ALTERED CORTICAL CALBINDIN-IMMUNOREACTIVE INTERNEURON POPULATIONS ASSOCIATED WITH SCHIZOPHRENIA

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Amy Contole Dupper

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Thesis written by
Amy Contole Dupper
B.A., The College of Wooster, 2010
M.A., Kent State University, 2013

Approved by:

Dr. Mary Ann Raghanti	Advisor
Dr. Richard Meindl	Interim Chair, Department of Anthropology
Dr. Raymond A. Craig	Associate Dean, College of Arts and Sciences

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ABSTRACT

Schizophrenia reduces the fitness of an individual but continues to remain prevalent in 1% of the human population around the world and is thought to be a consequence of human brain evolution. Recent research has indicated that many symptoms of schizophrenia are associated with cortical disinhibition. Inhibitory processes are responsible for focusing on specific tasks and blocking out excess signals. This study focused on calbindin-immunoreactive (CB-ir) interneurons, a subset of the GABAergic neurons, in Brodmann's areas 9 and 46. These areas have been implicated in auditory hallucinations as revealed by fMRI. Interneurons that colocalize with calbindin are decreased in schizophrenia, and it is thought that this decrease contributes to deficits in language and auditory processing. The density of these interneurons was compared to those of the surrounding areas (Areas 44 and 41) to determine if there was a selective disinhibition within areas 9 and 46. Tissue samples were analyzed using immunohistochemistry in order to visualize CB-ir interneurons. Results showed a decrease in CB-ir interneurons in layers V-VI of areas 46 and 41 in schizophrenic subjects compared to normal controls. Alternately, there was an increase in schizophrenic subjects of CB-ir interneurons in layers II-III of area 41 compared to normal controls. These results indicate abnormal CB-ir interneuron densities in both the frontal and temporal lobes of schizophrenic patients that may result in deficits in audition, attention, and audition.

CHAPTER 1

INTRODUCTION

One defining characteristic of human evolution is the dramatic increase in brain size relative to body size (Dean 2009; Crow 1997; Crow 2000; Crow 1997). However, this rapid encephalization may not have been without consequences. Some researchers have proposed that schizophrenia is a 'side effect' of human brain evolution (Crow 1997; Crow 2000; Crow 1997). Although schizophrenia reduces the fitness of the affected individuals, it continues to occur in 1% of all human populations (Crespi *et al.* 2007).

The first historical documentation of schizophrenia dates to the second millennium B.C. in Egypt (Kyziridis 2005). Initially referred to as 'dementia praecox', this disorder was characterized by inappropriate affect (responding in the absence of a particular stimulus), anhedonia (loss of pleasure), feeling unhappy or depressed, sleep disorders, abnormal psychomotor activity, and disturbances in attention and memory (Costello 1993). In 1908, Eugen Bleuler coined the term "schizophrenia," which is Greek for split (schizo) mind (phrenic) (Bleuler 1908).

Schizophrenia is a mental illness characterized by both 'positive' and 'negative' symptoms with an onset that does not occur until late adolescence (Andreasen 1995). Positive symptoms are exaggerations of normal behavior and include hallucinations, delusions, and excited motor activity (Javitt 2010). Negative symptoms, in contrast, are lost functions and include withdrawal from social situations, loss of volition, reduced

emotional responsiveness, and decreased verbal capacity (Jablensky 2010). Positive symptoms are usually more responsive to drug treatments compared to negative symptoms (Andreasen 1995).

A combination of genes and environment has been shown to contribute to schizophrenia (Torrey *et al.* 1997). A number of studies have analyzed the heredity of schizophrenia by documenting its frequency in monozygotic and dizygotic twins in different geographic areas (Kallmann 1946; Shields *et al.* 1967; Slater 1953). Shields *et al.* (1967) found that the concordance rate for developing schizophrenia was 9.1% for dizygotic twins and 59.2% for monozygotic twins. An English study found a similar concordance rate of 11.3% for dizygotic twins and 58.3% for monozygotic twins (Slater 1953). While there is an obvious genetic component underlying schizophrenia, the concordance rate for monozygotic twins was not 100%, suggesting that environmental factors also play a role.

There is a correlation between the development of schizophrenia and seasonality of births, as analyzed in a study encompassing 29 countries in the Northern Hemisphere and 10 countries from the Southern Hemisphere (Torrey *et al.* 1997). Individuals born in the winter and spring months had a higher incidence of schizophrenia (Torrey *et al.* 1997). Turnquist (1993) and Thiery *et al.* (2002) examined this link between schizophrenia and seasonality of birth, and found that variations in light and changing maternal hormone levels during fetal development may be implicated in the development of schizophrenia. Specifically, light/dark cycles caused changes in the mammalian neuroendocrine system, which signal the pineal gland to begin secreting melatonin

(Thiery *et al.* 2002). Melatonin triggers the production of maternal hormones such as luteinizing hormone-releasing hormone (LHRH), luteinizing hormone (LH), and follicle stimulating hormone (FSH), which all regulate estrogen production (Thiery *et al.* 2002). Variations in the production of these hormones could cause the production of estrogen to be altered, affecting brain development (Thiery *et al.* 2002).

During brain development, there is an overproduction in the birth of synaptic connections known as synaptogenesis (McGlashan *et al.* 2000). After birth, there is a gradual reduction in synaptic connections that lasts until the second decade of life in order to fine-tune the connections in the brain (McGlashan *et al.* 2000). Fluctuations in maternal hormone levels could affect neuronal pruning, which for humans lasts until adolescence (Turnquist 1993). Excessive neuronal pruning may result in a limited amount of synaptic connections throughout the brain, and schizophrenia could develop (Turnquist 1993). McGlashan *et al.* (2000) made a model correlating the extent of synaptic pruning and the severity of auditory hallucinations. They found that the greater the loss of neuronal connections, the more severe the symptoms in schizophrenia (McGlashan *et al.* 2000). There is also a relationship between the termination of synaptic pruning during adolescence and the introduction of schizophrenia symptoms early in the second decade of life, indicating that dysfunctional synaptic pruning may be a factor in the development of schizophrenia (Turnquist 1993).

In another study examining environmental factors in the development of schizophrenia, Watson and his colleagues (1984) discovered a relationship between infectious diseases, seasonality of birth, and the occurrence of schizophrenia. Individuals

in England who were exposed to diphtheria, pneumonia, and influenza while in utero had a significantly higher susceptibility to schizophrenia (Watson *et al.* 1984). These diseases usually occurred during the winter months, which provided an explanation for why children born during the winter or spring months had a higher incidence of schizophrenia (Watson *et al.* 1984). Taken together, these findings imply a combination of both genetic and environmental factors in the development of schizophrenia.

1.1 Evolution and Schizophrenia

1% of the modern human population suffers from schizophrenia (Crespi *et al.* 2007), and it is thought to be a human-specific disease (Dean 2009). Some have suggested that its emergence occurred prior to the evolution of modern *Homo sapiens*, and may have occurred in hominid ancestors such as *Homo erectus* (Nichols 2009). The hominid lineage is unique in that humans display habitual bipedality, a greatly enlarged brain, and the capacity for language, the latter two being implicated in the development of schizophrenia (Dean 2009). As the brain enlarged, greater amounts of energy were required to maintain human cognitive abilities (Dean 2009). Schizophrenia could develop if an inadequate amount of energy was available to the central nervous system (CNS) during development (Dean 2009).

1.2 Language and Schizophrenia

Crow (1997; 2000; 1997; 1995) argued that schizophrenia emerged as a byproduct in the capacity for language in *Homo sapiens*. As the brain expanded, hemispheric lateralization, in which one hemisphere is dominant over the other for specific cognitive tasks, became more important (Crow 1997). However, individuals with schizophrenia usually lack this hemispheric dominance required during language and audition, and display the resulting deficits (Crow 2000). Chimpanzees, bonobos, gorillas, and humans were shown to have the same lateralization of the left hemisphere, suggesting that hand gestures, a form of communication, arose in response to lateralization of area 44 (Cantalupo & Hopkins 2001). However, perceptual lateralization does not necessarily coincide with structural lateralization (Sherwood *et al.* 2003). Although many non-human primates have the same brain asymmetries, it may be the connections in the left hemisphere that specialize in language processing that make this characteristic unique to humans (Sherwood *et al.* 2003).

If the lateralization hypothesis were true, the capacity for language must have arisen very rapidly along with schizophrenia (Crow 1997). Contradictory evidence, such as the use of symbols to communicate among primates, indicates that language arose gradually over time (Aiello *et al.* 1993). Additionally, individuals diagnosed with schizophrenia do not always experience language deficits (Nichols 2009). Horrobin expands on Crow's hypothesis by asserting that the biochemical characteristics of schizophrenia are also those that are essential to human uniqueness (Horrobin 1998; Horrobin 1999). These characteristics, including increased metabolic intake to support a

larger brain and increasingly complex connectivity in the brain may be implicated in the many negative manifestations of schizophrenia (Horrobin 1998; Horrobin 1999). However, relatives of schizophrenics have exhibited high levels of creativity and intelligence, conferring a heterozygote advantage, which is why schizophrenia is thought to be essential to making us human (Horrobin 1999).

1.3 The Social Brain

A more recent hypothesis for the evolution of schizophrenia examines the development of the social brain (Brothers 1990; Burns 2006; Burns 2004). Increasingly complex social pressures during our evolution led to the growth of brain regions involved in higher cognition, allowing humans to function as highly complex social beings. Positron emission tomography (PET) and functional magnetic resonance imaging (fMRI) studies have determined that the social brain exists as complex interconnections linking the prefrontal lobes to the parietal and temporal lobes (Burns 2006). Areas of the brain that are categorized as belonging to the social brain are the dorsolateral prefrontal cortex (areas 9 and 46), the orbitofrontal cortex (areas 10, 11, and 47), the anterior cingulate cortex (areas 24, 32, and 33), the amygdala, the superior temporal gyrus (areas 41 and 42), and the parietal association cortex because these areas are activated when performing social cognitive tasks (Adolphs 2001; Barbas 2000; Devinsky *et al.* 1995; Haxby *et al.* 2002; Saxe & Kanwisher 2003). All of these brain regions were shown to be abnormal in schizophrenic patients (Yucel *et al.* 2002; Tamminga *et al.* 1992; Russell *et al.* 2000;

Sigmundsson *et al.* 2001; Sanfilipo *et al.* 2000; Gur *et al.* 2002). Sigmundsson *et al*. (2001) found decreased gray matter volume in schizophrenic brains in the anterior cingulate cortex, the middle frontal cortex, the left medial temporal lobe, the left superior temporal gyrus, the inferior frontal gyrus, and the insular cortex, which is similar to the findings of Sanfilipo *et al.* (2000) that found decreased gray matter in the superior temporal gyrus. In addition, Sanfilipo *et al*. (2000) found that large reductions in white matter volumes were indicative of severe negative symptoms in schizophrenia. Schizophrenics also showed decreased levels of metabolic rates in the hippocampus (Tamminga *et al.* 1992), the anterior cingulate cortex (Yucel *et al.* 2002; Tamminga *et al.* 1992), and the left middle/inferior frontal gyrus and insula (Russell *et al.* 2000) while performing a task. Metabolic rates were also decreased in the left amygdala and bilateral hippocampus when asked to discern different emotional states, signifying that perception of other's emotional states is impaired in schizophrenia (Gur *et al.* 2002). As our brain expanded and connections between the frontal, parietal and temporal lobes increased, our nervous system was left vulnerable to environmental and genetic factors (Brüne 2001), suggesting that schizophrenia is a disorder of the social brain.

1.4 Dysregulation of Neurotransmitters

Other hypotheses of schizophrenia focus on the dysregulation of neurotransmitters in the brain. The major inhibitory neurotransmitter in the brain is γ-aminobutyric acid (GABA), and the major excitatory neurotransmitter is glutamate (Carlsson 2006). For the brain to process information accurately, there must be a careful

balance between glutamate and GABA (Carlsson 2006). The glutamate hypothesis of schizophrenia states that schizophrenia is the result of an excess of glutamate in the brain (Carlsson 2006). This hypothesis was first proposed after the discovery that administration of phencyclidine (PCP) and ketamine generated schizophrenic-like symptoms by blocking the glutamate receptor NMDA (Egan *et al.* 2000), which plays a role in transmitting excitatory signals (Gordon 2010). A blockade of the NMDA receptor prevents the influx of calcium into the postsynaptic cell, causing glutamate to remain in the synapse at high levels and results in excessive production of glutamate (Gordon 2010). Excessive glutamate contributes to symptoms of psychosis, working-memory deficits, and social withdrawal, all symptoms of schizophrenia (Gordon 2010).

Experiments that have tested the glutamate hypothesis of schizophrenia have revealed that children, following a low dose of ketamine, did not exhibit the schizophrenic-like symptoms seen in adults following the same dose (Krystal *et al.* 1994). Similarly, rats that were given PCP and ketamine became more susceptible to psychotic effects as they matured (Olney & Farber 1995). These studies mirror the nature of schizophrenia to emerge during adolescence in humans (Egan *et al.* 2000).

Symptoms of schizophrenia often include an inability to filter out excess information in the brain, symptoms that may implicate deficits in the GABAergic system (Benes & Berretta 2001). Inhibition is a critical aspect of learning, as it is inhibitory processes that are responsible for blocking excess signals to the brain (Benes *et al.* 1996). Recent research has indicated that many symptoms of schizophrenia, including auditory hallucinations, are associated with cortical disinhibition of GABA (Carlsson 2006).

GABA colocalizes with calcium-binding proteins in cortical interneuron subpopulations (Beasley *et al.* 2002) and is synthesized from the enzyme glutamic acid decarboxylase (GAD) (Kalkman & Loetscher 2003). Glutamic acid activates GABA via NMDA receptors (Olney *et al.* 1999). When an NMDA receptor antagonist blocks NMDA receptors, GABA is not activated, causing excitatory inputs to be uninhibited in the brain (Olney *et al.* 1999). Cortical interneurons receive signals from external and internal sources, which result in a release of GABA (Benes & Berretta 2001). Intrinsic signals originate from other GABA-immunoreactive (ir) neurons in the prefrontal cortex, while extrinsic signals originate from outside the prefrontal cortex (Benes & Berretta 2001).

90% of the GABAergic neurons in the prefrontal cortex co-localize with non-overlapping subpopulations (Raghanti *et al.* 2010) of calcium-binding proteins: calbindin (CB), calretinin (CR), and parvalbumin (PV), giving a good estimation of the total GABA interneuron density within the brain (Eyles *et al.* 2002). Each type of calcium-binding protein can be identified by its specific biochemical phenotype through the use of immunohistochemistry: parvalbumin interneurons generally have a chandelier or basket shape; calretinin is represented by bipolar or bitufted interneurons; and calbindin is most often found in a double-bouquet morphology but can also have a multipolar morphology (Figure 1) (Eyles *et al.* 2002).

The present study explored the GABAergic hypothesis by quantifying the density of CB-ir interneurons in the schizophrenic brain compared to normal controls and compared to CB-ir interneuron densities among different brain areas within the same subject. While many previous studies have looked at calcium-binding protein densities in

specific areas throughout the brain, this study is unique in that it examined multiple cortical areas within the same individuals to determine if there were area-specific decreases.

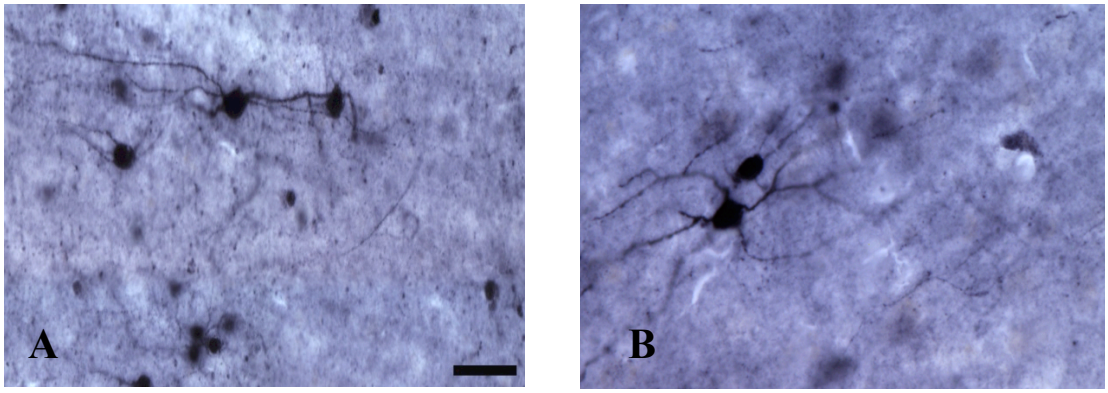

Figure 1. A typical double-bouquet CB-ir interneuron (A) and multipolar neuron (B) (scale bar = 25μm).

1.5 The role of Calbindin and GABA in Schizophrenia

Calcium-binding proteins belong to the EF-hand family of intracellular calcium acceptors (Hof *et al.* 1999). These EF-hand calcium-binding proteins either act as calcium 'triggers', which initiate a cascade of reactions or as calcium 'buffers', which act to decrease the free-floating calcium ions in the brain (Hof *et al.* 1999). Parvalbumin, calretinin, and calbindin are all classified as 'buffer' calcium-binding proteins (Hof *et al.* 1999). Calbindin was first discovered in the duodenum, kidney, and brain of birds and reptiles, and it has since been discovered in the kidney and brain of amphibians and mammals, and in the brains of fishes (Parmentier *et al.* 1987). It is believed that

calbindin functioned to translocate calcium to the kidneys and intestines when vertebrates migrated from the water onto the land (Parmentier *et al.* 1987). However, the brain of birds and mammals is not dependent on vitamin D, which means calbindin must fulfill an alternate role in the brain (Parmentier *et al.* 1987). The brain requires the uptake of calcium in order to send high electrical signals, which could be toxic to the cell, therefore, calbindin acts as a calcium buffer in order to protect the cell from the toxic effects of large amounts of calcium (Parmentier *et al.* 1987).

In the primate prefrontal cortex, CB-ir interneurons are involved in intercolumnar communication (Raghanti *et al.* 2010) and are characterized by varying neuron sizes and densities across all layers (Hof *et al.* 1999). In the monkey, great ape, and human prefrontal cortex, CB-ir interneuron density was greatest in the superficial layers II-III, with decreased density in deeper layers IV-VI, which give off ascending processes towards the upper layers of the prefrontal cortex (Condé *et al.* 1994; Chance *et al.* 2005; DeFelipe *et al.* 1989). The axons of CB-ir interneurons in the mammalian prefrontal cortex often synapse on apical dendrites of pyramidal neurons and provide inhibitory signals to these pyramidal neurons (Condé *et al.* 1994; Benes & Berretta 2001).

Studies examining the density of calcium-binding proteins have been done in order to gain a better understanding of the evolution of the mammalian brain. Extant members of the Xenarthra (giant anteater, lesser anteater, and two-toed sloth) and Afrotheria (rock hyrax and black and rufous giant elephant shrew) clades are crown species of evolutionary lineages that extend back before the K-T boundary at 65 mya (Sherwood *et al.* 2009). Immunohistochemistry was done to identify calcium-binding

proteins in the frontal cortex of species from these two clades (Sherwood *et al.* 2010). CB-, CR-, and PV-ir interneurons were all present in the brains of these crown taxa, even though CB-ir neurons tended to have a multipolar morphology rather than the usual double bouquet morphology that is observed in extant primates (Sherwood *et al.* 2010). These data suggest that calcium-binding proteins have been conserved over mammalian evolution, since the common ancestor of all mammals contained similar interneuron types that were retained in its mammalian descendants (Sherwood *et al.* 2010).

GABAergic deficits can be seen in the brain as a decrease in calcium-binding protein-expressing interneurons that colocalize with GABA (Ahn *et al.* 2011). Many studies have examined the density of calcium-binding proteins in the prefrontal cortex of schizophrenic subjects compared to control subjects, and there have been conflicting results. One study by Sakai *et al.* (2008) identified CB-, CR-, and PV-ir neurons in Brodmann's area 9 using immunohistochemistry and found that CB-ir interneuron density in layer II and PV-ir density in layer IV was reduced in schizophrenic patients compared to controls. A similar study found a reduction of CB-ir interneurons in layers II, III, and V, and a reduction of PV-ir interneurons in layer III in Brodmann's area 10, a region anterior to area 9, in schizophrenic subjects compared to normal controls (Beasley *et al.* 2002). Both studies did not observe a significant decrease in CR-ir neurons in schizophrenic relative to normal controls (Sakai *et al.* 2008; Beasley *et al.* 2002). CB- and PV-ir neurons were also decreased in areas 9 and 46 in all cortical layers for schizophrenic subjects compared to normal controls (Beasley *et al.* 2001). In contrast, Daviss & Lewis (1995) looked at CB- and CR-ir neurons in the prefrontal cortex of 5

matched pairs of schizophrenic and control subjects and found an increase of 50-70% in CB-ir neurons in Brodmann's areas 9 and 46 in both layers II-III of schizophrenic brains. Although these data differ somewhat in their findings, the main calcium-binding protein implicated in schizophrenic brains has been calbindin, which may contribute to deficits in language and auditory processing (Chance 2005).

1.6 Cortical Areas Implicated in Schizophrenia

The four cortical areas examined in this study were Brodmann's areas 9, 41, 44, and 46 from the right hemisphere (Figure 2). Areas 9 and 46 have been implicated in short-term working memory and auditory hallucinations common to many schizophrenic patients, while the right hemisphere area 44 is a homologue to Broca's area, and area 41 is the primary auditory cortex.

Areas 9 and 46

Area 9 lies within the regions of the superior and middle frontal gyri and contains a cell-rich layer VI, delineating this area from the anterior area 10, which is relatively cell-sparse (Casanova *et al.* 2008). Inferior to area 9 is area 46, which is located in the middle frontal gyrus (Zuffante *et al.* 2001). Together, areas 9 and 46 form part of the dorsolateral prefrontal cortex (DLPFC) and have been implicated in working memory in human and non-human primates (Zuffante *et al.* 2001). Information regarding the role these regions play in behavior has been obtained by lesioning these cortical regions in non-human primates (Levy & Goldman-Rakic 2000). When the dorsolateral cortex was

lesioned in adult rhesus monkeys, they exhibited a deficit in spatial working memory tasks but not non-spatial working memory tasks, revealing this area to be central to spatial working memory in primates (Goldman-Rakic 1987). Similarly, Diamond & Goldman-Rakic (1989) compared subjects with bilateral ablations to the prefrontal cortex to those with intact prefrontal cortices in their performance of Piaget's "A not B" task. This task is designed to measure the ability of a subject to remember where an object is hidden after a certain amount of time (Piaget 1937). Subjects (in this case, human infants and adult rhesus macaque monkeys) uncovered a hidden object from one of two locations after a delay of a few seconds (Diamond & Goldman-Rakic 1999). Human infants aged 7.5-9 months and rhesus monkeys with DLPFC lesions were able to retrieve the object after no delay in hiding the object, but these same subjects failed to recover the object after a short delay (Diamond-Goldman-Rakic 1989). Conversely, human infants older than 9 months were able to correctly find the object after a short delay of several seconds (Diamond & Goldman-Rakic 1989). This implicates the dorsolateral prefrontal cortex as playing a role in short-term memory (Diamond & Goldman-Rakic 1989).

One defining symptom of schizophrenia is auditory hallucinations, which have been shown to involve the DLPFC (Lennox *et al.* 2000; Shergill *et al.* 2001; Hoffman *et al.* 2007; Goldstein *et al.* 1999; Sommer *et al.* 2008; Sommer *et al.* 2001). Auditory hallucinations are experienced by 70% of schizophrenics and often interfere with their quality of life (Sommer *et al.* 2008). Auditory hallucinations are defined as hearing voices in the absence of an external stimulus (David 1999). Several hypotheses were proposed to explain why auditory hallucinations occur in schizophrenia. The first

hypothesis was termed the 'cerebral irritation' model, which stated that hallucinations are the result of an overproduction of excitability in cortical areas involved in sensory memory (David 1999). The second hypothesis, the 'disinhibition' model, asserted that a reduction of sensory input caused cortical activity to manifest as hallucinations (David 1999).

In order to test these hypotheses, functional MRI (fMRI) has been used to track blood flow during auditory hallucinations (Bunney & Bunney 1999). Several studies have shown an increase in blood flow to the left middle frontal gyrus (Brodmann's area 46) during auditory hallucinations (Lennox *et al.* 2000; Shergill *et al.* 2001; Hoffman *et al.* 2007; Goldstein *et al.* 1999). A similar study by Sommer *et al.* (2008) looked at areas of cerebral activation in schizophrenic patients while experiencing auditory hallucinations and when performing a word generation task. They found that Broca's area (44) in the left hemisphere was mainly activated during the word generation task, but the right inferior frontal area (9 and 47) was highly active during auditory hallucinations (Sommer *et al.* 2001; Sommer *et al.* 2008). Rather than hypofunctionality of the left hemisphere during auditory hallucinations, the left hemisphere appears to disinhibit the right hemisphere in schizophrenia when performing language tasks (Sommer *et al.* 2001).

Area 44

Area 44 in the right hemisphere (the homologue to Broca's area) is located in the inferior frontal gyrus (David 1999). Area 44 is involved in encoding vocal cues and forming sentences (Taglialatela *et al.* 2008). Both men and women show stronger

activation in the left inferior frontal gyrus when performing a language comprehension task (Frost *et al.* 1999). Chikazoe *et al.* (2007) measured the time in which participants' eyes reflexively orient toward a peripheral stimulus (a saccade trail) and then compared those results with their reaction times during an antisaccade trail, in which participants must voluntarily move the eye away from the stimulus. Eye movements were recorded using fMRI and revealed significant activation of the right inferior frontal gyrus when performing the antisaccade trial (Chikazoe *et al.* 2007). This study implicated the right inferior frontal gyrus in inhibitory control over oculomotor and manual responses (Chikazoe *et al.* 2007). A similar study assessing inhibitory responses used the go/no go and stop signal tasks, in which the subject responds to an arrow on a computer screen by pushing a left or right key as quickly as possible unless a beep is heard signaling the subject to inhibit the reaction (Aron *et al.* 2003). Patients with lesions to the right inferior frontal gyrus had slower stop signal reaction times compared to controls, showing that this area is important in inhibiting manual reactions (Aron *et al.* 2003).

Area 41

The final cortical area being examined in this study is area 41, which is also known as the primary auditory cortex (Mitelman *et al.* 2005). This area is located on the superior temporal plane, including about half of the medial portion of Heschl's gyrus (Pekkola *et al.* 2004). The primary auditory cortex was shown to be strongly activated during a pure tone stimulus, while both the primary auditory cortex and peripheral regions were activated by band-passed noise bursts, which proves that the auditory cortex

possesses a hierarchical organization (Wessinger *et al.* 2001). The core area of the auditory cortex is area 41, where sounds are processed and then radiate laterally and medially to outer regions (Wessinger *et al.* 2001). Lesions to the primary auditory cortex resulted in a deficit in perceiving the resolution of pitch, which supports previous findings that the primary auditory cortex is involved in processing tones (Zatorre *et al.* 2002).

These cortical areas were chosen because both areas 9 and 46 were strongly activated during auditory hallucinations in fMRI studies (Lennox *et al.* 2000; Shergill *et al.* 2001; Hoffman *et al.* 2007; Goldstein *et al.* 1999). The CB-ir interneuron densities in these areas in schizophrenic subjects were compared with those in control subjects. This study is unique in that surrounding cortical areas (areas 44 and 41) were chosen in order to compare within-subject CB-ir interneuron densities

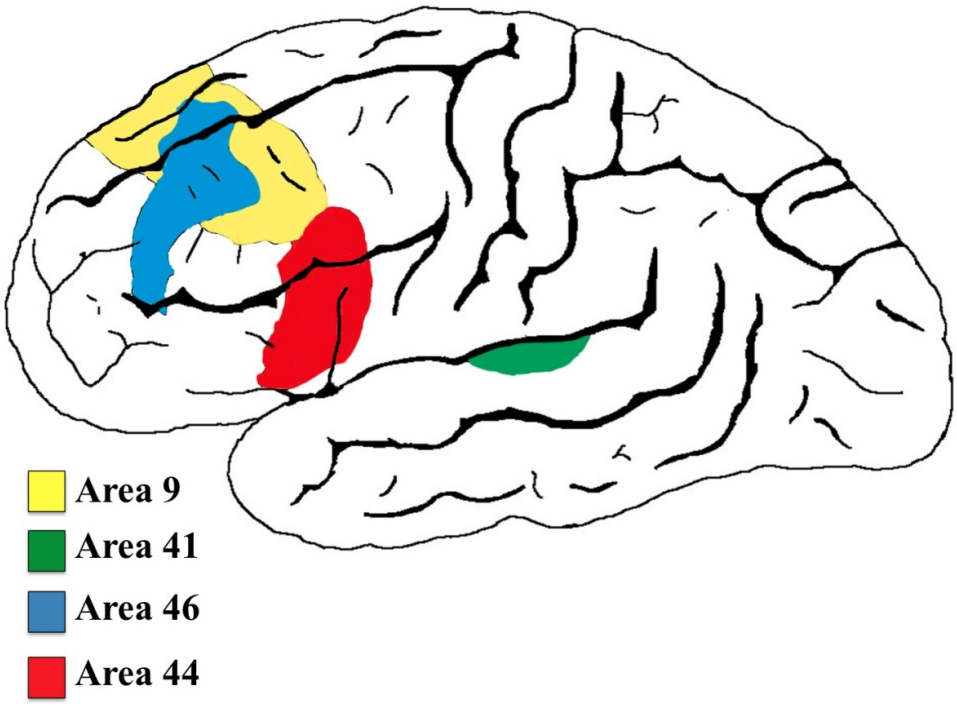

Figure 2. An illustration of the lateral view of the location of Brodmann's areas 9, 41, 46, and 44.

1.7 Hypotheses

Hypothesis: There will be a lower density of GABAergic CB-ir interneurons in the areas of the brain involved in auditory hallucinations (areas 9 and 46) relative to surrounding cortical areas in schizophrenic subjects.

Many studies have found differences in densities of neurons expressing calcium-binding protein subpopulations between schizophrenic and normal controls. Even though

there have been inconsistencies in the results, calbindin has repeatedly been implicated in the schizophrenic brain.

The present study examined the GABAergic hypothesis of schizophrenia, which asserts that GABA is reduced in certain cortical brain regions of schizophrenics, resulting in disinhibition. Specifically, the CB-ir interneuron densities between normal and schizophrenic individuals in cortical areas 9, 41, 44, and 46 were measured and analyzed. Areas 9 and 46 have been implicated in the auditory hallucinations common to many schizophrenics (Lennox *et al.* 2000; Shergill *et al.* 2001; Hoffman *et al.* 2007; Goldstein *et al.* 1999). Areas 41 and 44 were also examined in order to compare within the same individual those densities in areas implicated in schizophrenia with areas not implicated but functionally similar. Three cortical areas that were examined are located in the frontal cortex (areas 9, 44, and 46), while area 41 is located in the temporal cortex (Figure 2). While previous studies have looked at one cortical area and compared it to the same cortical area across subjects, this study measured the CB-ir interneuron densities of 4 cortical areas and compared the within subject interneuron densities in addition to comparing interneuron densities across schizophrenic and control groups.

CHAPTER 2

METHODS

2.1 Specimens

Brain samples were obtained from the Alzheimer's Disease and Schizophrenia brain bank at the Mount Sinai School of Medicine. The sample included four males and four females. Among the eight subjects, three (one male and two females) were diagnosed with schizophrenia, and five (three males and two females) were normal controls (Table 1). Cortical samples from all four cortical regions of interest were available for each individual, and all samples were obtained from the right hemisphere since increased metabolic activity during auditory hallucinations has been noted in this hemisphere (Sommer *et al.* 2001; Sommer *et al.* 2008).

Sex	Age	Diagnosis
Male	84	Control
Male	80	Control
Male	66	Control
Female	85	Control
Female	88	Control
Male	71	Schizophrenic
Female	70	Schizophrenic
Female	74	Schizophrenic

Table 1. Human subjects used in this study.

2.2 Immunohistochemistry

Cortical samples were frozen on dry ice and sectioned into 40 μm-thick sections using a sliding microtome. Each section was then placed into individual microcentrifuge tubes, which contained freezer storage solution (30% each distilled water, ethylene glycol, and glycerol and 10% 0.244 M PBS). Immunohistochemistry was performed on tissue samples in order to visualize CB-ir interneurons. Floating tissue sections were rinsed 10 × 5 in PBS and pretreated for antigen retrieval by incubating in 0.05% citraconic acid (pH 7.4) at 86° C for 30 minutes. Tissue sections were then cooled for 20 minutes and rinsed with PBS. Endogenous peroxidase was quenched using a diluted mixture of hydrogen peroxide (75% methanol, 2.5% hydrogen peroxide, and 22.5% ddH_2O) for 20 minutes. Tissue sections were rinsed in PBS and placed in dilution buffer made up of 4% normal serum, 0.6% Triton X-100, and PBS for 1 hour at room temperature. Sections were rinsed using PBS and incubated in a primary antibody (catalog #300, CB-D28, Swant, Switzerland) at a dilution of 1:8000 at 4°C on an orbital shaker for 48 hours. After being incubated in primary antibody for 48 hours, tissue sections were washed in PBS and placed in biotinylated secondary antibody (1:500) in a solution of PBS and 2% anti-mouse serum for 1 hour, after which the sections were rinsed in PBS. The tissue samples were then incubated in avidin-peroxidase complex (PK-6100, Vector Laboratories, Burlingame, CA) at room temperature for 1 hour. CB-ir was visualized using 3,3-diaminobenzidine (DAB) as a chromogen (SK-4100, Vector Laboratories) enhanced with nickel for 8 minutes. The reaction was stopped by placing

the sections in PBS. Sections were mounted on gelatin-coated slides, dehydrated, and coverslipped using DPX mountant.

2.3 Data Collection

All tissue samples were prepared at the same time, using the same procedures, which eliminated any histological inconsistencies across samples. Quantitative data was collected using an Olympus BX-51 photomicroscope equipped with a Ludl XY motorized stage, Heidenhain z-axis encoder, StereoInvestigator software (MBF Bioscience, Williston, VT, USA, version 10), and a digital camera that projects images onto a 24-inch LCD flat panel monitor. CB-ir interneurons were quantified in layers II-III and V-VI of each cortical area. Layers were delineated at low power (4X, 0.13 N.A.) and the sections were analyzed using optical disector probe with a fractionator sampling scheme at high magnification (40X, 0.75 N.A.). A guard zone of 2 μm was used at the top and bottom of each section, and section thickness was measured every 10^{th} sampling site. Counting frame was set at 150 × 150 μm with an optical dissector height of 7 μm. All CB-ir interneurons visualized by immunohistochemistry were included in this study. The mean number of sample sites was 174.45 (S.D.=49.32, range 110-360), and the number of neurons counted per layer/area/individual averaged 489.67 (S.D.=282.978, range 44-1251) (Table 2).

2.4 Statistical Analysis

A repeated-measures ANOVA was used to analyze differences between layer (II-III and V-VI) and area (9, 41, 46, and 44) controlling for treatment (between normal and schizophrenic patients). Statistical significance was set at 0.05 (one-tailed). Post-hoc testing was done using independent t-tests.

Area/Layer	Diagnosis	Mean	Standard Deviation
Area 9 II/III	Control	4.036	1.5449
	Schizophrenic	3.727	1.2440
Area 9 V/VI	Control	0.588	0.2308
	Schizophrenic	0.650	0.0656
Area 41 II/III	Control	9.230	1.3498
	Schizophrenic	11.757	1.4922
Area 41 V/VI	Control	5.822	0.9049
	Schizophrenic	4.303	0.8203
Area 44 II/III	Control	13.286	7.8136
	Schizophrenic	16.390	8.7870
Area 44 V/VI	Control	8.328	7.3621
	Schizophrenic	4.383	1.6095
Area 46 II/III	Control	10.816	4.1772
	Schizophrenic	7.467	1.6610
Area 46 V/VI	Control	6.436	3.2871
	Schizophrenic	2.417	1.2309

Table 2. Mean CB-ir interneuron density in neurons/mm³ for each area and layer examined between schizophrenics and controls.

CHAPTER 3

RESULTS

3.1 Descriptive Morphology

CB-ir interneurons were densest in layer II and upper layer III for both schizophrenic and normal controls, as noted by DeFelipe *et al.* (1989). Layers V-VI also contained CB-ir interneurons, but they were more sparsely scattered (Figure 3 and 4). Those CB-ir interneurons in layers II-III were small to medium sized with the characteristic double-bouquet morphology, with short projecting dendrites as noted by Sherwood *et al.* (2003). Deeper layers V-VI, in contrast, tended to contain larger CB-ir interneurons with a multipolar morphology, with dendrites projecting radially from the body and extending into more superficial layers (Figure 5).

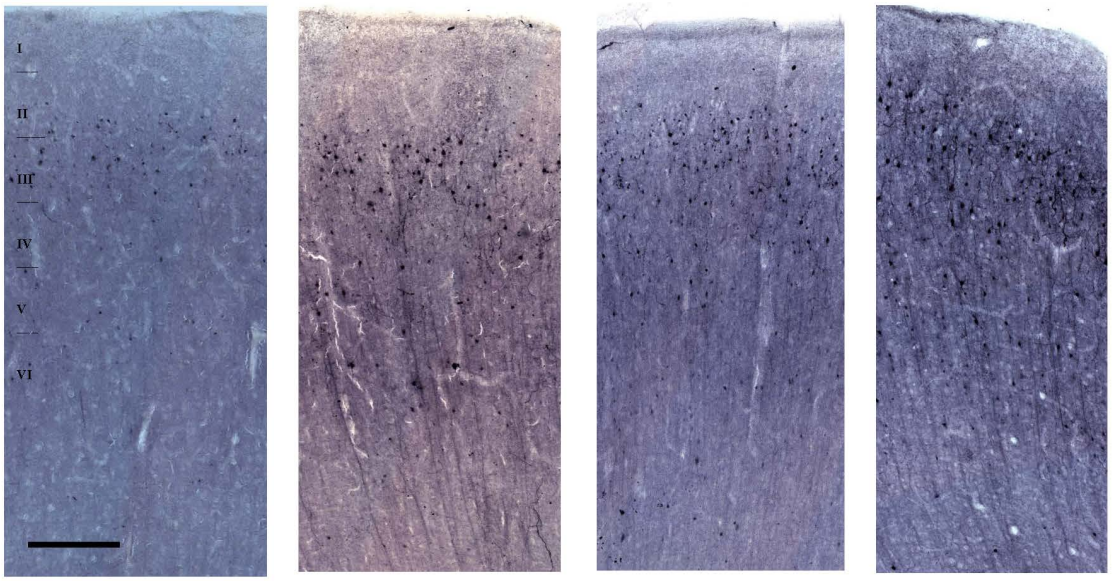

Figure 3. CB-ir interneurons in each cortical layer examined in normal controls (scale bar = 250μm).

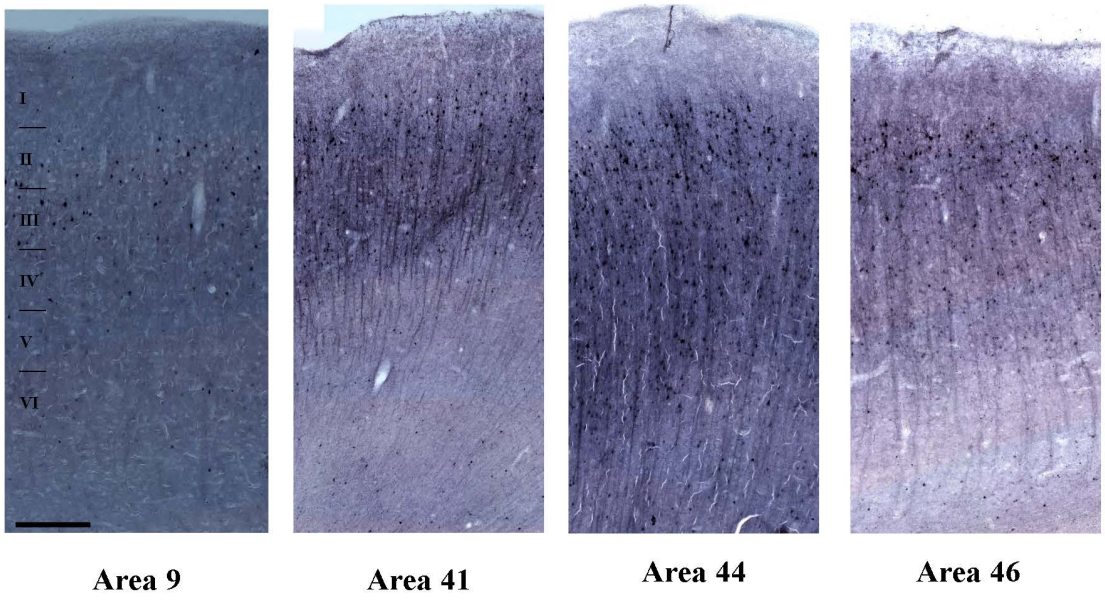

Figure 4. CB-ir interneurons in each cortical layer examined from schizophrenic patients (scale bar = 250μm).

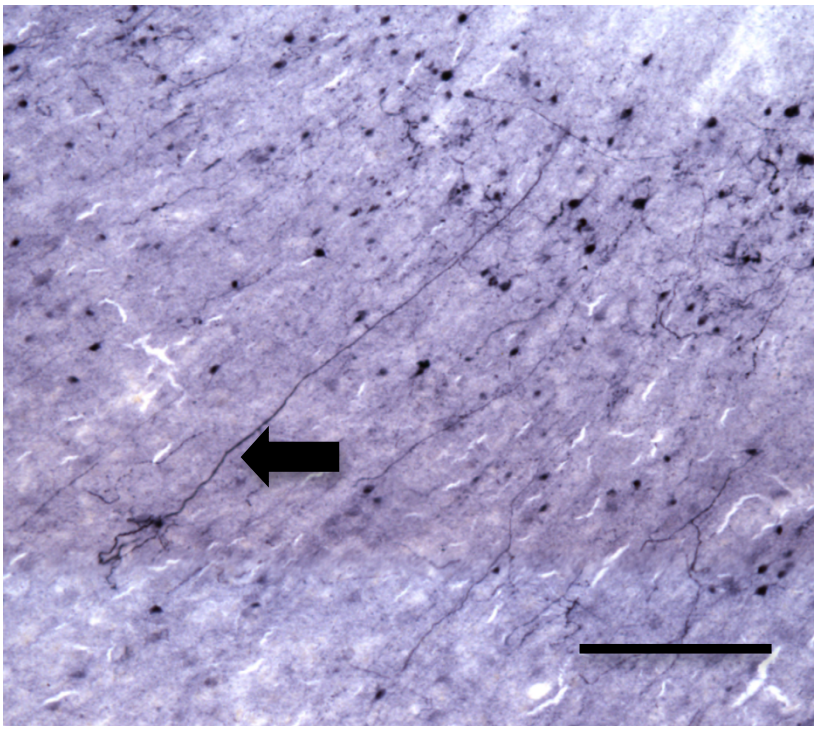

Figure 5. A picture of CB-ir interneurons, the arrow indicates an axon ascending from the cell soma in layer III vertically into layer I (scale bar = 250μm).

3.2 Quantitative Analyses

The repeated-measures ANOVA revealed differences in CB-ir interneuron density in both layer ($F_{2,6} = 75.49$; $p < 0.05$) and area ($F_{2,6} = 74.06$; $p < 0.05$) among normal controls and schizophrenics.

Post hoc testing demonstrated that there was a significant difference of CB-ir interneuron density between normal controls and schizophrenic subjects in layers II-III ($t_6 = -2.47$, $p = 0.02$, directional) and layers V-VI ($t_6 = 2.37$, $p = 0.03$, directional) of area 41.

A significant difference was also found for layers V-VI of area 46 (t_6 = 1.982, p = 0.048, directional; Figure 6). No significant differences in CB-ir interneuron density were found in area 9.

An independent samples t-test was also run measuring interneuron density between males and females, but only layers V-VI of area 46 attained statistical significance (t_6 = 3.009, p = 0.012, directional). Since only one variable out of 8 was significant, sex was not considered an important factor in explaining CB-ir neuron densities between schizophrenics and normal controls.

	Mean	Standard Deviation	Minimum	Maximum
Sample Site	179.33	56.17	110	360
Neuron Number	545.8	280.281	44	1251
Cegund	0.06	0.02366	0.03	0.15
CESH	0.051	0.02412	0.03	0.15

Table 3. Descriptive statistics for all areas examined in normal human controls. N=64 cortical sites for all 8 subjects.

	Mean	Standard Deviation	Minimum	Maximum
Sample Site	166.33	37.3296	117	268
Neuron Number	396.13	234.184	76	882
Cegund	0.0654	0.02254	0.04	0.12
CESH	0.0588	0.02262	0.03	0.11

Table 4. Descriptive statistics for all areas examined in Schizophrenic patients. N=64 cortical sites for all 8 subjects.

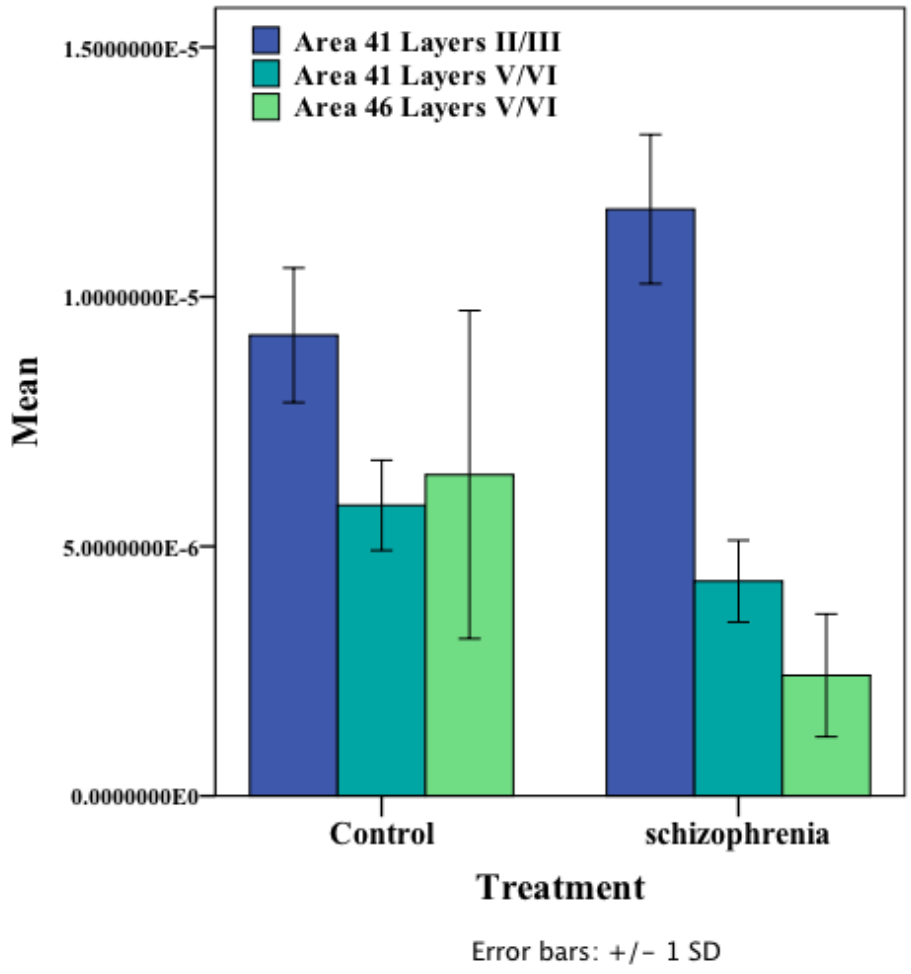

Figure 6. Mean CB-ir neuron densities for layers II/III in area 41 and layers V/VI in areas 41 and 46.

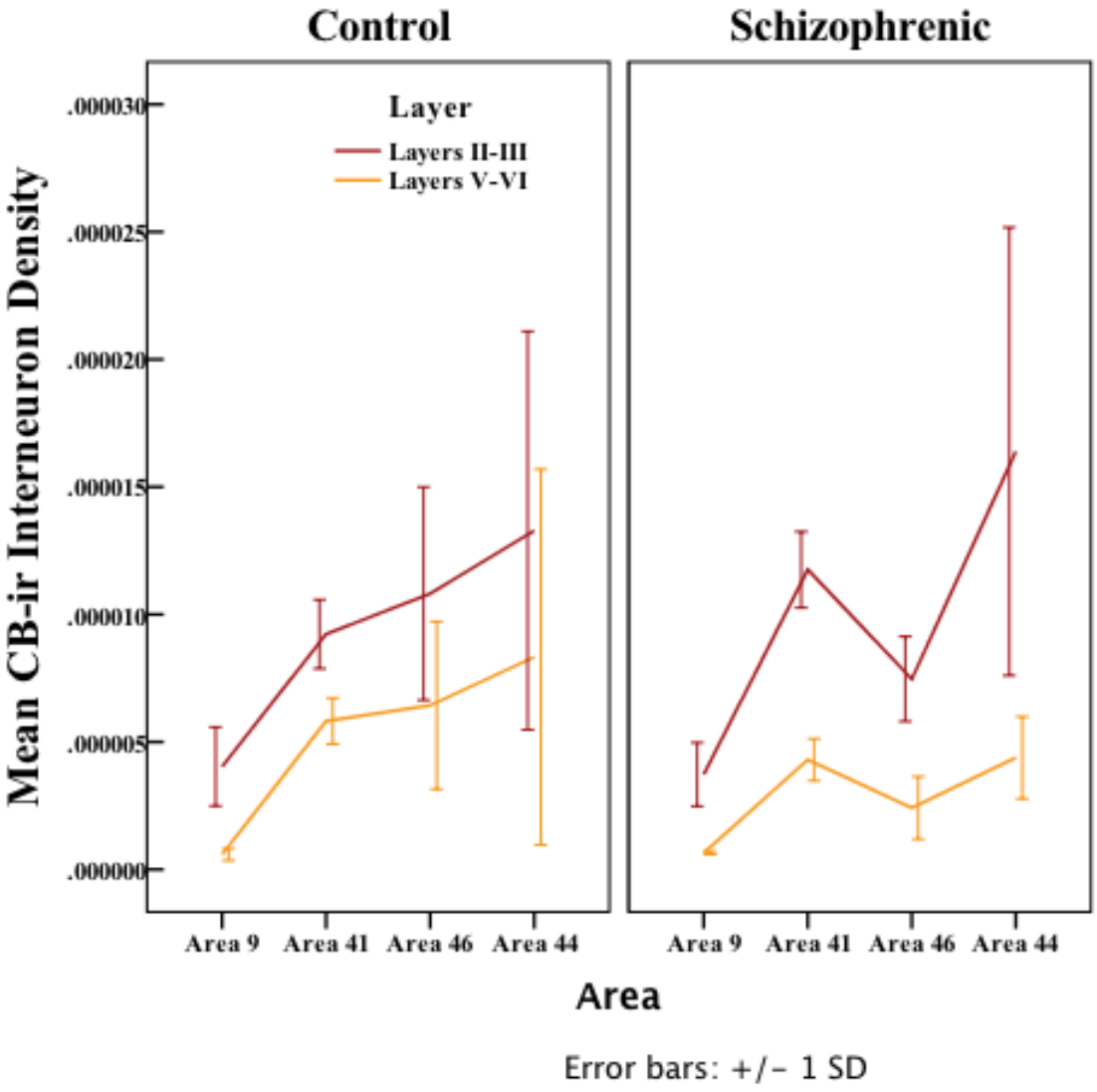

Figure 7. CB-ir interneuron densities in areas 9, 41, 44, and 46 for schizophrenics and normal controls.

CHAPTER 4

DISCUSSION

The present study found a significant decrease in the density of CB-ir interneurons in layers V-VI of areas 41 and 46, while CB-ir interneuron density was increased in layers II-III of area 41 in schizophrenic individuals compared to normal controls. The decreased CB-ir interneuron density in layers V-VI of areas 41 and 46 means that GABA is disinhibited. However, there is an increase of GABA in layers II-III in area 41 in schizophrenics. It was hypothesized that the density of CB-ir interneurons would be decreased in areas 46 and 9, based on previous fMRI studies indicating that these areas are active in schizophrenic subjects while experiencing auditory hallucinations (Lennox *et al.* 2000; Shergill *et al.* 2001; Hoffman *et al.* 2007; Goldstein *et al.* 1999). Contrary to this hypothesis, no significant differences were detected in area 9. The results of this study are similar to the findings reported by Iritani *et al.* (1999) that failed to detect a difference in the density of CB-ir neurons in Brodmann's area 9 between normal and schizophrenic humans.

While differences were not detected in Brodmann's area 9, CB-ir interneuron density was significantly decreased only in layers V-VI of Brodmann's area 46 and not in the more superficial layers II-III. This means there are a decreased number of inhibitory signals that synapse on the neurons in the upper cortical layers of area 46. This finding coincides with the results of previous studies that have found decreased interneuron

densities in area 46 in schizophrenic subjects (Beasley *et al.* 2001). The reason for reduced GABAergic interneuron densities could be explained by developmental deficiencies. Around 2 weeks after birth, CB-ir neurons reorganize in the brain and are more susceptible to toxic events, which target GABAergic neurons before the neurons are protected by calcium-binding proteins (Reynolds & Beasley 2001). Deficits in reorganization could also impact neuron connections to other areas of the brain (Bunney & Bunney 1999). Because the middle frontal gyrus has strong connections to the paralimbic and limbic system, which are involved in memory, attention, and motivation, developmental deficiencies could explain why all of these are impaired in people with schizophrenia (Goldstein *et al.* 1999).

There was no significant decrease in CB-ir interneuron density between schizophrenic and normal human controls in Brodmann's area 44 (Broca's homologue). Although other studies looking at neuron densities did not focus specifically on subpopulations of calcium-binding proteins, Nissl staining failed to reveal significant differences in interneuron density in area 44 between schizophrenic and normal controls (Selemon *et al.* 2003; Selemon 2001). Although a number of schizophrenic patients experience syntax difficulties, which most likely involve area 44, this was not correlated with a difference in overall neuronal density in this area (Selemon 2001). This current study's finding that Broca's homologue is not significantly different in terms of CB-ir neuron density also suggests that this area may not be involved in the pathophysiology of schizophrenia.

Contrary to the hypothesis of this study was the significant increase in CB-ir interneuron density in schizophrenic subjects compared to normal controls of layers II-III in area 41. Alternately, there was a significant decrease in neuronal density of layers V-VI in area 41 (Figure 6). Chance *et al.* (2005) conducted the first study focusing on CB-ir interneurons in the planum temporale, an area that includes areas 41, 42, and 22. They found decreased CB-ir density in the planum temporale of schizophrenic subjects (Chance *et al.* 2005). The superior temporal gyrus is one of the most variable structures in schizophrenia (Chance *et al.* 2008). The planum temporale, which is a part of the superior temporal gyrus, is often greatly reduced in size, especially in the left hemisphere, of schizophrenic subjects with a difference between males and females (Kasai *et al.* 2003). Chance *et al*. (2008) found decreased minicolumn number in schizophrenic females and increased minicolumn number in schizophrenic males. Another study found the primary auditory cortex to be activated during auditory hallucinations as revealed through fMRI (Dierks *et al.* 1999). Disrupted auditory processing has been observed in schizophrenic patients, and one way to test this is through delayed tone-mismatch performance, where two tones are given separated by a brief pause in between (Rabinowicz *et al.* 2000). Subjects must then detect a difference in tone between the two (Rabinowicz *et al.* 2000). Rabinowicz *et al*. (2000) found that patients diagnosed with schizophrenia had a much higher tone-matching threshold; a greater difference in tone was required for the subject to detect a difference, than normal controls. This study by Rabinowicz *et al.* (2000) also observed no significant difference between schizophrenic and normal controls in sensitivity of same modality distracters, which would be observed

in patients with damage to the prefrontal cortex. This current study supports previous studies that found abnormalities in the primary auditory cortex in patients with schizophrenia.

4.1 Future Directions

The current study looked at several areas in the prefrontal cortex and one in the auditory cortex in schizophrenic and normal human controls and compared the results of each area to the other areas being studied within the same subjects. One surprising finding was increased CB-ir interneuron density in area 41 layers II-III but decreased CB-ir interneuron density in area 41 layers V-VI in schizophrenic brains. It would be useful to also look at Brodmann's area 42 and other areas located in the auditory cortex in order to narrow down those that may be abnormal in schizophrenia. It is also interesting to note that all significant decreases in CB-ir interneuron density were limited to cortical output layers V-VI. Further studies focusing on the densities of subpopulations of calcium-binding proteins in these cortical areas are needed. Other studies have looked at the distribution of other subpopulations of calcium-binding proteins in the brains of schizophrenics and have found differences in density. Several studies found decreases in parvalbumin-immunoreactive (PV-ir) interneurons in layers III and IV of the prefrontal cortex of schizophrenic subjects (Beasley & Reynolds 1997; Beasley *et al.* 2002), while no difference in calretinin-immunoreactive (CR-ir) interneurons was observed (Beasley *et al.* 2002). PV-ir interneurons are also diminished during development, while CR-ir interneurons did not appear to have density reductions, suggesting that PV-ir interneurons

are susceptible to neurotoxic events early in life that could contribute to the development of schizophrenia (Reynolds & Beasley 2001). Based on these studies, it would be advantageous to examine calretinin and parvalbumin in addition to calbindin in order to see the full distribution of GABAergic neurons in schizophrenic brains.

No previous studies were found prior to this one that looked at calcium-binding interneuron densities in area 44 in the right hemisphere. Because fMRIs have noted increased blood flow to this brain region during auditory hallucinations, it would be interesting to conduct more studies that looked at all three interneuron subtypes in this area in order to gain a better understanding of the role this region plays in schizophrenia.

4.2 Conclusion

The results of the present study indicate that there are area and layer-specific alterations in the CB-ir interneuron subpopulation of GABAergic interneurons in the schizophrenic brain. In addition to absolute differences in densities within cortical areas 46 and 41, a different pattern across cortical areas emerged, indicative of a deficit of CB-ir interneurons within area 46 of the schizophrenic brain. The findings presented here support a hypothesis of area-specific disinhibition within the schizophrenic brain that coincides with altered cortical activity recorded during auditory hallucinations (Lennox *et al.* 2000; Shergill *et al.* 2001; Hoffman *et al.* 2007; Goldstein *et al.* 1999). These findings implicate GABAergic abnormalities in the prefrontal and primary auditory cortices in schizophrenia, which contributes to deficits in working memory, attention, motivation, and auditory hallucinations characteristic of this disorder.

REFERENCES

Adolphs, R. The neurobiology of social cognition. *Current Opinion in Neurobiology* **11,** 231-239 (2001).

Ahn, K. *et al.* Probing GABA receptor function in schizophrenia with Iomazenil. *Neuropsychopharmacology* **36,** 677-683 (2011).

Aiello, L.C. & Dunbar, R.I.M. Neocortex size, group size, and the evolution of language. *Current Anthropology* **34,** 184-193 (1993).

Andreasen, NC. Symptoms, signs, and diagnosis of schizophrenia. *Lancet* **346,** 477-481 (1995).

Aron, A.R. *et al.* Stop-signal inhibition disrupted by damage to right inferior frontal gyrus in humans. *Nature Neuroscience* **6,** 115-116 (2003).

Barbas, H. Connections underlying the synthesis of cognition, memory, and emotion in primate prefrontal cortices. *Brain Research Bulletin* **52,** 319-330 (2000).

Beasley, CL. *et al.* Selective deficits in prefrontal cortical GABAergic neurons in schizophrenia defined by the presence of calcium-binding proteins. *Society of Biological Psychiatry* **52,** 708-715 (2002).

Beasley, C.L. & Reynolds, G.P. Parvalbumin-immunoreactive neurons are reduced in the prefrontal cortex of schizophrenics. *Schizophrenia Research* **24,** 349-355 (1997).

Benes, FM. *et al.* Up-regulation of GABAA receptor binding on neurons of the prefrontal cortex in schizophrenic subjects. *Neuroscience* **75,** 1021-1031 (1996).

Benes, FM. and Berretta, S. GABAergic interneurons: implications for understanding schizophrenia and bipolar disorder. *Neuropsychopharmacology* **25,** 1-27 (2001).

Bleuler, E. Dementia praecox or the group of schizophrenias. Trans., J. Zinkin (1950). New York: International Universities Press (1908).

Brothers, L. The social brain: A project for integrating primate behavior and neurophysiology in a new domain. *Concepts in Neuroscience* **1,** 27-51 (1990).

Brüne, M. Social cognition and psychopathology in an evolutionary perspective. Current status and proposals for research. *Psychopathology* **34,** 85-94 (2001).

Bunney, WE. and Bunney, BG. Evidence for a compromised dorsolateral prefrontal cortical parallel circuit in schizophrenia. *Brain Research Reviews* **31,** 138-146 (2000).

Burns, JK. An evolutionary theory of schizophrenia: Cortical connectivity, metarepresentation, and the social brain. *Behavioral and Brain Sciences* **27,** 831-885 (2004).

Burns, JK. The social brain hypothesis of schizophrenia. *World Psychiatry* **5,** 77-81 (2006).

Cantalupo, C. & Hopkins, W.D. Assymmetric Broca's area in great apes: a region of the ape brain is uncannily similar to one linked with speech in humans. *Nature* **414,** 505 (2001).

Carlsson, A. The neurochemical circuitry of schizophrenia. *Pharmacopsychiatry* **39,** S10-S14 (2006).

Casanova, MF. *et al.* Neuronal distribution in the neocortex of schizophrenic patients. *Psychiatry Research* **158,** 267-277 (2008).

Chance, S.A. *et al.* Reduced density of calbindin-immunoreactive interneurons in the planum temporale in schizophrenia. *Brain Research* **1046,** 32-37 (2005).

Chance, S.A. *et al.* Auditory cortex asymmetry, altered minicolumn spacing and absence of ageing effects in schizophrenia. *Brain* **131,** 3178-3192 (2008).

Chikazoe, J. *et al.* Activation of right inferior frontal gyrus during response inhibition across response modalities. *Journal of Cognitive Neuroscience* **19,** 69-80 (2007).

Condé, F. *et al.* Local circuit neurons immunoreactive for calretinin, calbindin D-28k or parvalbumin in monkey prefrontal cortex: distribution and morphology. *Journal of Comparative Neurology* **341,** 95-116 (1994).

Costello, CG. *Symptoms of Schizophrenia*. John Wiley & Sons, Oxford (1993).

Crespi, B. *et al.* Adaptive evolution of genes underlying schizophrenia. *Proceedings of the Royal Society B* **274,** 2801-2810 (2007).

Crow, TJ. A theory of the evolutionary origins of psychosis. *European Neuropsychopharmacology* **5** (suppl.), 59-63 (1995).

Crow, TJ. Schizophrenia as failure of hemispheric dominance for language. *Trends Neuroscience* **20,** 339-343 (1997).

Crow, TJ. Is schizophrenia the price that *Homo sapiens* pays for language? *Schizophrenia Research* **28,** 127-141 (1997).

Crow, TJ. Schizophrenia as the price that Homo sapiens pays for language: a resolution of the central paradox in the origin of the species. *Brain Research Reviews* **31,** 118-129 (2000).

David, AS. Auditory hallucinations: phenomenology, neuropsychology and neuroimaging update. *Acta Psychiatrica Scandinavica* **99,** 95-104 (1999).

Daviss, S.R. & Lewis, D.A. Local circuit neurons of the prefrontal cortex in schizophrenia: selective increase in the density of calbindin-immunoreactive neurons. *Psychiatric Research* **59,** 81-96 (1995).

Dean, B. Is schizophrenia the price of human cerebral nervous system complexity? *Australian and New Zealand Journal of Psychiatry* **43,** 13-24 (2009).

DeFelipe, J. *et al.* Synapses of double bouquet cells in monkey cerebral cortex visualized by calbindin immunoreactivity. *Brain Research* **503,** 49-54 (1989).

Devinsky, O. *et al.* Contributions of anterior cingulated cortex to behaviour. *Brain* **118,** 279-306 (1995).

Diamond, A. & Goldman-Rakic, P.S. Comparison of human infants and rhesus monkeys on Piaget's AB task: evidence for dependence on dorsolateral prefrontal cortex. *Experimental Brain Research* **74,** 24-40 (1989).

Dierks, T. *et al.* Activation of Heschl's gyrus during auditory hallucinations. *Cell* **22,** 615-621 (1999).

Egan, MF. and Hyde, TM. Schizophrenia: neurobiology. *Kaplan and Sadock's Comprehensive Textbook of Psychiatry* 1129-1146 (2000).

Eyles, DW. *et al.* Neuronal calcium-binding proteins and schizophrenia. *Schizophrenia Research* **57,** 27-34 (2002).

Frost, J.A. *et al.* Language processing is strongly left lateralized in both sexes. *Brain* **122,** 199-208 (1999).

Goldman-Rakic, P.S. Circuitry of primate prefrontal cortex and regulation of behavior by representational memory. *Comprehensive Physiology* **5**, 373-401 (1987).

Goldstein, JM. *et al.* Cortical abnormalities in schizophrenia identified by structural magnetic resonance imaging. *Archives of General Psychiatry* **56,** 537-547 (1999).

Gordon, JA. Testing the glutamate hypothesis of schizophrenia. *Nature Neuroscience* **13,** 2-4 (2010).

Gur, R.E. *et al.* An fMRI study of facial emotion processing in patients with schizophrenia. *American Journal of Psychiatry* **159,** 1992-1999 (2002).

Haxby, J.V. *et al*. Human neural systems for face recognition and social communication. *Society of Biological Psychiatry* **51,** 59-67 (2002).

Hof, P.R. *et al.* Cellular distribution of the calcium-binding proteins parvalbumin, calbindin, and calretinin in the neocortex of mammals: phylogenetic and developmental patterns. *Journal of Chemical Neuroanatomy* **16,** 77-116 (1999).

Hoffman, RE. *et al.* Probing the pathophysiology of auditory/verbal hallucinations by combining functional magnetic resonance imaging and transcranial magnetic stimulation. *Cerebral Cortex* **17,** 2733-2743 (2007).

Horrobin, DF. Schizophrenia: the illness that made us human. *Medical Hypotheses* **50,** 269-288 (1998).

Horrobin, DF. Lipid metabolism, human evolution and schizophrenia. *Prostaglandins, Leukotrienes and Essential Fatty Acids* **60,** 431-437 (1999).

Iritani, S. *et al.* Calbindin immunoreactivity in the hippocampal formation and neocortex of schizophrenics. *Progress in Neuro-Psychopharmacology and Biological Psychiatry* **23,** 409-421 (1999).

Jablensky, A. The diagnostic concept of schizophrenia: its history, evolution, and future prospects. *Dialogs in Clinical Neuroscience* **12,** 271-287 (2010).

Javitt, D.C. Glutamatergic theories of schizophrenia. *Israel Journal of Psychiatry & Related Sciences* **47,** 4-16 (2010).

Kalkman, HO. and Loetscher, E. GAD_{67}: the link between the GABA-deficit hypothesis and the dopaminergic- and glutamatergic theories of psychosis. *Journal of Neural Transmission* **110,** 803-812 (2003).

Kallmann, F.J. The genetic theory of schizophrenia: an analysis of 691 schizophrenic twins' index families. *American Journal of Psychiatry* **103,** 309 (1946).

Kasai, M.D. *et al*. Progressive decrease of left superior temporal gyrus gray matter volume in patients with first-episode schizophrenia. *American Journal of Psychiatry* **160,** 156-164 (2003).

Krystal, JH. *et al.* Subanesthetic effects of the noncompetitive NMDA antagonist, ketamine, in humans: psychotomimetic, perceptual, cognitive, and neuroendocrine responses. *Archives of General Psychiatry* **51,** 199-214 (1994).

Kyziridis, TC. Notes on the history of schizophrenia. *German Journal of Psychiatry* **8,** 42-48 (2005).

Lawrie, SM. *et al.* Reduced frontotemporal functional connectivity in schizophrenia associated with auditory hallucinations. *Biological Psychiatry* **51,** 1008-1011 (2002).

Lennox, BR. *et al.* The functional anatomy hallucinations in schizophrenia. *Psychiatry Research: Neuroimaging Section* **100,** 13-20 (2000).

Levy, R. & Goldman-Rakic, P.S. Segregation of working memory functions within the dorsolateral prefrontal cortex. *Experimental Brain Research* **133,** 23-32 (2000).

McGlashan, TH. *et al.* Schizophrenia as a disorder of developmentally reduced synaptic connectivity. *Archives of General Psychiatry* **57,** 637-648 (2000).

Mitelman, SA. *et al.* Cotical intercorrelations of frontal area volumes in schizophrenia. *NeuroImage* **27,** 753-770 (2005).

Nichols, C. Is there an evolutionary advantage of schizophrenia? *Personality and Individual Differences* **46,** 832-838 (2009).

Olney, JW. and Farber, NB. Glutamate receptor dysfunction and schizophrenia. *Archives of General Psychiatry* **52,** 998-1007 (1995).

Olney, JW. *et al.* NMDA receptor hypofunction model of schizophrenia. *Journal of Psychiatric Research* **33,** 523-533 (1999).

Parmentier, M. *et al.* Human 27-kDa calbindin complimentary DNA sequence: evolutionary and functional implications. *European Journal of Biochemistry* **170,** 207-215 (1987).

Pekkola, J. *et al.* Primary auditory cortex activation by visual speech: an fMRI study at 3T. *NeuroReport* **16,** 125-128 (2004).

Piaget, J. The construction of reality in the child. *Basic Books*, New York, Original French edition (1937).

Rabinowicz, EF. *et al.* Auditory sensory dysfunction in schizophrenia: imprecision or distractibility? *Archives of General Psychiatry* **57,** 1149-1155 (2000).

Raghanti, MA. A comparative perspective on minicolumns and inhibitory GABAergic interneurons in the neocortex. *Frontiers in Neuroanatomy* **4,** 1-10 (2010).

Reynolds, G.P. & Beasley, C.L. GABAergic neuronal subtypes in the human frontal cortex—development and deficits in schizophrenia. *Journal of Chemical Neuroanatomy* **22**, 95-100 (2001).

Russell, T.A. *et al.* Exploring the social brain in schizophrenia: left prefrontal underactivation during mental state attribution. *American Journal of Psychiatry* **157,** 2040-2042 (2000).

Sakai, T. *et al.* Changes in density of calcium-binding-protein-immunoreactive GABAergic neurons in prefrontal cortex in schizophrenia and bipolar disorder. *Neuropathology* **28,** 143-150 (2008).

Sanfilipo, M. *et al.* Volumetric measure of the frontal and temporal lobe regions in schizophrenia: relationship to negative symptoms. *Archives of General Psychiatry* **57,** 471-480 (2000).

Saxe, R. & Kanwisher, N. People thinking about thinking people: the role of the temporo-parietal junction in theory of mind. *Neuroimage* **9,** 1835-1842 (2003).

Selemon, L.D. Regionally diverse cortical pathology in schizophrenia: clues to the etiology of the disease. *Schizophrenia Bulletin* **27,** 349-377 (2001).

Selemon, L.D. *et al.* Regional specificity in the neuropathologic substrates of schizophrenia: a morphometric analysis of Broca's area 44 and area 9. *JAMA Psychiatry* **60,** 69-77 (2003).

Shergill, SS. *et al.* Modality specific neural correlates of auditory and somatic hallucinations. *Journal of Neurology, Neurosurgery & Psychiatry* **71,** 688-690 (2001).

Sherwood, C.C. *et al.* Variability of Broca's area homologue in African great apes: implications for language evolution. *The Anatomical Record* **271A,** 267-285 (2003).

Sherwood, C.C. *et al.* Neocortical neuron types in Xenarthra and Afrotheria: implications for the brain evolution in mammals. *Brain Structure and Function* **213,** 301-328 (2009).

Sherwood, C.C. *et al.* Inhibitory interneurons of the human prefrontal cortex display conserved evolution of the phenotype and related genes. *Proceedings of the Royal Society B* **277,** 1011-1020 (2010).

Shields, J., Gottesman, I.I., & Slater, E. Kallmann's 1946 schizophrenic twin study in the light of new information. *Acta Psychiatrica Scandinavica* **43,** 385 (1967).

Sigmundsson, T. *et al.* Structural abnormalities in frontal, temporal, and limbic regions and interconnecting white matter tracts in schizophrenic patients with prominent negative symptoms. *American Journal of Psychiatry* **158,** 234-243 (2001).

Slater, E. Psychotic and neurotic illnesses in twins. *Special Report Series, Medical Research Council (London)* **278** (1953).

Sommer, I.E.C. *et al.* Language lateralization in schizophrenia, an fMRI study. *Schizophrenia Research* **52,** 57-67 (2001).

Sommer, I.E.C. *et al.* Auditory verbal hallucinations predominantly activate the right inferior frontal area. *Brain* **131,** 3169-3177 (2008).

Sommer, I.E. and Diederen, K.M. Language production in the non-dominant hemisphere as a potential source of auditory verbal hallucinations. *Brain* **132,** 1-2 (2009).

Taglialatela, J.P. *et al.* Communicative signaling activates 'Broca's' homolog in chimpanzees. *Current Biology* **18,** 343-348 (2008).

Tamminga, C.A. *et al.* Limbic system abnormalities identified in schizophrenia using positron emission tomography with fluorodeoxyglucose and neocortical alterations with deficit syndrome. *Archives of General Psychiatry* **49,** 522-530 (1992).

Thiery, JC. *et al.* Neuroendocrine interactions and seasonality. *Domestic Animal Endocrinology* **23**, 87-100 (2002).

Torrey, E.F. *et al.* Seasonality of births in schizophrenia and bipolar disorder: a review of the literature. *Schizophrenia Research* **28,** 1-38 (1997).

Turnquist, K. Second-trimester markers of fetal size in schizophrenia (letter). *American Journal of Psychiatry* **150,** 1571-1572 (1993).

Watson, C.G. *et al.* Schizophrenic birth seasonality in relation to the incidence of infectious diseases and temperature extremes. *Archives of General Psychiatry* **41,** 85-90 (1984).

Wessinger, C.M. *et al.* Hierarchical organization of the human auditory cortex revealed by functional magnetic resonance imaging. *Journal of Cognitive Neuroscience* **13,** 1-7 (2001).

Yucel, M. *et al.* Anterior cingulated activation during Stroop task performance: a PET to MRI coregistration study of individual patients with schizophrenia. *American Journal of Psychiatry* **159,** 251-254 (2002).

Zatorre, R.J. *et al.* Structure and function of auditory cortex: music and speech. *TRENDS in Cognitive Sciences* **6,** 37-46 (2002).

Zuffante, P. *et al.* Working memory deficits in schizophrenia are not necessarily specific or associated with MRI-based estimates of area 46 volumes. *Psychiatry Research: Neuroimaging Section* **108,** 187-209 (2001)