2021-06-22

Limbic Morphometry in Individuals with Schizophrenia and Their Nonpsychotic Siblings

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A thesis submitted to the faculty of
Brigham Young University
in partial fulfillment of the requirements for the degree of

Master of Science

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ABSTRACT

Limbic Morphometry in Individuals with Schizophrenia
and Their Nonpsychotic Siblings

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The limbic system is hypothesized to play a critical role in pathophysiology of schizophrenia, with abnormalities thought to contribute to the expression of various aspects of the cognitive deficits and clinical symptoms. Psychosis is understood as highly heritable and family members, specifically non-affected siblings, while not displaying overt signs of the disorder, often exhibit features similar to those observed in patients, though to a lesser degree. The overarching aim of this project was to investigate the integrity of limbic circuitry in a sample of patients with schizophrenia and their non-affected siblings and examine its potential relationship with various clinical features of the illness. Cortical thickness of the entorhinal, parahippocampal, cingulate, and orbitofrontal cortices; as well as subcortical surface shape of the hippocampus and amygdala were the focus of this study. Findings from this study reveal relative similarity in limbic integrity between individuals with schizophrenia and their non-affected siblings, which are both disparate from healthy individuals. This suggests aspects of the neurobiological underpinnings of psychosis, particularly limbic regions, are genetically influenced regardless of symptom expression and are latent features in non-affected family members. Relationships between positive symptomatology and shape abnormalities of subcortical structures suggest a potential substrate for clinical characteristics in psychosis not evident in non-ill siblings.

Keywords: schizophrenia, unaffected siblings, brain imaging, limbic circuitry, morphometric similarity, clinical symptoms

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Limbic Morphometry in Individuals with Schizophrenia and Their Nonpsychotic Siblings

Psychosis is defined as an impaired relationship with reality that is characterized by a constellation of clinical symptoms, cognitive dysfunction, and functional impairment (American Psychiatric Association, 2013). Despite our current understanding of the behavioral features involved in psychosis, a complete characterization of the clinical symptoms and cognitive deficits from a neurobiological perspective is still lacking. Common cognitive issues experienced by those with psychosis include deficits in working memory, attention, and problem solving (Bowie & Harvey, 2006; Gold & Harvey, 1993; Dickinson et al., 2007), while the clinical manifestations can include prominent negative, positive and disorganized symptoms. The connections between cognitive issues/clinical symptoms with brain abnormalities in schizophrenia have been a popular research topic for decades (Andreasen, 1985), with cognitive impairment even being proposed as a contributor to these irregularities (Kahn & Keefe, 2013). Cognition has also been shown to have a genetic link amongst non-affected family members and individuals with schizophrenia (Toulopoulou et al., 2010; Sniekers et al., 2017; Smeland et al., 2017). Therefore, by having this valuable insight as to how their minds operate, these elements help us to derive a better understanding of schizophrenia.

The limbic system is a complex set of structures involved in motivation, emotion, learning, and memory, and it is theorized that abnormalities of the limbic system play a role in the expression of deficits within these cognitive domains for schizophrenia (Bachus & Kleinman, 1996; Gur et al., 2007; Heckers, 2000; Schneider et al., 1998; Shenton et al., 1992). Recent literature has documented these specific areas of the brain—amygdala, hippocampus, cingulate gyrus, hypothalamus and thalamus—and their relationships with psychosis (Aydin et al., 2019; Busch et al., 2019; McHugo et al., 2019). More specifically, certain structures within the

limbic system have been associated with specific clinical symptoms in past studies: positive symptoms and medial temporal regions—amygdala, hippocampus, parahippocampal, and entorhinal cortices (Bogerts, 1997; Velakoulis et al., 2006; Prasad et al., 2004), negative symptoms and cingulate (Bersani et al., 2014) and orbitofrontal regions (Sanfilipo et al., 2000; Gur et al., 2007; Kanahara et al., 2013; Walton et al., 2018), and disorganization and parahippocampal (Ohnuma, 1997; Prasad, Rohm, & Keshavan, 2004), cingulate (Ohnuma, 1997; Lahti et al., 2006), and orbitofrontal cortices (Nakamura et al., 2008). Furthermore, limbic system structures are highly interconnected with other cerebral circuitry, thus communicating with additional regions implicated in schizophrenia (Wong et al., 2020; de Zwarte et al., 2019; Kesby et al., 2018). Overall, there is strong evidence linking involvement of the limbic system in the complex pathophysiology of psychosis.

Psychosis is understood as a highly heritable condition (Lee et al., 2017; American Psychiatric Association, 2013) and family members of patients with schizophrenia, specifically non-affected siblings, while not displaying overt signs of the disorder, often exhibit cognitive impairments similar to those observed in patients, though to a lesser degree (Wisner et al., 2011; Harms et al., 2007; Delawalla et al., 2006). These impairments in siblings have been utilized as potential intermediate phenotypes in studies of the genetics of schizophrenia (Diaz-Asper et al., 2008; Mamah et al., 2008; Egan et al., 2001; Gottesman & Gould, 2003; Kremen et al., 1994; Moran et al., 2013). Some work has demonstrated reduced emotional regulation and memory processing in the neural circuits of non-affected siblings, indicating that they may be essential pieces of vulnerability for psychosis (van der Meer et al., 2014; Steel et al., 2002). However, only a few studies (Staal et al., 2000; Moran et al., 2013) comparing individuals with schizophrenia to their unaffected siblings have focused on multiple cortical and subcortical limbic

structures—this is what makes our study unique. Collectively, these elements of analysis strongly exhibit that structural deficits in psychosis are genetically influenced, and signify a dimensional theory of the disorder (de Zwarte et al., 2019). Genetic and environmental factors have also been recorded to examine the general heritability of limbic tracts. As a result, the greatest heritability was found for pathways that are thought to subserve socialization, emotion, memory, and linguistic cognition. This suggests that, in regards to the limbic network, inheritance might be based upon the structure of discrete systems, and is greater for fronto-temporal systems for multifaceted social reactions and emotion (Budisavljevic et al., 2016). Therefore, examining neurobiological features of non-affected siblings, who are potential carriers of psychosis genes, is a meaningful approach for investigating putative endophenotypes of schizophrenia.

As stated above, the limbic system is hypothesized to play a critical role in schizophrenia, with siblings of patients with schizophrenia offering a vital resource for distinguishing between trait—signifies the properties of the biological/behavioral processes that act as a precursor and/or cause in the pathophysiology—and state markers—exhibits the status of clinical displays—(Chen et al., 2009). The overarching aim of this project is to investigate the integrity of limbic circuitry in non-affected siblings of patients with schizophrenia in a regional U.S. sample, and examine its potential relationship with various clinical features of the illness. The goal is to quantify specific limbic regions in magnetic resonance space involved in the suspected pathophysiology of clinical symptoms and compare these regions between patients with schizophrenia, their non-affected siblings, healthy control participants and their healthy siblings. Finally, we aim to investigate whether the integrity of these regions relates to clinical symptom burden. Findings from this study would further our understanding of the role of the limbic system in psychosis in general, as well as whether specific clinical symptoms are tied to a particular

interaction among these limbic regions. As a result—especially due to the lack of utilization of limbic surface mapping in non-affected siblings in the psychosis-imaging literature—findings from the study could also allude to neurological differences between siblings, and the potential role of genetics in brain structure/functioning in psychosis populations.

In summary, this project proposes to investigate neuroimaging markers of limbic morphology in schizophrenia relative to non-affected siblings of patients, and describe its role in the expression of clinical symptomatology. The **first aim** is to quantify imaging features of the entorhinal cortex, parahippocampal gyrus, cingulate cortex, orbitofrontal cortex, hippocampus, and amygdala in a sample of schizophrenia subjects and compare them against a group of non-affected siblings and control subjects. It is *hypothesized* that cortical thickness of the entorhinal cortex, parahippocampal gyrus, cingulate cortex, orbitofrontal cortex, and shape deformation of the hippocampus and amygdala of the siblings will be more similar in form to the patients when compared to the control participants. So, if these structures are similar between patients and their siblings, despite the difference in their symptom presentations, then it will confirm the strength of utilizing genetic underpinnings as a clinical tool. The **second aim** of the study is to determine whether abnormalities in limbic circuitry involved in psychosis relate to positive, negative and disorganized symptoms exclusively in individuals with schizophrenia and their siblings. It is *hypothesized* that positive symptoms will relate with medial temporal regions (hippocampus, amygdala, parahippocampal and entorhinal cortex), negative symptoms with cortical thickness of the cingulate and orbitofrontal cortex, and disorganization with cortical thickness of the parahippocampal gyrus, orbitofrontal, and cingulate cortex.

Methods

Participants

From an initial pool of 216 subjects, this study included 130 anonymized participants (those who had completed patient and sibling MRI scans of satisfactory quality) recruited as part of an NIH-funded longitudinal study of schizophrenia at the Conte Center for the Neuroscience of Mental Disorders at Washington University in St. Louis, as was approved by the University IRB. These individuals were separated into four distinct groups: individuals with schizophrenia (SCZ; n=25), their unaffected full-siblings (SCZ-SIB; n=25), healthy comparison participants (CON; n=40), and their respective full-siblings (CON-SIB; n=40). SCZ were required to meet DSM-IV criteria for schizophrenia, and to be determined as clinically stable, measured by requiring symptom global severity scores to not change for a minimum of two weeks before participation. Inclusion criteria for CON and CON-SIB included no family history of schizophrenia spectrum disorders, while SCZ-SIBs' inclusion criteria was defined as having a sibling with a schizophrenia spectrum disorder and not having experienced psychosis themselves. Several SCZ-SIB, CON, or CON-SIB subjects had psychiatric diagnoses other than schizophrenia spectrum disorders—CON subjects were not allowed to currently have a diagnosis of an Axis I major mood disorder, whereas SCZ-SIB and CON-SIB did not have this restriction. CON-SIB were recruited as an additional comparison group, particularly for the SCZ-SIB group, thus allowing a history of an Axis I major mood disorder as well. Participants were excluded if they had a history of head trauma with loss of consciousness, neurological or unstable medical disorders, or recent (i.e., within the previous month) substance abuse or dependence (according to the DSM-IV). Informed consent was obtained from all participants. This cohort of subjects was also used in a number of previous studies relating to the brain morphology of schizophrenia

(Harms et al., 2007; Mamah et al., 2008; Delawalla et al., 2006). Each group's demographic profile is listed below in Table 1.

Measures

Psychiatric diagnoses were assessed with the Structured Clinical Interview for DSM- IV, Axis I Disorders (SCID-I). In order to assess clinical symptomology, measures of particular areas of psychosis were obtained via two different methods. First, the summations of specific SCID-I item scores were utilized to assess a lifetime history of psychopathology traits for negative symptoms, hallucinations, disorganized thoughts, and delusions. For measuring current state clinical symptoms, the Scales for Assessment of Positive and Negative Symptoms (SAPS and SANS), the Structured Interview for Prodromal Syndromes (SIPS) and the Chapman Psychosis Proneness Scales (CPPS) were utilized. The positive symptom score was comprised of SAPS hallucinations and delusions item scores*;* SIPS, suspiciousness, grandiosity, unusual thought content, and perceptual abnormalities scores; and the CPPS Magical Ideation and Perceptual Aberration scores (Mamah et al., 2008). The negative symptom score was comprised of SANS anhedonia, alogia, affective flattening, and avolition item scores*;* SIPS decreased ideational richness, avolition, decreased experience of self, social isolation, deterioration in role functioning, and decreased expression of emotion scores; and the CPPS Social Anhedonia and Physical Anhedonia scores (Mamah et al., 2008). The disorganization score was comprised of SAPS bizarre behavior and thought disorder item scores; SANS attention scores; and SIPS disorganized communication, personal hygiene, odd behavior, trouble with attention, and bizarre thinking scores (Mamah et al., 2008). The measures used included the same items as in Mamah et al., 2008, and Harms et al., 2007.

Image Acquisition and Processing

In terms of the magnetic resonance data, structural T1-weighted images of the whole brain were acquired on a Siemens Magnetom Vision 1.5T imaging system using 3D MPRAGE sequence (TR = 10 ms, TE = 4 ms, Flip angle = 30°, ACQ = 1, Matrix = 256 × 256, Scanning time = 5.6 min). Cortical thickness was estimated using the FreeSurfer (FS) toolkit v6.0 (Fischl et al., 1999), which is a software package for brain segmentation and cortical surface reconstruction. The pipeline involves generation of a surface across segmented tissue types to approximate the cortical mantle and allows calculation of average cortical thickness (mm) for various regions of interest (ROIs). No manual edits to the surfaces were required. Bilateral measurements for the parahippocampal, entorhinal, cingulate (caudal anterior, isthmus, posterior, and rostral anterior) and orbitofrontal (lateral and medial) ROIs were derived from the default FS parcellation atlas (Desikan et al., 2006).

Subcortical limbic (amygdala and hippocampus) shape features were estimated using the high-dimensional computational approach known as FreeSurfer-initiated Large Deformation Diffeomorphic Metric Mapping (FS+LDDMM). This pipeline involves an assortment of coordinate systems created to embody the morphology of selected brain structures. The process has demonstrated increased accuracy relative to other subcortical procedures (e.g., FreeSurfer; Khan et al., 2008; Grenander & Miller, 1994, 1998). The FS+LDDMM approach strives to reconstruct the anatomical boundaries of the various subcortical brain regions by generating an extension or reduction of a surface shape (Qui et al., 2010; Khan et al., 2008). It incorporates, volume-centered brain tissue division, template shape instillation, typical surface momentum maps, and random field representation (Qui et al., 2010). Utilizing the subcortical segmentation volume from FreeSurfer, it first initiates a linear registration/alignment of a subject's

image to a template image of a selected structure, in this case either the amygdala or hippocampus, via anatomical landmarks and warped using diffeomorphic mapping of voxel intensities. A tessellated graph is then overlaid on the subject image with vertices that directly align with vertices on the template surface. Diffeomorphic transformations permit individual surface points to freely correspond with one another, which assist in conserving the distinctive aspects of the shape of the structure (Khan et al., 2008).

Lastly, vertex-wise t-distribution maps were created to assess shape deformation patterns in the different group comparisons for the amygdala and hippocampus. Warmer colors signify outward deformation while cooler colors signify inward deformation for perpendicular change. These deformations are interpreted as depictions of localized volume reduction or amplification within the brain (Hanko et al., 2019). Multiple comparison correction was accomplished through the application of random field theory (Adler, 1981).

Design and Statistical Analysis

Demographics and Clinical Symptoms

Group differences in demographic variables were assessed by calculating F values using one-way analysis of variance (ANOVA) models for continuous data (participant age, parental socioeconomic status, and total alcohol consumption in last two years) and the χ^2 value from a chi-square comparison for categorical data (gender, race, handedness, lifetime substance dependence, and clinical diagnoses). In terms of clinical measures, for lifetime symptomology, total summation scores of the different items for each domain were utilized. For current symptomology, from the raw scores of the questionnaires, z-scores were calculated utilizing the standard deviation and mean from the original population, and then averaged to generate the categories of positive

symptoms, negative symptoms, and disorganized symptoms. Then Cronbach's alpha was computed to determine the reliability of the set of current symptomology test items for positive symptoms (a=0.88), negative symptoms (0.91), and thought disorganization (0.73) (Mamah et al., 2008).

Cortical Structures

For analysis of group differences in cortical thickness of limbic cortical ROIs (entorhinal, parahippocampal, lateral and medial orbitofrontal, and cingulate cortices), a repeated measures multivariate analyses of variance (RM-MANOVA) model was performed with hemisphere as the within-subjects effect, and group status as a between-subjects effect; group-by-hemispheres interaction effects were also assessed. For the significant group effects, follow-up two-group RM-ANOVA models were used to assess specific group contrasts.

Subcortical Structures

For the analyses on shape deformation, separate principal components analyses (PCA) were conducted for both the hippocampus and amygdala to reduce the dimensionality of surface points resulting in an orthonormal set of principal components (PC) that represent variation in shape of the structures (Beg et al., 2005). The first 10 eigenvectors of the PCA accounted for ~87% of the overall shape variance for each hemisphere of each structure, and were used for subsequent statistical modeling. Despite other studies from the same dataset choosing to utilize the first 15 eigenvectors (Harms et al., 2007; Mamah et al., 2008), the risk of potentially increasing the noise within the statistical analyses was not worth the small additional amount of coverage the next five eigenvectors offered (< 3% in total). To assess for group differences in both the hippocampus and amygdala, separate MANOVA models were conducted on the principal components (PC) scores

of each subcortical structure for each hemisphere, with the PC scores as dependent variables, group status as a fixed factor, and gender as a covariate. Significant group effects were further evaluated with follow-up MANOVA models to identify the specific between group differences in shape variances.

Clinical Relationships

Finally, Pearson bivariate correlation coefficients were calculated to examine the relationship between clinical symptoms scores (for both lifetime and current symptomology) and brain imaging measures. This included average cortical thickness for each hemisphere of entorhinal, parahippocampal, lateral orbitofrontal, medial orbitofrontal, and cingulate regions in addition to the first PC scores of the amygdala and the hippocampus. These were conducted separately for SCZ and SCZ-SIB groups.

Results

Demographics and Clinical Symptoms

For detailed demographic information, refer to Table 1. In regards to gender, the groups displayed a variance in distribution ($\chi 2=18.40$, $p< 0.01$) but not for race ($\chi 2=3.4$, $p = 0.75$), handedness ($\chi 2=1.5$, $p = 0.69$), and parental socioeconomic status ($F_{(3,126)}= 0.40$, 126, $p = 0.80$). There was also a statistically significant variation amongst age distribution ($F_{(3,126)}= 3.68$, $p = 0.01$), however, across groups, the differences in mean age were less than 3 years—SCZ (22.6 years old), SCZ-SIB (22.3 years old), CON (21.2 years old), and CON-SIB (20.0 years old) (Harms et al., 2007). The average illness duration for the SCZ was 4.60 years, with a standard deviation (SD) of 4.40 years. Lastly, the groups also differed in their history of mood disorder ($\chi 2= 25.50$, $p =0.00$) (with 52% of SCZ, 52% of SCZ-SIB, 35% of CON-SIB, and 5% of CON having a mood disorder), but not anxiety disorder ($\chi 2= 5.30$, $p = 0.15$) (Harms et al., 2007; Mamah et al., 2008)

Regarding clinical data in SCZ, for lifetime symptoms, the average positive symptoms score was 29.08, SD=4.81 (delusions: 16.80, SD=3.28; hallucinations: 12.28, SD=2.48). For negative symptoms, the mean was 5.88, SD=1.94; and for disorganized symptoms, the mean was 8.76, SD=1.48. In regards to current symptoms for SCZ, the mean for positive symptoms was a z-score of 1.32, SD=1.04; negative symptoms was a z-score of 1.20, SD=0.73; and disorganization was a z-score of 0.92, SD=1.11. Finally, for SCZ-SIB, the mean for positive symptoms was a z-score of -0.14, SD=0.44; negative symptoms was a z-score of -0.08, SD=0.59; and disorganization was a z-score of -0.07, SD=0.54. Lastly, a chi-squared test was used to assess the relationship between gender and group (Table 6.), only the ration between SCZ-SIB and CON was non-significant ($\chi 2=1.39$, $p=0.24$). Consequently, gender was included as a covariate in specific SCZ-SIB vs. CON contrasts.

Cortical Structures

Descriptive statistics (Table 2.) and cortical thickness analyses (Table 3.) are summarized in the tables below. Results of the RM-MANOVA models revealed a significant main effect for hemisphere in the parahippocampal ($F_{(1,126)}$=33.52, p=0.00), lateral orbitofrontal ($F_{(1,126)}$=147.97, p=0.00) and medial orbitofrontal cortices ($F_{(1,126)}$=4.13, p=0.04). Significant main effects for group were observed in the cingulate ($F_{(3,126)}$=3.74, p=0.01). Furthermore, significant group-by-hemisphere interactions were observed in the parahippocampal gyrus ($F_{(3,126)}$=3.14, p=0.03). Follow-up two-group RM-ANOVA models were used to assess specific effects of each group contrast. In the cingulate cortex, significant differences were noted between SCZ-SIB and CON (right hemisphere: $F_{(2,62)}$=4.14, p=0.05), where CON was thicker in comparison. For the parahippocampal gyrus, no group contrasts were significant.

Surface Analyses of the Hippocampus and Amygdala

The MANOVA analyses exhibited both a significant group-by-eigenvector interaction as well as a significant gender-by-eigenvector interaction throughout the structures. In addition, follow-up comparison models revealed significant group-by-eigenvector interactions between only SCZ vs. CON contrasts—left hippocampus ($F_{(10,53)}$=9.64, p= 0.00); right hippocampus ($F_{(10,53)}$=7.91, p= 0.01); left amygdala ($F_{(10,53)}$=6.37, p= 0.01); right amygdala ($F_{(10,53)}$=7.34, p= 0.01). Throughout the analyses of the subcortical PCA data, gender was included as a covariate given it significantly contributed to the models.

Visual inspection of amygdala shape maps (Figure 1) revealed a number of prominent group differences. For all SCZ contrasts, there were observable inward deformations in the left

posterior, ventral, and dorsal regions. Also, specifically within the SCZ vs CON contrast, there were some inward deformations on both the dorsal and ventral regions of the right hemisphere; and lateral outward deformations on the left hemisphere. For SCZ-SIB vs. CON, there was some slight outward deformation in lateral regions of both hemispheres, as well as the ventral region for the left hemisphere. In regards to CON vs. CON-SIB contrast, there were ventral, dorsal, and posterior outward deformations for each hemisphere when compared to CON-SIB. Only the SCZ vs. CON comparison survived RFT correction for multiple comparisons, which showed pronounced inward deformation in ventral and posterior regions of the left amygdala, suggesting localized volume reduction (Figure 2).

Inspection of hippocampal surface maps (Figure 3) also revealed a number of between group differences. For all SCZ contrasts, there were observable inward deformations in the dorsal and ventral regions of both hemispheres (with the more extensive deformation occurring in the CON contrast). Furthermore, bilateral posterior and anterior outward deformations were noted in the CON contrast, as well as some lateral, and ventral outward deformations in both the left hemisphere with SCZ-SIB, and bilaterally with CON). For SCZ-SIB vs. CON, there was a slight detection of outward deformation for lateral, ventral, and dorsal regions of the right hemisphere, as well as the anterior region of the left hemisphere. For CON vs. CON-SIB, inward deformations ventral regions of the right hemisphere and bilateral dorsal regions and were observed. No group contrasts survived correction for multiple comparisons.

Correlation with Clinical Symptoms

In reference to lifetime clinical symptomatology, significant positive correlations were observed in SCZ between the severity of hallucinations and left hippocampal (Pearson's r=0.59,

p<0.00), right hippocampal (r=0.51,p=0.01), and the left amygdalar shape (r=0.47, p=0.02). Lastly, for lifetime symptoms there was a significant positive relationship amongst thought disorganization and cortical thickness of the right parahippocampal gyrus (r=0.42, p=0.04) in the SCZ group. No significant correlations were noted between clinical symptoms and brain measures in the SCZ-SIB group.

For current clinical symptomatology, in SCZ a significant positive correlation was observed between the severity of positive symptoms and left hippocampal (r=0.54, p=0.01) and the right hippocampal shape (r=0.50, p=0.01). For negative symptoms, a positive correlation with the right lateral orbitofrontal cortex was noted (Pearson's r=0.42,p=0.04) in the SCZ group. In SCZ-SIB, a significant positive correlation with current positive symptoms and left entorhinal was noted (Pearson's r=0.47,p=0.02). Furthermore, a significant negative correlation between disorganization and cortical thickness of the lateral orbitofrontal cortex of the left (Pearson's r=-0.41,p=0.04) and the right hemisphere (Pearson's r=-0.42,p=0.04) was observed for SCZ-SIB.

Discussion

This project sought to investigate neuroimaging markers of limbic morphology in schizophrenia relative to non-affected SCZ-SIB, as well as describe its role in the expression of clinical symptomatology. Our first hypothesis predicted that cortical thickness of the entorhinal, parahippocampal, cingulate cortex, orbitofrontal cortex, as well as the surfaces of the hippocampus and amygdala of the SCZ-SIB will be similar in form to the SCZ when compared to the CON. In terms of examination of cortical limbic regions there was no consistent or clear pattern within the mean sizes of the cortical group surface analyses—not amongst the hemispheres, or regions (e.g., medial temporal region). Results of the RM-MANOVA models revealed significant main effects for hemisphere in just the parahippocampal, lateral orbitofrontal, and medial orbitofrontal cortices. For group main effects, we only observed significant scores within the cingulate. While the right hemisphere was the only significant hemisphere of the cingulate, due to the conflicting evidence found in the literature (Fornito et al., 2008; Mitelman et al., 2005; Choi et al., 2005), we are hesitant to make any hemispheric assumptions until the limitations of the study have been addressed. Lastly, significant group-by-hemisphere interactions were observed in just the parahippocampal gyrus. Interestingly, despite these findings for the parahippocampal gyrus, no group contrasts were significant in its follow-up ANOVAs. This indicates that group does not explain how the dependent variables differ from the independent variables for this structure. Out of all of the cortical structures, the cingulate cortex was the only cortical region to have a significant group contrast—It also was the only cortical region to consistently display SCZ and SCZ-SIBs as being the most morphologically similar out of the contrasts; and for both hemispheres. Therefore, these findings surrounding the cingulate cortex align with previous limbic system findings of psychosis (Raznahan et al., 2011)—they both support the cingulate cortex being similar in structure between

SCZ and SCZ-SIB. The rest of the cortical findings contrasted with the previous findings of current literature because they did not display similarities in form between SCZ and SCZ-SIB groups (Staal et al., 2000; Moran et al., 2013). Interestingly, the data displayed a number of contrasts where, in comparison to the SCZ group, the SCZ-SIBs were more similar to the CON or CON-SIB groups. However, there is a potential element of the study that may explain this finding. Siblings of both CON and SCZ were allowed to be included in the study even with a history of a major mood disorder according to the DSM-IV—which may be a potential explanation for those similarities. Many major mood disorders commonly occur as comorbidities with psychosis, and they are also observed to have a number of similar symptoms and genetic underpinnings (American Psychiatric Association, 2013). As a result, these variables make for a more complicated data set. These group comparisons may propose that the existence of a major mood disorder does not have a significant connection with cortical schizophrenia-like limbic structural malformations. However, this requires further study. When reviewing the subcortical data, the results did demonstrate a lack of differences between SCZ and SCZ-SIB groups; therefore, our findings were aligned with the current literature (Staal et al., 2000; Moran et al., 2013). However, SCZ-SIB did not significantly differ from CON either, suggesting potential abnormalities may be intermediary between the groups. Also, while none of their contrasts across structures and hemispheres were significant, their similarities were not as high as expected. This could be due to a number of the limitations that are later discussed. Lastly, when assessing surface shape maps of the amygdala, only the SCZ vs. CON comparison survived RFT correction for multiple comparisons; displaying localized volume reduction of the left amygdala. Past literature heavily supports this observation in individuals with schizophrenia (Breier et al., 1992; Rajarethinam et al., 2001; Keshavan et al., 2002; Barta et al., 1990). Therefore, we are

inclined to believe that not only is it a general indicator of volume loss, but it is also a distinct feature of schizophrenia. This is especially true considering the large role that emotions play in the different aspects of psychosis (e.g., delusions, hallucinations, negative symptoms).

Regarding relationships with clinical symptoms, we hypothesized various correlations amongst limbic structures. First, we hypothesized that positive symptoms would relate with medial temporal regions (amygdala, hippocampus, parahippocampal, and entorhinal cortices). Next, we hypothesized that negative symptoms would correlate with the cingulate and orbitofrontal cortices. Lastly, we hypothesized that disorganized symptoms would relate with the parahippocampal gyrus, the orbitofrontal cortex, and the cingulate cortex. So, in accordance with our hypothesis, we observed a positive relationship between SCZ severity of positive symptoms with the medial temporal region for both lifetime and current (mainly with the hippocampus); which, except in terms of direction for the hippocampus, supports previous literature (Velakoulis et al., 2006). This suggests that an increase in size of structures like the hippocampus results in an increase in the addition of abnormal behavior and thoughts. Our findings aligned with other previous studies (Sanfilipo et al., 2000; Gur et al., 2007; Kanahara et al., 2013; Walton et al., 2018) by illustrating a positive correlation between the severity of current negative symptoms for SCZ and the orbitofrontal cortex. This is not surprising, considering that, “The cortical volume of the orbitofrontal cortex…inversely predicted interdependent self-construal,” (Kitayama et al., 2017). Also, for the severity of lifetime disorganized symptoms within the SCZ group, there was an observed positive relationship with the parahippocampal gyrus—which, except in terms of direction, aligns with the current literature (Ohnuma, 1997; Prasad, Rohm, & Keshavan, 2004). This suggests that an increase in size of the parahippocampal gyrus results in an increase in the amount of disorganized behavior and speech. However, some of our findings were not in

agreement with previous literature—for the SCZ group, we did not observe any significant correlations between negative symptoms and the cingulate (Bersani et al., 2014); and we did not detect any significant associations between disorganized symptoms and the cingulate (Ohnuma, 1997; Lahti et al., 2006), as well as the orbitofrontal cortices (Nakamura et al., 2008). Regarding hypotheses in the SCZ-SIB group, we observed relationships between current positive symptoms (only hallucinations) and aspects of medial temporal regions, as well as current disorganization and the orbitofrontal cortex. By finding significant clinical measures for SCZ-SIB, our data exhibits a detectible clinical comparison between SCZ and SCZ-SIBs. However, SCZ-SIB did differ from the SCZ group by displaying a negative correlation between disorganization and the lateral orbitofrontal cortex. Also, while both SCZ and SCZ-SIB had significant positive correlations within the medial temporal regions for current positive symptoms, SCZ was significant in the hippocampus while SCZ-SIB was significant in the entorhinal cortex. Therefore, the genetic aspect of psychosis produced similar, but not identical, structural anatomies amongst siblings. Also, since there was a significant relationship between the structures and their current psychopathology severity, this implies a concept of "state-independence". Nevertheless, this topic necessitates further study.

One limitation that may be contributing to these findings is the study's small sample size. Small sample size affects the reliability of the findings because it has a greater risk of being significant just by chance. Another potential limitation involves the regional geographic aspect of our subject pool. Due to this, these findings have a lower generalizability, especially in regards to race. Our inclusion of individuals who are regularly taking atypical antipsychotics may also limit these findings, as a result of their cited negative effect on limbic structure (Lieberman et al., 2005; Chakos et al., 2005). This could have emphasized a potentially greater limbic structural deficit

than there actually was. Likewise, as previously stated, another limitation could be the inclusion of siblings of both SCZ and CON that have a history of major mood disorder. Also, this study is limited due to not taking into account substance use in our analyses. This could be potentially valuable data to consider given the long-term effects of substances on cognition (D'Souza et al, 2009; Thoma & Daum, 2013). Another limitation is the lack of a canonical correlation analysis as well as a multiple comparisons correction for the clinical correlations to account for the complex nature of the data. By not including these methods, the study is not able to maximize the differences between groups when there are multiple intercorrelated outcome variables. Lastly, the study could have benefited from a volume analysis to compare to the subcortical surface analyses. While surface analyses may provide significant advantages over volume-based analyses (Anticevic et al., 2008), a volume analysis is a widely used method utilized within neuroimaging literature, making it ideal for replication and comparison. It also reduces inter-subject variability (Woods et al., 1998), and it is geometrically applicable to most subcortical regions (Anticevic et al., 2008). Therefore, the study could only benefit from including them. Future studies should examine each of these limitations.

In conclusion, our findings exhibited a similarity between individuals with schizophrenia and their non-affected siblings in regards to their cortical structures (the cingulate cortex's thickness), subcortical structures (amygdala and hippocampus shape), and clinical symptomatology. Aspects of the neurobiological underpinnings of psychosis, particularly limbic regions, appear genetically influenced regardless of symptom expression and are latent features in non-affected family members. Relationships between positive symptomatology and shape abnormalities suggest a potential substrate for clinical characteristics in psychosis not evident in non-ill siblings. So, overall these findings can help provide us with a better understanding of the

illness so that we can potentially help influence strategies aimed at the diagnosis, management, and treatment of schizophrenia.

Table 1. Demographics and Clinical Characteristics

Characteristics/Profiles	SCZ n=25	SCZ-SIB n=25	CON-SIB n=40	CON n=40	F or χ^2	p
Age (yrs)	22.6 (3.2)	22.3 (3.5)	20.0 (3.5)	21.2 (3.6)	3.68	0.01
Gender *n* (%)					χ^2=18.4	0.0004
Female	5 (20)	15 (60)	29 (72.5)	18 (45)		
Male	20 (80)	10 (40)	11 (27.5)	22 (55)		
Race *n* (%)					χ^2=3.4	0.75
African American	8 (32)	8 (32)	8 (20)	8 (20)		
Caucasian	17 (68)	17 (68)	31 (77.5)	31 (77.5)		
Other	0 (0)	0 (0)	1 (2.5)	1 (2.5)		
Handedness *n* (%)					χ^2=1.5	0.69
Right	21 (84)	23 (92)	37 (92.5)	35 (87.5)		
Left	4 (16)	2 (8)	3 (7.5)	5 (12.5)		
Parental Socioeconomic Status[a]	2.9 (1.2)	2.9 (1.0)	3.1 (0.9)	2.9 (1.0)	0.4	0.8
Illness duration (yrs)	4.6(4.4)	n/a	n/a	n/a	-	-
Current Antipsychotic (AP) (%)[b]						
Atypical	87	0	0	0	-	-
Typical	4.3	0	0	0	-	-
Atypical & Typical	8.7	0	0	0	-	-
Lifetime Typical AP use (%)[c]	30.4	0	0	0	-	-
Duration of Typical AP use (months)[d]	11.0 (7.0)	n/a	n/a	n/a	-	-
Lifetime Substance Dependence *n* (%)						
Alcohol	7 (28)	3 (12)	0 (0)	2 (5)	χ^2=15.7	0.001
Sedative/Hypnotic	0 (0)	0 (0)	0 (0)	0 (0)	-	-
Cannabis	9 (36)	3 (12)	4 (10)	1 (2.5)	χ^2=15.9	0.001
Stimulant	0 (0)	1 (4)	1 (2.5)	0 (0)	χ^2=2.3	0.52
Opioid	0 (0)	0 (0)	0 (0)	0 (0)	-	-
Cocaine	0 (0)	1 (4)	1 (2.5)	0 (0)	χ^2=2.3	0.52
Hallucinogen	0 (0)	1 (4)	1 (2.5)	0 (0)	χ^2=2.3	0.52
Total Alcohol Consumption in Last 2 yrs (kg)	9.1 (14.8)	7.6 (13.0)	3.5 (6.7)	5.2 (8.9)	1.72	0.17
Current Axis I Diagnosis *n* (%)						
Psychotic Disorder	25 (100)	0 (0)	0 (0)	0 (0)	χ^2>100	<0.0001
Mood Disorder	2 (8)	0 (0)	3 (7.5)	0 (0)	χ^2=5.2	0.16
Anxiety Disorder	4 (16)	4 (16)	4 (10)	1 (2.5)	χ^2=4.5	0.21
Lifetime Axis I Diagnosis *n* (%)						
Psychotic Disorder	25 (100)	0 (0)	0 (0)	0 (0)	χ^2>100	<0.0001
Mood Disorder	13 (52)	13 (52)	14 (35)	1 (2.5)	χ^2=25.5	<0.0001
Anxiety Disorder	5 (20)	6 (24)	6 (15)	2 (5)	χ^2=5.3	0.15

[a]Range is 1–5, with higher values indicating lower socioeconomic status. Minor differences in how the siblings reported their parental information account for the small differences in parental socioeconomic status (Mamah et al., 2008).

[b]Atypical antipsychotics used (number of subjects) were risperidone, aripiprazole, olanzapine, clozapine, ziprasidone, and quetiapine. Typical antipsychotics used were haloperidol, thiothixene and loxapine. Two SCZ were receiving an unknown study medication and were not used in calculating percentages (Mamah et al., 2008).

[c]Refers to lifetime use of scheduled typical antipsychotic for >1 week. Typical antipsychotics used on an "as needed" basis were not included (Mamah et al., 2008).

[d] Only SCZ with lifetime use of typical antipsychotics were used in calculating the mean. n/a=not applicable (Mamah et al., 2008).

Table 2. Descriptive Statistics for Cortical Data (mm3)

Structure	Entorhinal	Parahippocampal	Medial Orbitofrontal Cortex	Lateral Orbitofrontal Cortex	Average Cingulate Cortex	
	Mean (SD)	**Mean (SD)**	**Mean (SD)**	**Mean (SD)**	**Mean (SD)**	**n**
Left Hemisphere						
SCZ	3.30 (0.27)	2.70 (0.22)	2.50 (0.19)	2.64 (0.14)	2.54 (0.10)	25
SCZ-SIB	3.19 (0.26)	2.78 (0.35)	2.51 (0.18)	2.64 (0.13)	2.54 (0.12)	25
CON	3.35 (0.28)	2.77 (0.27)	2.54 (0.16)	2.68 (0.16)	2.58 (0.14)	40
CON-SIB	3.29 (0.28)	2.80 (0.31)	2.54 (0.15)	2.66 (0.15)	2.62 (0.14)	40
Total	3.29 (0.28)	2.77 (0.29)	2.53 (0.16)	2.66 (0.15)	2.58 (0.13)	130
Right Hemisphere						
SCZ	3.28 (0.34)	2.67 (0.27)	2.50 (0.14)	2.75 (0.10)	2.54 (0.11)	25
SCZ-SIB	3.2 (0.38)	2.59 (0.26)	2.56 (0.16)	2.78 (0.15)	2.52 (0.11)	25
CON	3.27 (0.28)	2.68 (0.28)	2.56 (0.14)	2.82 (0.12)	2.59 (0.13)	40
CON-SIB	3.31 (0.32)	2.68 (0.26)	2.57 (0.15)	2.83 (0.17)	2.62 (0.13)	40
Total	3.27 (0.32)	2.66 (0.27)	2.55 (0.15)	2.80 (0.14)	2.57 (0.13)	130

Table 3. Cortical RM-MANOVA model and follow-up ANOVA results

	Hemisphere		Group		Hemisphere x Group	
Structure	*F*	*p*	*F*	*p*	*F*	*p*
Entorhinal	Λ = 1.00	0.49	1.20	0.31	Λ = 0.98	0.54
Left Parahippocampal	Λ = 0.79	**<0.001**	0.36	0.78	Λ = 0.93	**0.03**
SCZ vs SCZ-SIB	1.01	0.32				
SCZ vs CON	1.28	0.26				
SCZ-SIB vs CON	0.02	0.90				
CON vs CON-SIB	0.21	0.65				
Right Parahippocampal	Λ = 0.79	**<0.001**	0.36	0.78	Λ = 0.93	**0.03**
SCZ vs SCZ-SIB	1.26	0.27				
SCZ vs CON	0.02	0.89				
SCZ-SIB vs CON	1.87	0.18				
CON vs CON-SIB	0.01	0.94				
Medial Orbitofrontal Cortex	Λ = 0.97	**0.04**	0.98	0.40	Λ = 0.99	0.60
Lateral Orbitofrontal Cortex	Λ = 0.46	**<0.001**	1.19	0.32	Λ = 0.98	0.48
Left Average Cingulate Cortex	Λ = 1.00	0.64	3.74	**0.01**	Λ = 0.99	0.71
SCZ vs SCZ-SIB	0.00	0.97				
SCZ vs CON	1.30	0.26				
SCZ-SIB vs CON	1.10	0.30				
CON vs CON-SIB	1.73	0.19				
Right Average Cingulate Cortex	Λ = 1.00	0.64	3.74	**0.01**	Λ = 0.99	0.71
SCZ vs SCZ-SIB	0.17	0.69				
SCZ vs CON	2.66	0.11				
SCZ-SIB vs CON	4.14	**0.05**				
CON vs CON-SIB	0.86	0.36				

Table 4. Subcortical MANOVA models

	Group x Eigenvector		Gender x Eigenvector	
Structure	*F*	*p*	*F*	*p*
Left Hippocampus	Λ = 0.72	**<0.001**	Λ = 0.61	**<0.001**
SCZ vs SCZ-SIB	2.95	0.09	14.46	**<0.001**
SCZ vs CON	9.64	**<0.001**	19.58	**<0.001**
SCZ-SIB vs CON	1.61	0.21	21.88	**<0.001**
SCZ vs CON-SIB	0.96	0.33	25.42	**<0.001**
Right Hippocampus	Λ = 0.71	**<0.001**	Λ = 0.59	**<0.001**
SCZ vs SCZ-SIB	3.80	0.06	11.48	**<0.001**
SCZ vs CON	7.91	**0.01**	8.79	**<0.001**
SCZ-SIB vs CON	1.38	0.25	7.76	**0.01**
CON vs CON-SIB	1.54	0.22	9.96	**<0.001**
Left Amygdala	Λ = 0.73	**<0.001**	Λ = 0.56	**<0.001**
SCZ vs SCZ-SIB	1.41	0.24	7.27	**0.01**
SCZ vs CON	6.37	**0.01**	8.50	**0.01**
SCZ-SIB vs CON	1.12	0.29	9.76	**<0.001**
SCZ vs CON-SIB	2.61	0.11	8.93	**<0.001**
Right Amygdala	Λ = 0.77	**0.02**	Λ = 0.53	**<0.001**
SCZ vs SCZ-SIB	2.33	0.13	4.98	**0.03**
SCZ vs CON	7.34	**0.01**	3.21	0.08
SCZ-SIB vs CON	1.75	0.19	1.31	0.26
CON vs CON-SIB	0.01	0.94	1.15	0.29

Table 5. Chi-square Test Between Gender and Group

Group Comparison	**χ2**	***p***
Everyone	18.39	0.00
SCZ vs. SCZ-SIB	8.33	0.00
SCZ vs. CON	4.21	0.04
SCZ-SIB vs. CON	1.39	0.24
CON vs. CON-SIB	6.24	0.01

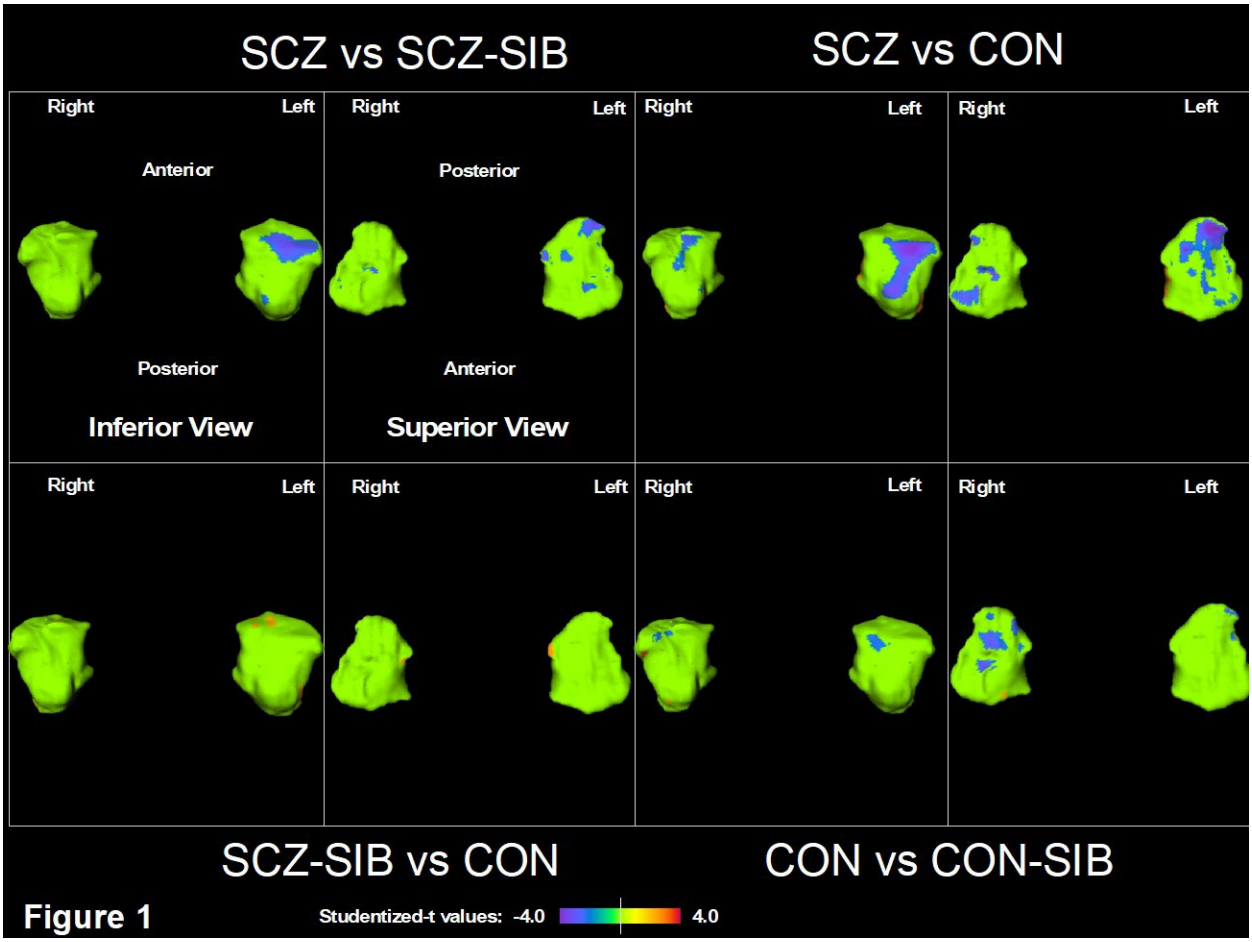

Figure 1. Amygdala surface shape contrast t-maps between: schizophrenia (SCZ) patients and siblings of patients (SCZ-SIB); SCZ and control (CON) participants; SCZ-SIB and CON; CON and siblings of control (CON-SIB) participants. Studentized-t values with cooler colors ($t<0$) indicate inward shape differences and warmer colors ($t>0$) indicate outward shape differences.

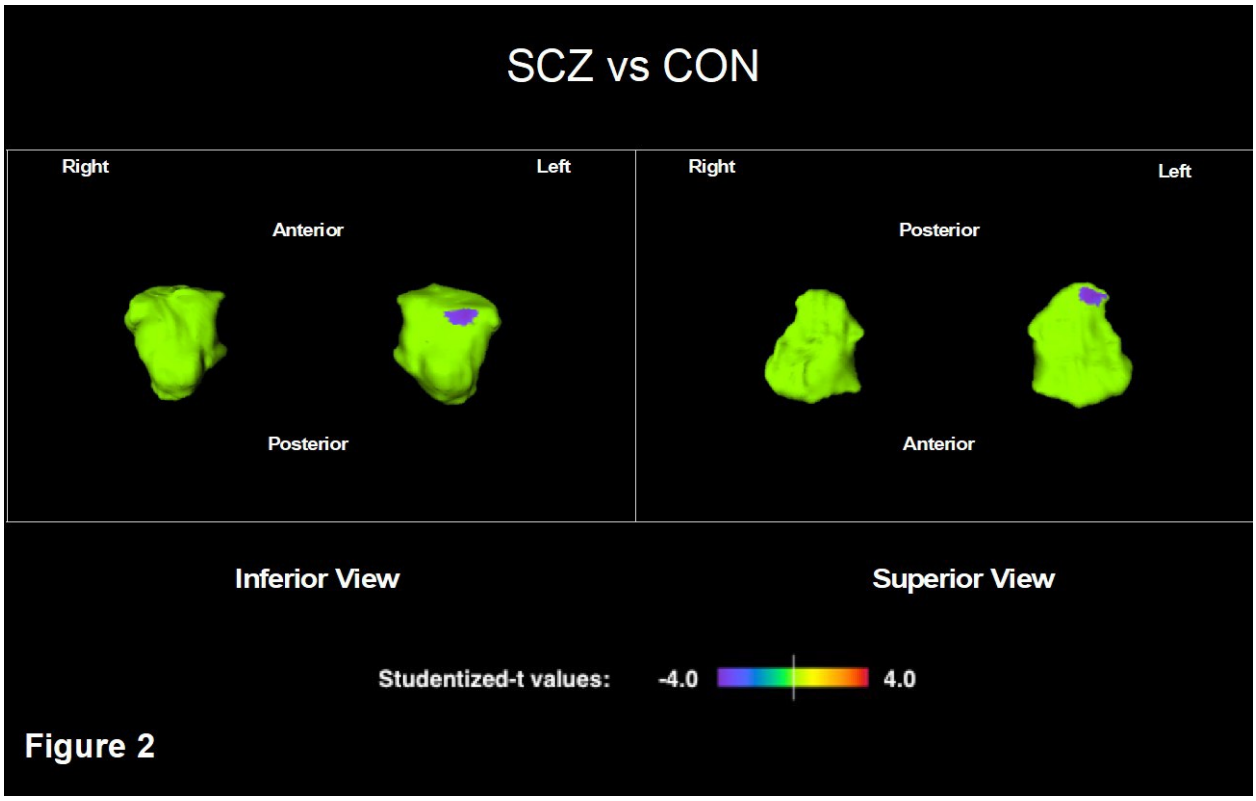

Figure 2. Amygdala surface shape contrast t-maps after multiple comparison correction via random field theory (Adler, 1981). Contrast maps are between: schizophrenia (SCZ) patients and control (CON) participants. Studentized-t values with cooler colors ($t<0$) indicate inward shape differences and warmer colors ($t>0$) indicate outward shape differences.

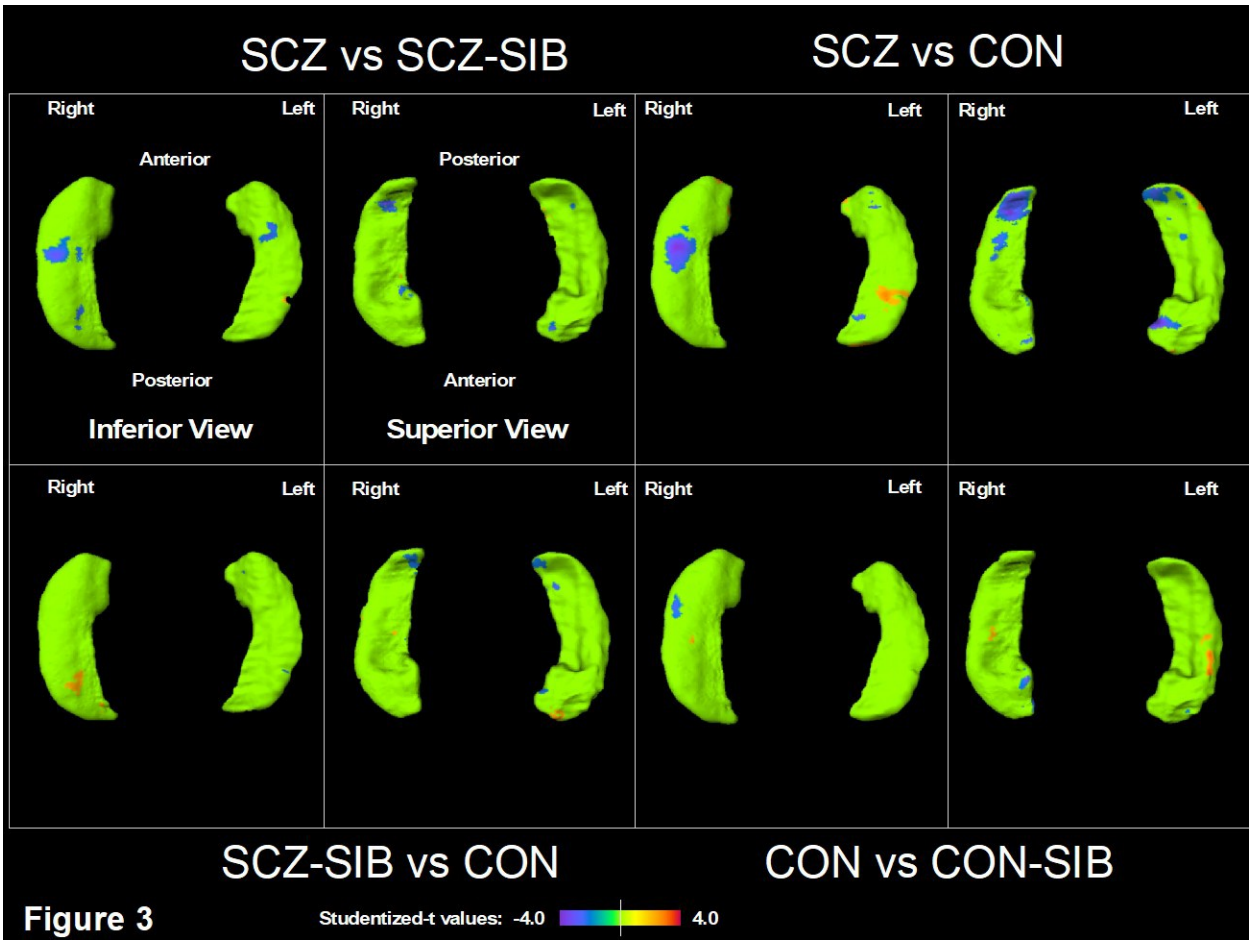

Figure 3. Hippocampus surface shape contrast t-maps between: schizophrenia (SCZ) patients and siblings of patients (SCZ-SIB); SCZ and control (CON) participants; SCZ-SIB and CON; CON and siblings of control (CON-SIB) participants. Studentized-t values with cooler colors ($t<0$) indicate inward shape differences and warmer colors ($t>0$) indicate outward shape differences.

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