

Hippocampal neuroanatomy in first episode psychosis: A putative role for Glutamate and Serotonin receptors

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Abstract

Disrupted serotonergic and glutamatergic signaling interact and contribute to the pathophysiology of schizophrenia, which is particularly relevant for the hippocampus where diverse expression of serotonin receptors is noted. Hippocampal atrophy is a well-established feature of schizophrenia, with select subfields hypothesized as particularly vulnerable due to variation in glutamate receptor densities. We investigated hippocampal anomalies in first-episode psychosis (FEP) in relation to receptor distributions by leveraging 4 sources of data: (1) ultra high-field (7-Tesla) structural neuroimaging, and (2) proton magnetic resonance spectroscopy (1H-MRS) of glutamate from 27 healthy and 41 FEP subjects, (3) gene expression data from the Allen Human Brain Atlas and (4) atlases of the serotonin receptor system. Automated methods delineated the hippocampus to map receptor density across subfields. We used gene expression data to correlate serotonin and glutamate receptor genes across the hippocampus. Measures of individual hippocampal shape-receptor alignment were derived through normative modeling and correlations to receptor distributions, termed Receptor-Specific Morphometric Signatures (RSMS). We found reduced hippocampal volumes in FEP, while CA4-dentate gyrus showed greatest reductions. Gene expression indicated 5-HT1A and 5-HT4 to correlate with AMPA and NMDA expression, respectively. Magnitudes of subfield volumetric reduction in FEP correlated most with 5-HT1A ($R=0.64$, $p=4.09E-03$) and 5-HT4 ($R=0.54$, $p=0.02$) densities as expected, and replicated using previously published data from two FEP studies. Right-sided 5-HT4-RSMS was correlated with MRS glutamate ($R=0.357$, $p=0.048$). We demonstrate a putative glutamate-driven hippocampal variability in FEP through a serotonin receptor-density gated mechanism, thus outlining a mechanistic interplay between serotonin and glutamate in determining the hippocampal morphology in schizophrenia.

Introduction

The hippocampus is a brain structure widely implicated in neurological and psychiatric disorders (Small et al., 2011). As part of the memory circuit with several intricate subregions folded onto themselves, it remains a key structure extensively studied for its varied roles in cognition--namely memory, spatial navigation, and perception (Lisman et al., 2017; Zeidman and Maguire, 2016). Beyond the subfields, there is evidence for functional organization along a “long axis” gradient of specialization with the anterior (or ventral) responsible for anxiety-like behavior, emotion and stress, and posterior (or dorsal) hippocampus for cognitive functions (Fanselow and Dong, 2010; Strange et al., 2014; Vos de Wael et al., 2018). In schizophrenia, the hippocampus shows early atrophy of select subfields such as the CA1 and subiculum with extension to the rest later in the disease (Ho et al., 2017; Schobel et al., 2013).

In first-episode psychosis (FEP) and schizophrenia, there are consistent reports of hippocampal volumetric reductions across human neuroimaging studies. Recent work using higher field MRI shows CA4/DG as the earliest subfield affected (Nakahara et al., 2018) while earlier studies demonstrate preference for the CA1 and subiculum subfields (McHugo et al., 2018; Narr et al., 2004; Sauras et al., 2017; Szeszko et al., 2003; Verma et al., 2009). The anterior hippocampus is thought to be the primary site of pathology in schizophrenia given that lesions in the region cause increased dopaminergic transmission and model features of schizophrenia (Lipska et al., 1993; Small et al., 2011; Strange et al., 2014), while there is also evidence for abnormalities in the posterior hippocampus (Strange et al., 2014). In general, studies have focused on the grey matter content of the hippocampus--however, the hippocampus is covered by bundles of white matter that form projections to the rest of the brain. These white matter projections include the alveus, fimbria, and fornix that envelop the hippocampal formation (Amaral et al., 2018). The

involvement of these structures in psychosis is presently unclear, with contradictory evidence (Abdul-Rahman et al., 2011; Kuroki et al., 2006; Luck et al., 2010).

Regarding the mechanism for neuroanatomical changes of the hippocampus in psychosis, two major pathways are suspected. Firstly, glutamatergic excess causing hypermetabolism and excitotoxic damage (Schobel et al., 2013) is considered to be a key process in early schizophrenia (Kegeles et al., 2012; Marsman et al., 2013; Merritt et al., 2016; Poels et al., 2014) that affects grey matter volume, especially of the CA1 (Aoyama et al., 2011). CA1 and subiculum may be particularly vulnerable due to the relatively high density of NMDA and AMPA receptors compared to other subregions (Coultrap et al., 2005). Secondly, trophic effects mediated by serotonin, especially 5-HT_{1A} receptor promoting neurogenesis and 5HT₄ promoting neuronal survival (Cho and Hu, 2007), may be disrupted in schizophrenia (Eggers, 2013). These 2 pathways are likely to interact as well, with glutamatergic activity being reduced by 5HT_{1A} (Johnston et al., 2014; Kasamo et al., 2001; Tada et al., 2004) or increased by 5HT_{2A}/5HT₄ activation (Araneda and Andrade, 1991; Chapin et al., 2002; Mlinar et al., 2006), as shown in preclinical studies.

Human imaging studies have yet to clarify the role of 5HT system and glutamate receptors on the hippocampal atrophy seen in FEP. Magnetic Resonance Spectroscopic (MRS) measures of hippocampal glutamate do not reliably relate to hippocampal volume in FEP (Briend et al., 2020; Klär et al., 2010; Kraguljac et al., 2013; van Elst et al., 2005). Possible reasons for this lack of relationship between hippocampal glutamate and volume include the difficulty in quantifying glutamate resonance in lower field strengths of MRS, as well as the mixed nature of clinical samples with varying length of illness and treatment exposure—2 key clinical factors that affect hippocampal glutamate as well as volume (Briend et al., 2020). Furthermore, regional glutamate levels often predict the volume of distant brain regions more robustly than predicting the local

grey matter volume in psychosis(Plitman et al., 2014), possibly due to disrupted trophic effects of functional connections.

In this work, we first extend the study of hippocampal subfields in FEP to include white matter subregions and probe the serotonin-dependent nature of hippocampal atrophy in FEP. To this end, we sought to (1) replicate previous volumetric studies in FEP examining the hippocampal subfields and shape, and (2) leverage an in-vivo atlas of the serotonin receptor system(Beliveau et al., 2017) to test whether patterns of atrophy in FEP correlate with known distribution of serotonin receptor expression in healthy individuals. We hypothesized that the CA4/DG and CA1 would be amongst the most impacted in FEP, in line with previous studies. Amongst the white matter tracts, the fornix has the strongest evidence for volumetric changes and we expected to replicate this finding(Abdul-Rahman et al., 2011; Kuroki et al., 2006; Luck et al., 2010; Qiu et al., 2010). We further sought to replicate our sub-field morphometric findings in early psychosis by curating data from 3 published studies that examined the hippocampus in psychosis.

Secondly, we explore if serotonin-specific hippocampal atrophy is influenced by the putative glutamatergic excess in early stages of psychosis. To this end, we first determined the co-expressive relationship between serotonin and glutamate receptors using the gene expression data from the Allen Human Brain Atlas(Hawrylycz et al., 2012) (AHBA). We then related in vivo 7-tesla MRS glutamate concentration from the ACC to serotonin-dependent atrophic patterns in the hippocampus in our FEP sample. We hypothesized that the serotonin receptor subtypes that are highly expressed in the hippocampal subfields most affected in FEP will be spatially co-expressed with glutamate receptors. Thus, we aim to provide preliminary and indirect evidence from human imaging data to link the 2 major neurochemical pathways of hippocampal atrophy in psychosis.

Methods

Participant and data acquisition. Methods detailing participant recruitment, acquisition of structural and MRS brain imaging are available in Supplementary Methods. Briefly, healthy control (HC) subjects and FEP participants were recruited with lifetime antipsychotic treatment of less than 14 days. All subjects underwent structural brain imaging at 7T resolution as described in our prior reports (Dempster et al., 2020; Limongi et al., 2020), and glutamate levels were estimated using ¹H-MRS from a single voxel placed on dorsal anterior cingulate cortex (dACC; more information in Supplement). The MAgE T Brain algorithm was used for hippocampal morphometry (Amaral et al., 2018; Chakravarty et al., 2013; Pipitone et al., 2014; Winterburn et al., 2013) to measure subfield volumes and shape (Chakravarty et al., 2015; Lerch et al., 2008; Raznahan et al., 2014)—details are available in Supplementary Methods.

Statistical analysis. We employed multiple linear regression with total hippocampal GM and WM volumes as dependent variables, examining the main effect of diagnosis accounting for age, gender as covariates. Volumetric analyses were conducted at three levels of granularity: the “whole” hippocampus (including GM and WM subfields), using summed volumes of hippocampal grey matter (GM) and white matter (WM) separately, then at the level of the individual subfields. GM included the CA1, subiculum, CA4DG, CA2CA3, and the SR/SL/SM (stratum radiatum/stratum lacunosum/stratum moleculare; termed stratum henceforth), and WM included the alveus, fimbria, fornix, and mammillary bodies.

Mapping hippocampal serotonin receptor distributions. We used a previously published atlas of the serotonin receptor subtypes (5-HT1A, 5-HT1B 5-HT2A, 5-HT4) and the serotonin transporter (5-HTT) (Beliveau et al., 2017) derived from PET images of 210 healthy subjects to examine 1) Average density of serotonin receptors within subfields, and 2) Serotonin receptor

densities mapped onto hippocampal surfaces, with aim of correlating structural differences observed (between HC and FEP) with serotonin receptor densities (Supplementary Figure 1). Generation of the serotonin atlas is described in the original work--briefly, 210 healthy control subjects underwent PET scanning with 5 tracers to quantify brain-wide density of the serotonin receptors. PET data were collected at approximately 2mm resolution with structural imaging at ~1mm for accurate mapping and normalization. The subject maps were aligned to the MNI152 template and averaged to produce a map of serotonin receptor densities at 1mm isotropic voxels. The atlas demonstrated high correlations with postmortem data and mRNA expression using the AHBA (Beliveau et al., 2017).

We generated a MAGeT Brain segmentation of the hippocampal subfields and surface models on the MNI152 template by using the same template library used for segmentation of the subjects in MAGeT Brain (as described in Supplement) and inspected for accuracy. We obtained average serotonin receptor densities per subfield, and for each vertex on hippocampal surfaces generated for the MNI152 template (Supplementary Figure 2). We tested for correlations between 1) Effect sizes quantifying volumetric difference with hippocampal subfields ($-\log_{10}$ of p value) and shape differences (t-statistic), and 2) Serotonin receptor densities at the level of the subfields (average density per subfield) and on hippocampal surfaces.

We tested the reliability of our results by repeating all of the above including volumetric analyses and correlations between structural changes and receptor densities using an alternate set of hippocampal subfield definitions using the FreeSurfer software (Iglesias et al., 2015) (Supplementary Methods).

Reproducibility across different samples. We further tested the reliability of subfield-to-receptor correlations using data from published studies examining hippocampal subfields in psychosis. Studies 1 (41 FEP, 39 HC) (Li et al., 2018) and 2 (58 FEP, 76 HC)(Baglivo et al., 2018) included first-episode psychosis subjects, and Study 3 with chronic schizophrenia (155 SCZ, 79 HC)(Ho et al., 2017) where mean duration of illness was 7 years. Samples in Study 1 was medication naive, Study 2 excluded patients with antipsychotic treatment greater than 3 months, and mean daily dose was 212.3 (CPZ equivalent) for Study 3. Study 3 was included to compare findings in a FEP population vs. chronic schizophrenia, to test whether our results were FEP-specific and that structure-receptor correlations in chronic schizophrenia would likely reflect medication related changes.

All three studies used the same hippocampal subfield module within FreeSurfer, while version differed--Studies 1 and 2 with 6.0 and Study 3 used version 5.3. Both versions utilize the same computational atlas with minor differences in outputs(Iglesias et al., 2015). P-values of subfield volumetric comparisons between healthy controls and psychosis groups were $-\log_{10}$ transformed. Studies 2 and 3(Baglivo et al., 2018; Ho et al., 2017) reported findings from select subfields including GC-ML-DG, CA1, CA3, CA4, molecular layer, hippocampal tail, and presubiculum (only for Study 2) while Study 1 reported on all subfields. We restricted further analyses for all three studies within the 8 subfields stated above. We repeated correlations between subfield effect sizes and serotonin receptor densities, expecting to replicate findings with strongest correlations for 5-HT_{1A} and 5-HT₄ within Studies 1 and 2 but not in Study 3.

Serotonin-glutamate receptor subtype co-expression patterns. We used the AHBA to quantify gene expression correlations between serotonin and glutamate receptor (NMDA and AMPA) genes to find which serotonin receptor subtype best correlated with glutamate receptors.

The methods and rationale for this approach of finding glutamate receptor proxies are outlined in Supplementary Methods.

Normative modelling to examine individual morphometric patterns. We generated normative maps of hippocampal shape to examine whether the structure-receptor correlations translate to the level of the individual. Hippocampal shape measures were regressed against age and sex in the entire cohort (including HC and FEP). Residuals for the FEP group were then z-scored against the HC mean and standard deviation at each vertex. The resulting surface z-score map is then correlated against the distribution of 5 serotonin receptors, yielding 10 Pearson's correlations (5 per hemisphere) which we term Receptor-Specific Morphometric Signatures (RSMS). The RSMS value for each receptor is a coefficient between -1 and +1, with +1 indicating a perfect spatial correlation between the distribution of that specific receptor (e.g. 5HT4) and the observed pattern of structural deviation within hippocampus in a patient with FEP. This provided a quantitative index for the receptor-specific morphometric deviation in the hippocampus for various 5HT receptors. For the 5HT receptors with spatial distribution matching the volumetric reductions across the subfields in FEP (i.e. 5HT1A and 5HT4), we tested the relationship between RSMS and ACC glutamate levels. ACC glutamate levels covary with hippocampal glutamate in schizophrenia (Gallinat et al., 2016; van Elst et al., 2005). Based on the results from preceding analysis, we hypothesized that ACC glutamate levels will relate to RSMS measures for 5HT1A and 5HT4.

Results

The final sample consisted of 68 subjects (27 HC, 41 FEP) passing image preprocessing quality control. 18 subjects (20.9% of initial cohort) were excluded due to errors in automated hippocampal analysis either due to global MR quality or segmentation failure locally. Group-wise demographic data are available in Supplementary Table 2--there were no significant differences in age (FEP=22.90 (4.71); HC=21.93 (3.54)) or sex distribution between groups. Patients had a median 1.45 Defined Daily Dose of antipsychotic exposure (i.e. <2 days of lifetime exposure)

Volume reduction

At the level of the whole hippocampus (GM and WM combined), there were reduced volumes in left ($t[64]=-2.155$, $p=0.035$), and right ($t[64]=-2.588$, $p=0.012$) hippocampus. We did not see significant volumetric differences at the level of the GM and WM between HC and FEP (Figure 1a) at the Bonferroni-corrected threshold of $p=0.0125$ (Left GM ($t[64]=-2.22$, $p=0.030$); Right GM ($t[64]=-2.552$, $p=0.013$); Left WM ($t[63]=-2.425$, $p=0.0182$; after excluding one HC outlier with $<3SD$) and Right WM ($t[64]=-2.402$, $p=0.0193$)), though all measures were significant at an uncorrected level. At subfield level, none were significant after Bonferroni correction ($p < 2.78E-03$), while 6 (bilateral CA4DG, right fimbria, stratum, CA1, and CA2-CA3) were significant at the uncorrected ($p < 0.05$) threshold with a preference for right hemisphere structures (Figure 1c). CA4-DG show greatest difference amongst subfields, with both the left ($t[64]=-2.70$, $p=8.91E-03$) right ($t[64]=-2.87$, $p=5.50E-03$), reduced in FEP compared to HCs (Figure 1c).

Vertex-wise analysis of shape differences between HC and FEP did not yield any findings that survived FDR correction. However, examining subthreshold vertices shows bilateral anterior hippocampus differences (Figure 1d). At the peak vertex in the right anterior hippocampus, there was significant inward displacement in FEP compared to HC ($t[64]=-3.103$, $p=2.85E-03$) (Figure 1d).

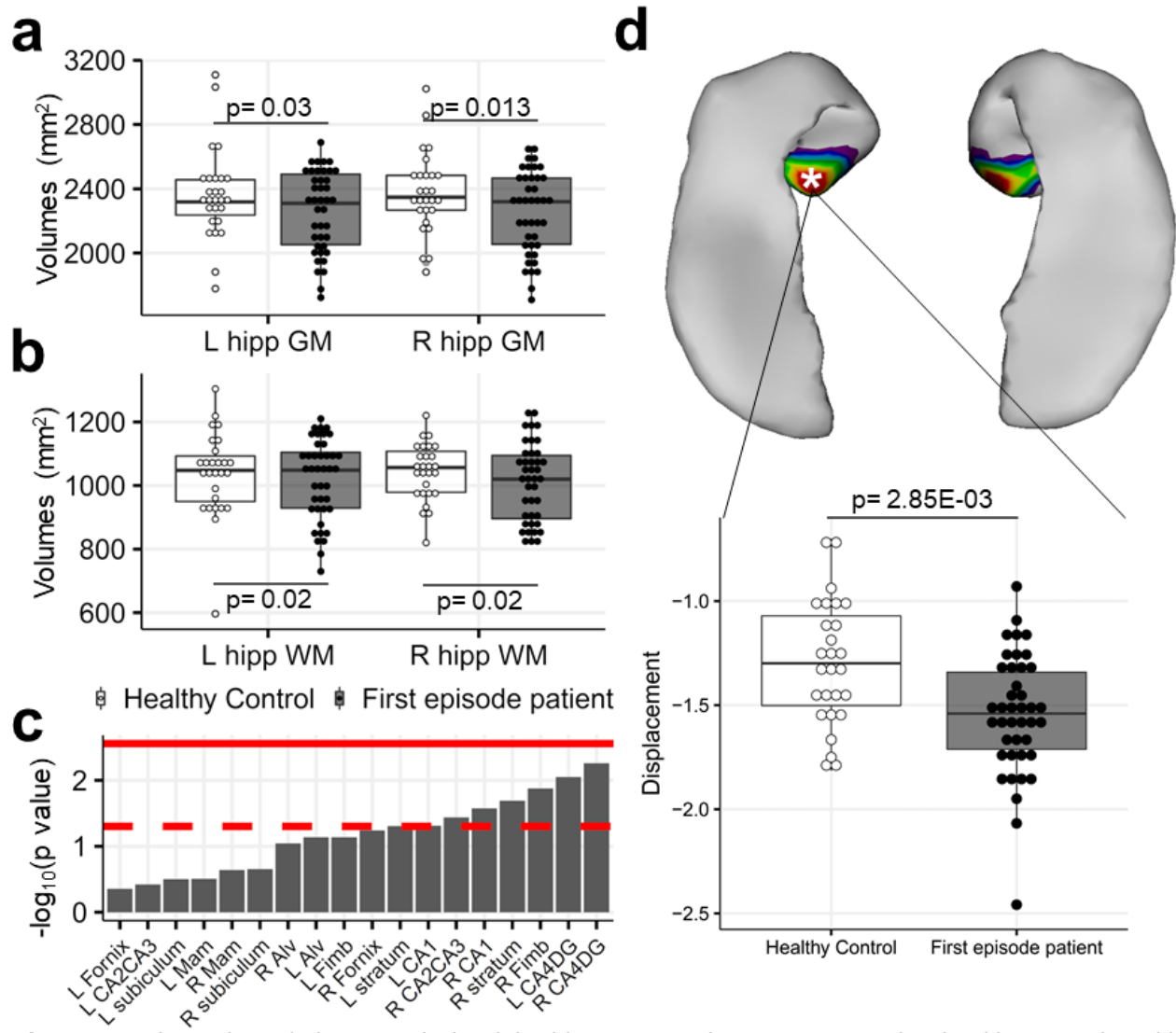


Figure 1. Volumetric and shape analysis of the hippocampus in FEP compared to healthy controls. a-b) Distribution and comparison of hippocampal GM and WM volumes. P-values for left hippocampal WM are after excluding 1 outlying HC subject. c) Subfield-wise comparisons between HC and FEP. Solid red line indicates the Bonferroni-corrected p-value threshold for 18 subregions, while the dotted red line indicates $p=0.05$. d) Shape analysis demonstrating bilateral shape differences of the anterior hippocampus in FEP compared to HC.

5HT-receptor expression and subfield morphometry

We tested correlations between 1) Subfield effect sizes resulting from multiple linear regression ($-\log_{10}$ of the p value), and 2) Mean density of serotonin receptor subtypes. 5-HT1A showed the strongest correlation (Pearson's $R = 0.64$, $p = 4.09E-03$, and the second strongest correlation was 5-HT4 ($R = 0.54$, $p = 0.02$). 5-HT1B, 2A, and 5-HTT showed modest positive correlations ($R = 0.40, 0.50, 0.43$, and $p = 0.10, 0.03, 0.07$ respectively (Figure 2a). We repeated the structure-receptor density correlation analysis using t-statistics from hippocampal shape analysis, which revealed similar findings. For the left hippocampus, 5-HT1A density demonstrated the strongest correlations to structural effect sizes ($R = -0.119$, $p = 4.827E-05$), followed by 5-HTT ($R = 0.161$, $p = 3.658E-08$) (Figure 2b). 5-HT4 was not significantly correlated ($R = 0.050$, $p = 0.0913$), as was 5-HT1B and 2A ($R = 0.039, 0.042$, $p = 0.182, 0.154$) (Figure 2b). The right hippocampus showed somewhat contrasting findings of 5-HT2A density correlating significantly ($R = -0.168$, $p = 3.723E-09$) as well as 5-HTT ($R = 0.120$, $p = 2.564E-05$), with 5-HT1A, 1B, and 4 not well correlated ($R = -0.004, -0.038, 0.003$, $p = 0.886, 0.185, 0.920$) (Figure 2c).

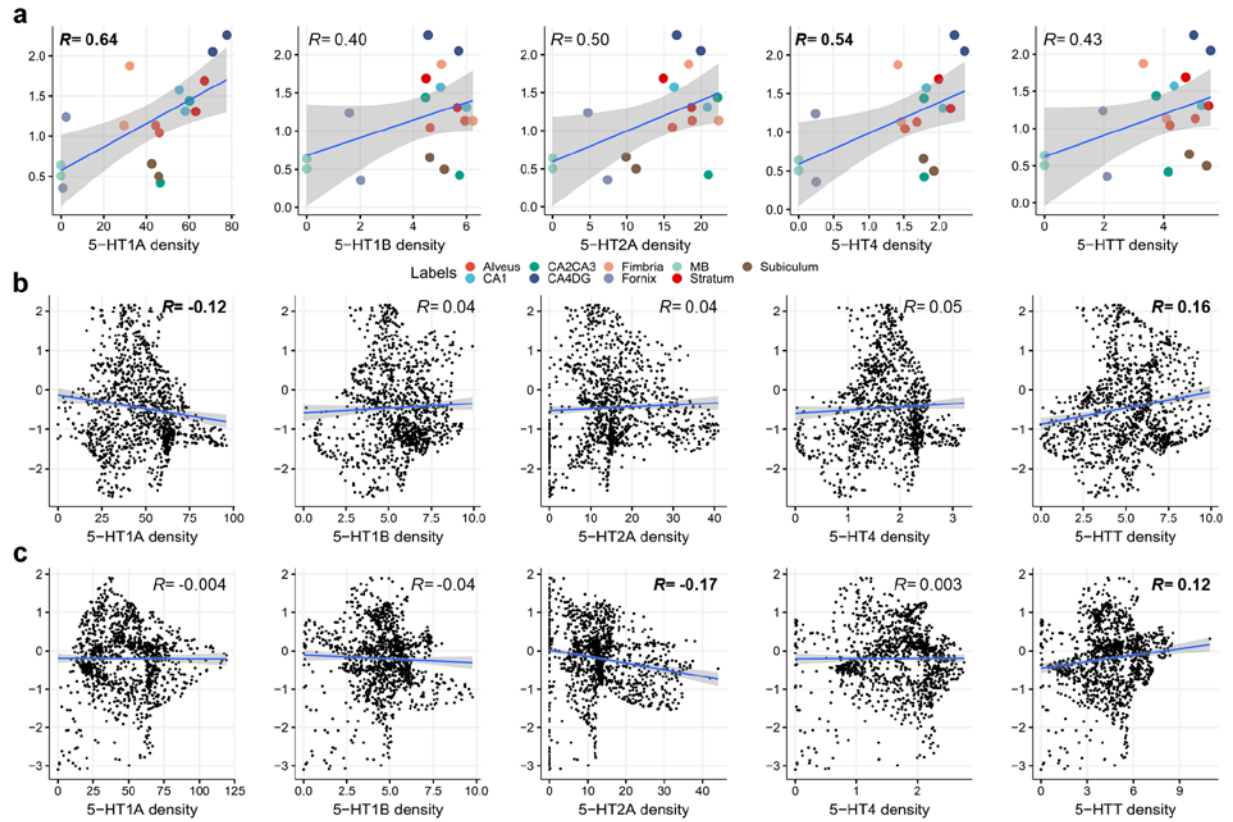


Figure 2. Linking serotonin receptors to volumetric differences. a) Correlations between effect sizes (HC vs. FEP) measured as $-\log_{10}(p \text{ value})$ and average receptor densities across the hippocampal subfields. b-c) Correlations between hippocampal shape effect sizes (HC vs. FEP) measured as t -statistics and receptor densities per vertex, for the left (b) and right (c) hippocampus.

Validation based on Freesurfer pipeline

We repeated our volumetric analysis and subfield effect size-receptor correlations using a different hippocampal subfield analysis pipeline through FreeSurfer. 61 subjects (38 FEP, 23 HC) passed quality control (Supplementary Figure 3). Volumetric analysis demonstrates the left ($t = -2.05$, $p = 0.045$) and right ($t = -2.74$, $p = 8.26E-03$) hippocampi to be reduced in FEP (Supplementary Figure 4). FreeSurfer subfields analyses show the R CA1 ($t = -2.90$, $p = 5.24E-03$), molecular layer, hippocampal tail, and fimbria with the strongest effect sizes (Supplementary Figure 4). None of the subfield results survive multiple testing correction. In repeating the subfield-serotonin correlations, we partly reproduce our results for 5-HT1A ($R = 0.372$, $p = 0.074$) and 5-HT4 ($R = 0.292$, $p = 0.167$) demonstrating strongest correlations (Figure 3, top row, and Supplementary Table 3).

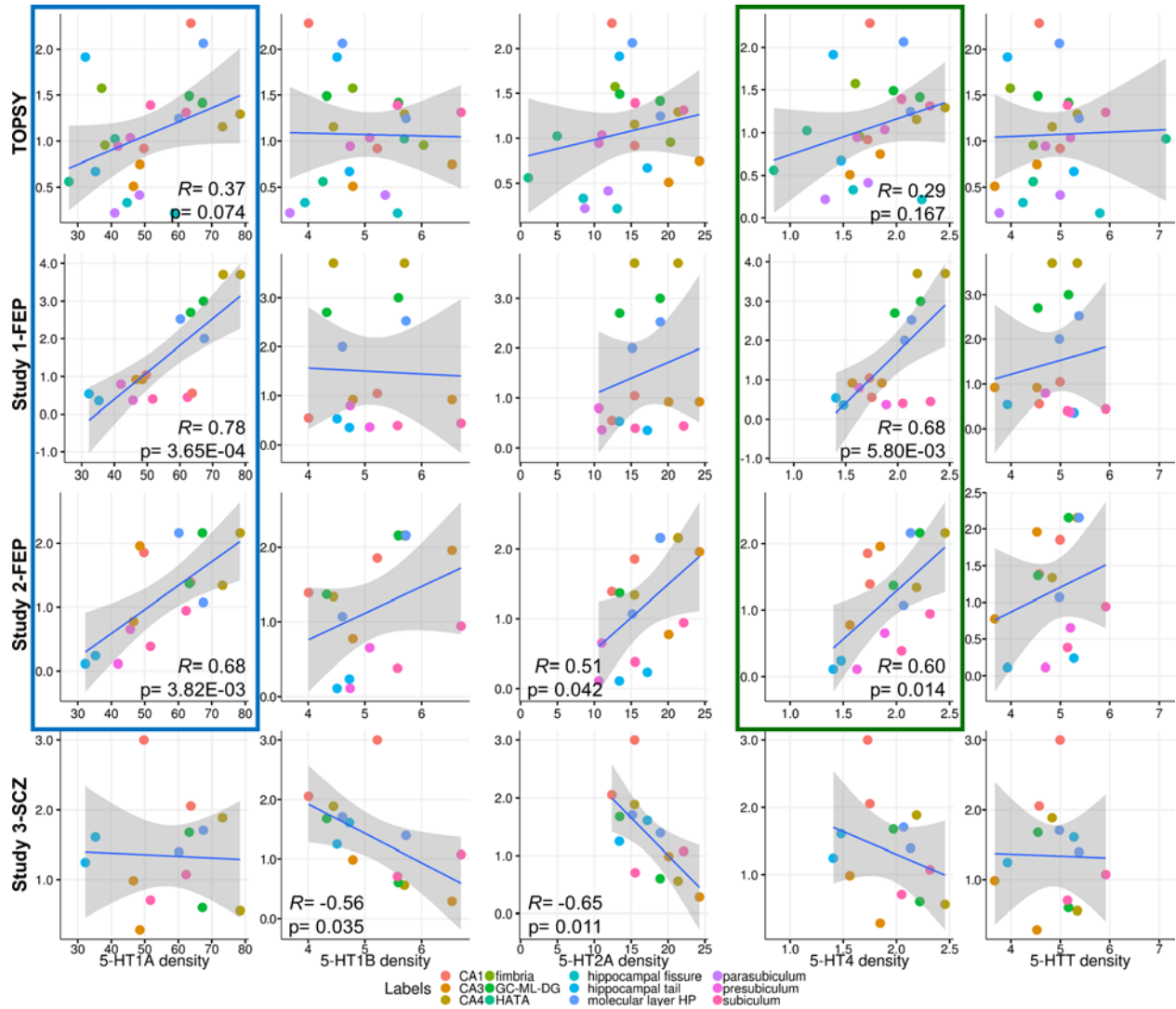


Figure 3. Correlations between effect sizes measured as $-\log_{10}(p \text{ value})$ on the y-axis and average receptor densities across the hippocampal subfields as defined by FreeSurfer. Studies 1 and 2 consisted of data from FEP subjects while Study 3 studied chronic schizophrenia. Effect sizes from our cohort and Studies 1-2 all demonstrate strongest correlations with 5-HT1A density (blue box) and 5-HT4 (green box), consistent with the hypothesis.

Validation based on other published datasets

We then sought replication across different samples using data from 3 recently published studies. Here, we demonstrate replication of our hypothesis with hippocampal subfield effect sizes correlating most strongly with the distribution of 5-HT1A and 5-HT4 as in our own sample--evident for Study 1 (5-HT1A: $R=0.78$, $p=3.65E-04$, 5-HT4: $R=0.66$, $p=5.80E-03$) and Study 2 (5-HT1A: $R=0.68$, $p=3.82E-03$, 5-HT4: $R=0.60$, $p=0.0138$) (Figure 3). Study 3 effect sizes

showed significant correlations for 5-HT1B ($R=-0.57$, $p=0.035$) and 5-HT2A ($R=-0.65$, $p=0.011$). Full table of Pearson's correlations and p-values are available in Supplementary Table 3.

5HT-glutamate gene expression correlations

Next, we examined gene expression correlations between serotonin receptors and glutamate receptor expression (Figure 4a). We first examined the hierarchical clustering based on gene-gene correlations, finding HTR1A and HTR4 to cluster closely with serotonin genes (Figure 4A). HTR1A clustered with GRIA1, and HTR4 with GRIN1 respectively. Examining the correlations themselves, HTR1A and HTR4 had the highest correlations amongst serotonin genes to glutamate genes (Supplementary Table 4)--with HTR1A-GRIA1 ($R=0.882$), and HTR4-GRIN1 ($R=0.935$) being the only correlations higher than $R=0.8$ to glutamate genes (Supplementary Table 4). Significance testing was done via Pearson's R correlation with FDR for multiple testing correction. Only HTR1A-GRIA1 and HTR4-GRIN1 correlations survived FDR correction at $q < 0.05$ (Supplementary Table 5). HTR1A (encoding the 5-HT1A subtype) was highly positively correlated with GRIA1-3 (AMPA subunits), and HTR4 (encoding the 5-HT4 subtype) with GRIN1, GRIN2A-C (NMDA subunits) (Figure 4b). In addition, we found SLC6A4 (5-HTT) to negatively correlate (with Pearson's $R \sim -0.50$) with most glutamate receptor genes (Supplementary Figure 5). Therefore, 5-HT1A and 5-HT4 receptor distributions demonstrate strongest and consistent correlations to volumetric differences (Figures 2-3) which may be reflected in their correlations with AMPA and NMDA glutamate receptor densities.

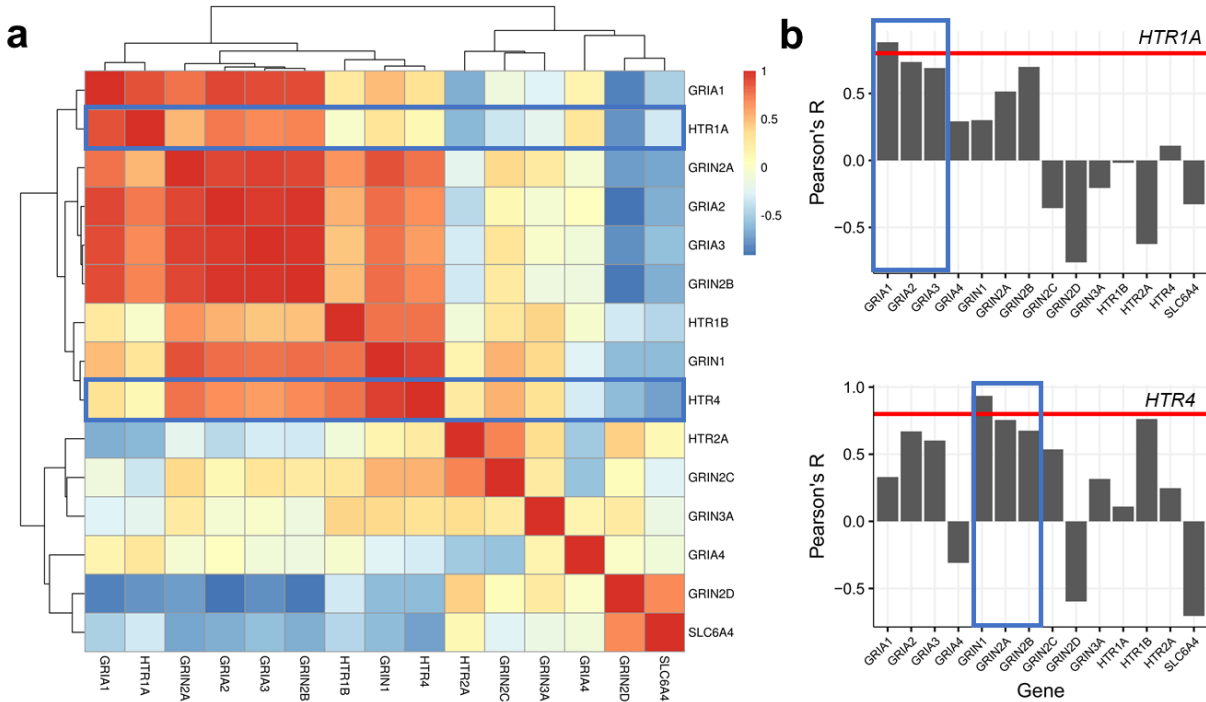


Figure 4. Linking serotonin and glutamate receptors through gene expression analysis. a) Gene expression correlations between 13 genes sampled in the AHBA for AMPA, NMDA subunits and serotonin receptor subtypes. Blue boxes are 2 candidate genes (HTR1A and HTR4) for predicting glutamate receptor expression. b) HTR1A and HTR4 expression was positively correlated with GRIA1-3 and GRIN1-2 genes (outlined in blue) that code for AMPA and NMDA receptor subunits respectively. Red line indicates Pearson's $R=0.80$. HTR1A-GRIA1 and HTR4-GRIN1 were the only correlations that survived FDR correction ($q < 0.05$).

MRS glutamate and hippocampal structure

Lastly, we used normative maps of hippocampal shape to generate individual measures of structure-receptor alignment, termed RSMS (Supplementary Figure 6a, Supplementary Figure 7 for distributions). Amongst the FEP group, 31 patients had MRS data available. Testing for correlations between 5-HT1A and 5HT4-RSMS with MRS measures of glutamate showed significant correlation between the right 5-HT4-RSMS with glutamate ($R= 0.357$, $p= 0.048$) (Supplementary Figure 6b). The left 5-HT4-RSMS was not significantly correlated with MRS measures, nor was 5-HT1A-RSMS ($p > 0.10$).

Discussion

Neuroimaging studies in schizophrenia have long since demonstrated structural changes in early schizophrenia. Here, we replicated previous findings of regional selectivity in volume loss within FEP patients. For the first time, we linked hippocampal structural changes in psychosis to the distribution of serotonin receptors 5-HT_{1A} (at the subfield and vertex level) and 5-HT₄ (at the subfield level). We replicated this association using published data from two FEP studies with respect to 5-HT_{1A} and 5-HT₄ specific correlations at the level of subfields. Lastly, we demonstrate a spatial correlation between 5HT_{1A} and 5HT-4 with AMPA and NMDA receptor expression respectively in healthy template data, providing a mechanistic link for how glutamatergic excess could affect hippocampal shape in FEP. More directly, we observe that a higher ACC glutamate level in FEP relates to 5-HT₄-specific morphometric signature of hippocampal shape, highlighting NMDA-mediated glutamate-5HT interactions in hippocampal morphometric changes. Apart from providing evidence for the glutamatergic mechanism for the morphological changes in schizophrenia, these results uncover receptor distribution as a critical mediator of the interindividual heterogeneity in the neuroanatomy of schizophrenia.

In our structural analyses, we replicate previous findings of reduced hippocampal volumes bilaterally, without significant asymmetry in findings (Figure 1). Interestingly, the WM also demonstrated similar reductions bilaterally. At the level of subfields, we found the CA4/DG subfield to be the most affected bilaterally in FEP--in line with more recent studies using high-resolution imaging(Nakahara et al., 2018), while other studies using standard resolution generally report CA1 as the key subfield. Methodological differences likely contribute to this discrepancy between findings with the choice of hippocampal subfield delineation affecting outcomes. For example, our own FreeSurfer-based analysis showed CA1 to be the most affected in FEP rather than CA4/DG. Definitions of the hippocampal subfields differ between

methods used in this study as well as the computational aspects of the algorithms themselves, highlighting the caution needed in interpreting the sequential structural changes in the subregions (for instance, the differences among Lieberman(Lieberman et al., 2018) (primacy of CA1); Tamminga(Tamminga et al., 2012) (primacy of CA3) and Harrison(Harrison and Eastwood, 2001) (primacy of CA4)). However, there is notable consistency across methods (Freesurfer, MAgE parcellations, and vertex-wise analysis) in localizing the FEP-specific changes to the anterior hippocampus and subregions more prominent in the anterior hippocampus such as the CA1, CA4/DG. Another significance of our findings is that we were amongst a few studies (roughly 3 out of 23) that studied medication-naïve FEP patients (Supplementary Table 5). Given antipsychotic medications may alter brain structure, our cohort with 1.45 median DDD days of antipsychotic exposure contributes a clearer perspective towards grey matter changes in early psychosis. We also highlight hippocampal white matter with reductions as a whole (Figure 1b) and within subregions including the fimbria, alveus and fornix (Figure 1c), in keeping with histopathological studies(Eastwood and Harrison, 2003; Heckers et al., 1991) and neuroimaging observations(Abdul-Rahman et al., 2011; Baumann et al., 2016; Luck et al., 2010; Qiu et al., 2010). These were not unexpected findings since grey and white matter subfields form closely related pathways originating from the entorhinal cortex(Amaral et al., 2018), and are often impacted in a prion-like progressive pathology along the circuit(Amaral et al., 2018; Miller et al., 2015; Small et al., 2011).

Using gene expression analysis, we found 5-HT1A and 5-HT4 to correlate with AMPA and NMDA subunits respectively. There is robust evidence for serotonin and glutamate receptor co-localization and interaction within the hippocampus and specifically within CA1, dentate gyrus and ventral hippocampus which would explain increased susceptibility to glutamate-mediated changes. Both 5-HT1A(Pompeiano et al., 1992) and 5-HT4(Tanaka et al., 2012; Vilario et al., 1996) expression has been shown to be enriched in the ventral pole (anterior

hippocampus)(Tanaka et al., 2012) and dentate gyrus(Segi-Nishida, 2017). Higher NMDA and AMPA expression has been reported in the anterior CA1 than CA3(Coultrap et al., 2005; Han et al., 2016). 5-HT4 receptor increases excitability of hippocampal pyramidal neurons by reducing long-term depression (LTD), a key NMDA-mediated synaptic plasticity mechanism(Hagena and Manahan-Vaughan, 2017). 5HT4 receptors play a crucial role in the potentiation of pyramidal CA3-CA1 synapses of the trisynaptic pathway(Teixeira et al., 2018), and promote dendritic spine formation(Segi-Nishida, 2017), explaining the observed co-expression with NMDA receptors.

We confirmed our hypothesis that the pattern of morphometric anomalies in the hippocampus correlate with the distribution of glutamate receptors through serotonin receptor proxies. We replicate these findings based on data from two additional FEP studies(Baglivo et al., 2018; Li et al., 2018), specific to early psychosis rather than chronic schizophrenia (Figure 3). These group-level findings are further supported by analyses using individual measures. Through normative modelling and assessing individual morphometric patterns and alignment to serotonin receptor maps, we further illustrate a glutamate-dependent mechanism for hippocampal atrophy. Right 5-HT4-RSMS, indicating resemblance of an individual's hippocampal shape to 5-HT4 (or NMDA) receptor distribution, was positively correlated with MRS measures of glutamate (Supplementary Figure 6b). This suggests that structural changes in the right hippocampus, specifically, is more closely related to excess glutamate through an NMDA receptor-dependent mechanism.

In testing the subfield-receptor correlations using published results from a chronic schizophrenia population, we found that structural anomalies correlated with 5-HT1B and 5-HT2A with a reversal of correlations--subfields with higher expression of receptors had less pronounced structural deficits. These contrasting structural changes secondary to antipsychotic medications

considering neuroleptics tend to antagonize 5-HT_{1B} and 5-HT_{2A}(Audinot et al., 2001; Meltzer, 1999). We speculate possible neuroprotective effects of 5-HT_{2A} antagonism as chronic 5-HT_{2A} blockade was shown to promote hippocampal neurogenesis(Jha et al., 2008). Further, antipsychotics reduce NMDA receptor availability in CA1 and CA2 the long term(Krzystanek et al., 2015), which may contribute to the correlation reversal as excitotoxicity-related structural damage becomes less prominent in chronic stages of schizophrenia.

Extant neuropathological evidence indicates that reduction in dendritic spines as well as the number of GABAergic interneurons, rather than reduction in pyramidal cell numbers, is the likely substrate of hippocampal volume reduction in schizophrenia(Heckers and Konradi, 2015). In this context, two competing interpretations could be made from our observations. Functionally, interneuron reduction may result in a disinhibition effect, with consequent high glutamatergic output at mPFC synapses, explaining the observed relationship between ACC glutamate levels and hippocampal volume changes in regions expected to have a higher NMDA expression. This notion is supported by ketamine related effects in CA1 and further propagation of hypermetabolism with repeated glutamatergic surge observed in mice(Schobel et al., 2013). Alternatively, reduced dendritic spines in anterior hippocampus may be secondary to prefrontal glutamatergic excess leading to excitotoxic damage, and may serve to compensate for the resulting hypermetabolism in hippocampus.

We note several limitations of this study, including the relatively small sample size and cross-sectional analyses. The size of both our study groups (FEP and HC) were limited due to accessing medication-naïve FEP individuals (max treatment 3 days of antipsychotics), with matched HCs based on parental education. While our sample was closely matched with respect to age, sex, and socioeconomic status, this restricted our power to detect significant differences. We acknowledge the possibility of medication effects on the relationship between serotonin

system and hippocampus. This is less likely in the setting of our medication-naïve patients (median lifetime daily dose exposure <1.45 days), and consistent findings across 2 additional FEP samples (Figure 3), suggesting that in early psychosis the receptor-specific findings are consistent regardless of treatment duration. The automated methods for analyzing the hippocampus and subfields is a potential source of discrepancy--however we attempt to show consistency between two different algorithms (MAGeT Brain and FreeSurfer) in comparing our results to previous studies. Both the AHBA and the serotonin atlases were based on healthy subjects, which we note as a limitation as serotonin and glutamate receptors may be altered in psychosis. Finally, the MRS voxel was placed on the dACC; not the hippocampus. Previous multi-voxel MRS studies in schizophrenia report a high degree of correlation across distant regions in the same subject (Kumar et al., 2020), and there is evidence for stronger ACC-hippocampus glutamate correlations in schizophrenia compared to controls (See Figure 3 in Gallinat and colleagues (Gallinat et al., 2016)). Regional glutamate measures are more likely to predict volume changes in functionally connected brain regions than the local grey matter volume (see Plitman and colleagues (Plitman et al., 2014) for a review), possibly as glutamate resonance within a MRS voxel often depends on the amount of available grey matter tissue, thus limiting our ability to find MRS metabolite-structure correlations within the voxel. Furthermore, short-term exposure to antipsychotics seem to have limited effect on ACC than hippocampal glutamate measures (Kraguljac et al., 2019). Nevertheless, given the lack of more direct evidence from a hippocampal voxel placement for MRS, we urge caution when generalizing these results. Regarding the largely negative findings for RSMS, it is likely that the lack of hippocampal MRS measures contributed, and that not all hippocampal abnormalities could be ascribed to glutamate pathways. Another limitation of the shape-based RSMS approach is that shape analysis only takes into account the “outer” surface of the hippocampus, and does not include the inner hippocampal subfields which may have limited the significance of the results.

By highlighting the role of 5HT1A and 5HT4 in glutamate-mediated hippocampal volume reduction in psychosis, our study raises the question of repurposing or employing existing agents with preferential 5HT1A (e.g. lurasidone, cariprazine) and 5HT4 affinity (e.g. prucalopride) as neuroprotective agents in schizophrenia. Hippocampal morphometry may provide the much-needed treatment engagement targets for the putative procognitive agents affecting 5HT1A and 5HT4 system. We advocate for the use of structural MRI, as well as MRS in clinical trials evaluating such agents, to generate the conclusive experimental evidence for the glutamatergic hypothesis of hippocampal dysfunction in schizophrenia.

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Conflict of Interest

LP receives book royalties from Oxford University Press and income from the SPMM MRCPsych course. In the last 5 years, his or his spousal pension funds held shares of Shire Inc., and GlaxoSmithKline. LP has received investigator initiated educational grants from Otsuka, Janssen and Sunovion Canada and speaker fee from Otsuka and Janssen Canada, and Canadian Psychiatric Association. LP, MM and KD received support from Boehringer Ingelheim to attend an investigator meeting in 2017. JT received speaker honoraria from Siemens Healthcare Canada. All other authors report no potential conflicts of interest.

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