SCHIZOPHRENIA: THE HOUSE OF CARDS DISORDER

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Abstract

Schizophrenia is a highly heritable disease with 200,000 cases per year in the US and a cost of $62.7 billion. It is a neurological disorder that produces positive, negative and cognitive symptoms. The papers used in this literature review include the most recent studies of schizophrenia. This review covers the still largely elusive genetic causes and neurological pathologies, diagnostic criteria, and the current treatments for schizophrenia.

Introduction

Schizophrenia as a disorder began to develop as a diagnosis in the early 20th century (Tandon *et al*, 2009). The symptoms and characteristics of the disease vary across countries and cultures. Diagnosis today uses criteria from the DSM-V, a manual published by the American Psychiatric Association that provides the standard criteria for the classification of mental disorders (American Psychiatric Association). In schizophrenia there is no leading factor to the cause of the disease. It is widely accepted that it is a highly heritable disease, and while there is promise in the field of genetic schizophrenia, years of research have yet to yield an answer. It is clearly a neurological disorder, producing hallucinations of an extraordinary measure in patients, and yet the precise neural pathology remains a mystery. How can a disease that is so well characterized and recognized so soundly as a neurological disorder, have so little evidence to support it? Below will be a general overview of the disease of schizophrenia as it is classified today, a look into the genetics of the disease, the neurological pathology believing to underlie schizophrenia, and current treatments that are used today while

more answers are searched. The aim is not to provide you with a definite answer of what causes schizophrenia, but to bring about a better understanding of what *is* schizophrenia.

Features and Diagnosis of Schizophrenia

The original conceptualization of schizophrenia came from the works of Kraepelin, Bleuler and Schneider (reviewed by: Tandon *et al,* 2009), and the differences in their ideas is what lead to changes in the definition of schizophrenia over the years. The most recent Diagnostic and Statistical Manuel of Mental Disorders (DSM-V) states the following diagnostic criteria for schizophrenia.

A. Two or more of the following symptoms, each of which must be present for a significant portion of time during a 1-month period. At least one of the symptoms must be either 1, 2, or 3
    1. Delusions
    2. Hallucinations
    3. Disorganized speech
    4. Grossly disorganized or catatonic behavior
    5. Negative symptoms
B. For a significant portion of time since the onset of these symptoms, level of functioning in one or more major areas is below that before the onset.
C. There must be continuous signs of disturbance for at least 6 months. Of those 6 months, there needs to be at least 1 month of Criteria A symptoms.
D. Other disorders with psychotic features like Schizoaffective disorder and depressive or bipolar disorder have been ruled out.
E. The disturbance cannot be attributed to the physiological effects of a substance, such as drug abuse, or another medical condition.

F. If there is a history of autism spectrum disorder or communication disorder from childhood, the diagnosis of schizophrenia is only made is there are prominent delusions or hallucinations.

Taken from DSM-V: Schizophrenia Spectrum and Other Psychotic Disorders by the American Psychiatric Association, from dsm5.org

The three general classifications of schizophrenic symptoms are positive, negative and cognitive, although there are also the less defined classifications of mood, disorganization and motor symptom dimensions. The positive symptoms of schizophrenia are associated with impaired reality, and include symptoms such as delusions and hallucinations. The delusions that are traditionally linked to schizophrenia are those that involve delusions of control or thought insertion, although delusions of reference (when an individual experiences a coincidence event and believes it to have strong personal signification). The delusions that a patient experiences are influences by their life and the socio-cultural setting in which they find themselves. The hallucinations that occur in schizophrenia can be in any of the five senses. The most common are auditory hallucinations and usually characterized as threatening voices speaking to the individual. The "formal" onset of schizophrenia is marked by reality distortion, and positive symptoms tend to emerge in adolescence or early adulthood. The neural mechanisms believed to lead to positive symptoms involve dopaminergic mesolimbic over-activity ("Clinical features and conceptualization", 4). The negative symptoms of schizophrenia involve a loss of affective (the experience of feeling) and conative (how one acts on feelings) function. Examples of negative symptoms are abulia, the loss of motivation; alogia, poverty of speech; avolition, lack of initiative; and reduced social drive. Primary negative symptoms are considered fundamental to schizophrenia, while secondary negative symptoms are

caused by "extrinsic" factors like the environment and depression (5). The cognitive symptoms of schizophrenia were not characterized in the early classifications of the disease, but there have been consistent reports over the past century of intellectual deficits in patients. The cognitive deficit seen in schizophrenia is of a generalized nature, however there are impairments in episodic memory, processing speed, verbal fluency, attention, and executive functions and working memory. The cognitive deficits of schizophrenia are found in the pre-morbid phase of the disease and persist throughout the course of the illness (6).

There are three/four phases of schizophrenia that can be defined. The premorbid phase in which there are just subtle cognitive, motor or social deficits. These are assumed to demonstrate the precursors or risk factors to developing schizophrenia, although this is an untested belief ((Tandon *et al*, 2009, "Clinical Features and Conceptualization"). The "symptoms" that are characteristic of the premorbid phase include poor academic achievement, delays in motor development and emotional detachment (7). The prodrome phase of schizophrenia includes "subthreshold psychotic symptoms." These fall in the range of cognitive deficits, negative symptoms, mood symptoms and a decline in function to a greater extent that any seen in the previous phase. This phase can last from a few months to years before the first psychotic episode (8). During this time there may begin to be the emergence of positive symptoms, and it has been found that the more severe the positive symptoms are during this time, the higher the risk of "converting" to schizophrenia (Yung *et al.*, 2008). The prodromal phase is sometimes considered to be an extension of the premorbid phase, which is really just characteristic of the time before the first onset of psychosis. The real onset of schizophrenia is the second (or third) phase of

schizophrenia and it is difficult to define due to the variations of what the definition of onset is in schizophrenia. This is usually classified though by the first psychotic episode that is consistent with Criterion A. This tends to occur in adolescence or early adulthood, and there is a general trend that earlier onset of the disease is characterized by greater cognitive deficits and severe negative symptoms. After the first psychotic episode the course of schizophrenia varies across patients with periods of exacerbations and remissions. As schizophrenia progresses, there has been a general observation that positive symptoms become less severe while negative symptoms become more prevalent, and the cognitive symptoms tend to remain stable (9).

The heterogeneity of schizophrenia has been one of the key factors in the difficulty of diagnosing the disorder, and has led to the narrowing of symptoms that define a broad range of psychotic disorders. Tandon *et al.* (2009) makes note of eight "traditional" subtypes of schizophrenia as defined by Kraepelin (Kraepelin *et al.* 1990, Kraepelin *et al.* 1971). The first is the catatonic type, characterized by psychomotor disorders such as rigidity, excitement and posturing. The second is the disorganized type with incoherence, disorganized behavior, and "grossly inappropriate affect." The paranoid type of schizophrenia involves a preoccupation with organized delusions or frequent hallucinations that revolve around a common theme. Schizoaffective type is characterized by the presence of other mood disturbances along with psychosis. The undifferentiated type of schizophrenia is when a patient presents psychotic symptoms that meet the criteria for schizophrenia, but does not have any characteristics pertaining to a particular subtype. The sixth type of schizophrenia is residual, in which there is the diagnosis of at least one prior phase of schizophrenia along with a current period in

which the patient is free from prominent psychotic symptoms but still displays minimal "residual" symptoms. The seventh type is single schizophrenia in which there is an absence of key positive symptoms, but there is a gradual onset of amotivation in the patient. This means that there is a disconnect in the patient in associating their behavior with the outcomes that arise from the behavior. The final type of schizophrenia is latent schizophrenia, characterized by off behaviors and "marked aloofness" (13).

Before we observe the genetic neurological components of schizophrenia, an interesting note was made in the conclusion of Tandon *et al.'s* review of the clinical features of schizophrenia. In it they state, "schizophrenia is very unlikely to be a unitary disease entity, and yet it appears to be one of the best validated psychiatric diagnoses" (16). Taking a step back, one can see the astonishing truth behind this statement. Clinically, schizophrenia is difficult to diagnose due to the varying ages of onset, and the symptoms manifest differently in each patient. There are other psychotic disorders marked by periods of psychosis that are not classified as schizophrenia, and it is possible for patients to go undiagnosed for years, if they do not themselves seek out medical assistance.

Genetics of Schizophrenia

Given the struggle to simply classify the characteristics of schizophrenia as a disease, the high heritability of the disease appeared to be the key to unlocking the secrets of schizophrenia. The original approach to identify associated alleles was through gene linkage and association studies but the data produced from these studies were conflicting and did not provide enough evidence to support the association of a single gene as

leading to susceptibility of schizophrenia. One of the problems with linkage and association studies has been the need of thousands of marker to be able to screen the entire genome, a resource that many studies have not had access to. Linkage analyses are also imprecise in that they have found many positive results for possible candidate genes, yet these results are difficult to replicate in follow-up studies. Linkage analyses require enormous sample sizes (50,000 sibling pairs) to counter the "implausibly large number of genes implicated" (Sullivan *et al.,* 2005). A meta-analysis of candidate genes performed by Farell *et al.* (2014) looked to find support for historical candidate genes identified with linkage studies as genetic risk factors for schizophrenia. This large-scale analysis had four components: a meta-analysis of the candidate gene studies, a Psychiatric Genomic Consortium (PGC) mega-analysis, evaluations of specific candidate genes by experts who largely studied or discovered the genes, and survey ratings from schizophrenia geneticists. A large amount of historical candidate genes (21/25) were found to not have support as genes for schizophrenia, but the study found support for two candidate genes, TNF and NOTCH4, that have been further studied. The identification of these genes as potential candidate genes for schizophrenia arose from previous linkage studies associating these chromosomal regions to schizophrenia as well as from miscellaneous hypotheses. The meta-analysis performed by Farell *et al*. showed the uncertainty of the positive findings of linkage analysis studies. These studies rely heavily on the psychiatric diagnosis of schizophrenia and the idea that mutations in a single gene are causative. However, this may not be true. Nevertheless, only animal models of the disease will ultimately pave the way towards an understanding of the biology of the disease.

The use of animal models to improve our understanding of the changes that precipitate of schizophrenia, rather than a focus on treating the symptoms, is a prerequisite to enable the development of new and more effective therapeutic strategies (Jones *et al.* 2011). The complex and unclear nature of gene–gene and gene–environment interactions in the etiology of schizophrenia means that the challenge to develop more reliable predictive animal models of this disorder is one that can be compared to the initial sequencing of the human genome. Like the human genome, it will likely require a truly multi-disciplinary approach combining the expertise of psychiatry, neuroscience, genetics and computer/bio engineering.

The current antipsychotic medications mentioned above that are used to treat schizophrenia focus primarily on dopamine systems and appear to act as dopaminergic antagonists. With the limited success of linkage and association studies, the genes involved in dopaminergic and serotonergic transmission were thought to be good targets for candidate gene studies. Increased dopamine transmission in the basal ganglia is attributed to psychosis while decreased transmission in prefrontal areas can lead to cognitive decline (Johnson *et al.* 2008). The SNARE complex is critical in mediating synaptic vesicle fusion with the presynaptic membrane in exocytosis (Südhof, 2013)

The SNARE complex is composed of three proteins: SNAP-25, synaptobrevin and syntaxin-1A. The SNARE complex interacts with many other proteins, such as complexins and synaptotagmin, which help to mediate presynaptic vesicle fusion. While the specific contribution of defective SNARE proteins to schizophrenia is not entirely clear, in their article about presynaptic function Waiters *et al* (2011) noted that impaired glutaminergic transmission in the hippocampus and prefrontal cortex is strongly

associated with schizophrenia, and could possibly lead to alterations in the dopaminergic circuits in the midbrain. Johnson *et al* (2008) proposed that there might be a link between the synaptic hypothesis of schizophrenia and the neurodevelopmental hypothesis.

The synaptic hypothesis states that mutations in genes that influence synaptic transmission, particularly those involved in presynaptic exocytosis like SNAREs, may be involved in the underlying pathology of schizophrenia. Association studies have provided strong evidence for SNAP-25 and syntaxin-1A as candidate genes for schizophrenia. It is believed that the SNARE complex may act as a common final pathway where the functional synaptic abnormalities observed in schizophrenia are modulated (Johnson *et al* 2008). The effects of the SNARE complex can be seen in mouse models where the SNARE proteins have been disrupted, leading to behavioral changes that may be animal correlates of human schizophrenic behavior.

One of these models is the so-called "blind-drunk" mouse, which has a single nucleotide polymorphisms (SNP) in the SNAP-25 gene. Jeans *et al.* (2007) examined effects of a dominant mutation in codon 67 of SNAP-25, a T to C transition that causes an amino acid substitution from isoleucine to threonine. This mutation causes the formation of two additional hydrogen bonds in the SNARE complex leading to an increase in stability. To observe the cellular affects of the SNP, whole-cell voltage clamp recordings were taken in the somatosensory cortex of the mice. The mutation causes significant impairment in the constitutive release of glutamate due to failure to replenish the readily releasable pool of the synapse. The "blind-drunk" mutation exhibits an ataxic gait and has been shown to have pre-pulse inhibition (PPI) deficits.

Deficits in PPI are well documented in schizophrenia patients, and have been seen in unaffected family members as well. This deficit in pre-pulse inhibition is thought to imply an abnormality in the sensorimotor gating mechanism, which is regulated by dopamine, thus providing further possible support for the dopamine hypothesis. The blind-drunk mutants should show a significantly impaired PPI of the acoustic startle response, an important endophenotype for schizophrenia, in comparison with the wild-type mice (Jeans *et al.* 2007).

The ataxia of the mutants was quantified using a rotarod test, and it was observed that the mean latency to fall was much lower in the mutants than for their wild-type counterparts. While ataxia is not a phenotype observed in the human manifestation of schizophrenia, it has an important implication that will be discussed in more detail later on. The mutant mice also display anxiety behavior during the light/dark test and apathetic behavior in the "playground paradigm test," phenotypes that appear to be correlates of some of the negative symptomatologies of schizophrenia. With this study, the blind-drunk mouse has emerged as a strong rodent model for schizophrenia, and has important implications for the role of SNAP25 in schizophrenia. The human gene for SNAP-25 is located in the chromosomal region 20p12.3, which has been suggested as a strong candidate region by a recent meta-analysis (Jeans *et al.* 2007).

Another successful mouse model implicating the SNARE complex in schizophrenia are the complexin I and complexin II knockout mice (Johnson *et al*. 2008). Complexins are regulator proteins of the SNARE complex that bind to it in a competitive manner to facilitate Ca^{2+} dependence of synaptic vesicle fusion. Complexin I knockout mice display ataxia while complexin II knockout mice have a variety of subtle

neurological abnormalities like cognitive and motor deficits. Complexins are not directly involved in synapse formation or brain development but they are important regulators of exocytosis and changes in their expression can lead to changes in LTP in the hippocampus (Johnson *et al* 2008).

While ataxia is not a symptomatology commonly associated with schizophrenia, it has been observed in both the blind-drunk mice and the complexin I knockout mice. Nevertheless, both models are taken as strong correlates for strong candidate genes of schizophrenia with a phenotype that is not normally observed. This has lead to a conceptual implication about the role of mouse models in schizophrenia and how to compare the phenotypes observed in the mutant models with those seen in schizophrenic patients.

There is a quick side note to be made of the use of mice models in genetic studies to characterize potential candidate genes of schizophrenia. There is a distinction between phenotypes that mimic known symptoms of schizophrenia and those that are not observed in patients at all. Researchers need to determine whether "non-disease symptoms" like, for example, the motor effects observed in the mutant mice are a species-specific effect that does not apply to humans, or a consequence of a not fully mimicked human mutation such that the mutant protein in mouse is differently expressed in comparison to the mutant human gene. One must take into consideration the fact that the characteristic features of schizophrenia, in fact the main criteria for the diagnosis of schizophrenia, are difficult to measure in mice, at least for now. Currently, it is not possible to determine if a mouse is experiencing hallucinations and delusions; so far, only negative symptoms like apathy can be physically measured. While the behaviors observed can be implied to be

representative of some of these characteristics, these are simply deductions. It is not possible to ask a mouse how it is doing or for the mouse to voice any hallucinations it may be having. With that being said, mouse models are useful to study candidate genes and their role for neural circuits in a way that is not possible with post-mortem brain studies, MRI studies of patients or "induced pluripotent stem cells" (iPSCs) taken from patients.

A newer approach has been an integrated pathway-based approach in attempts to identify possible associations between various genomic regions and schizophrenia Juraeva *et al*. (2014). As a quick background, a single nucleotide polymorphism, SNP, is when there is a change of a single nucleotide in the genomic sequence of an individual. This occurs in the genomic DNA, in particular in "coding" and "non-coding" regions of DNA. Such SNPs may affect the amino acid sequence of a protein if they affect a non-redundant nucleotide of the "triplet code" encoding specific amino acids, or change DNA elements that influence the expression of a gene or how it will be spliced. Alternatively, SNPs may have no effect on health or the fitness of the organism.

SNPs have been implicated in our susceptibility to disease, and in particular they have been a great area of focus in the study of the genetic basis for schizophrenia. Using an integrated hierarchal approach, Juraeva *et al*. (2014) identified pathways with susceptibility to schizophrenia and detected genes involved in these pathways that may be affected, and determined the functional consequences of the SNPs in the affected genes/regulatory regions. The study identified 14 significant pathways associated with schizophrenia, one of which was a cell adhesion pathway that had also been identified in a study by O'Dushlaine *et al.* (2013).

The strongest evidence for association with schizophrenia were CACNB2 and CTCF. CACNB2 is a gene that codes for a voltage-dependent calcium channel expressed in the heart and brain. In a recent study, CACNB2 was also implicated with genome-wide significance for autism spectrum disorder, bipolar disorder and ADHD ("Identification of risk loci," 2013). CTCF is a transcription regulator protein that is believed to be an important modulator of conformational changes in chromatin (Juraeva *et al* 2014). It is also believed to be involved in neural differentiation. This study has shown that calcium channel signaling, synapse formation and the modulation of transcription regulation implicated in neuronal diversity are important in the development of schizophrenia, and in particular CACNB2 and CTCF were found to be strong candidate genes for schizophrenia.

The latest work in the genetic study of schizophrenia has been focused on variations in the major histocompatibility complex (MHC) locus. The recent work of Sekar *et al.* (2015) provides one of the most notable discoveries in the search for candidate susceptibility genes. As has been stated above, there are hundreds of loci in the human genome that contain SNPs associated with schizophrenia, yet the strongest relationship has been with genetic markers at the MHC locus, located on chromosome 6. Sekar *et al.* (2015) focused primarily on the C4 gene, which demonstrated a prominent peak of association with schizophrenia and appears to be a distinct genetic influence from the previously implicated MHC locus.

The C4 gene encodes complement component 4, which has two distinct genes, C4A and C4B. An expression analysis was performed to demonstrate that RNA expression of *C4A* and *C4B* increased in proportion to the copy number of the genes and

that the expression of *C4A* is roughly three times greater than that of *C4B*. This information was used to create genetic predictors of *C4A* and *C4B* expressions in the brain, allowing for a way to demonstrate an association with schizophrenic patients. From the analysis of SNP data, it was shown that the more strongly a SNP correlated with the predicted C4A expression, the more it was associated with schizophrenia. To further define this association, the schizophrenia risk levels for the common C4 structural alleles were measured and demonstrated risks of 1.00-1.27, showing that the effect of each C4 allele on schizophrenia is through its effect on the expression of C4A. To solidify these results, the schizophrenic association with all 13 combinations of C4 structure and MHC SNP haplotypes were measured. Each of the C4 alleles had a characteristic level of risk for schizophrenia, regardless of where it appeared.

As has been stated above, C4 is a critical component of the classical complement cascade, an immune pathway that is innate in humans and works to recognize and eliminate pathogens that are present. In past studies, it has been found that in the brain there are genes involved in the classical complement cascade that also function in the pruning of synapses. From the preliminary genetic findings and the implication of the role of other complement cascade genes in neuronal networks, Sekar *et al.* (2015) made the assumption that C4A expression might be elevated in the brain of schizophrenic patients. In fact, median gene expression was 1.4 times greater in five regions of schizophrenic brains. To determine the distribution of C4 in the brain, postmortem immunocytochemistry demonstrated C4+ cells (cells that contain C4) in both grey and white matter, and a greater number in the hippocampus. C4 expression was prevalent particularly on neurons and synapses, implying that it is either produced there or

deposited there, perhaps by astrocytes. Sekar *et al.* (2015) then narrowed the range of C4 expression down to a primary cortical neuron, and demonstrated that the majority (75%) of C4 immunoreactivity was localized to neuronal processes, of which 65% could be seen in dendrites and 35% was observed in axons. With the presence of C4 in neurons, and in particular at synapses, implies that it could work with other genes of the classical complement cascade to promote the pruning of synapses.

In a mouse model, deficiencies in C4 lead to reduced C3 immunostaining in the dorsal lateral geniculate nucleus of the thalamus. In the classical complement cascade, C3 works to target subsets of synapses and is required for pruning by microglia. The results from the mice models suggest that C4 plays a role in the pruning of synapses by the classical complement cascade, and therefore is involved in the refinement of synapses that occurs during development.

How does work on C4 expression and association with schizophrenia as presented by Sekar *et al.* (2015) relate to the pathology of schizophrenia as a disease? In humans, late cortical maturation (and therefore pruning) is still occurring into early adulthood. During this period, from adolescence to early adulthood, the clinical symptoms of schizophrenia typically develop, usually accompanied with a decline in cognitive function as well. As will be discussed in detail in a later section of the paper, there has been a pathological finding that in the brains of schizophrenic patients, there is a significant loss of cortical grey matter and a reduced number of synapses onto cortical pyramidal neurons (Garey, 2010). With recent studies, it has been implicated that neuron-microglia interactions via the complement cascade could lead to excessive pruning, and thus contribute in part to the cortical thinning observed in schizophrenia patients.

However, it remains to be seen whether cortical thinning is a cause or consequence of the disease.

Neuroscience behind Schizophrenia

Just as determining the genetic basis for schizophrenia has important implications in its heritability and characterization, establishing a neurobiological basis is critical in validating the diagnosis of schizophrenia and, in being able to outline causative mechanisms, define specific targets that can be targeted for a potential treatment. There has been difficulty in narrowing down specific neurological abnormalities in the human brain that are associated with schizophrenia, as there is a difficulty in narrowing down the specific genetic components of the disease. One of the reasons that it is so difficult to pinpoint the precise neurological causes of schizophrenia is that it is still unclear whether it is the disease itself that is causing anatomical/neurological differences or whether neurological differences lead to schizophrenia. To begin, let's discuss the anatomical differences observed in the brains of schizophrenic patients.

Previous anatomical studies were performed post-mortem on schizophrenic patients. These studies would look at the overall brain volume of patients and pathological brain tissue samples. It should be noted that the patients would have suffered from the disease for years, and long-term treatment with antipsychotics could have influenced results as well. The emergence of the new technology of magnetic resonance imaging (MRI), studies have shifted from overall brain volume to specific relationships between brain structure and neuro-cognition in schizophrenia. In a review of studies from 1991- 2003, Antonova *et al*. (2004) compiled what they assumed to be the most "consistent and compelling" findings.

The idea that schizophrenia affects the brain was introduced early in the history of the disease, and from the resulting research there is a general consensus that almost every cortical and subcortical brain structure is abnormal in schizophrenia. The ventricular volume of patients with schizophrenia is increased, and in particular an enlarged third ventricle has been associated with cognitive deficits of abstraction/flexibility, language, and attention and concentration. This increase in ventricular volume results due to the degeneration of the tissue surrounding the ventricles. It should be pointed out though that in male patients this apparent relationship between lateral ventricle size and cognitive function is disrupted, with smaller LV (versus larger LV) being associated with better cognitive functioning as compared to female patients and normal controls. It has been proposed that the increase in third ventricle volume could be an indication of the nature of the cognitive deficit, and that it is actually the pathology of the thalamus that could be causing this deficiency in schizophrenia. The thalamus is key to the cortico-striatal-thalamo-cortical and cortico-cerebella-thalamo-cortical circuitry. Disruption of these circuits may cause deficits in abstraction/flexibility and attention/concentration (Bornstein *et al.* 1992).

Another brain area implicated in the pathology of schizophrenia is the prefrontal cortex (Keshavan *et al.,* 2008). The prefrontal cortex is involved in processes that include planning complex cognitive behavior, decision-making, and controlling social behavior. In general, the PFC is implicated in executive function, that is, differentiating between different thoughts, predicting outcomes and expectations based on actions, working towards a defined goal, and social "control". In schizophrenic patients, the archicortical PFC has been found to correlate with executive function, attention, and verbal and visual

memory. While results have varied in studies, the general consensus has arisen that the archicortical PFC, but not the paleocortex, is involved in executive function and motor control (Keshavan *et al.* 2008).

Another study of the PFC to note is that of Zuffante *et al.* (2001), which measured Brodmann area 46 and working memory in male schizophrenic patients and healthy male controls. The reason for attempting to find volume differences in Brodmann area 46 is its role in sustaining attention and working memory, which is roughly correlated to the dorsolateral prefrontal cortex. Patients with schizophrenia demonstrate impaired spatial and non-spatial working memory skills. Zuffante *et al.* (2001) did not find any association between Brodmann area 46 volume and working memory ability in schizophrenic patients (Zuffante *et al.* 2001). This implies that these deficits in working memory may arise from other factors, one such factor being that the areas that support working memory in the brain are disrupted. It has also been proposed that there is a disrupted connectivity, characterized by white matter abnormalities, within the "working memory network," that leads to the impaired working memory that can be seen in schizophrenic patients. Studies on prefrontal cortex abnormalities in schizophrenia have not been definite to date and some are contradictory to each other, as have been studies on other areas of the brain (Keshavan *et al.* 2008).

It is clear that there are structural abnormalities in the brains of patients with schizophrenia, and there has been significant evidence to support abnormalities in the ventricles and prefrontal cortex of patients, but the results are still not as solid as one would hope. The technology used to study the brain today has become incredibly advanced in the past few years, especially with the emergence of magnetic resonance

imaging to allow researchers to study the brains of patients while they perform various tasks, but the images are not exactly precise. MRI as a method is still limited in its resolution (ranging from 1-4 mm), and with some brain structures and abnormalities being so small, they are difficult to distinguish in an MRI. The images produced by an MRI need to be analyzed to produce a clear image, and how the researcher analyzes the data influences the results that are produced. To be able to attain a clear picture on the anatomical differences that arise in schizophrenic patients, there needs to be an improvement in the imaging technology available.

The other option is post-mortem studies of the brains of schizophrenic patients, but these show the long term effects of the disease and it is possible that the long-term administration of antipsychotics as influence the architecture of the brain. Since there seems to be pruning or better axonal degeneration involved, it is likely that the true abnormal structures/pathologies have been lost. To fully understand the pathology of the disease, it is necessary to demonstrate anatomical and/or functional changes just before or at the onset of the first episode of schizophrenia.

In a recent long-term study, Lieberman *et al.* (2014) found that haloperidol use, a first generation antipsychotic; resulted in decreased gray matter volume, while olanzapine, a second generation antipsychotic, did not show these same changes (Keshavan *et al*, 2008). Changes in the anatomy of the brain due to schizophrenia are difficult to diagnosis and there are many patients who are left undiagnosed for months or years after their first episode. The ideal study would be a longitudinal study in schizophrenic patients and healthy relatives from childhood until death to observe the pre-existing abnormalities as well as the long-term effect schizophrenia as a disease has

on a brain. Such a study is costly and at the moment an unrealistic task for researchers to undertake.

Mismatch negativity (MMN) is a component of the event-related potential that occurs when there is an odd stimulus in a uniform sequence of stimuli. This occurs in all sensory systems, but it is most commonly been used to study audition. In the auditory system, MMN demonstrates a pre-attentive stage of information processing. In schizophrenic patients, the amplitude of MMN has been reduced, it appears to have a relation to prolonged illness duration, and the heritability is high (around 68%). Deficits in the MMN are related to impairments in sensory memory, and one source of MMN has been found to be the dorsolateral prefrontal cortex, which has been implicated with schizophrenia in previous studies (Umbricht *et al*. 2005). It is hence possible that MMN is the neurological basis for the language impairment that is observed in schizophrenic patients.

Kantrowitz *et al.* (2015) looked at the neural mechanisms that lead to deficits in auditory emotion recognition, a prevalent feature in schizophrenia and a component of cognitive impairment. Auditory emotion recognition (AER) is the ability we have as humans to recognize emotion in the speech of others, and deficits in AER are usually associated with an inability to interpret tonal features of speech. In the study, the tonal features of speech were recreated using frequency modulation tones (FM) that mimic the "prosodic contours" of specific emotional stimuli. The MMN of FM tones was assessed as was the event-related potential/resting-state function connectivity (rsfMRI) in schizophrenic patients and controls. Patients demonstrated a reduction in MMN across RM stimulus type and had impairments in AER and FM tone discrimination. The rsfMRI

analysis demonstrated reduced auditory-insula connectivity, which together with MMN reduction to FM tones accounted for roughly 50% of the variance in AER performance. These findings demonstrate that the social cognitive deficits in schizophrenia arise from impaired pre-attentive processing of tonal information (MMN reduction) and from a reduction in the connectivity between the auditory cortex and the insula. This supports a previous model that interactions between the auditory cortex and the insula are critical in processing emotion-related stimuli, and Kantrowitz *et al* (2015) were able to add to the model by demonstrating a correlation between impaired AER ability and impaired functional (auditory-insula) connectivity. Both of these two factors together contribute in an independent way to the overall pattern of AER deficit in schizophrenic patients (Kantrowitz *et* al, 2005).

In the central nervous system, neural oscillations can be observed with electroencephalography (EEG). An EEG records electrical activity of the brain through electrodes placed on the scalp. These neural oscillations are produced by either individual neurons or from interactions between neurons. The "waves" that arise from activity of neural tissue can be characterized by their frequency, amplitude and phase. There are five characterized frequency bands that have been identified. The best-known frequency band is alpha activity (8-13 Hz), which originates from the occipital lobe and arises from synchronous activity of thalamic pacemaker cells. Alpha waves are present during different stages of the wake-sleep cycle, and one of the most "common" is during the relaxed mental state that occurs during wake wen eyes are closed. A second form of alpha waves occurs during REM sleep, and is located in a frontal-central area of the brain rather than the occipital lobe. Another frequency band are delta waves that occur around 0-4 Hz.

Delta waves are usually associated with slow-wave sleep, found in Stage 3 REM sleep. Beta waves have a range of 13 – 30 Hz and are split into three sections: Low beta waves (13-16 Hz), beta waves (16-20 Hz) and high beta waves (20-30 Hz). The frequency band of beta activity is associated with normal waking consciousness, and in the motor cortex beta waves are associated with muscle contractions. Theta waves are associated with a frequency of 4 – 7.5 Hz, and activity is increased during memory encoding and retrieval. The last type of neural oscillation that has been observed are gamma waves, with a range of 30-80 Hz. Up to this point, gamma waves are thought to have the most extensive function, and have been thought to underlie the mechanisms of attention, working memory, visual perception, and sensory selection. In studies on gamma oscillations, a general mechanism has been proposed in which gamma waves are responsible for the coupling of functional brain areas during associative learning (Miltner *et al.* 1999). In recent years, it has been thought that synchronous oscillations could be underlying cognitive functions like working memory and consciousness.

It is known that schizophrenia may arise from abnormalities in neuronal circuitry, and an idea is that there is a disruption in the synchrony of neural oscillations. Spencer *et al.* (2004) used visual Gestalt stimuli to demonstrate that in schizophrenic patients, compared to healthy individuals, the gamma band oscillation that occurs in lower. The Gestalt stimuli are based on the principles of grouping that were proposed by Gestalt psychologists. These principles demonstrate the ways that humans naturally perceive objects in organized patterns. The reason these stimuli were used in the study is because they produce a gamma band oscillation that is "phase-locked" to reaction time, and thus can be used to deduce processes that may be leading to the conscious perception of the

stimuli. It is this phase-locking aspect of the oscillation observed in schizophrenic patients that Spencer *et al.* (2005) believe is correlated to the core symptoms of schizophrenia. The lower frequency in the oscillation suggests that there is some problem in the underlying neural circuitry such that it is unable to synchronize at a higher frequency. A possible explanation for this disruption in neural circuitry is attributed to decreased connectivity, demonstrated through the reduced brain volumes of schizophrenic patients. Another explanation is that there is reduced excitatory input to pyramidal cells, which is supported by the recent Sekar 2015 paper that demonstrated increase C4 expression in schizophrenic patients, a gene involved in the pruning that leads to reduced synapses onto pyramidal neurons. Spencer proposes that neural synchrony is sensitive to the integrity of neural circuitry, and this impaired circuitry results in distortions of perception and failures of cognitive integration that are characteristic of schizophrenia (Spencer *et al.* 2004).

As more research is done to find the neurological basis of schizophrenia, the picture beginning to emerge is that schizophrenia emerges from fine disruptions of neuronal circuitry. This disruption results in part from a reduction in dendritic, axonal and synaptic processes, which is observed in the reduced brain volume of schizophrenic patients. There is a finer disruption that is occurring across neuronal circuitries, and these disruptions accumulate to the materialization of the disease in young adults. It is also interesting to note that many of the factors contributing to schizophrenia are highly heritable, yet the underlying genetics are extremely complex.

Awadalla et al. (2010) hypothesized that deleterious de novo mutations may play a role in cases of autism spectrum disorders and schizophrenia, two etiologically

heterogeneous disorders with significantly reduced reproductive fitness. Awadalla et al. (2010) presented a direct measure of the de novo mutation rate (mu) and selective constraints from de novo mutations estimated from a deep re-sequencing dataset generated from a large cohort of ASD and schizophrenia cases (n = 285) and population control individuals (n = 285) with available parental DNA. A survey of approximately 430 Mb of DNA from 401 synapse-expressed genes across all cases and 25 Mb of DNA in controls found 28 candidate de novo mutations, 13 of which were cell line artifacts. Awadalla et al. (2010) calculated a direct neutral mutation rate (1.36 x 10(-8)) that was similar to previous indirect estimates, but they observed a significant excess of potentially deleterious de novo mutations in ASD and schizophrenia individuals. Awadalla et al. (2010) concluded that their results emphasized the importance of de novo mutations as genetic mechanisms in ASD and schizophrenia and the limitations of using DNA from archived cell lines to identify functional variants. From the conclusions Awadalla et al. (2010) drew, the traditional genetic approach needs to be modified, and while there has been such an effort to separate various neurological disorders, we may discover more if we look for the similarities between them.

One final neurological factor to consider in schizophrenia before examining the current treatments for schizophrenia is the NMDA receptor. The NMDA receptor is a ligand-gated channel that is activated by the neurotransmitter glutamate in the presence of the cofactor glycine and/or serine and depolarization of membrane the receptor resides in. NMDA receptors are crucial in long-term potentiation (LTP) and long-term depression (LTD), especially in terms of spine formation and the “elaboration” of dendrites. LTP and LTP are involved in the process of learning and memory. When there is an increase

in activity, there is strengthening of synapses (LTP), while LTD is a reduction in the efficacy of synapses due to specific activity. Drugs such as phencyclidine (PCP) inhibit the NMDA receptor and produce responses similar to the hallucinations that are associated with schizophrenia. The antipsychotic medications used to treat schizophrenia increase flow through NMDA receptors, which lead many to believe that schizophrenia may arise due to a deficit in these receptors (Kandel, 214). The original idea was that the decreased function of NMDA receptors was a result of increased levels of dopamine. This so-called "Dopamine hypothesis" of schizophrenia was very popular in the earlier studies of the disease since dopaminergic blockade was demonstrated to have an antipsychotic effect, and postmortem studies of schizophrenic patients showed increased D_2 receptor binding (Keshavan, 2008). Studies on the dopamine hypothesis have been inconclusive, and the focus has shifted to the NDMA receptor deficit being an element that leads to dysfunction in the cortical circuit, resulting in the downstream effect of dopamine-mediated psychosis (Coyle 2012, 920).

Moghaddam *et al.* (2003) used in vivo dialysis to show that sub-anesthetic doses of ketamine resulted in increased extracellular glutamate and release of endogenous dopamine in the PFC of rats. Later studies demonstrated that the resulting glutamate that was released is due to GABAergic interneurons that are activated by NMDA receptors. These GABAergic interneurons are responsible for inhibition in pyramidal neurons and are important for NMDA hypofunction (Coyle, 2012). Since the NMDA receptor is critical for activity-dependent synaptic growth (LTP/LTD), loss of NMDA function leads to less spines, a factor that is again noted in the gross anatomical studies of the brains of schizophrenic patients.

Treatments of Schizophrenia

The traditional treatment of schizophrenia has been with the use of antipsychotic medication. The first antipsychotic released was chlorpromazine, and revolutionized the way that schizophrenia was treated. There are two classes of antipsychotic medications, first- and second-generation agents ("Treatment and prevention Past, present, and future"). First-generation antipsychotic agents, like haloperidol, are effective in reducing positive symptoms in patients, although they are relatively unaffected against negative cognitive symptoms. The side effects associated with first-generation antipsychotics are extrapyramidal symptoms, such as dystonia or akathisia, and tardive dyskinesia (jerky movements). Second-generation antipsychotics, such as clozapine, are considered "atypical". These medications do not cause the side effects of first-generation antipsychotics, but they have their own side effects, the most serious of which is a decrease in white blood cell count known as agranulocytosis. The generation of these second-generation antipsychotics arouse due to the desire to produce a "safer clozapine" with less short-term and long-term motor side effects. Recent studies however have shown that there is no difference between the two classes of antipsychotic medications, and neither is associated with better cognitive or social outcomes ("Treatment and prevention past, present and future", 2-3). Both classes of antipsychotic medications are effective against the positive and disorganization symptoms of schizophrenia. The primary mechanism of antipsychotic medications is the blockade of the dopamine D_2 receptor, which is one of the reasons the dopamine hypothesis of schizophrenia had such a strong hold for a long period of time. In the treatment of schizophrenia, the patient's

response in the first few weeks of antipsychotic therapy is predictive of what their long-term response will be ("Treatment and prevention Past, present, and future", 3-6).

The use of antipsychotics alone is not enough in the treatment of schizophrenia. Antipsychotics only reduce the positive symptoms, meaning that the negative symptoms still persist, as do the impairments in cognitive function and social functioning. "Psychotherapies" and social treatments have been used in combination with antipsychotics to provide schizophrenic patients with an overall better quality of life. One such approach is a psychoeducational intervention that provides patients and their families with more information about the disorder and ways to cope with the illness. Meta-analyses have demonstrated the effectiveness of this approach, showing a decrease in the relapse rates of patients. These analyses have also come to the general conclusion that interventions that include family members are more effective than those without (Lincoln *et al.*, 2007). Other supplementary treatments to the use of antipsychotic medications are assertive community treatment, where the clinical aspect of treatment is provided to the patient in a multidisciplinary approach. This has been shown to reduce hospitalizations, although this effect is more prevalent in patients who have a high baseline rate of hospitalization to begin with.

Cognitive Behavior Therapy is based on the hypothesis that symptoms of delusions and hallucinations come from irrational attributions, and patients can reduce these symptoms by rationally appraising their experience of them. Supported employment for schizophrenic patients provides ongoing job support, tailored job placement and an integration of vocational and mental health services to help patients maintain a somewhat independent life ("Treatment and prevention past, present and

future"). These non-pharmacological treatments can be used in conjuncture with antipsychotics to provide the best quality of life possible for patients given the limits of what we have available today.

Discussion

We have come far in our understanding of schizophrenia as a disease, even if that journey has been confounding at many points, and yet we are still far from having any answer to what schizophrenia actually is. The high heritability of the disease clearly points to a strong genetic component, and yet there is likely no single "schizophrenia gene" but only many. There is definite brain loss in post mortem brains of schizophrenic patients, but there has not been a definite brain area that can be correlated to the symptoms and deficits of schizophrenia.

The picture that is beginning to emerge is that schizophrenia arises from an accumulation of SNPs and small disruptions in neural circuitry. Schizophrenia as a disease reveals the delicate nature of our neuronal circuitry, and how a cascade effect of a lack of synaptic plasticity or loss of synapses can lead to a severe disconnect from normal function and reality.

The majority of neural circuits necessary for normal function are initially present (as the onset is late) but they stop working in synchrony in early adolescence. It is almost like when making a house of cards. You start on a flat, stable surface and as you build up the cards, you make sure that they are all aligned and symmetrically placed to ensure the most stable base for the next layer. In schizophrenia, its as if you are starting on a surface with a tablecloth on top of it, it is still sturdy, but it is not as solid a foundation as a table

itself. Then, as you begin to build your cards up, you shift them all slightly to the left, or you allow for various gaps in-between the cards. One or two of these "errors" might not disrupt the integrity of your house of cards too much. If you continue to add these imperfections, a point will come when the foundation is too weak, and the house of cards will collapse. Schizophrenia as it manifests itself is similar to a house of cards. It is the accumulation of all of the factors summed above that lead to the characteristics and pathology of the disease. It is possible that there is a factor that is underlying all of these small difference, such as the cards found at the bottom of a house of cards can "make or break" the entire structure, but at the moment this factor is but a speculation. A continuous pursuit of research in schizophrenia is the key to unlocking the secrets held in schizophrenic patients.

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