Title: Brain Structural Changes in Schizophrenia Patients Compared to the Control: A MRI-Based Cavalieri's Method

Running title: Stereological analysis of brain MRIs in schizophrenia patients

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Abstract

Introduction: Schizophrenia is a severe psychotic brain disorder. One of the probable mechanisms can be volumetric changes in some brain regions. Therefore, the aim of the present study was to estimate quantitative analysis of the brain by magnetic resonance imaging (MRI) in patients with schizophrenia compared to the controls.

Methods: This case-control study was conducted on MRI scans of 20 patients with schizophrenia and 20 healthy controls in Zahedan, Southeastern Iran. MRIs with 4 mm slice thickness and 5 mm intervals in coronal and sagittal planes were captured. Then, quantitative parameters including volume and volume density of various brain regions were estimated in both groups using Cavalieri's point counting method. Data analyses performed using Mann-Whitney U test.

Results: The findings of this investigation revealed that volumes of gray matter, hippocampus, and gray/white matter in patients with schizophrenia were significantly lower than the controls ($p < 0.05$). The volumes of lateral ventricles in patients with schizophrenia (36.60 ± 4.32 mm³) were significantly higher than the healthy individuals (30.10 ± 7.98 mm³). However, there were no statistically significant changes in the total volume of brain, cerebral hemispheres, white matter, brain stem, cerebellum, and corpus callosum between the two groups ($p > 0.05$).

Conclusion: Volumetric estimations on brain MRI-based stereological technique can be helpful for elucidation of structural changes, follow-up the treatment trends, and evaluating the therapeutic situation in schizophrenia patients. Volumetric alternations in specific brain areas might be linked to cognitive impairments and severity of symptoms in patients with schizophrenia. Further researches are needed in this regards.

Keywords: Schizophrenia, Stereology, Magnetic Resonance imaging, Quantitative changes, Cavalieri's method

1. Introduction

Schizophrenia is a progressive, debilitating and severe neuropsychiatric disorder that affects approximately 0.5-1% of the population worldwide (Kim, Yang, & Jeong, 2015; Van Os & Kapur). Schizophrenia typically emerges in the adolescent years or early adulthood, between 18 and 25 years old, and it is frequently recognized as a chronic and lifelong disease (Insel, 2010). It is characterized by positive symptoms (hallucinations, delusions, paranoia); negative symptoms (anhedonia, social withdrawal, behavioral disorders); and cognitive dysfunction including memory impairment, impotence to maintain attention and disruption in executive functions (Meyer, 2013; Tandon, Nasrallah, & Keshavan, 2009). Thus far, several factors have been suggested to be involved in the development and progression of the disease, such as alternations and disconnection in myelin; genetic factors; the number of dopaminergic neurons and oligodendrocytes; volumetric changes in different areas of the brain; and neurodegenerative, neuroinflammation, and neurodevelopmental deficiencies. All these factors can lead to structural and functional changes in the brain of patients with schizophrenia (Jaaro-Peled, Ayhan, Pletnikov, & Sawa, 2010; Roussos & Haroutunian, 2014). Despite extensive studies and substantial advances in genetic, neurochemical and neurobiological theories presented on schizophrenia (Insel, 2010; Jaaro-Peled et al., 2009), the exact development, progression and its underlying pathogeneses of this complex psychological disorder are unknown and challenging for basic researchers and clinicians (Murray & Lewis, 1987). As mentioned above, one of the contributing factors in the pathology of schizophrenia is volumetric changes, which can lead to structural disconnection and neurophysiological alternations in the brain of patients (Tepest et al., 2013).

In numerous investigations, stereological technique is the recommended approach for the estimation of quantitative parameters of the brain in normal aging, neurodegenerative diseases and schizophrenia (Heidari, Moghtaderi, Mahmoudzadeh-Sagheb, & Gorgich, 2017; Pakkenberg, Scheel-Krüger, & Kristiansen, 2009). These exact and unbiased findings help us to obtain a better perception of underlying mechanisms and alternations in development and progression in different phases (acute, chronic) of the disease (Kipp, Kiessling, Hochstrasser, Roggenkamp, & Schmitz, 2017).

Quantitative volumetric brain measurements on MRI scan in patients with neurodegenerative disease owing to selective regional atrophy are beneficial for clinicians to ascertain disease progression and to evaluate volume alternations and response to treatment (Ciumas,

Montavont, & Ryvlin, 2008; Heidari, Moghtaderi, et al., 2017). A previous study conducted by our team on brain MRI scans of methamphetamine abusers showed that volume loss was significant in some areas of the brain in drug abusers compared to that in controls (Heidari, Mahmoudzadeh-Sagheb, Shakiba, & Gorgich, 2017). Another volumetric study based on Cavalieri's point counting method on brain MRI scans of patients with Parkinson's disease revealed that volume reduction in some regions of the brain in these patients was significant compared to that in the controls. These studies suggested that quantitative evaluation of MRI scans might be beneficial for clinical applications and to analyze clinical manifestations in patients (Heidari, Mahmoudzadeh-Sagheb, Moghtaderi, Ramazanpour, & Gorgich, 2020; Heidari, Moghtaderi, et al., 2017). Therefore, the main goal of the current study is estimate volumetric analysis of brain MRI scans in patients with schizophrenia and compare the results with the controls.

2. Methods

2.1. Study Design and Subjects

In the current case-control study, we evaluated volumetric alternations on brain MRI scans of 40 subjects in two groups: patients with schizophrenia (n=20) and gender and age-matched healthy controls (n=20). Schizophrenia was diagnosed based on Diagnostic and Statistical Manual of Mental Disorders IV, Text Revision (DSM-IV TR), criteria by an expert psychiatrist (M. Shakiba). The schizophrenia group included patients (14 males, 6 females) who had a history of the disease for at least 12 months without a history of other neurological diseases such as epilepsy, Parkinson's and Alzheimer's disease, mental retardation, and head trauma and of taking psychoactive substances. The healthy control group included those who had no background history of psychiatric and neurological disorders, underlying disease, and drug abuse. In addition, alcoholics and smokers were excluded from the study. All the subjects were enrolled by the convenience sampling method from individuals who were referred to the psychiatric clinic of Baharan Psychiatric Hospital, Zahedan, Southeastern Iran.

2.2. MRI Protocol and Volumetric Estimations

Stereological estimations and quantitative measurements of brain regions in both groups were done based on Cavalieri's principle. According to this principle for unbiased estimation of volume of an object it must be sectioned into a series of parallel planes with a fixed distance. To avoid bias the first section must be placed at a random situation in a constant interval of

length and the serial sections which are acquired from the object must be transit via the entire area (Alper et al., 2006). In the present study the method of stereological estimation was conducted by the following manner:

At first, for estimation of volumetric parameters of various areas in the participants, FLAIR (Fluid Attenuated Inversion Recovery) successions of structural brain MRI scans taken in two diverse anatomical axis (sagittal, coronal) with 4 mm slice thickness and 5 mm intervals were prepared. The brain structural MRIs from patients with schizophrenia and controls were captured using three-dimensional (3D) high-resolution T1-weighted MR 1.5 T scanner system (GE systems, Paris). Next, point-counting grids that contained organized points superimposed on MRI scans and points hit to the desired regions of the brain were computed by Cavalieri's point-counting method as described in our previous studies (Heidari et al., 2020; Heidari, Mahmoudzadeh-Sagheb, et al., 2017; Heidari, Moghtaderi, et al., 2017). (Figure 1).

Subsequently, the results obtained from quantitative estimations (volumes and volume densities) of various brain regions were compared between schizophrenia and control groups. All volumetric calculations were performed using Cavalieri's point-counting formula, and the results were reported as cm³.

$$v = \frac{\sum_{i=1}^m p \times a/p \times t}{M^2}$$

Where, v is the estimated volume of any desired object, $\sum P$ is the sum of the number of points hitting that object in all slices, a/p is the area associated with each point in the stereological grid, t is the mean distance between the captured slices, and M is the linear magnification of the image (Heidari et al., 2020; Heidari, Mahmoudzadeh-Sagheb, et al., 2017; Heidari, Moghtaderi, et al., 2017).

In the next step, an estimate of the volume density (V_v) of the brain components in the reference space (total brain) was obtained using the formula $V_v = P(\text{part})/P(\text{ref})$, where, $P(\text{part})$ is the number of test points that fall in each component profile and $P(\text{ref})$ is the number of points that hit the total brain (Heidari et al., 2020).

2.3. Statistical Analysis

The collected data were reported as mean $\pm$ standard error (SE), and nonparametric Mann-Whitney U-test was applied to characterize volumetric differences between the two groups. SPSS software for Windows (version 21, Chicago, IL, USA) was used for all statistical analyses. The significance level was set at $p < 0.05$.

3. Results

The mean age of patients with schizophrenia and healthy controls was 60.4 ± 7.09 and 61.3 ± 6.91 years, respectively. There was no significant difference in gender between patients and healthy participants. The ratio of males to females in patients with schizophrenia and healthy participants was 14 to 6.

Comparison of the results of volumetric analysis in patients with schizophrenia and controls revealed that there was a statistically significant increase in cerebral ventricles volume and volume density, right ventricle volume and volume density, and left ventricle volume density ($p < 0.05$). The total volume of lateral ventricles in schizophrenia patients and healthy subjects was $36.60 \pm 4.32 \text{ mm}^3$ and $30.10 \pm 7.98 \text{ mm}^3$, respectively.

On the other hand, gray matter volume, white matter/gray matter volume, and total volume and volume density of each hippocampus were significantly lower in patients with schizophrenia than in healthy participants ($p < 0.05$).

However, there were no statistical significant changes in the total volume of the brain and volume of cerebral hemispheres, white matter, brain stem, cerebellum, and corpus callosum between the two groups ($p > 0.05$). Additional details of volumetric changes in different brain areas of each group are presented in Table 1.

4. Discussion

In the current study, a significant reduction was found in volumes of gray matter and hippocampus in patients with schizophrenia compared to that in controls. On the other hand, despite a decrease in total volume of brain and volume of cerebral hemispheres and white matter in patients with schizophrenia compared to those in healthy subjects, this volume reduction was not statistically significant. In addition, the volumes of lateral ventricles were significantly increased in patients with schizophrenia compared to those in healthy participants.

The findings of Chung et al. showed a substantial increase in lateral ventricle volume of patients with schizophrenia as compared to that in controls. They also found a significant inverse association between the expansion of ventricular volume and gray matter thickness in individuals with clinical high risk for psychosis. They stated that lateral ventricular system enlargement is associated with significantly steep rates of cortical reduction (Chung et al., 2017). Another study conducted by Meduri et al. using morphometrical and morphological analyses of the lateral ventricles, delineated that lateral ventricle total volume, right and left ventricle total volume and volume density, and left ventricle total volume in patients with schizophrenia were significantly higher than those in the control group (Meduri et al., 2010). Morphologically, the enlargement of the ventricular system is the most significant deficit in patients with schizophrenia compared to that in the controls (Shenton, Dickey, Frumin, & McCarley, 2001). Therefore, the results of our research team confirmed the previous findings about lateral ventricular enlargement in patients with schizophrenia. We speculate that increased ventricular volume in patients suffering from schizophrenia probably is one of the fundamental findings in brain MRI of these patients, which occurs tandem with cortical and subcortical reduction of gray matter (basal ganglia) volume (Hashimoto et al., 2018; Meduri et al., 2010). Nevertheless, reduction in white matter volume of adjacent of lateral ventricles (Price et al., 2006) and hyperdopaminergic situations with its significance neurotoxic effects (Abi-Dargham, 2014) can affect expansion of these spaces.

The human hippocampus is one of the important brain structures with nearly 10 million glutamatergic and γ -amino butyric acid (GABA)-ergic neurons. It plays a substantial role in the regulation of emotion, affect, and cognitive functions. On the basis of neuroimaging and postmortem studies, the hippocampus is considered a key region in the early pathophysiology of schizophrenia (Konradi et al., 2011). Evidence suggests an abnormality in GABA-ergic inhibition of hippocampal pyramidal cells, impaired hippocampal interneurons, and a region-specific upregulation of GABA (A) receptor binding in patients with schizophrenia (Konradi et al., 2011). Falkai et al., in their stereological postmortem study, showed a significant reduction in glial cells and neuron numbers in subregions of the left side hippocampus in CA4 and DG, respectively, in patients with schizophrenia compared to those in healthy controls. In addition, they found that this cellular decline in substructure of the hippocampus (CA4/DG) occurs along with decreased volume of the total hippocampus (Falkai et al., 2016). Another study by Calvo et al. demonstrated that total volume of right and left hippocampi in patients with schizophrenia were significantly lesser than that in the controls. They concluded

that the hippocampus volume loss in early stages of disease could increase vulnerability of patients to severe mental illness (Calvo et al., 2018). Reduced volume of the hippocampus subregions (CA1 and CA4/DG) occurs even in the first episode of schizophrenia and is widespread more along with disease progression. Interestingly, volume loss in different subregions of the hippocampus due to the involvement of its anterior or posterior part is associated with severity of symptom (Nakahara, Matsumoto, & van Erp, 2018). Our results regarding quantitative changes and reduction in hippocampus volume in patients with schizophrenia were consistent with those reported in the above-mentioned studies. Therefore, it seems that the decline in hippocampus volume occurs in the first episode of schizophrenia, and a series of metabolic and structural factors including neuronal hyperactivation in particular GABA-ergic ones, levels of different neurotransmitters, decreased numbers of neurons and oligodendrocytes plays fundamental roles in this event (Falkai et al., 2016; Lieberman et al., 2018; Nakahara et al., 2018).

Despite our findings regarding the total volume of brain and the cerebral hemispheres that did not show any statistically significant reductions in patients with schizophrenia compared to that in the controls, many previous studies on patients with schizophrenia conducted using neuroimaging indicated that these patients had a decreased cortical brain volume compared to that in healthy individuals (Haijma et al., 2012; Pantelis et al., 2003). This issue that the reduction in cortical brain volume in patients with schizophrenia occurs due to antipsychotic drug effects or neuropathological processes is still under debate (Ho, Andreasen, Ziebell, Pierson, & Magnotta, 2011). Vita et al. illustrated that antipsychotic drugs affected the cortical thickness and reduced volume of the cortical brain (Vita, De Peri, Deste, Barlati, & Sacchetti, 2015). Another study conducted by Zhang et al. showed that cortical gray matter volume in patients with schizophrenia was significantly lesser than that in normal subjects. In addition, they claimed and supported that reduction in cortical volumes of the brain in the patients is associated with taking more antipsychotic medicines and related to the inflammatory basis (Zhang et al., 2016). Other experimental studies reported that antipsychotic drugs could lead to neurodegeneration and negative changes in the brain volume through induction of oxidative damage and reduction in the expression of growth factors (neurotrophins). These factors play critical roles in neuronal survival and differentiation in the brain (Pillai, Parikh, Terry Jr, & Mahadik, 2007). However, our results related to the total volume of the brain and cerebral hemispheres did not show any significant difference between the two groups, but a significant reduction was observed in the volume of

gray matter in patients with schizophrenia compared to that in the controls. This event is feasible due to volumetric changes in subcortical structures of the brain in patients with schizophrenia. With regard to the lack of reduced brain cortical volume in our study, we agree that various factors are involved in volumetric changes, including duration of disease, levels of inflammatory mediators, and exposure to antipsychotic drugs (Hashimoto et al., 2018; Zhang et al., 2016). Furthermore, reduction in the brain volume might be exaggerated in selected areas of the brain in some patients with schizophrenia due to disease heterogeneity (Kim, Kim, & Jeong, 2017; Zhang et al., 2016).

Kim et al. demonstrated significant reduction in volume of white matter in patients with schizophrenia compared to that in healthy subjects, especially in superior frontal gyrus (SFG), superior temporal gyrus (STG), and inferior temporal gyrus (ITG). In addition, they found that there was a negative correlation between volume of white matter in STG and disease duration. Lastly, their results suggested that any abnormality and loss of white matter volume in STG could be related to the psychopathology of schizophrenia (Kim et al., 2017). Our results did not show a statistically significant reduction in total volume and volume density of white matter in patients with schizophrenia compared to those in controls. In addition, we did not observe a significant difference in the volumes of the corpus callosum, but there was significant increase in the volume density of corpus callosum in patients with schizophrenia compared to that in controls. However, results of other studies are contrary to changes in white matter and corpus callosum volumes in the present study (Arnone, McIntosh, Tan, & Ebmeier, 2008; de Moura et al., 2018; Del Re et al., 2016). The findings of Moura et al., which are in line with our findings, showed no significant alternations in the corpus callosum volume in both patients and healthy subjects. Their results showed that long-term exposure to antipsychotic drugs led to greater increase in volume in some regions of the corpus callosum volume (posterior part) (de Moura et al., 2018). Amone et al., in their meta-analysis, explained that volume reduction in the corpus callosum region is more prominent in the first episode of schizophrenia, whereas in patients with chronic schizophrenia, showed relatively greater corpus callosum volume (Arnone et al., 2008). In addition to these results, Del Re et al. reported no substantial changes in follow-up of patients with first episode of schizophrenia as compared to those in the controls (Del Re et al., 2016). On the basis of the aforementioned results, we proposed that volumetric changes in white matter and corpus callosum probably occur in specific areas of the brain and are associated with clinical severity symptoms. Variables that can affect the volumes of white matter and corpus callosum in the

brain are duration of illness and chronic intake of antipsychotic medications. Another possible explanation for these that are contrasting with those in different studies could be the heterogeneity in subjects' enrolment with different severity of disease and the small sample size. Nevertheless, the role of compensatory processes for the structural and volumetric changes in different regions of the brain should not be forgotten (Heidari, Mahmoudzadeh-Sagheb, et al., 2017; Heidari, Moghtaderi, et al., 2017).

Some limitations that we faced in this study were the low sample size, evaluation of some variables such as duration of illness, number of episodes, and use of antipsychotic drugs. These are suggested to be considered in designing future studies.

5. Conclusion

In conclusion, according to our findings it seems that volumetric estimations on brain MRI-based stereological technique can be helpful for elucidation of structural changes, follow-up the treatment trends, and evaluating the therapeutic situation in schizophrenia patients. Other volumetric changes can vary in the different areas of the brain depending on duration of the disease, antipsychotic therapy, and inflammatory status of the patients. These changes might be linked to cognitive impairments and the severity of clinical symptoms in patients with schizophrenia. Finally, these findings can be beneficial in assessing antipsychotic treatments and dysfunctional connectivity in patients with schizophrenia. Furthermore, elucidation of the different pathways of various structural abnormalities related to schizophrenia is required for recognizing and determining the role of discrete pathophysiological phenomena in the development and progress of mental illness. In this regards further studies with a larger sample size and more variables are recommended.

Ethical Considerations

Compliance with ethical guidelines

The Institutional Ethics Committee of the Zahedan University of Medical Sciences (IR.ZAUMS.Rec.1390-2391) approved this study.

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Authors' Contributions

Z. Heidari and HR. Mahmoudzadeh-Sagheb co-designed the study, supervised all the experiments and analyzed the results. M. Shakiba participated in study design and collecting and selection of samples. EA.C. Gorgich participated in literature review, data analysis, and drafted the manuscript. All authors read, modified and approved the final version of the manuscript.

Conflict of Interest

The authors declared that there was no conflict of interest.

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References

- Abi-Dargham, Anissa. (2014). Schizophrenia: overview and dopamine dysfunction. *The Journal of clinical psychiatry*, 75(11), e31-e31.
- Alper, Fatih, Kantarci, M, Altunkaynak, E, Varoglu, AO, Karaman, Adem, Oral, E, & Okur, Adnan. (2006). Quantitative magnetic resonance imaging of brainstem volumes, plaques, and surface area in the occipital regions of patients with multiple sclerosis. *Acta Radiologica*, 47(4), 413-418.
- Arnone, D, McIntosh, AM, Tan, GMY, & Ebmeier, KP. (2008). Meta-analysis of magnetic resonance imaging studies of the corpus callosum in schizophrenia. *Schizophrenia research*, 101(1-3), 124-132.
- Calvo, Ana, O'Hanlon, Erik, Coughlan, Helen, Kelleher, Ian, Clarke, Mary, & Cannon, Mary. (2018). O6. 1. Hippocampal volume in adolescents with persistent psychotic experiences: a longitudinal population-based MRI study. *Schizophrenia Bulletin*, 44(Suppl 1), S89.
- Chung, Yoonho, Haut, Kristen M, He, George, van Erp, Theo GM, McEwen, Sarah, Addington, Jean, . . . Mathalon, Daniel H. (2017). Ventricular enlargement and progressive reduction of cortical gray matter are linked in prodromal youth who develop psychosis. *Schizophrenia research*, 189, 169-174.
- Ciomas, Carolina, Montavont, Alexandra, & Ryvlin, Philippe. (2008). Magnetic resonance imaging in clinical trials. *Current opinion in neurology*, 21(4), 431-436.
- de Moura, Mariana TM, Zanetti, Marcus V, Duran, Fabio LS, Schaufelberger, Maristela S, Menezes, Paulo R, Scazufca, Marcia, . . . Serpa, Mauricio H. (2018). Corpus callosum volumes in the 5 years following the first-episode of schizophrenia: Effects of antipsychotics, chronicity and maturation. *NeuroImage: Clinical*, 18, 932-942.
- Del Re, Elisabetta C, Konishi, Jun, Bouix, Sylvain, Blokland, Gabriëlla AM, Mesholam-Gately, Raquelle I, Goldstein, Jill, . . . Seidman, Larry J. (2016). Enlarged lateral ventricles inversely correlate with reduced corpus callosum central volume in first episode schizophrenia: association with functional measures. *Brain imaging and behavior*, 10(4), 1264-1273.
- Falkai, Peter, Malchow, Berend, Wetzstein, Katharina, Nowastowski, Verena, Bernstein, Hans-Gert, Steiner, Johann, . . . Bogerts, Bernhard. (2016). Decreased

- oligodendrocyte and neuron number in anterior hippocampal areas and the entire hippocampus in schizophrenia: a Stereological Postmortem Study. *Schizophrenia bulletin*, 42(suppl_1), S4-S12.
- Haijma, Sander V, Van Haren, Neeltje, Cahn, Wiepke, Koolschijn, P Cédric MP, Hulshoff Pol, Hilleke E, & Kahn, René S. (2012). Brain volumes in schizophrenia: a meta-analysis in over 18 000 subjects. *Schizophrenia bulletin*, 39(5), 1129-1138.
- Hashimoto, Naoki, Ito, Yoichi M, Okada, Naohiro, Yamamori, Hidenaga, Yasuda, Yuka, Fujimoto, Michiko, . . . Narita, Hisashi. (2018). The effect of duration of illness and antipsychotics on subcortical volumes in schizophrenia: Analysis of 778 subjects. *NeuroImage: Clinical*, 17, 563-569.
- Heidari, Zahra, Mahmoudzadeh-Sagheb, Hamidreza, Moghtaderi, Ali, Ramazanpour, Negar, & Gorgich, Enam Alhagh Charkhat. (2020). Structural changes in the brain of patients with relapsing-remitting multiple sclerosis compared to controls: a MRI-based stereological study. *Ir J Med Sci* 189, 1421–1427.
- Heidari, Zahra, Mahmoudzadeh-Sagheb, Hamidreza, Shakiba, Mansour, & Gorgich, Enam Alhagh Charkhat. (2017). Stereological Analysis of the Brain in Methamphetamine Abusers Compared to the Controls. *Int J High Risk Behav Addict*, 6(4).
- Heidari, Zahra, Moghtaderi, Ali, Mahmoudzadeh-Sagheb, Hamidreza, & Gorgich, Enam Alhagh Charkhat. (2017). Stereological Evaluation of the Brains in Patients with Parkinson's disease Compared to Controls. *Rev Romana Med Lab*, 25(3), 265-274.
- Ho, Beng-Choon, Andreasen, Nancy C, Ziebell, Steven, Pierson, Ronald, & Magnotta, Vincent. (2011). Long-term antipsychotic treatment and brain volumes: a longitudinal study of first-episode schizophrenia. *Archives of general psychiatry*, 68(2), 128-137.
- Insel, Thomas R. (2010). Rethinking schizophrenia. *Nature*, 468(7321), 187.
- Jaaro-Peled, Hanna, Ayhan, Yavuz, Pletnikov, Mikhail V, & Sawa, Akira. (2010). Review of pathological hallmarks of schizophrenia: comparison of genetic models with patients and nongenetic models. *Schizophrenia bulletin*, 36(2), 301-313.
- Jaaro-Peled, Hanna, Hayashi-Takagi, Akiko, Seshadri, Saurav, Kamiya, Atsushi, Brandon, Nicholas J, & Sawa, Akira. (2009). Neurodevelopmental mechanisms of schizophrenia: understanding disturbed postnatal brain maturation through neuregulin-1–ErbB4 and DISC1. *Trends in neurosciences*, 32(9), 485-495.

- Kim, Gwang-Won, Kim, Yun-Hyeon, & Jeong, Gwang-Woo. (2017). Whole brain volume changes and its correlation with clinical symptom severity in patients with schizophrenia: A DARTEL-based VBM study. *PloS one*, 12(5), e0177251.
- Kim, Gwang-Won, Yang, Jong-Chul, & Jeong, Gwang-Woo. (2015). Emotional effect on cognitive control in implicit memory tasks in patients with schizophrenia. *NeuroReport*, 26(11), 647-655.
- Kipp, Markus, Kiessling, Maren C, Hochstrasser, Tanja, Roggenkamp, Caroline, & Schmitz, Christoph. (2017). Design-based stereology for evaluation of histological parameters. *Journal of Molecular Neuroscience*, 61(3), 325-342.
- Konradi, Christine, Yang, C Kevin, Zimmerman, Eric I, Lohmann, Kathryn M, Gresch, Paul, Pantazopoulos, Harry, . . . Heckers, Stephan. (2011). Hippocampal interneurons are abnormal in schizophrenia. *Schizophrenia research*, 131(1-3), 165-173.
- Lieberman, JA, Girgis, RR, Brucato, G, Moore, H, Provenzano, F, Kegeles, L, . . . Corcoran, CM. (2018). Hippocampal dysfunction in the pathophysiology of schizophrenia: a selective review and hypothesis for early detection and intervention. *Molecular psychiatry*, 23(1), 1764–1772.
- Meduri, Mario, Bramanti, Placido, Ielitto, Giuseppe, Favaloro, Angelo, Milardi, Demetrio, Cutroneo, Giuseppina, . . . Pandolfo, Gianluca. (2010). Morphometrical and morphological analysis of lateral ventricles in schizophrenia patients versus healthy controls. *Psychiatry Research: Neuroimaging*, 183(1), 52-58.
- Meyer, Urs. (2013). Developmental neuroinflammation and schizophrenia. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, 42, 20-34.
- Murray, Robin M, & Lewis, Shôn W. (1987). Is schizophrenia a neurodevelopmental disorder? *British Medical Journal (Clinical Research Ed.)*, 295(6600), 681.
- Nakahara, Soichiro, Matsumoto, Mitsuyuki, & van Erp, Theo GM. (2018). Hippocampal subregion abnormalities in schizophrenia: A systematic review of structural and physiological imaging studies. *Neuropsychopharmacology reports*, 38(4), 156-166.
- Pakkenberg, B, Scheel-Krüger, J, & Kristiansen, LV. (2009). Schizophrenia; from structure to function with special focus on the mediodorsal thalamic prefrontal loop. *Acta Psychiatrica Scandinavica*, 120(5), 345-354.
- Pantelis, Christos, Velakoulis, Dennis, McGorry, Patrick D, Wood, Stephen J, Suckling, John, Phillips, Lisa J, . . . Soulsby, Bridget. (2003). Neuroanatomical abnormalities

- before and after onset of psychosis: a cross-sectional and longitudinal MRI comparison. *The Lancet*, 361(9354), 281-288.
- Pillai, Anilkumar, Parikh, Vinay, Terry Jr, Alvin V, & Mahadik, Sahebarao P. (2007). Long-term antipsychotic treatments and crossover studies in rats: differential effects of typical and atypical agents on the expression of antioxidant enzymes and membrane lipid peroxidation in rat brain. *Journal of Psychiatric Research*, 41(5), 372-386.
- Price, Gary, Cercignani, Mara, Bagary, Manjinder S, Barnes, Thomas RE, Barker, Gareth J, Joyce, Eileen M, & Ron, Maria A. (2006). A volumetric MRI and magnetization transfer imaging follow-up study of patients with first-episode schizophrenia. *Schizophrenia Research*, 87(1-3), 100-108.
- Roussos, Panos, & Haroutunian, Vahram. (2014). Schizophrenia: susceptibility genes and oligodendroglial and myelin related abnormalities. *Frontiers in cellular neuroscience*, 8, 5.
- Shenton, Martha E, Dickey, Chandlee C, Frumin, Melissa, & McCarley, Robert W. (2001). A review of MRI findings in schizophrenia. *Schizophrenia research*, 49(1-2), 1-52.
- Tandon, Rajiv, Nasrallah, Henry A, & Keshavan, Macheri S. (2009). Schizophrenia, "just the facts" 4. Clinical features and conceptualization. *Schizophrenia research*, 110(1), 1-23.
- Tepes, Ralf, Schwarzbach, Christopher J, Krug, Barbara, Klosterkötter, Joachim, Ruhrmann, Stephan, & Vogele, Kai. (2013). Morphometry of structural disconnectivity indicators in subjects at risk and in age-matched patients with schizophrenia. *European archives of psychiatry and clinical neuroscience*, 263(1), 15-24.
- Van Os, J, & Kapur, S. Schizophrenia *Lancet* 2009, 374, 635–645. 10, S0140-6736.
- Vita, Antonio, De Peri, Luca, Deste, Giacomo, Barlati, Stefano, & Sacchetti, Emilio. (2015). The effect of antipsychotic treatment on cortical gray matter changes in schizophrenia: does the class matter? A meta-analysis and meta-regression of longitudinal magnetic resonance imaging studies. *Biological Psychiatry*, 78(6), 403-412.
- Zhang, Y, Catts, VS, Sheedy, D, McCrossin, T, Kril, JJ, & Weickert, C Shannon. (2016). Cortical grey matter volume reduction in people with schizophrenia is associated with neuro-inflammation. *Translational psychiatry*, 6(12), e982.

Table 1: Stereological indices in various regions of the brain in patients with schizophrenia and the control group.

Stereological indices	Schizophrenia group (n=20)	Control group (n=20)	Difference percentage (%)	<i>p</i> -value
Total brain volume	1207.50±105.48	1316.10±119.57	-8.28	NS
Cerebral hemispheres				
Total volume (mm ³)	816.30±68.33	872.60±78.29	-6.40	NS
Volume density (%)	68.10±8.67	66.70±7.87	2.10	NS
Left Cerebral hemisphere				
Total volume (mm ³)	441.90±23.54	424.70±51.08	4.08	NS
Volume density (%)	37.00±5.30	34.60±2.15	6.94	NS
Right Cerebral hemisphere				
Total volume (mm ³)	480.40±41.22	460.30±55.15	4.34	NS
Volume density (%)	37.50±2.44	35.10±4.49	6/84	NS
Cerebellum				
Total volume (mm ³)	134/80±14/14	133/30±17/91	0.75	NS
Volume density (%)	11/30±1/53	10/20±1/32	10.78	NS
Cerebral ventricles				
Total volume (mm ³)	36.60±4/32	30.10±7.98	21.59	0.037*

Volume density (%)	3.00±0/41	2.30±0.57	30.43	0.003*
Left ventricle				
Total volume (mm ³)	18/70±2/41	15/20±5/12	23/03	NS
Volume density (%)	4/40±0/77	3/70±1/80	18/92	0.006*
Right ventricle				
Total volume (mm ³)	17/90±1/98	14/90±3/58	20/13	0/036*
Volume density (%)	3/80±0/50	3/30±0/84	15/15	0.005*
Brain stem				
Total volume (mm ³)	22/30±2/67	21/70±4/41	4/70	NS
Volume density (%)	1/90±0/21	1/60±0/25	18/75	NS
Corpus callosum				
Total volume (mm ³)	22.90±1.04	22.70±1.06	1.32	NS
Volume density (%)	1.90±0.17	1.70±0.14	11.70	0.022*
Gray matter volume				
Total volume (mm ³)	321.4±35.55	395.50±38.66	-18.73	0.0001**
Volume density (%)	26.80±4.07	30.40±4.67	-11.84	NS
White matter volume				
Total volume (mm ³)	630.9±64.65	635.60±33.67	-0.70	NS
Volume density (%)	52.60±6.90	48.60±4.41	-8.23	NS
White matter/Gray matter volume	0.52±0.05	0.63±0.08	-0.17	0.001*
Hippocampus				
Total volume (mm ³)	8.40±0.15	11.50±0.29	-26.95	0.01*

Volume density (%)	0.7±0.12	0.88±0.21	-20.45	0.034
Left hippocampus				
Total volume (mm ³)	4.60±0.69	5.70±0.93	-19.29	0.004*
Volume density (%)	1.00±0.17	1.24±0.22	-19.35	0.003*
Right hippocampus				
Total volume (mm ³)	4.50±0.59	5.90±1.40	-23.72	0.012*
Volume density (%)	1.02±0.15	1.39±0.56	-26.61	0.041*

NS: not significant, * P<0.05, **P<0.0001

Figure 1. The points counting technique has been demonstrated on a sagittal brain MRI using stereological method.