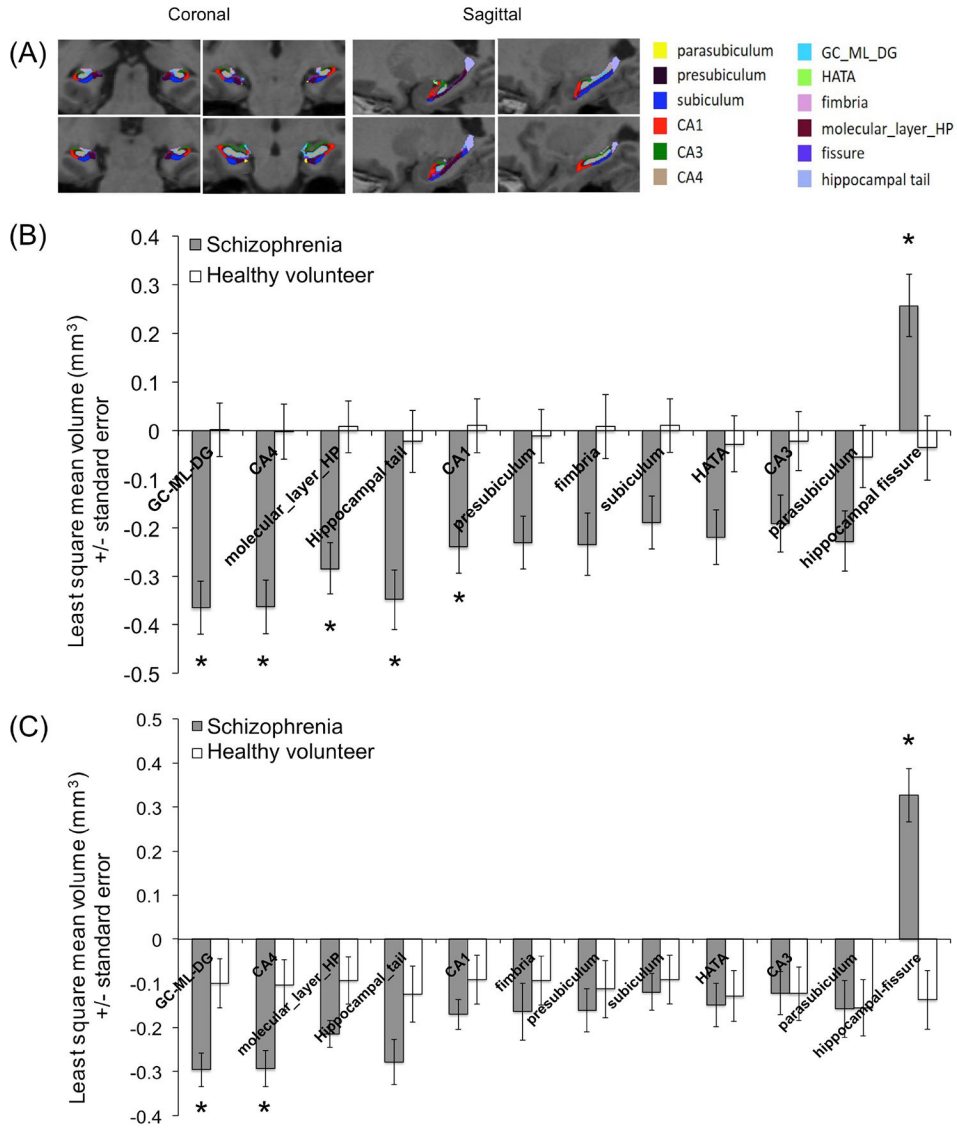


Figure 1. Hippocampal formation subregion segmentations and least square mean normalized volumes.

(A) Left shows hippocampal formation segmentations on 4 coronal slices from anterior (top) to posterior (bottom). Right row shows hippocampal formation segmentations on 4 left hemisphere sagittal slices from medial (top) to lateral (bottom). (B) Least square mean normalized hippocampal subregion volumes \pm standard error by diagnosis (Model 1). The hippocampal subregion volumes are normalized to the control mean of left and right hemisphere subregion volumes, and statistical analyses controlled for ICV. (C) Least square mean normalized hippocampal subregion volumes \pm standard errors by diagnosis (Model 2). The hippocampal subregion volumes are normalized to the control mean of left and right hemisphere subregion volumes, and statistical analyses controlled for left and right hippocampus volume. Schizophrenia (gray bars). Healthy volunteers (white bars). * significant Bonferroni corrected group contrasts ($p < 0.004$).

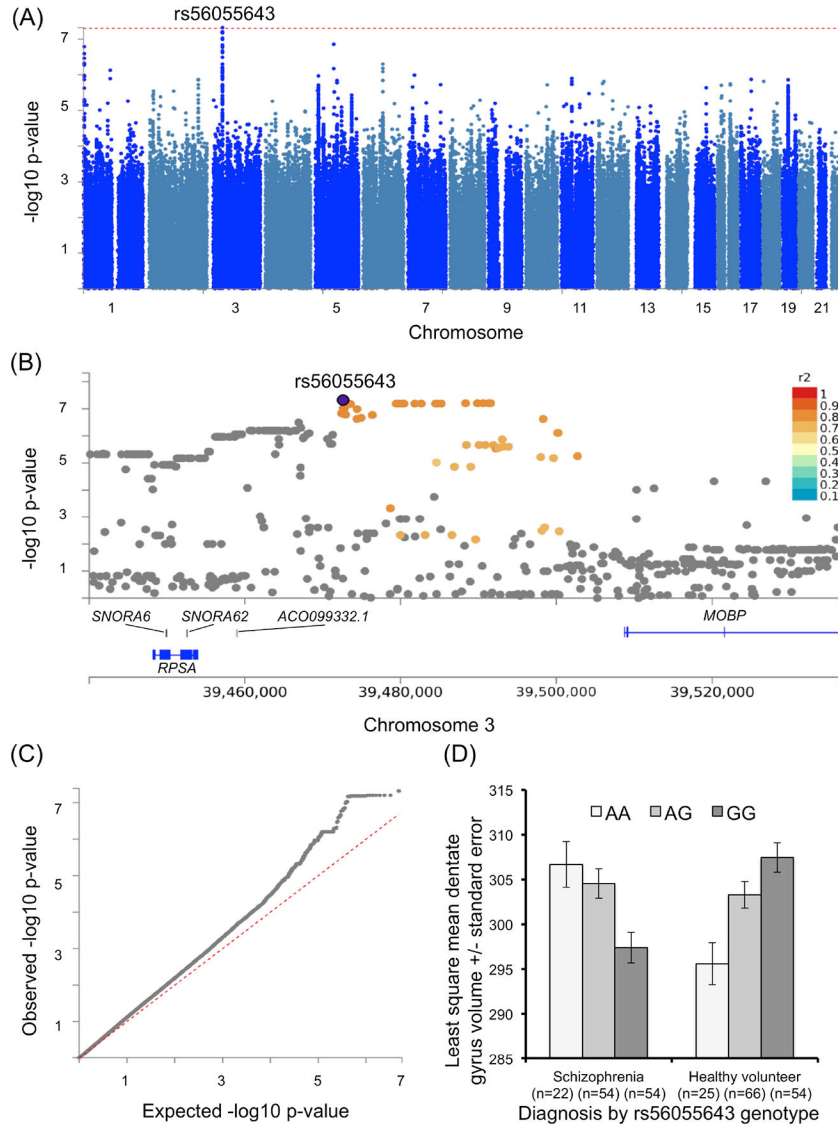


Figure 2. Genome-wide association with dentate gyrus volume. (A) A manhattan plot displays the association p value for each SNP in the genome (displayed as $-\log_{10}$ of the p-value). Red line displays $p=5 \times 10^{-8}$ line. (B) Detailed manhattan plot around top SNP (rs56055643) along with gene mapping. SNPs in genomic risk loci are color-coded as a function of their maximum r^2 to the one of the independent significant SNPs in the locus, as follows: red ($r^2 > 0.8$), orange ($r^2 > 0.6$), green ($r^2 > 0.4$), blue ($r^2 > 0.2$), and grey ($r^2 \leq 0.2$). (C) Quantile-quantile plot for GWAS results. The empirical and theoretical distributions are shown as black and red line, respectively. (D) Depiction of the diagnosis by rs56055643 interaction on dentate gyrus volume (\pm standard error).

Table 1.

Sample demographics and clinical characteristics

	Schizophrenia (n=176)	Healthy volunteer (n=173)	Statistic	p-value
Mean age (SD)	38.9 (11.5)	37.6 (11.3)	$t_{346}=1.11$	0.27
Gender (M/F)	132/44	123/50	$\chi^2_1=0.36$	0.39
Handedness ^a (bilateral/left/right)	4/12/159	2/7/164	FET	0.40
Subject socioeconomic status ^b (SD)	4.6 (0.9)	5.7 (0.9)	$t^{347}=-11.18$	<0.0001
Parental socioeconomic status ^b (SD)	5.6 (1.7)	5.9 (1.5)	$t_{347}=-1.25$	0.21
NAART	29.6 (12.3)	39.8 (11.5)	$t_{343}=-7.92$	<0.0001
Ethnicity			FET	0.14
American Indian or Alaskan Native	3	3		
Asian	22	15		
Black or African American	35	19		
Native Hawaiian or Pacific Islander	2	2		
White	113	134		
Smoking status			$\chi^2_2=66.9$	<0.0001
Current smoker	79 (45%)	14 (8%)		
Ex-smoker	33 (19%)	32 (19%)		
Never-smoker	63 (36%)	127 (73%)		
Smoking – current pack-years	6.9 (13.1)	1.9 (7.1)	$t_{345}=4.44$	< 0.0001
Smoking – lifetime pack-years	10.7 (16.3)	2.3 (8.6)	$t_{344}=6.02$	< 0.0001
Age at onset	21.9 (7.8)			
Duration of illness	17.1 (11.6)			
PANSS positive	15.4 (5.2)			
PANSS negative	14.3 (5.4)			
PANSS general	28.2 (7.4)			
PANSS composite	1.1 (6.3)			

FET=Fisher's Exact Test

^abased on Edinburgh Handedness Inventory^bbased on the Education Level of the Hollingstead Socioeconomic Status Scale; NAART = North American Adult Reading Test; PANSS = Positive and Negative Syndrome Scale.

Table 2.

Effects on hippocampal formation subregion volumes based on multivariate mixed-model regression analyses

Effect	<i>Model 1</i>				<i>Model 2</i>			
	nDF	dDF	F-value	p-value	nDF	dDF	F-value	p-value
Diagnosis	1	636	18.19	< 0.0001	1	634	1.95	0.16
Region	11	618	3.39	0.0001	11	622	3.47	0.0001
Diagnosis × Region	11	618	4.06	< 0.0001	11	622	4.15	< 0.0001
Hemisphere	1	507	30.76	< 0.0001	1	637	11.71	0.0007
Diagnosis × Hemisphere	1	507	1.19	0.28	1	615	1.31	0.25
Hemisphere × Region	11	629	32.19	< 0.0001	11	627	31.30	< 0.0001
Diagnosis × Hemisphere × Region	11	629	1.82	0.05	11	627	1.77	0.06
Site	6	637	15.39	< 0.0001	6	631	1.50	0.18
Diagnosis × Site	6	631	2.18	0.04	6	625	0.89	0.50
Site × Region	66	618	2.54	< 0.0001	66	622	2.61	< 0.0001
Diagnosis × Site × Region	66	618	1.11	0.26	66	622	1.14	0.21
Sex	1	652	11.84	0.0006	1	632	39.26	< 0.0001
Age	1	652	0.10	0.75	1	628	26.30	< 0.0001
ICV	1	652	293.09	< 0.0001				
Hippocampus volume					1	657	3504.73	< 0.0001

Model 1 statistically controls for individual differences in intracranial volume (ICV). Model 2 statistically controls for individual differences in hippocampal volume. nDF = nominator degrees of freedom; dDF = denominator degrees of freedom; p-values < 0.05 are listed in **bold**.

Table 3.

Gene-based analysis based on dentate gyrus genome-wide association results

Gene	CHR	Start	End	Gene based p-value
<i>SLC25A38</i>	3	39424839	39438842	9.59E-8
<i>RPSA</i>	3	39448180	39454033	8.88E-8
<i>MOBP</i>	3	39508689	39570970	3.27E-5

CHR: Chromosome; Start: Start location of gene in base pairs; End: End location of gene in base pairs; Gene based p-value: gene-based test p-value based on GCTA. The association p-value for a set of SNPs (± 50 Kb of UTRs) from an approximated distribution of the sum of χ^2 -statistics over the SNPs was calculated based on the GWAS data and LD correlations between SNPs from 1000 Genomes Project samples as a reference.

Genome wide significance was defined at $p = 0.05/24,765$ (the number of total genes) = 2.0×10^{-6} .