IDENTIFYING SNP CORRELATES OF A SCHIZOPHRENIA SUBTYPE CHARACTERIZED BY POOR PREMORBID FUNCTIONING AND PERCEPTUAL ORGANIZATION DEFICITS

by

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ABSTRACT OF THE DISSERTATION

Identifying SNP Correlates of a Schizophrenia Subtype Characterized by Poor Premorbid Functioning and Perceptual Organization Deficits

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Schizophrenia has been defined as a neurodevelopmental disorder (Weinberger, 1987; Lewis & Levitt, 2002; Javitt & Coyle, 2004). The etiology of schizophrenia is thought to be comprised of a combination of genetic and environmental components. Although the estimated heritability of schizophrenia is 80% (Sullivan et al., 2003); well-known (e.g. psychosocial stressors) and unknown environmental factors have a significant role in the development of this disorder. These factors subsequently lead to epigenetic and neurochemical changes affecting brain structure and function; thus also characterizing schizophrenia as a neural circuitry disorder (Friston & Firth, 1995; Benes, 2000; Lisman et al., 2008).

Visual perceptual organization and premorbid social-sexual functioning impairments are significant cognitive and social deficit traits that are found in many individuals with schizophrenia, and that are strongly correlated with each other. However, the relationship between these phenotypes and the genetic etiology of schizophrenia is not known. Furthermore, clarification of this issue is complicated by heterogeneity of perceptual and social history functioning in the disorder. Therefore, the

aim of this study was to reduce heterogeneity compared to past studies by determining association of this combined phenotype to single nucleotide polymorphisms (SNPs) in genes associated with glutamatergic and GABAergic neurotransmitter systems; the primary hypothesized neurocircuitry of visual perceptual organization in schizophrenia. A combination of clinical assessments, a battery of visual perceptual organization tasks and saliva samples were ascertained in order to determine links between selected phenotypes and genotypes.

The observed relationships among perceptual, clinical, demographic variables are consistent with past findings suggesting a possible patient subtype with poor premorbid social sexual functioning, significant disorganized symptoms, and abnormal visual perceptual functioning. While the contribution of specific SNPs to these phenotypes has not been definitively clarified, the psychosocial developmental and perceptual organization factors analyzed are suggested to interact in schizophrenia spectrum populations and thought to be related to the genetic liability of schizophrenia. Overall, the study data suggest that further study of well defined phenotypes and potentially associated genetic variants may be useful for future studies in schizophrenia spectrum populations that aim to develop personalized treatments.

DEDICATION

I would like to dedicate this dissertation to the family members who undertook the task of ensuring my perseverance throughout graduate school:

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My brothers, Justin and Jerry, for giving me hugs, snacks and laughs when I needed them.

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Portions of this dissertation that pertain to the contour integration and Ebbinghaus illusion task are under review in the journal *Frontiers in Psychology* for the Frontiers Research Topic: Visual Dysfunction in Schizophrenia: A View into the Mechanisms of Madness. The reference for the submitted paper is Joseph, J., Bae, G., Silverstein, S.M. Gender, symptom, and developmental factors associated with perceptual organization impairments in schizophrenia. The contour interpolation task data and analyses will also be submitted as an article by Keane, B.P., Joseph, J., Silverstein, S.M. Perceptual and conceptual disorganization in schizophrenia: Two sides of the same coin? The submissions noted above represent contributions from all listed coauthors. I, as the dissertation author, have made original and meaningful contributions to the fundamental experimental design, data collection and analyses that are presented in this dissertation.

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LIST OF ABBREVIATIONS

3'UTR - 3' Untranslated Region

5'UTR - 5' Untranslated Region

AA - African American

AFC - Alternative Forced Choice

CAU - Caucasian

CEU - CEPH (Utah residents with ancestry from northern and western Europe)

Chr - Chromosome

CNTRACS - Cognitive Neuroscience Test Reliability and Clinical applications for Schizophrenia

CNV - Copy Number Variation

Cy2 - Cyanine dye 2

Cy3 - Cyanine dye 3

dbSNP - database SNP

DIGS - Diagnostic Interview for Genetic Studies

DNA - Deoxyribonucleic Acid

DTNBP1 - Dystrobrevin Binding Protein 1

DV - Dependent Variable

EDTA - Ethylenediaminetetraacetic acid

GABA - γ-aminobutyric acid

GAD1 – Glutamate Decarboxylase 1

GWA - Genome Wide Association

HICLAS - Hierarchical Classes Analysis

Hz - Hertz

HWE - Hardy Weinberg Equilibrium

IBS - Identity by State

IQ - Intelligence Quotient

IV - Independent Variable

JOVI - Jittered Orientation Visual Integration

LED - Light Emitting Diode

MAF - Minor Allele Frequency

MANCOVA - Multivariate Analysis of Covariance

MDS- Multidimensional Scaling Analysis

NCBI - National Center for Biotechnology Information

NMDA - *N*-methyl-*d*-aspartate

NONSYN - Nonsynonymous

NRG1 - Neuregulin1

PANSS - Positive and Negative Syndrome Scale

PAS - Premorbid Adjustment Scale

PCR - Polymerase Chain Reaction

SILS - Shipley Institute of Living Scale

SNP - Single Nucleotide Polymorphism

SYN - Synonymous

TCAG- Toronto Centre for Applied Genomics

YRI - Yoruba in Ibadan, Nigeria

Chapter 1: Introduction

1.1 Schizophrenia is a Major Public Health Concern

Schizophrenia is a psychiatric disorder characterized by symptoms in one or more of three domains: positive (psychotic), negative (deficit) and cognitive symptoms. Schizophrenia affects approximately 1% of the population worldwide, with prevalence similar across different countries (McGrath et al., 2004).

In addition to significant functional disability (e.g., high rates of unemployment and reduced capacity for independent living), people with schizophrenia have an approximately threefold increased risk of premature death and this risk has increased substantially in recent years (McGrath et al., 2008). This appears to be for two main reasons: symptom severity leading to suicide, and medical comorbidities such as cardiovascular disease and diabetes (Prince et al., 2007) which are thought to be accounted for by unhealthy lifestyles, lack of access to good health care, and side effects of medication (e.g., weight gain). High rates of smoking and substance abuse also contribute to early mortality. Despite some improvements in treatment methods and increased life expectancy of the general population, the average life span for individuals with serious mental illness in the US is only 56 years (Insel, 2010).

The total annual combined direct and indirect cost of schizophrenia in the US is estimated to be $67.2 billion (Wu et al., 2005). As a result, schizophrenia has become a major public health concern. Efforts that focus on interventions that identify individuals with the earliest manifestation (Insel, 2009) of schizophrenia and/or reduce probability of relapse, should lead to better clinical and economic outcomes in the management of schizophrenia. Unfortunately, none of the current treatments are completely effective

and few patients achieve complete remission, with even fewer achieving complete remission for extended time periods. In addition, rates of treatment noncompliance and likelihood of relapse is very high in this patient population (Lindenmayer et al., 2009; Clayton et al., 2010; Panish et al., 2013).

One reason for the relatively slow progress in uncovering the etiology of schizophrenia (a prerequisite for designing more effective treatments) is the heterogeneity inherent to the condition. Schizophrenia is now thought to be a class of syndromes rather than a single disease. Given this, it is clear that future studies need to better identify the syndromes within schizophrenia. The purpose of this project, therefore, was to investigate the possible genetic contribution of selected single nucleotide polymorphisms to a clinical presentation characterized by several related traits: perceptual organization impairment, poor premorbid (i.e., pre-diagnosis) social functioning, and a tendency to express disorganized symptoms when psychotic. The following sections will review the relevant literature pertaining to cognition, heterogeneity, visual perceptual grouping, premorbid social functioning, hypothesized perceptual organization neurotransmitter systems, single nucleotide polymorphisms and cognitive endophenotypes in schizophrenia.

1.2 Cognitive Deficits in Schizophrenia

Cognitive deficits are a hallmark of schizophrenia, which is typically diagnosed in late adolescence or early adulthood, although evidence of premorbid social, cognitive, and academic abnormalities have been reported prior to onset in many studies (Murray et al., 1992; Schenkel & Silverstein, 2004; Mesholam-Gately et al., 2009). There are multiple cognitive impairments associated with schizophrenia and these include but are not limited to: working memory, executive functioning, auditory or visual perception and memory,

etc. A number of studies have also established that cognitive symptoms may be the most disabling for many individuals with schizophrenia because they lead to poor treatment and functional outcomes (e.g., reduced benefit from psychosocial treatment, reduced ability to work, poorer academic functioning, poorer social skills, etc.) (Addington & Addington, 1993; McGurk & Mueser, 2004; Green, 2006). Therefore cognitive deficits have become a primary target for treatment (Gold, 2004; Dickinson et al., 2010; Keefe et al., 2011; Goff et al., 2011; Keefe et al., 2012; Minzenberg & Carter, 2012).

1.3 Schizophrenia is a Heterogeneous Disorder

Schizophrenia is a disorder that is heterogeneous in nature. Specific cognitive, behavioral and genetic abnormalities have only been found in subpopulations of patients studied (Carpenter & Buchanan, 1994; Heinrichs, 2001; Sebat et al., 2009; Raffard & Bayard, 2012). The small effect sizes observed for cognitive, genetic and pharmacological studies of schizophrenia may be due to this heterogeneity. Reducing heterogeneity by focusing on subsets of patients characterized by multiple cognitive and clinical factors can improve our understanding of the role of specific psychological and biological characteristics in different syndromes within schizophrenia (Bleuler, 1911/1950; Silverstein, 2008) and may lead to the design of treatments with greater specificity.

Assessment of specific cognitive impairments in schizophrenia has had limited success since performance is confounded in many paradigms by multiple cognitive processes (Knight & Silverstein, 2001; MacDonald & Carter, 2002; Carter, 2005; Silverstein, 2008) and other factors such as low motivation, medication-related sedation, anxiety, etc. (Chapman & Chapman, 1978; Silverstein, 2008). Thus far, most cognitive impairments have not been convincingly demonstrated in schizophrenia to be independent of a

generalized deficit. Some notable exceptions include context processing in working memory (MacDonald et al., 2005) and altered visual perceptual organization ability (Place and Gilmore, 1980; Silverstein & Keane, 2011).

1.4 Perceptual Organization Deficits and Poor Premorbid Functioning in Schizophrenia

Perceptual organization is the process of binding stimulus features into a global form early in visual processing (typically within the first 200 ms after stimulus exposure) (Place & Gilmore, 1980; Silverstein et al., 1996; Silverstein et al., 2000). Perceptual organization deficits have consistently been found in schizophrenia (e.g., Place & Gilmore, 1980; Wells & Leventhal, 1984; Silverstein et al., 1996; Phillips & Silverstein, 2003; Uhlhaas & Silverstein, 2005; Silverstein & Keane, 2011) and have been associated with a more severe prognosis, poor premorbid functioning, and also with disorganized symptoms (Silverstein & Knight, 1998; Silverstein et al., 2000; Uhlhaas & Silverstein, 2005; Silverstein & Keane, 2011) but not positive or negative symptoms (Knight & Silverstein, 2001).

It has been hypothesized that deficits in perceptual organization are part of a widespread impairment in coordination of cognitive and neural processes in schizophrenia (Phillips & Silverstein, 2003). Cognitive coordination is the process that binds components of mental representations based on contextual relationships. It involves modulating feedforward activity based on various types of context (e.g., temporal, spatial, memory, expectations, etc.) without changing the nature of the feedforward signals themselves (Phillips & Singer, 1997; Olypher et al., 2006; Kay & Phillips, 2010). Cognitive disorganization, or impaired cognitive coordination, is believed to be a core deficit in the

disorganized syndrome of schizophrenia (Phillips & Silverstein, 2003) based on past findings of significant relationships between perceptual organizations deficits and increased cognitive and/or behavioral disorganization, but not positive or negative symptoms (Uhlhaas & Silverstein, 2005; Silverstein & Keane, 2011).

However, specificity is needed in applying the cognitive coordination construct (Phillips & Silverstein, 2003-open peer commentary by Daniel C. Javitt). Disorganization is considered a heritable symptom dimension in schizophrenia (Reitkerk et al., 2008) and it is linked to patients with histories of poor premorbid adjustment (Wickham et al., 2001). Moreover, disorganized symptoms and perceptual organization impairments that correlate with these have been hypothesized to be rooted in altered modulatory activity, based on hypofunction at NMDA and GABA receptors (Phillips & Silverstein, 2003). However, the extent to which genetic changes related to NMDA and GABA circuitry functioning predict variance in premorbid functioning, perceptual organization deficits, and disorganized symptoms has not yet been determined. This study was designed to allow for an opportunity to identify genetic correlates of a very narrowly defined phenotype, where previous literature suggests a unique genetic basis (Toulopoulou et al., 2007).

Perceptual organization impairments have consistently been associated with disorganized symptoms and poor premorbid social functioning (Place & Gilmore, 1980; Knight, 1984; Uhlhaas & Silverstein, 2005; Silverstein & Keane, 2011). Poor premorbid samples have been traditionally defined by deficits in social competence, flat affect inappropriate affect and poor response to treatment, and premorbid status was assessed by using scales of premorbid adjustment that ascertain levels of social functioning such as friendships,

dating, and marriage (Knight, 1984). Poor premorbid social functioning has been found to increase the risk for developing psychosis (Dragt et al., 2011) and emergence of disorganized symptoms (Wickham et al., 2001; Schenkel et al., 2005a).

In addition, recent reports suggest that variation in premorbid functioning may be useful to account for heterogeneity in schizophrenia independent of symptom factors (Cole et al., 2012). Therefore, the simultaneous study of premorbid social functioning, perceptual organization, and disorganized symptoms may be valuable for discovering potential biomarkers for neurodevelopmental traits (Uhlhaas et al., 2008) and treatment response (Silverstein & Keane, 2011), which may ultimately allow for a determination of whether there is a subtype of patient characterized by these impairments.

1.5 Neurotransmitter Circuitry Linking Perceptual Organization and Schizophrenia

Although many neurotransmitter systems including dopamine, glutamate, GABA, serotonin and acetylcholine are implicated in schizophrenia, there is evidence to suggest that many cognitive and physiological deficits in schizophrenia have a basis in the glutamatergic/GABAergic system. The strongest support for altered glutamatergic system function in schizophrenia comes from evidence of NMDA receptor hypofunction (Lisman et al., 2008). Pharmacological blockade of the NMDA receptor with antagonists such as PCP or ketamine exacerbates many symptoms of schizophrenia and can induce schizophreniform behaviors in individuals without a psychiatric diagnosis (Javitt & Zukin, 1991). Therefore, many recent human and animal studies have used NMDA receptor antagonists to model schizophrenia (Coyle, Tsai & Goff, 2003; Javitt, 2004; Anticevic et al., 2012; Driesen et al., 2013).

Hypofunction of NMDA receptors results in reduced activity in GABAergic inhibitory interneurons (Lewis & Moghaddam, 2006; Gonzalez-Burgos & Lewis, 2008), which leads to reduced inhibition onto pyramidal cells, and hence excessive glutamate release in cortical regions (Javitt & Zukin, 1991; Olney & Farber, 1995; Moghaddam et al., 1997; Roopun et al., 2008; Benes, 2009; Behrens & Sejnowski, 2009; Bitanihirwe et al., 2009; Moghaddam & Javitt, 2012). The loss of inhibition also leads to a hyperstimulation in primary corticolimbic networks which are suggested to be associated with both positive and negative symptoms of schizophrenia (Lisman et al., 2008). The dysfunction of these receptors leads to overstimulation in both the glutamatergic and dopaminergic systems. Therefore, the dopaminergic and glutamatergic systems are interrelated in schizophrenia (Carlsson & Carlsson, 1990; Javitt & Zukin, 1991; Flores & Coyle, 2003).

Of particular relevance to this proposal, GABAergic interneurons are involved in synchronizing oscillations (Bartos et al., 2007). Neural synchrony is involved in mediating temporal patterns of cognitive processing, and reduced synchrony has been related to perceptual organization deficits in schizophrenia (Uhlhaas & Singer, 2006; Uhlhaas, 2013). A loss of parvalbumin positive GABAergic interneurons in particular has been found to reduce neural oscillations (Lodge et al., 2009; Spencer, 2009; Woo et al., 2010) and lead to cognitive symptoms (Cho et al., 2006) in schizophrenia, including perceptual deficits.

1.6 Gender Differences in Schizophrenia

A limitation of many perceptual and cognitive studies in schizophrenia is that few account for individual performance differences or heterogeneity. However, varied findings among schizophrenia patients suggest that individual and subset differences

should be highlighted in current and future studies of the disorder. For example, gender differences have been associated with a number of factors related to schizophrenia including premorbid functioning (Childers & Harding, 1990; Goldberg et al., 2011; Ochoa et al., 2012), age of onset (Walker et al., 2002; Goldberg et al., 2011; Zhang et al., 2012), symptomatology (Goldstein et al., 1988; Walker et al., 2002; Zhang et al., 2012), relapse rate (Ochoa et al., 2012), cognitive ability (Weiser et al., 2000; Hoff & Kremen, 2002), treatment response (Angermeyer et al., 1990; Buchsbaum et al., 1992; Keks et al., 2002), and substance abuse (Mahoney et al., 2010). A number of reports theorize an influence of gender in the development of schizophrenia pathogenesis (Goldstein et al., 1989, 1990a, 1990b, 1998; Seeman & Lang, 1990; Hoff & Kremen, 2002; Walker et al., 2002; Goldberg et al., 2011; Walder et al., 2012; Walder et al., 2013). Of note, gender differences have been reported in non-clinical populations on the Ebbinghaus illusion (Phillips et al., 2004) on which schizophrenia patients have demonstrated significant impairment (Uhlhaas et al., 2006).

1.7 Single Nucleotide Polymorphisms in Schizophrenia

Molecular genetics currently supports a complex polygenic model of schizophrenia that involves many common SNPs that are shared with other psychiatric diseases but are not common to most non-psychiatric diseases (Purcell et al., 2009; Williams et al., 2010). Genome Wide Association (GWA) studies utilize a high throughput sequencing method and are useful for reporting initial findings. The advantage of GWA study is that possible association in many genes can be determined quickly. However, based on the current data in HapMap, more than a million SNPs are needed to effectively tag genes (Altshuler et al., 2008). In addition, some of the SNPs that are missing from current array platforms

are also not in linkage disequilibrium with any SNPs used as tags. Since many hypotheses are being tested in GWA studies, a large number of samples and multiple testing corrections are needed. Overall association to schizophrenia susceptibility has been difficult to study even as new GWA analyses techniques are being employed. Therefore, new strategies to determine susceptibility genes are needed.

Common SNPs associated with cognitive traits account for a small amount of variance and require targeted strategies for gene investigation (Kempf & Weinberger, 2009). *A priori* selected SNPs take advantage of increased statistical efficiency of association analysis of complex diseases and the biological understanding of phenotype, tissues, genes and proteins that may be involved in the disease (Tabor et al., 2002). The *a priori* approach is considered most valuable for determining allelic expression and/or association to endophenotypes. Therefore, this approach was thought to be particularly useful for reducing heterogeneity by characterizing the genetic basis of a syndrome characterized by cognitive (perceptual), psychosocial developmental (premorbid functioning), and clinical features (disorganization symptoms). However, SNPs that capture many putative causal alleles have different statistical properties that those that capture a single site (de Bakker et al., 2005) and this among the factors noted above is an important consideration before undertaking a GWA or *a priori* SNP study.

1.8 Cognitive Endophenotypes in the Study of Schizophrenia

Current literature linking genotypes to phenotypes indicates that genetic risk variants do not suggest a linear relationship with current diagnostic categories. The approach of using symptoms within a broader diagnostic construct suggests that they are relevant to neuropsychiatric diseases generally, but may or may not be specific to schizophrenia.

This is of concern since genetic effects are extensively reduced by modest phenotypic error (Ioannidis et al., 2009). In contrast, intermediate phenotypes for schizophrenia and other disorders are characterized by a firmer grounding in each disorder's neurobiology. Recent studies have focused on finding possible cognitive and clinical endophenotypes that correlate to susceptibility genes. It is thought to be easier to identify genes associated with specific endophenotypes, than to a diagnosis of schizophrenia, since endophenotypes are thought to be more closely related to the underlying neuropathology than are symptoms, whose etiology may be more multifactorial (Cannon & Keller, 2006; Braff et al., 2007).

However, no cognitive test has yet to be considered an endophenotype with high specificity for molecular mechanisms related to schizophrenia (DeLisi, 2009). A major hindrance in the study of cognitive endophenotypes is that patients are heterogeneous with regards to their cognitive abilities/deficits, and clinical presentations (e.g., symptoms, developmental trajectory). In addition, the neurodevelopmental and state dependent contributions to cognitive task performance are not well known. Elucidating the role of specific genetic variants associated with cognitive and clinical traits is an important step towards improving the risk factors for schizophrenia.

Premorbid cognitive deficits are thought to be relatively stable over the course of schizophrenia (Reichenberg et al., 2010; Allott et al., 2011) and may also be related to the neurobiological basis of schizophrenia (Keshavan et al., 2010), supporting the idea that impairments related to poor premorbid functioning, such as perceptual organization deficits, could be potential endophenotypic markers for genetic study. It is important to note that although level of perceptual organization impairment has been suggested to

covary with level of disorganized symptoms, and to decrease during short-term inpatient treatment, it is still present even among clinically stable outpatients with few disorganized symptoms (Silverstein et al., 1996; Uhlhaas et al., 2005; Silverstein et al., 2009).

1.9 Study Aim

The aim of this study was to determine whether: 1) specific genetic alterations related to glutamate and GABA transmission are associated with cognitive and clinical features that have themselves been linked in past studies: poor premorbid social functioning, perceptual organization impairment, and disorganized symptoms; and then 2) investigate the genetic correlates of this combined phenotype. This was completed by focusing on a domain of cognition, perceptual organization, where impairment in schizophrenia has been associated with both poor premorbid social functioning and disorganized symptoms. *A priori* SNPs in Neuregulin 1 (NRG1), Dystrobrevin Binding Protein 1 (DTNBP1) and Glutamate Decarboxylase 1 (GAD1) genes were analyzed to determine if the presence of specific genotype subgroups correlated with the incidence of clinical or perceptual deficits.

1.10 Hypothesis

The proposed study was designed to address the heterogeneity of cognition and genetics in schizophrenia by using cognitive tasks that minimize generalized deficit confounds, and by selecting SNPs within genes that have a common relation to the NMDA and GABAergic pathways, and that are thought to implement the perceptual processes required by the chosen tasks. Clinical heterogeneity was accounted for in the study population by characterizing all patients on the basis of level of premorbid functioning.

Although there have been studies with negative association (Schirmbeck et al., 2008), premorbid functioning is thought to have a genetic basis (Gornick et al., 2005; Addington et al., 2005; Addington et al., 2008). Therefore, I hypothesized that abnormalities in genes related to NMDA and GABA functioning would be associated with perceptual organization impairment, poor premorbid social functioning, and disorganized symptoms.

1.11 Candidate Genes for SNP Selection

NRG1 was first identified as a possible candidate gene for schizophrenia by Steffanson et al. (2002). Subsequent studies have also suggested a significant association between NRG1 gene and schizophrenia. Therefore, neuregulin1 is considered a potential schizophrenia susceptibility molecule. The NRG1 gene is relevant both to schizophrenia neurocircuitry and the study hypothesis since Nrg1/ErbB4 signaling regulates GABAergic transmission in the adult cerebral cortex, subsequently influencing inhibitory cortical function (Rico & Marin, 2011). It is also implicated in the regulation of NMDA receptors in the prefrontal cortex (Gu et al., 2005).

DTNBP1 was first identified as a possible candidate gene in schizophrenia by Straub et al. (2002). Many other protein and gene expression studies have also implicated dystrobrevin as another possible schizophrenia susceptibility molecule. Dystrobrevin is involved in regulation of surface expression of the NMDA NR2A receptor subunit in the hippocampus (Tang et al., 2009) and is expressed at high levels in the cerebral cortex and hippocampus (Blake et al., 1998). DTNBP1 may affect cognitive function through multiple biochemical pathways (Guo et al., 2009). DTNBP1 has also been shown to have a general influence on cognitive ability (Burdick et al., 2006; Burdick et al., 2007; Zinstock et al., 2007; Zhang et al., 2010).

GAD is the primary enzyme involved in the synthesis of GABA in inhibitory neurons. GAD has two different isoforms, GAD65 and GAD67. GAD67, a GABA synthesizing enzyme, is regulated by the GAD1 gene and GAD67 protein expression has been shown to be reduced in the brains of schizophrenia patients (Guidotti et al., 2000; Addington et al., 2005). GABA concentrations have been found to be reduced in the visual cortex in schizophrenia (Yoon et al., 2010), making it a potentially relevant gene for visual perceptual organization. In addition, GAD67 has also been shown to be reduced in other cortical areas of patients with schizophrenia (Lewis et al., 1999; Coyle, 2006), making GAD1 a likely candidate gene for schizophrenia.

Based on data from the studies described above and others, the three genes we have selected code for proteins that are relevant to perceptual organization circuitry. Although a recent study demonstrated that postnatal deletion of NMDA NR1 from parvalbumin positive corticolimbic GABAergic interneurons in mice resulted in a schizophrenia-related phenotype of increased firing of cortical excitatory neurons and reduced neuronal synchrony (Belforte et al., 2010), the specific SNPs chosen *a priori* for this study are most relevant to perceptual organization deficits in schizophrenia because they are non-synonymous SNPs or SNPs associated with premorbid functioning (Gornick et al., 2005; Addington et al., 2005; Addington et al., 2007). Recent studies have also suggested that studying surrounding molecules (e.g. targeting the glycine site of the NMDA receptor) may be better for development of potential therapeutic targets than the NMDA receptor itself (Javitt, 2004; Javitt et al., 2008).

Chapter 2: Materials and Methods

2.1. Study Sample Characteristics

The study was approved by the UMDNJ Institutional Review Board and written consent was obtained for all study participants. The study sample consisted of female and male Caucasian and African American patients who met DSM IV-TR (American Psychiatric Association, 2000) criteria for schizophrenia or schizoaffective disorder. Individuals with current substance abuse, mental retardation, neurological disorders, or other affective disorders were excluded from the study.

Patients were recruited from three levels of care within a vertically integrated system (Smith et al., 1999): 1) acute partial hospital (most recent inpatient discharge was within the past 6 months and includes full daily structure and treatment); 2) extended partial hospital (where the last inpatient discharge ended over 6 months ago, but daily treatment and structure are still required); and 3) outpatient (where last inpatient discharge was over two years ago and patients require regular visits to psychiatric care providers). Patients ranged in age from 21 to 64.

2.2. Clinical Assessments

2.2.1 Diagnostic Interview for Genetics Studies

The Diagnostic Interview for Genetic Studies (DIGS) (Nurnberger et al., 1994) was administered in order to determine study eligibility. Psychiatric diagnoses for all participants were confirmed via review of UMDNJ-UBHC medical records.

2.2.2 Positive and Negative Syndrome Scale

Symptoms occurring 2 weeks prior to testing were assessed using the PANSS (Kay et al., 1986). Study participants were dichotomized based on their conceptual disorganization item score (Uhlhaas et al., 2006a, 2006b). All participants with a score of 2 or lower (i.e., within the normal range) were included in non-disorganized schizophrenia group (n = 36) and participants with a score of 3 or greater (i.e., mild or greater) comprised the disorganized schizophrenia group (n = 58). PANSS syndromes were based on the five factor model of Lindenmayer et al. (1994), which includes positive, negative, cognitive/disorganized, excitement, and depression factors. This model has shown stability across age ranges, cultures, and is relatively free of medication effects (Lindenmayer et al., 1994). A separate disorganization factor described by Cuesta & Peralta, (1995) was also derived and included scores for the following symptoms: poor attention, conceptual disorganization and inappropriate affect.

2.2.3 Premorbid Adjustment Scale

Psychosocial adjustment/functioning and academic development were evaluated using the PAS (Cannon-Spoor et. al., 1982). Each item was scored on a scale of 0 (good) to 6 (poor) from early childhood until one year prior to the onset of first psychotic symptoms or one year prior to first psychiatric hospitalization if exact onset could not be determined. Scores on 5 domains of functioning: social isolation, peer relationships, scholastic performance, adaptation to school, social-sexual aspects of life and an overall mean score of all domains (Cannon-Spoor et al., 1982) were calculated.

2.2.4 Medication Form

Study participants were taking atypical and/or typical antipsychotic medications with stable medication dosage. Antipsychotic medication dosages were converted to

chlorpromazine equivalents based on published standards (Andreasen et al., 2010). Correlations of antipsychotic dosages to task performance were determined.

2.2.5 Nicotine Assessments

There are numerous reports of increased use of nicotine in schizophrenia (Williams & Gandhi, 2008). Effects of these substances on arousal and cognition are significant, and may complicate the interpretation of clinical and cognition data in schizophrenia (George et al., 2002; Harris et al., 2004; Jacobsen et al., 2004; LeDuc & Mittleman, 1995; Silver et al., 2002). Recent and typical tobacco use was assessed with a smoking history and the Fagerström Test for Nicotine Dependence (Heatherton et al., 1991) in order to determine the effects of nicotine use or dependence on perceptual task performance.

2.2.6 Shipley Vocabulary Subtest B

The vocabulary subtest of the Shipley Institute of Living Scale (Zachary, 1991) was administered to participants in order to estimate IQ by converting raw scores into a WAIS-R IQ score.

2.2.7 Visual Acuity Assessment

A Snellen chart was used in order to ascertain visual acuity estimates for each eye separately (with the other eye covered) and then both eyes.

2.2.8 Handedness Assessment

Handedness was ascertained with a questionnaire about hand preference for different daily tasks including: writing, throwing, using scissors, etc. Participants were noted as right handed or left handed based on these responses.

2.3. Perceptual Stimuli

Previous studies of perceptual organization in schizophrenia have used simple stimuli with controlled conditions to improve our understanding of the developmental and neural basis of perceptual organization in schizophrenia (Silverstein & Keane, 2011). Therefore, the tasks described below are thought to be useful in the study of neural circuitry, genetics and possible treatments in schizophrenia. Prior studies of perceptual organization suggest that it occurs at multiple stages of processing and on multiple forms of representations (Fink et al., 1997; Lumer & Rees, 1999; Altmann et al., 2003). Therefore, four different tasks were included to assess a range of perceptual organization stages. All tasks were presented on twenty or 22 inch LED monitors with a frame rate of 60 Hz. Subjects were seated ~2 feet from the screen to maintain a visual angle of ~1 degree for each target stimulus. The brightness for all monitors (gamma was set to 1.0 and the white point was set to 6500K) was calibrated using Spyder software. The order of presentation of the tasks and conditions were counterbalanced across participants.

2.3.1 Contour Integration-JOVI Task

The Jittered Orientation Visual Integration (JOVI) task is a test of contour integration that determines a participant's ability to integrate Gabor elements into a perceptual whole. Gabor elements are sinusoidal luminance distributions that are Gaussian modulated (Silverstein et al., 2000; Uhlhaas et al., 2006). That is, Gabor elements show lower contrast at the edges compared to the center, and luminance varies from white to black in a gradually alternating fashion (Figure 1). Gabor elements are considered to activate orientation-selective feature detectors in the primary visual cortex (Angelucci & Bullier,

2003), and are therefore a useful means to study their integration in early visual processing.

The stimuli presented for this task are based on the study methods of Silverstein et al. (2012). Participants were shown static Gabor elements arranged in an oblong shape forming a contour embedded in a display of randomly oriented Gabor elements. The degree of orientation jitter of contour Gabor elements varied for each condition: (0°, 7-8°, 9-10°, 11-12°, 13-14°, 15-16°). For all stimuli, the ratio of the density of adjacent background elements to the density of adjacent contour elements was 0.9. Because adjacent background elements were, on average, closer together than adjacent contour elements, perceptual organization processes used to process contours are independent of density cues for this task (Silverstein et al., 2000).

All JOVI stimuli trials were presented for 2 seconds followed by a 1 second inter stimulus interval during which responses were no longer recorded. There were 48 stimulus trials per jitter condition which were presented in blocks of 12 trials. In addition, two types of catch stimuli (i.e., where no errors are expected) using 0° jitter were administered during each block to assess for attention lapses. One catch trial type had curved lines drawn through the contours to highlight the salience of the contour, and the other contained contour elements without any background elements to eliminate effects of distractor noise. The JOVI is a symmetric 1 alternative forced choice task where subjects responded whether the narrow end of the oblong contour was pointing left or right (Figure 1). The task and stimuli patterns were created using E-prime (Psychology Software Tools, Pittsburgh, PA).

2.3.2 Ebbinghaus Illusion Task

The Ebbinghaus illusion assesses integration of non-target information during target perception, and the resulting change in size perception of the target is thought to result from size constancy (Doherty et al., 2008, 2010). In this task (Figure 2), the perceived size of a center circle is modified by the presence of outer context circles: typically, larger context circles make the center circle appear smaller than its actual size, while smaller context circles lead to an enlarged appearance of the center circle (Phillips et al., 2004; Uhlhaas et al., 2006).

The stimuli used for this task were developed by Phillips, Doherty and colleagues (Phillips et al., 2004; Doherty et al., 2008, 2010). Participants were shown two black circles that were presented on a white background. One circle was 100 pixels in diameter and the other circle varied by 2, 6, 10, 14, or 18 pixels. These circles were presented in the presence of three different surrounding contexts: no outer context, misleading context (outer context circles should impair inner circle size discrimination by altering the perceived sizes of the center circles in a direction opposite to their actual sizes) and helpful context (outer context should aid inner circle size discrimination by amplifying the actual size difference). No context and misleading context conditions had 80 trials: 16 at each level of inner circle size difference. The Helpful context condition had 16 trials, all at a 2 pixel difference. Stimuli were presented for 2 seconds with 200 ms inter stimulus interval in a random order. The Ebbinghaus illusion task is a 2-alternative forced choice task where subjects responded whether the left center circle or right center circle was larger (Figure 2). The task and stimuli patterns were created using C++.

2.3.3 Contour Interpolation

Study participants were shown a configuration of four pac-men that formed illusory/interpolated contours or a control condition that does not form a contour (Figure 3). For each trial of the interpolation experiment, contrast, retinal size, and relative spacing between adjacent elements was based on the paradigm used by Ringach and Shapley (1996). In the illusory condition, the task was for subjects to determine whether these pac-men form a "fat" or "thin" square. In the control condition, the task was to indicate whether all the pac-men are rotated clockwise or counterclockwise. Distractor lines appeared with the figure on some trials. These lines impair performance on the illusory/interpolation condition trials independent of strategy (Keane et al., 2010).

The experiment consists of two blocks depending on whether the illusory contours are present or absent. Half the trials of each block involved distractor lines. Stimuli were presented for 117 ms in counterbalanced blocks of: 64 practice trials (without distractor lines), 42 (non-practice) trials without distractor lines, and 42 trials with distractor lines. Subjects received the illusory condition in one block and the control condition in the other. Block ordering was counterbalanced across subjects. The stimulus patterns were created in MATLAB with the Psychophysics Toolbox (Brainard & Pelli, 1997), and the Palamedes Toolbox (Prins & Kingdom, 2009) provides the Bayesian staircase procedures that determined threshold and slope for each subject.

2.3.4 Numerosity Task

This task is an abridged version of the Place and Gilmore experiment and is based on the Gestalt principles of similarity and proximity (Wells & Leventhal, 1984). Place and Gilmore investigated these phenomena by testing whether schizophrenia patients respond

to organizational/grouping qualities of a stimulus. Subjects without schizophrenia are thought to preferentially organize information by grouping stimuli according to their similarity and proximity. Subjects with schizophrenia do not employ this grouping strategy as efficiently as non-psychiatric controls. Therefore, patients with schizophrenia are thought to rely primarily on serial processing (Place & Gilmore, 1980).

In the current study, subjects were presented sets of stimuli (short line segments) arranged in a regular hexagonal pattern (7 mm lines that are 15 mm apart). In one condition, all stimuli were presented horizontally or vertically (homogeneous condition). In the other condition, the stimuli were presented with a combination of horizontal and vertical stimuli (heterogeneous-non adjacent condition). All stimuli were presented for 15 milliseconds. The homogeneous and heterogeneous oriented stimuli were presented in random order (Figure 4). Subjects determined the number of lines shown in each stimulus trail from a range of 0 to 6 lines. There are three practice blocks, each containing 10 stimuli and 3 experimental blocks which contain a total of 264 stimuli trials. The stimuli were created in MATLAB using the Psychophysics Toolbox (Brainard & Pelli, 1997). The computerized program determined the response time and accuracy for each stimulus set.

2.4 DNA Extraction and Genotyping

We selected a total of 8 SNPs from 3 candidate genes (DTNBP1, NRG1 and GAD1) shown in Table 1. The criteria stated below were used to determine the specific SNPs selected:

- SNPs selected had known MAF of 5% or greater in Caucasians and were targeted by the HapMap project.
- All Selected SNPs had previously shown allelic variation.
- All SNPs had shown positive association in schizophrenia.
- All SNPs selected are associated with the NMDA or GABAergic neurotransmitter systems as alterations in these systems have previously been shown to be related to cognitive deficits in schizophrenia.
- SNPs were also considered based on the continuously updated meta-analysis of genetic studies for schizophrenia, available at www.szgene.org (Allen et al., 2008) and association to premorbid functioning (Gornick et al., 2005; Addington et al., 2005; Addington et al., 2008) (see Tables 3 and 4).

DNA from saliva samples of consenting participants was extracted and genotyped at the University of Toronto Center for Applied Genomics using Oragene kits (DNA Genotek Inc, Ottawa, Ontario, Canada) after all clinical and cognitive testing was completed at UMDNJ-UBHC. All DNA extraction and genotyping was done blind to psychiatric diagnoses. An Autopure LS Gentra/Qiagen DNA extractor running puregene chemistry was used to extract the DNA from the saliva samples. The total DNA was quantified using a flurometer and Hoescht dye. The genomic DNA was hydrated in 10 mM Tris-HCL pH 8.0, 1 mM EDTA and stored at 4°C.

107 DNA samples were genotyped using the GoldenGate® Genotyping Assay (Illumina Inc. San Diego, California, USA) according to the manufactures protocol (Fan et al.,

2006) at The Centre for Applied Genomics (Toronto, Ontario, Canada). Compatibility of the selected SNPs for the Illumina custom panel design including design score, design rank, MAF and validation status were determined. The SNPs selected were verified using the Database of Single Nucleotide Polymorphisms (dbSNP). Bethesda (MD): National Center for Biotechnology Information, National Library of Medicine dbSNP database (version 128, Jan 2008) http://www.ncbi.nlm.nih.gov/SNP/.

SNPs were uploaded to Illumina's Assay Design Tool (ADT) (http://www.illumina.com/) for probe design on a custom panel (GS0013878-OPA). The extracted DNA samples were processed in 96-well plates and each plate also contained 4 genotyping control samples. A total of 5 µl of 50 ng/µl in 10 mM Tris-HCL pH 8.0, 1 mM EDTA of genomic DNA underwent an allele specific oligonucleotide hybridization followed by extension and ligation. A universal PCR step for the 8 loci followed with primers labeled with either Cy3 (primer 1) or Cy2 (primer 2). The amplified products were then hybridized to a GoldenGate Genotyping Universal BeadChip and scanned using the Illumina iScan (Illumina, San Diego, CA).

2.5 Data Analyses

The demographic, clinical and perceptual data were compared between male and female participants and African American and Caucasian participants using independent samples t-tests (Table 1, 2 and Figures 3-6). χ^2 square tests were performed to analyze categorical variables (Table 1 and 2). Spearman correlations were performed to examine the associations among premorbid functioning, disorganization, antipsychotic dosage, smoking status and perceptual scores. In cases where significant predictors of task performance included those consistent with *a priori* hypotheses, hierarchical regression

analyses were employed in order to determine the extent to which significant predictors accounted for variance in the dependent variable over and above that of other predictors. In cases where significant predictors were those that were not predicted, stepwise regression was used to determine the order in which these predictors accounted for variance in the dependent variable.

PAS

Psychosocial adjustment and academic development were evaluated using the Premorbid Adjustment Scale (PAS) (Cannon-Spoor et. al., 1982). Each item was scored on a scale of 0 (good) to 6 (poor) from early childhood until one year prior to the onset of first psychotic symptoms or one year prior to first psychiatric hospitalization if exact age of onset could not be determined. Scores on 5 domains of functioning: social isolation, peer relationships, scholastic performance, adaptation to school, social-sexual aspects of life and an overall mean score (Cannon-Spoor et al., 1982) were calculated. Prior literature has assessed the association of perceptual deficits and premorbid functioning by using the Phillips or Zigler-Phillips scale of premorbid adjustment (Zigler & Phillips, 1961). These scales primarily used questions about friendships, dating, and marriage. For current assessments, these measures correspond to the premorbid social sexual functioning factor of the PAS, a more current and widely used scale. Because our primary interest was in premorbid social-sexual functioning, only scores on this subscale of the PAS were used for statistical analyses.

PANSS

Symptoms occurring 2 weeks prior to testing were assessed using the Positive and Negative Syndrome Scale (PANSS) (Kay et al., 1986). PANSS syndromes were analyzed

based on the five factor model of Lindenmayer et al. (1994), which includes positive, negative, cognitive, excitement, and depression factors.

As disorganization has been shown to be a strong correlate of both perceptual disorganization and premorbid functioning, disorganized symptoms were characterized in two ways. The first method is based on our previous study where significant differences in perceptual organization ability were observed when patients were dichotomized based on the PANSS conceptual disorganization item score (Uhlhaas et al., 2006a, 2006b). All participants with a score of 2 or lower were considered the non-disorganized schizophrenia group (n = 36) and participants with a score of 3 or greater comprised the disorganized schizophrenia group (n = 58) for an independent samples t-test. For Spearman correlations, the conceptual disorganization item was kept continuous.

Contour Integration

For the contour integration task, the mean score across all jitter conditions was the performance index. A previous study suggested higher test-retest reliability for the overall mean score compared to threshold values (Silverstein et al., 2012). Subjects who were unable to identify the direction of the contour at a rate less than 75%[1] for the control stimuli trails were excluded from all perceptual data analysis.

Contour Interpolation

An entropy-based Bayesian adaptive staircase procedure jointly estimated threshold and slope (α and β) over the course of trials for each condition (Kontsevich & Tyler, 1999). This procedure assumed a cumulative log-Weibull shape and a stimulus independent error rate (lapse rate) of 3%—the same error rate discovered in other lower-level perceptual tasks that involve contextual modulation (e.g., Dakin et al., 2005).

Performance was indexed by threshold scores for each condition. For the purposes of this study, the score from the illusory contour without distractor lines condition was used as the most direct measure of perceptual organization.

Ebbinghaus Illusion

For the Ebbinghaus illusion task, two difference score indices were computed: 1) [(Percent Correct Helpful Context 2 Pixel Condition) – (Percent Correct Misleading Context 2 Pixel Condition)] and 2) [(Percent Correct No Outer Context 2 Pixel Condition) – (Percent Correct Misleading Context 2 Pixel Condition)].

Numerosity Task

For the numerosity task, the effects previously reported in schizophrenia could not be replicated. In addition, ceiling effects on task performance were observed. Therefore, the task was discontinued and no further analyses were performed.

Practice Effects, Reliability and Correlation

The contour integration task had shown good internal consistency, test-retest reliability and minimal practice effects in previous studies (Pennefather et al., 1999; Strauss et al., in press). Scores on the contour integration task and Ebbinghaus illusion were previously shown to be significantly and negatively correlated among schizophrenia patients (Uhlhaas et al., 2006). Patients who showed deficits in contour integration are more accurate at judging the size of the inner circles for the Ebbinghaus illusion. Correlations among all perceptual tasks were determined as part of this study. It was expected that the scores on all psychophysical tasks would be significantly correlated with each other.

SNP Data Analysis

The genotype data from the University of Toronto Center for Applied Genomics was analyzed with GenomeStudio v.2011 using the default parameters. Only SNPs with GenCall scores greater than 0.25 were called and samples were excluded from analyses if genotype call rates were below 85%. Each SNP cluster plot was manually inspected. One SNP failed the call rate threshold, and one SNP lacked allelic variation in the study sample, leading to 6 SNPs of good quality for further analysis at UMDNJ-UBHC.

Integration of Phenotypic and Genotypic Data

Data reduction

For the remaining 6 SNPs chosen on an *a priori* basis as being most likely to be linked to the dependent variables (DVs), each subject was characterized by a score that represents the genotype for each SNP. That is, for any single independent variable, a subject was represented by 00, 10, or 11 based on genotype (6 pairs of zeros and ones). In order to reduce the number of independent variables (IVs), a Hierarchical Classes Analysis (HICLAS; Van Mechelen, DeBoeck & Rosenberg, 1995) was used to identify patterns in the data, both among the participants (row bundles) and alleles/genotypes (column bundles). HICLAS is a general structural model for two-way two-mode matrices with binary (0, 1) entries. Following this step, each subject was assigned to the class or cluster that was the closest match to their observed scores. These clusters were then used as the IVs in a series of MANCOVAs.

HICLAS examination of the strength of relationships between variables

For each cluster solution MANCOVA, the IV represented the patient cluster; the DVs were: 1) overall score on the Premorbid Adjustment Scale 2) the perceptual organization indices; and 3) the PANSS P2 Conceptual Disorganization item. The MANCOVAs clarified whether the clusters generated based on genotypes were associated with differences in the primary DVs. MANCOVAs were also performed, using each genotype rather than cluster as the 3-level IV, if allelic variation was present for each SNP. A 3 cluster HICLAS solution was successful in categorizing patients into fewer than 6 subgroups based on the genotype data. However, due to racial variation of genotypes, MANCOVAs with genotype as the 3-level IV for each SNP were performed as an additional measure. Due to the exploratory nature of the study, the *p* levels reported for all of the MANCOVAs are uncorrected.

[1] In the correlational analyses, all reported variables that showed a significant correlation when using a 75% threshold remained correlated with the 90% threshold. All perceptual indices still suggest significant differences based on gender with a 90% correct threshold: Total JOVI Score $t(83) = 2.484$, $p = .015$; Ebbinghaus Helpful-Misleading Index: $t(57.746) = 2.894$, $p = .005$; Ebbinghaus No outer-Misleading Index: $t(83) = -2.564$, $p = .012$. Therefore the perceptual organization differences reported are considered robust to moderate attention deficits and thresholds were not changed for analyses with genotypic data.

Chapter 3: Results

3.1 Demographic and clinical differences

3.1.1 Gender differences in study sample

Female and male participant demographic and clinical characteristics are shown in Table 1. Females had significantly better premorbid social sexual functioning, were more likely to have been married and have outpatient status than males. There was a non-significant trend for fewer conceptual disorganization symptoms and greater left-handedness in the female study sample. Gender differences were also observed for PAS premorbid social sexual functioning subscale scores. Female participants had better premorbid social sexual functioning compared to males: $t(87) = -2.71$, $p = .008$, $d = -0.57$. There were no significant differences between genders in overall PAS score: $t(87) = -1.32$, $p = .191$ (Figure 5). There was a non-significant trend of gender differences for conceptual disorganization symptoms: $t(92) = -1.79$, $p = .077$.

3.1.2 Racial differences in study sample

African American and Caucasian participant demographic and clinical characteristics are shown in Table 2. African American participants were younger, had a lower education level, had lower Shipley vocabulary subtest scores, were more likely to be married and more likely to be in acute or extended partial hospital programs than Caucasian participants. Non significant trend level differences were observed for better premorbid social sexual functioning, higher levels of conceptual disorganization symptoms, and more typical antipsychotic medication use in the African American study sample.

3.2 Contour Integration Results

3.2.1 Significant Correlations and Differences

Gender differences were observed for total JOVI performance with females scoring higher than males: $t(94) = 3.02$, $p = .003$, $d = 0.68$ (Figure 5).

JOVI task performance was lower in the disorganized group (PANSS conceptual disorganization item score of 3 or higher) compared to the non-disorganized group (PANSS conceptual disorganization item score of 2 or lower) (Figure 8): $t(93) = 2.18$, $p = .032$; $r(95) = -.27$, $p = .008$.

The set of variables significantly correlated with lower total JOVI scores included: male gender ($r(96) = -.29$, $p = .004$), poor premorbid social-sexual functioning ($r(96) = -.30$, $p = .004$), right handedness ($r(96) = -.25$, $p = .014$), marital status ($r(96) = .22$, $p = .032$) and higher conceptual disorganization ($r(94) = -.27$, $p = .008$). Marital status was also significantly correlated with premorbid social-sexual scores $r(90) = -.33$, $p = .002$.

Hierarchical regression analyses were employed to determine the extent that newly correlated factors (gender and handedness) could predict contour integration performance compared to previous correlated factors (premorbid social functioning and disorganized symptoms) in our study sample. The factor that explained the most variance in JOVI performance was premorbid social sexual functioning: $R^2 = .13$, $F = 13.00$, $p = .001$. While gender, disorganized symptoms and handedness factors contributed to total JOVI score $R^2 = .16$, $F = 5.37$, $p = .002$, premorbid social sexual functioning still added a unique variance over and above these other factors: R^2 *change* $= .05$, $F = 5.56$, $p = .021$.

3.2.3 Contour Integration Non Significant Differences

No significant correlations were observed between total JOVI score and age: $r(96) = -.12$, $p = .231$; current smoking status: $r(96) = -.03$, $p = .754$; or chlorpromazine equivalents of antipsychotic medication dosage: $r(94) = -.11$, $p = .304$.

Although premorbid social-sexual functioning domain scores were related to contour integration task performance, overall mean PAS scores did not significantly correlate with scores on contour integration: $r(89) = -.052$, $p = .629$.

3.3 Ebbinghaus Illusion

3.3.1 Significant Correlations and Differences

On the Ebbinghaus illusion task, females were less context sensitive than males: Helpful-Misleading: $t(60) = -2.80$, $p = .010$, $d = -0.58$; Control-Misleading: $t(94) = -2.23$, $p = .028$, $d = -0.47$ (Figure 4).

The set of variables significantly correlated with greater sensitivity to Ebbinghaus illusion performance were: female gender: Helpful-Misleading: $r(96) = .261$, $p = .010$, Control-Misleading: $r(96) = .250$, $p = .014$; higher PANSS negative factor scores: Helpful-Misleading $r(94) = .286$, $p = .005$; Control-Misleading: $r(94) = .297$, $p = .004$; and higher Shipley Subtest B scores: Helpful-Misleading: $r(96) = .215$, $p = .036$; Control-Misleading: $r(96) = .187$, $p = .069$.

3.3.2 Ebbinghaus illusion performance varies based on gender and PANSS negative factor scores

Since there was no *a priori* hypothesis as to how these factors would contribute to variance in Ebbinghaus illusion index scores, a stepwise regression was performed. The stepwise analyses indicated that the combination of these factors accounted for significant variance in Ebbinghaus illusion scores: Helpful-Misleading Index: $R^2 = .17$, $F = 5.92$, $p = .001$; No outer-Misleading Index: $R^2 = .14$, $F = 4.77$, $p = .004$. However, Shipley subtest scores did not contribute significantly to prediction of Ebbinghaus scores over and above gender and negative symptoms: Helpful-Misleading Index: B (gender) =

.87, $t(94) = 1.61$, $p = .11$; No outer-Misleading Index: B (gender) = .47, $t(94) = 1.51$, $p = .14$.

3.3.3 Ebbinghaus Illusion Non Significant Differences

No significant differences were observed between the disorganized vs. non disorganized groups on the Ebbinghaus illusion indices: Helpful-Misleading $t(92) = -.191$, $p = .849$; Control-Misleading $t(92) = -.56$, $p = .577$.

No significant correlations were observed between Ebbinghaus task scores and age: Helpful-Misleading $r(96) = .06$, $p = .572$ and Control-Misleading $r(96) = -.07$, $p = .512$ current smoking status; Helpful-Misleading $r(96) = -.11$, $p = .293$; Control-Misleading $r(96) = -.04$, $p = .688$; or chlorpromazine equivalents of antipsychotic medication dosage: Helpful-Misleading $r(94) = -.02$, $p = .881$; Control-Misleading $r(94) = .02$, $p = .815$.

Although premorbid social sexual functioning domain scores were related to contour integration task performance, PAS social sexual factor scores were not significantly correlated with Ebbinghaus indices: Helpful-Misleading Index: $r(90) = .115$, $p = .281$; Control-Misleading Index: $r(90) = -.018$, $p = .864$. Overall PAS scores were also not significantly correlated with Ebbinghaus indices: Helpful-Misleading Index: $r(89) = -.174$, $p = .102$; Control-Misleading Index: $r(89) = -.173$, $p = .105$.

No significant differences were observed Ebbinghaus Helpful-Misleading Index based on race: $t(91.99) = -1.253$, $p = .213$ and the Control-Misleading Index of the Ebbinghaus illusion: $t(91.99) = -1.483$, $p = .112$

3.4 Contour Interpolation

3.4.1 Significant Correlations and Differences

Contour interpolation illusory without distractor line task performance was lower in the disorganized group (PANSS conceptual disorganization item score of 4 or higher) compared to the non-disorganized group (PANSS conceptual disorganization item score of 3 or lower): $t(93) = -2.48, p = .015$.

The set of variables significantly correlated with poorer illusory without distractor line performance included: increased age: $r(97) = .336, p = .001$; poorer premorbid social sexual functioning scores: $r(90) = .238, p = .024$; and higher conceptual disorganization scores: $r(95) = .202, p = .049$. Since age was significantly correlated with contour integration illusory with distractor lines, it was considered a covariate for the HICLAS and genotype MANCOVA.

However, there was no *a priori* hypothesis as to how these factors would contribute to variance in illusory without distractor lines scores, therefore, a stepwise regression was performed. The stepwise analyses indicated that the combination of these factors accounted for significant variance in threshold scores for this condition: $R^2 = .18, F = 8.84, p = .004$. However, PAS social sexual factor scores did not contribute significantly to prediction of illusory without distractor line scores over and above age and conceptual disorganization item scores: B(PAS social sexual factor) $= .18, t(90) = 1.70, p = .092$.

3.4.2 Non Significant Correlations and Differences

No significant correlation was observed between illusory without distractor line thresholds and chlorpromazine equivalents of antipsychotic medication dosage: $r(95) = .10, p = .320$; current smoking status: $r(97) = .14, p = .178$; gender: $r(97) = -.03, p = .770$; or race: $r(97) = -.02, p = .886$.

Although premorbid social sexual functioning domain scores were related to contour interpolation illusory performance with distractor lines, overall PAS scores were not significantly correlated: $r(90) = .12, p = .268$.

3.5 Genotype Data for Selected SNPs

The allele and genotype frequencies for Caucasian and African American participants are shown in Table 5 and 6. SNP locus rs11542313 had a poor genotype call rate and rs16876589 lacked allelic variation in both the Caucasian and African American study sample and was excluded from further analyses. SNP locus rs17470454 lacked allelic variation in African American participants and was excluded from the MANCOVA in African Americans.

Identity-by-state (IBS) distance of each participant was calculated by TCAG for each non study SNP that had a call rate of 95% or higher and Hardy-Weinberg equilibrium test p values larger than $1*10^{-5}$). Multidimensional scaling (MDS) analysis were performed on the matrix of the IBS pairwise distances in PLINK (http://pngu.mgh.harvard.edu/~purcell/plink). The plots show the values of each subject's position on first and second dimensions (Figure 9) and the position on the first and fourth dimensions (Figure 10). The MDS plots suggest that the participant genotype samples can be assigned to 2 clusters that roughly correspond to Caucasian and African American.

3.6 Integration of Phenotypic and Genotypic Data

3.6.1 HICLAS MANCOVA

The HICLAS analyses generated two possible cluster solutions with optimal fit scores. A 3 cluster solution has a Jaccard Index = .793 and a 7 cluster solution has a Jaccard Index = .866. The MANCOVAs of these cluster solutions are shown in Table 9. While the

contour illusory without distractor lines threshold index suggest possible association to HICLAS clusters, there was no significant associations of clusters if multiple testing corrections were applied for either cluster solution.

3.6.2 Genotype MANCOVA

Since the MDS plots suggested 2 subgroups corresponding to Caucasian and African Americans, these samples were separated for SNP genotype 3 level IV MANCOVAs. The MANCOVAs of Caucasians (Table 7) and African Americans (Table 8) are shown. Locus rs1978340 genotypes were associated with conceptual disorganization scores in Caucasians and rs3924999 genotypes were associated with Total JOVI score in African Americans but would not be significant after multiple testing corrections.

3.7 Other Significant Differences

Gender differences were also observed for PAS social sexual functioning. Female participants had better premorbid social sexual functioning compared to males: $t(87) = -2.71$, $p = .008$, $d = -0.57$. There were no significant differences between gender and overall premorbid functioning score: $t(87) = -1.32$, $p = .191$ (Figure 5).

There were also significant differences in premorbid social sexual functioning based on diagnosis of schizophrenia versus schizoaffective disorder in our study sample: $t(87.87) = -2.50$, $p = .014$. Since PAS social sexual functioning scores vary based on gender, independent samples t test were performed separately in males and females. Male participants with a diagnosis of schizoaffective disorder had better PAS social sexual scores than participants with schizophrenia $t(48.03) = -2.12$, $p = .039$. Female participants did not differ significantly on PAS social sexual scores based on diagnosis: $t(32) = -.49$, $p = .628$.

Chapter 4: Discussion

4.1 Summary of Study Findings

This study replicated a significant previous finding: impaired contour integration was associated with poorer premorbid functioning and increased disorganization symptoms (Silverstein et al., 2000; Schenkel et al., 2005b; Uhlhaas et al., 2005). In the current study, female gender was also associated with superior contour integration performance. However, hierarchical regression results indicated that premorbid social sexual functioning is a better predictor of contour integration performance than gender, handedness or disorganized symptoms in the sample. This suggests that the gender differences observed on contour integration performance are mostly in agreement with the hypothesis of a greater neurodevelopmental basis in males with schizophrenia (Goldstein et al., 1998), and with gender differences in premorbid functioning (Goldberg et al., 2011; Ochoa et al., 2012), and neurocognitive task performance (Hoff & Kremen, 2002; Johnson et al., 2005).

The gender differences on Ebbinghaus illusion task performance have not been previously reported. Moreover, unlike in our past studies with this illusion, task performance was not related to premorbid functioning or disorganization symptoms. One possible explanation for the different findings is that this study used the largest sample in any study of perceptual organization in schizophrenia to date, and so these results may be more representative. In addition, association of Ebbinghaus performance to negative symptoms in a non-inpatient population is a novel finding. The direction of gender differences in this study on the Ebbinghaus illusion where females with schizophrenia were less context sensitive that males with schizophrenia, is in opposition to findings in

the non-psychiatric population (Phillips et al., 2004) suggesting a possible interaction of gender, Ebbinghaus illusion performance and schizophrenia spectrum diagnosis. The findings noted should be considered exploratory and require replication.

4.2 Implications

Although it is controversial whether dichotomizing patients based on clinical and perceptual factors will reflect a specific etiology (Gottesman & Shields, 1982; Knight, 1984), disorganized symptoms, poor premorbid functioning and cognitive deficits such as perceptual organization, have been shown to predict a poor outcome in schizophrenia spectrum populations (Zigler & Phillips, 1961; Strauss & Carpenter, 1972; Knight, Roff, Barrnett, & Moss, 1979; Silverstein et al., 1998; Uhlhaas et al., 2006; Green, 2006; Bearden et al., 2011). Whether these relationships reflect a specific patient subtype or simply strong relationships between selected variables remains an open question. However, the data ascertained support past hypotheses that a subtype of patient characterized by these factors exists and signifies the importance of including perceptual, developmental, symptom, demographic and genetic alterations in order to better characterize heterogeneity in schizophrenia.

The results from this study do not indicate a significant correlation between contour integration and the Ebbinghaus illusion as reported by Uhlhaas et al. (2006a). This may indicate that the contour integration task and the Ebbinghaus illusion assess different stages of visual perceptual organization in schizophrenia. A possible interpretation of these findings is that contour integration reflects relatively more bottom-up than top-down contributions to perception (i.e., more stimulus-driven and lower level aspects of perception), whereas the Ebbinghaus illusion, by incorporating both grouping and size

constancy principles, involves a relatively greater influence of top-down factors including memory and past experience [see Purves and Lotto, (2011) for an account of the illusion consistent with this hypothesis, and Dima et al. (2009) for fMRI dynamic causal modeling data indicating reduced top-down involvement in another task involving experience-based modulation of perception in schizophrenia].

4.3 Study Limitations

A significant limitation of this study is that it is exploratory in nature and does not have power to show significant differences based on HICLAS subgroups. The study sample size is considerable for studies of perceptual organization, but can be considered insufficient for including multiple individual difference predictor subgroups and candidate genes or SNPs. Despite a potential lack of power, this study can provide estimates of the degree to which common variants account for variability in specific developmental, cognitive and clinical features, which can be helpful information in designing future studies that are appropriately powered.

While there were significant differences in premorbid social sexual functioning scores, overall premorbid functioning scores did not correlate with the perceptual measures in the study sample. This may be due to the fact that a very chronic sample was assessed and lacked the range of overall premorbid function that may be relevant to detecting relationships with other variables in a schizophrenia spectrum population. Although a number of factors potentially relevant to schizophrenia illness severity (perceptual grouping deficits, poor premorbid functioning, disorganized symptoms, male gender and genetic variants) were explored in this study, there are other potentially significant factors such as number of psychotic episodes, duration of untreated psychosis, family history,

trauma history, obstetrical complications, and maternal illness during pregnancy, etc. that were not taken into consideration to account for heterogeneity in the current study.

Trait versus state dependent effects on perceptual organization abilities cannot be comprehensively accounted for since inpatient participants were not included in the current study. These distinctions are important for determining whether perceptual organization tasks can be considered cognitive biomarkers or endophenotypes. It is important to note that many past studies of perceptual organization in schizophrenia used inpatients, and there is data to suggest that resistance to the Ebbinghaus illusion is related to psychotic state (Uhlhaas et al., 2006, Silverstein et al., submitted). Therefore, it is possible that the outpatient and partial hospital schizophrenia spectrum sample ascertained does not have sufficient range in Ebbinghaus illusion scores to detect relationships observed in past studies.

Psychiatric controls, non psychiatric controls, and first degree relatives were not included in the current study since our primary aim was to determine whether the phenotypes studied could generate more homogeneous subgroups for association studies in schizophrenia. Previous versions of the perceptual grouping tasks have been tested in patient and non psychiatric controls and these groups have shown significant differences compared to schizophrenia, suggesting specificity for the latter (Silverstein and Keane, 2011). However, the addition of these other populations will be necessary in order to determine whether visual perceptual organization performance and associated factors are restricted to schizophrenia spectrum diagnoses and also to verify the hypothesized heritability of perceptual grouping.

It is well recognized that false positive association in SNP studies can result for a number of reasons: individual SNPs being in linkage disequilibrium to actual causal variants, lack of appropriate multiple testing corrections or population stratification. While this study attempted to maximize the inclusion of non-synonymous SNPs, multiple testing corrections were not utilized and Ancestry Informative Markers were not included in the custom Illumina platform design, although information on grandparental origin was obtained in order to match ethnicity as much as possible. In addition, the Multidimensional scaling analyses using non hypothesized SNPs suggests two rough racial groups corresponding to Caucasian and African American in the study sample. However, within-race stratification is not accounted for in this study and may be a significant potential confound.

The *a priori* SNP association study design is limited in result replication and inclusion of all possible causative genes and polymorphisms (Tabor et al., 2002). Other disadvantages of such studies are that they cannot determine a possible disease mechanism (Kellendonk et al., 2009) or suggest regions of linkage or heritability. These limitations were not addressed due to the exploratory nature of the study and should be accounted for in future studies to determine true positive association. However, it is important to note that many models now being used to study molecular pathways in schizophrenia were generated based on genome wide and candidate gene SNP association studies.

4.4 Future Studies

The results of our study may be useful for future studies assessing gender, premorbid function and perceptual organization in at-risk, prodromal, and first episode populations. Level of chronicity was not taken into consideration for the current study. Since the

perceptual organization tasks employed have distinct developmental and neurocircuitry maturation trajectories (Kovács et al., 1999; Cisbra et al., 2000; Káldy & Kovács, 2003; Doherty et al., 2008), future studies may consider the spatiotemporal characterization of perceptual organization in early versus later stages of schizophrenia to determine if specific perceptual organization deficits in schizophrenia are primarily accounted for by altered maturation of perceptual organization circuitry or illness related perceptual organization degeneration.

While the perceptual organization tasks studied were assumed to be a possible endophenotype, this assumption relies on perceptual grouping deficits being stable and representing an underlying trait (Braff et al., 2007). While performance on some perceptual tasks such as contour integration have a significant state independent contribution, recent studies on Ebbinghaus illusion performance suggest it may be primarily mediated by state dependent effects (Silverstein et al., submitted). Future studies can help better elucidate psychotic state dependent effects associated with perceptual organization performance in schizophrenia by considering the inclusion of inpatients and exploration of epigenetic alterations or variants that may be regulators of gene expression. These factors are also significant in schizophrenia etiology and may be relevant to perceptual processes, but were considered outside the scope of the current study.

Although the gender differences observed in our study are exploratory and might be accounted for by premorbid functioning and severe illness sampling biases, future studies of perceptual grouping may help clarify the possible association of gender differences and perceptual task performance with biological factors in both schizophrenia spectrum

disorders and the non psychiatric population. Wisner et al. (2011) reported an interaction between gender, cognition and depression in unaffected siblings of schizophrenia patients suggesting the possible heritability of the interaction of gender and cognition. Markham et al., (2012) suggest that gender differences in prefrontal cortical function may be relevant for understanding neuropsychological task performance in schizophrenia. In addition, Malaspina et al. (2012) suggested an interaction of cognitive deficits, gender and olfactory differences. Therefore, future studies of perceptual deficits, and their possible association with sexually dimorphic genetic variants (Straub et al., 2007; Zhang et al., 2011) or neurohormonal circuitry (Walker et al., 2004; Walder et al., 2013) previously implicated in schizophrenia may be informative.

Overall, the study findings suggest that investigating the degree to which impairment at an early stage of perceptual organization is associated with genetic variants and predict a set of related psychosocial developmental (poor premorbid functioning), demographic (gender) and symptom (disorganized) factors may lead to characterization of a relatively homogenous group from which specific genetic alterations can be meaningfully examined in future studies. This study demonstrated that perceptual organization performance could not be accounted for by factors such as antipsychotic dosage, age, or smoking behaviors. Because previous studies indicate that patients with the characteristics noted above also have a poorer response to treatment and a more chronic illness course (Silverstein et al., 1998; Uhlhaas et al., 2006), improved insight and the development of personalized behavioral and pharmacological treatment strategies for this patient population is especially important.

Table 1. Male and Female Demographic and Clinical Characteristics.

Demographics	**Female (n = 35)**	**Male (n = 61)**	***p***
Age	47.71 (10.95)	46.44 (10.19)	.568
Estimated Age of Onset	21.56 (7.70)	22.60 (6.80)	.498
Education Level	12.44 (2.15)	12.71 (2.85)	.638
Shipley Vocabulary Subtest Score	88.49 (12.84)	90.23 (13.82)	.543
PAS Overall Average Score	2.50 (.65)	2.73 (.88)	.191
PAS Social Sexual Average Score	3.76 (2.46)	5.68 (4.24)	.008
PANSS Conceptual Disorganization Score	2.51 (1.20)	3.00 (1.31)	.077
Race (African American: Caucasian)*	13:22	31:30	.196
Handedness (Left: Right)*	8:27	5:56	.062
Current Smoker (Yes: No)*	21:14	32:29	.240
Current Antipsychotic Medications (Typical: Atypical: Both)*	5:27:3	6:45:8	.675
Current Psychiatric Program (Outpatient: Partial: Acute)*	21:12:2	27:16:18	.022
Marital Status (Single: Married: Divorced: Engaged)*	19:5:10:1	50:4:6:1	.035
*chi square test			

Table 2. African American and Caucasian demographic and clinical characteristics.

Demographics	**African American (n = 44)**	**Caucasian (n = 52)**	***p***
Age	42.91 (10.31)	50.29 (9.36)	.001
Estimated Age of Onset	21.26 (6.85)	23.04 (7.29)	.228
Participant Education Level	11.875 (2.43)	13.231 (2.62)	.010
Shipley Vocabulary Subtest Score	84.14 (13.58)	94.21(11.53)	.001
PAS Overall Average Score	2.64 (.86)	2.64 (.77)	.995
PAS Social Sexual Average Score	4.17 (3.61)	5.61 (3.81)	.071
PANSS Conceptual Disorganization	3.07 (1.32)	2.60 (1.23)	.078
Gender (Female: Male)*	13:31	22:30	.196
Handedness (Left: Right)*	6:38	7:45	.980
Current Smoker (Yes: No)*	22:22	24:28	.707
Antipsychotic Meds (Typical: Atypical: Both)*	8:29:7	3:43:4	.065
Marital Status (Single: Married: Divorced: Engaged)*	35:6:2:1	34:3:14:1	.024
Current Psychiatric Program (Outpatient: Partial: Acute)*	14:15:15	34:13:5	.002
*chi square test			

Table 3. SNPs Selected for Analysis.

Locus	**Gene**	**Ch**	**Location**	**MAF Hap Map CEU**	**MAF Hap Map YRI**	**SZGene Meta Analyses/ Significance**	**Premorbid Functioning Association**
rs10503929	NRG1	8	Coding NONSYN Met-Thr	0.225	0.0	Yes/yes	Yes (IQ only)
rs3924999	NRG1	8	Coding NONSYN Gln-Arg	0.358	0.013	Yes/no	No
rs17470454	DTNBP1	6	Coding NONSYN Pro-Ser	0.05	0.004	No/no	No
rs1047631	DTNBP1	6	3'UTR	0.18	0.167	Yes/no	Yes (PAS total only)
rs3213207	DTNBP1	6	Intron	0.117	0.022	Yes/yes	Yes (PAS sociability domain only)
rs1978340	GAD1	2	5'UTR	0.25	0.084	No/No	Yes (Hollis scale only)
rs16876589	DTNBP1	6	Coding NONSYN Gly-Asp	Not Available	Not Available	Yes/no	Unknown
rs11542313	GAD1	2	Coding SYNON His-His	0.25	Not Available	No/No	No

Table 4. SZgene meta analyses of available selected SNPs in Caucasians.

Locus	**MAF SZ**	**MAF NON PSYCH**	**Genotypes SZ**	**Genotypes NON PSYCH**
rs10503929	0.17	0.19	TT 0.686 TC 0.285 CC 0.029	TT 0.653 TC 0.310 CC 0.037
rs3924999	0.40	0.40	GG 0.359 AG 0.479 AA 0.161	GG 0.363 AG 0.472 AA 0.165
rs17470454	0.06	0.06	CC 0.887 TC 0.189 TT 0.004	CC 0.884 TC 0.112 TT 0.005
rs1047631	0.14	0.15	AA 0.735 GA 0.245 GG 0.020	AA 0.728 GA 0.250 GG 0.022
rs3213207	0.11	0.12	AA 0.791 GA 0.196 GG 0.013	AA 0.766 GA 0.219 GG 0.015

Table 5. Allele and genotype frequency in Caucasian study participants.

Locus	**Alleles**	**MAF CAU**	**Genotype Frequencies CAU**	**Hardy Weinberg Equilibrium p value**
rs10503929	T/C	0.15	CC 0.018, TC 0.263, TT 0.719	1
rs3924999	T/C	0.359	CC 0.456, TC 0.368, TT 0.175	0.171
rs17470454	G/A	0.018	AG 0.035, GG 0.965	1
rs1047631	G/A	0.125	AA 0.75, AG 0.25	1
rs3213207	G/A	0.123	AA 0.789, AG 0.175, GG 0.035	0.160
rs1978340	T/C	0.272	CC 0.526, TC 0.404, TT 0.07	1
rs16876589*	T/C	0	CC 1.0	1
rs11542313*	n/a	n/a	n/a	n/a

*excluded from further analyses

Table 6. Allele and genotype frequency in African American study participants.

Locus	Alleles	MAF AA	Genotype Frequencies AA	Hardy-Weinberg Equilibrium p value
rs10503929	T/C	0.012	TT 0.977, TC 0.023	1
rs3924999	T/C	0.126	CC 0.773, TC 0.205, TT 0.023	0.492
rs17470454*	G/A	0	GG 1.0	1
rs1047631	G/A	0.216	AA 0.614, AG 0.341, GG 0.045	1
rs3213207	G/A	0.035	AA 0.955, AG 0.023, GG 0.023	0.033
rs1978340	T/C	0.159	CC 0.727, TC 0.227, TT 0.045	0.601
rs16876589*	T/C	0	CC 1.0	1
rs11542313*	n/a	n/a	n/a	n/a

*excluded from further analyses

Table 7. Genotype association to clinical and perceptual indices in Caucasian participants.

Locus	Disorganization	PAS Social Sexual	Total JOVI	Ebbinghaus Index 1	Ebbinghaus Index 2	Interpolation Illusory No Line
rs10503929	.258	.972	.350	.724	1.000	.142
rs3924999	.859	.639	.811	.599	.532	.273
rs17470454	.116	.954	.681	.813	.720	.071
rs1047631	.688	.892	.667	.802	.870	.185
rs3213207	.716	.206	.433	.842	.665	.233
rs1978340	.019	.085	.569	.609	.606	.109

Note: All *p* values are uncorrected.

Table 8. Association of genotype to clinical and perceptual indices in African American participants.

Locus	P2 PANSS	PAS Social Sexual	Total JOVI	Ebbinghaus Index 1	Ebbinghaus Index 2	Interpolation Illusory No Line
rs10503929	.735	.107	.079	.840	.908	.080
rs3924999	.505	.184	.013	.982	.935	.118
rs17470454	n/a	n/a	n/a	n/a	n/a	n/a
rs1047631	.526	.068	.146	.812	.784	.191
rs3213207	.993	.054	.091	.664	.376	.254
rs1978340	.907	.084	.114	.096	.222	.188

Note: All *p* values are uncorrected.

Table 9. Association of HICLAS clusters to clinical and perceptual indices.

HICLAS Cluster	**P2 PANSS**	**PAS Social Sexual**	**Total JOVI**	**EBBING Index 1**	**EBBING Index 2**	**INTER Illusory No Line**
3 Cluster Solution*	.496	.331	.179	.852	.701	.013
7 Cluster Solution*	.326	.056	.154	.370	.344	.013

*Note: *p* values are uncorrected.

Figure 1. JOVI Task Stimuli. This figure illustrates the stimuli used for the JOVI task. The top left panel of the figure is an example of a lower jitter degree condition presented to participants (7-8°). The top right panel of the figure shows the highest jitter degree presented (15-16°). The bottom left and right panels show the catch stimuli included in each block to account for moderate participant attention level.

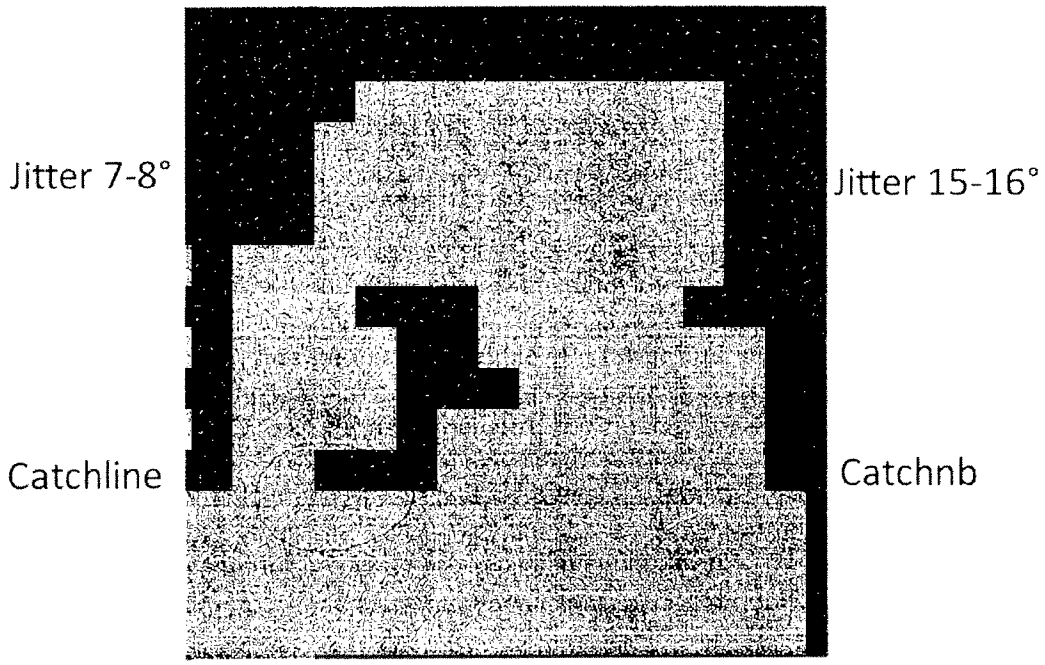


Figure 2. Ebbinghaus Illusion Stimuli. This figure illustrates the stimuli used for the Ebbinghaus illusion task. The top panel shows an example of the no outer context trials, the middle panel shows an example of the helpful context trials and the bottom panel is an example of the misleading context trials. In all three panels, the left center circle is 2 pixels smaller than the right center circle.

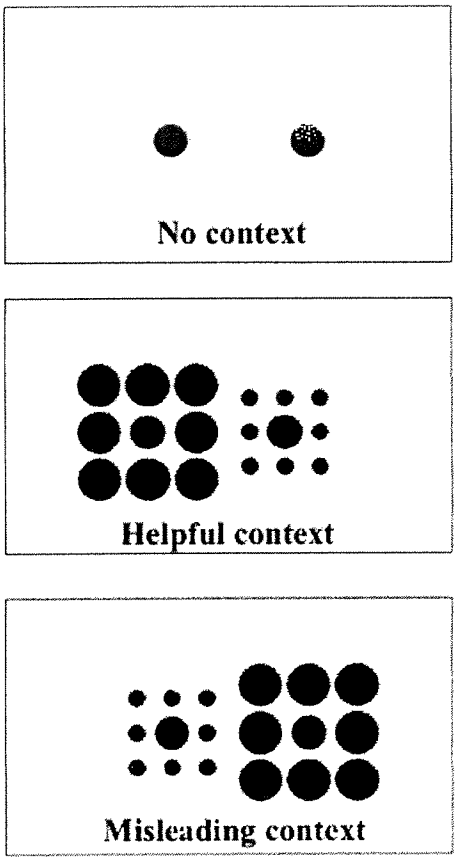


Figure 3. Contour Interpolation Task. This figure shows the type of stimuli that will be used in the Interpolation task. In the illusory condition (top left portion of figure), subjects discriminate between fat and thin squares that are perceived with illusory/interpolated contours. In the control condition (top right portion of figure), subjects will discriminate between configurations of Pac-men (Kanizsa figures) that are rotated either clockwise or counter-clockwise. Distractor lines added to the illusory and control conditions (bottom portion of figure), should reduce performance in the illusory condition, but should have no effect in the control condition.

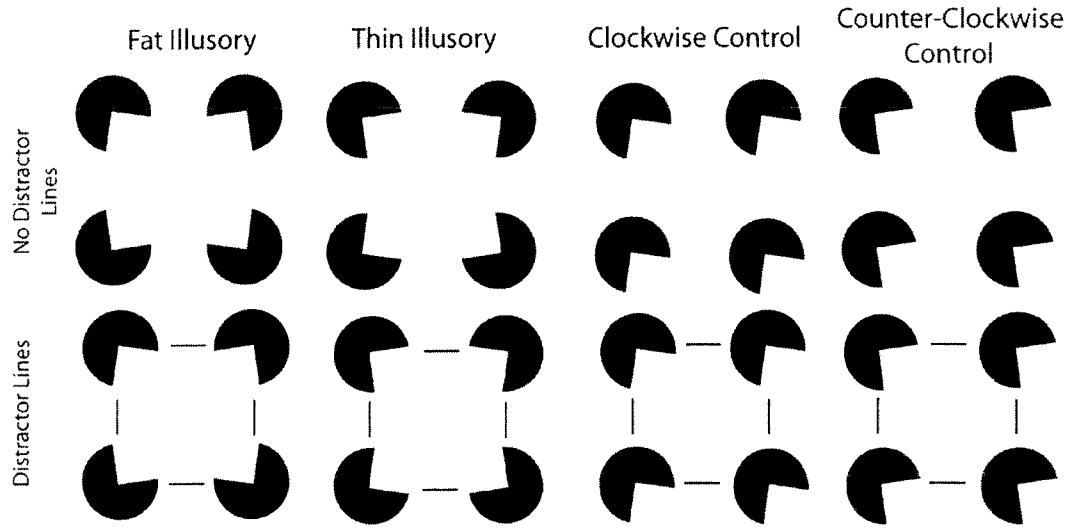


Figure 4. Numerosity Task. This figure shows the type of stimuli that will be presented during the task. The top panel is an example of the homogeneous condition, where are of the stimuli will be presented in the same direction, either horizontally (as shown) or vertically. The bottom panel is an example of the heterogeneous non-adjacent condition, where stimuli will be presented in a vertical-horizontal-vertical pattern (as shown) or vice versa. In this condition, no two identical stimuli are adjacent to each other.

Homogeneous Condition

Heterogeneous Non-Adjacent
Condition

Figure 5. Gender differences for contour integration (JOVI) task performance. Female participants have a higher mean score compared to males: $t(94) = 3.022$, $p = .003$, $d = 0.68$. Error Bars represent 95% Confidence Interval.

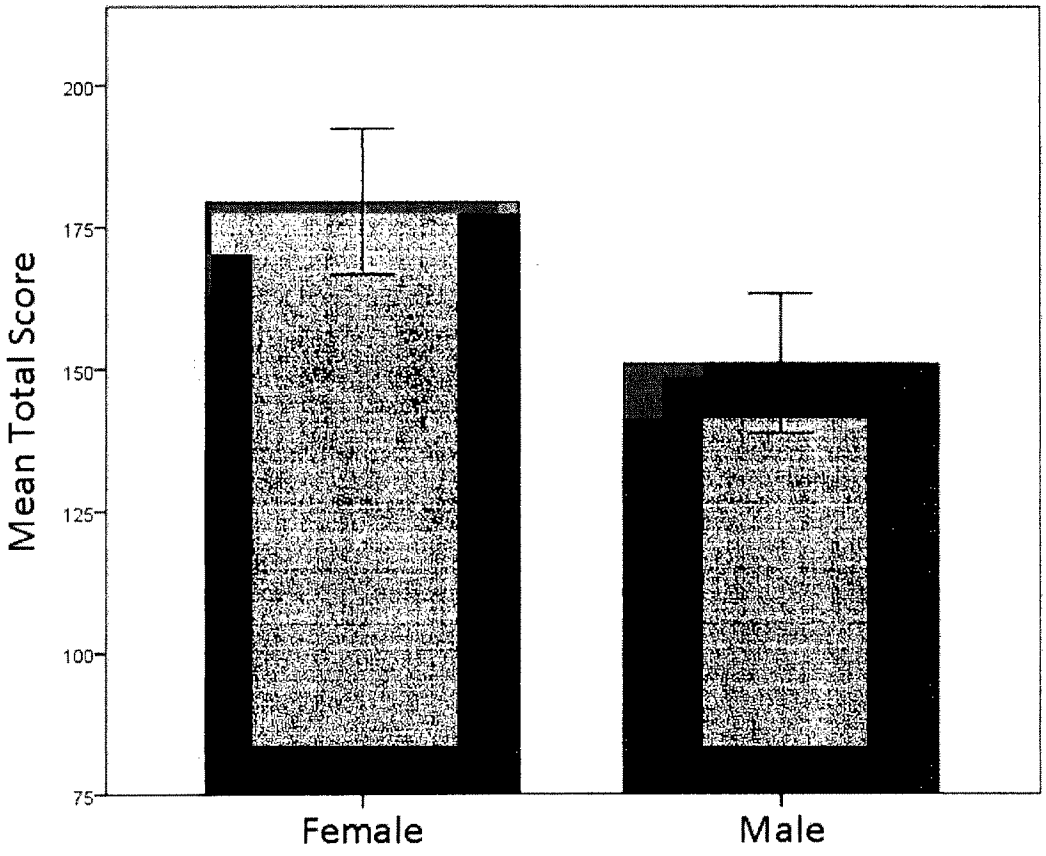


Figure 6. Gender differences for Ebbinghaus illusion performance. Female participants are less susceptible to effects of outer context compared to males: Helpful-Misleading: $t(60) = -2.797$, $p = .010$, d = -0.579; Control-Misleading: $t(94) = -2.231$, $p = .028$, $d = -0.470$. Error Bars represent 95% Confidence Interval.

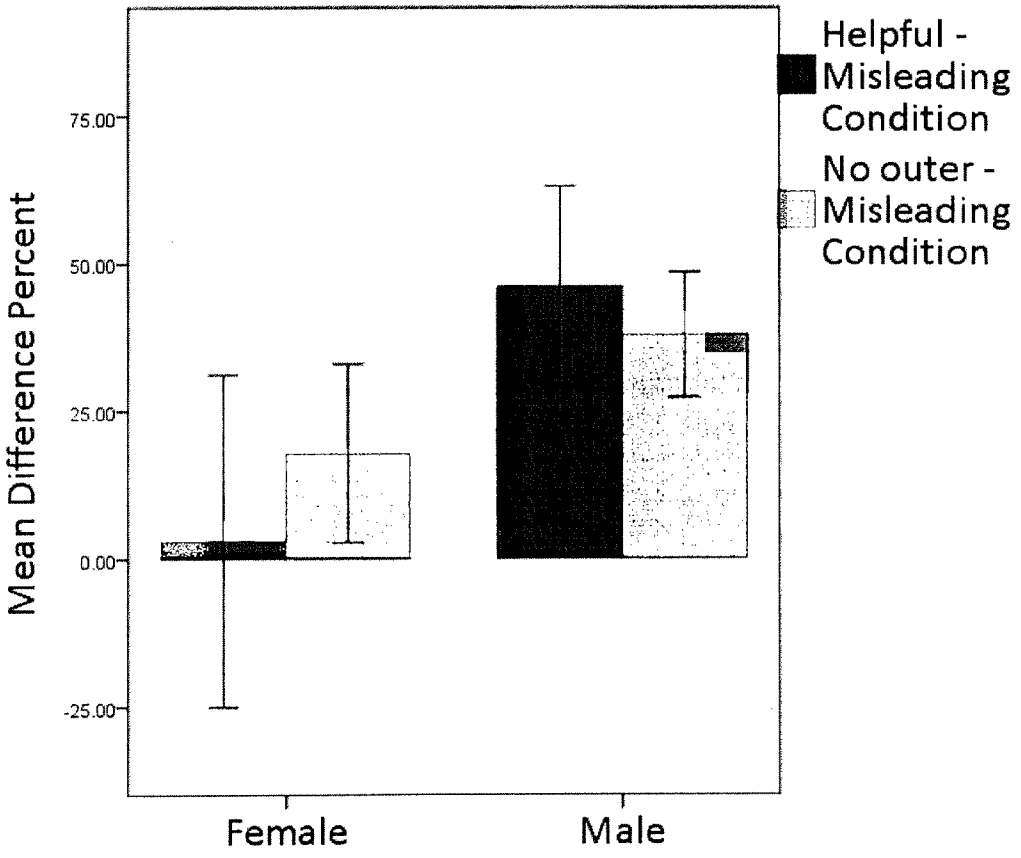


Figure 7. Differences in premorbid social sexual functioning based on gender. Female participants have higher PAS social sexual scores compared to males: PAS social sexual average: $t(87) = -2.709$, $p = .008$, $d = -0.573$; PAS Overall Average: $t(87) = -1.319$, $p = .191$, $d = -0.304$. Error Bars represent 95% Confidence Interval.

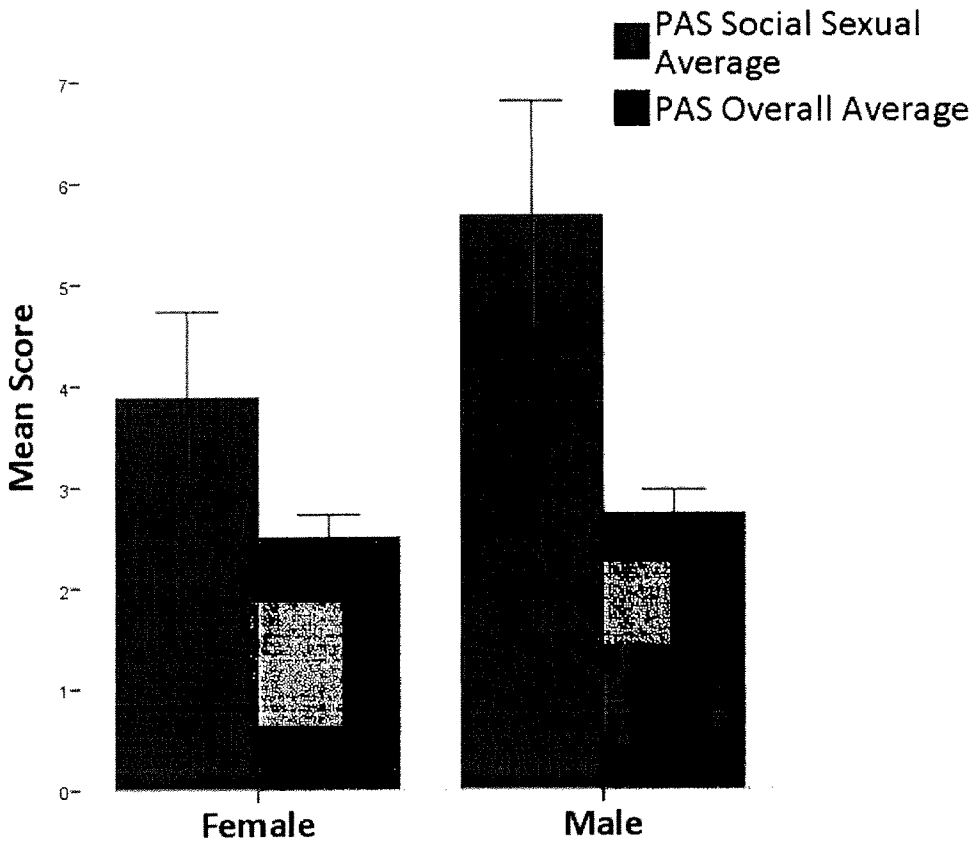


Figure 8. Differences in contour integration (JOVI) task performance based on disorganization symptoms. Participants with higher PANSS conceptual disorganization item scores have reduced contour integration performance: $r(95) = -.270$, $p = .008$; $t(93) = 2.179$, $p = .032$. Error Bars represent 95% Confidence Interval.

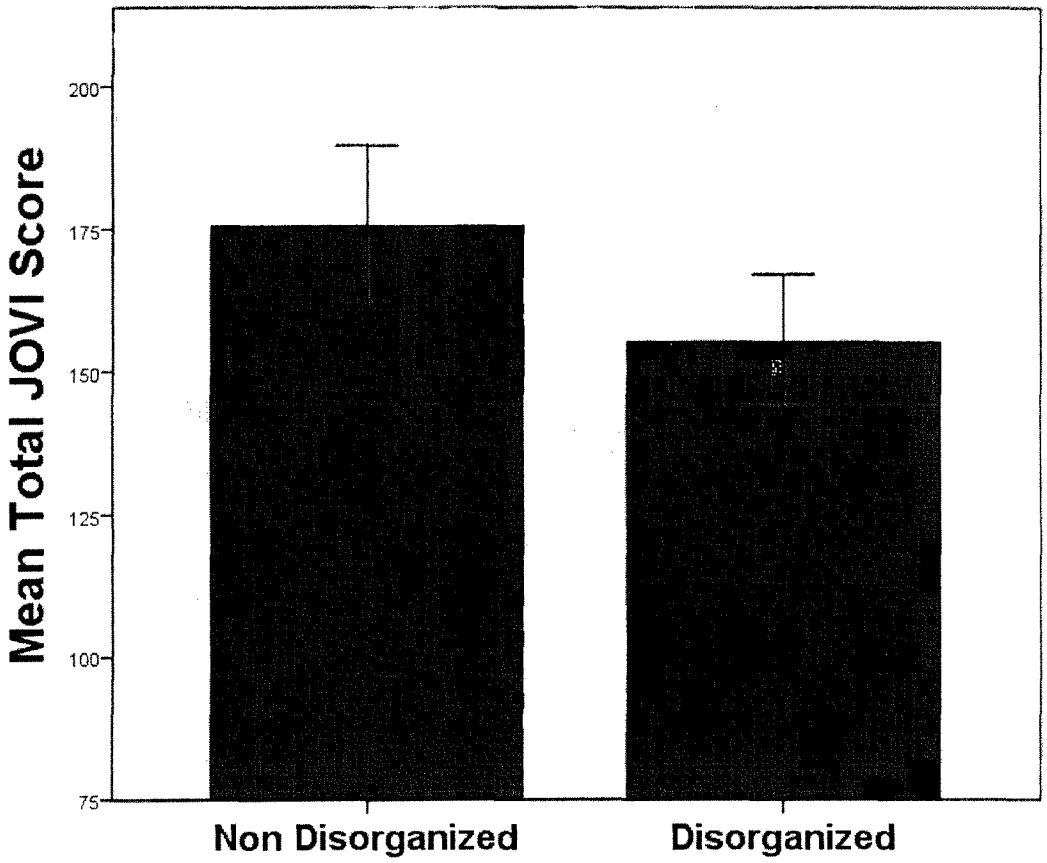


Figure 9. Multidimensional scaling plot of Second versus First Dimension of Identity by State distances of non-study SNPs. The plot points represent each participant. The red dots are Caucasian participants and the black dots are African American participants.

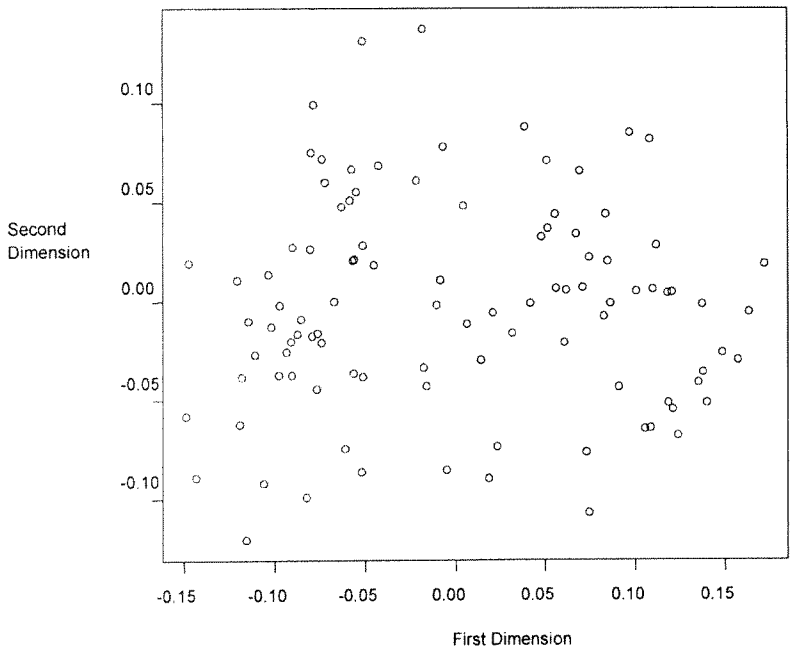
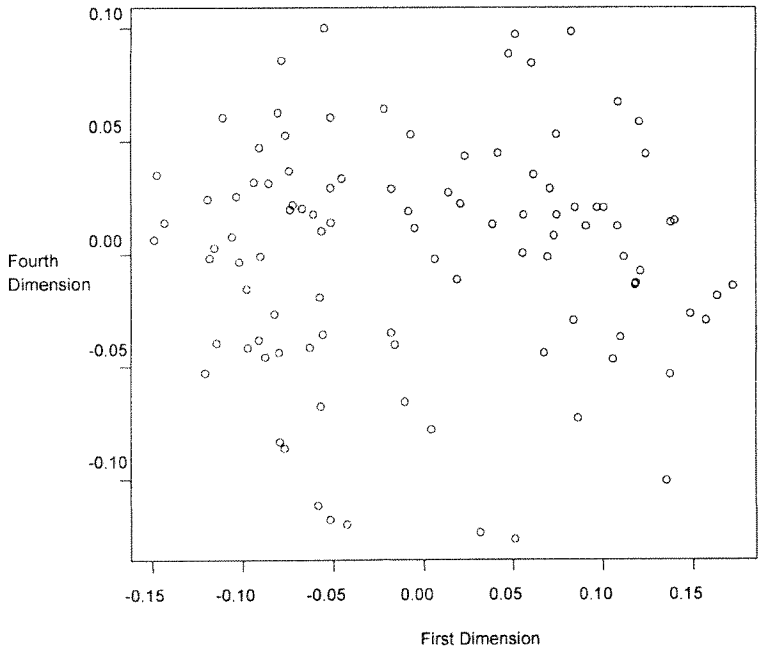


Figure 10. Multidimensional scaling plot of Fourth versus First Dimension of Identity by State distances. The red dots are Caucasian participants and the black dots are African American participants.



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CURRICULUM VITAE

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Article: Korstanje, R., Desai, J., Lazar, G., King, B., Rollins, J., Spurr, M., **Joseph, J.**, Kadambi, S., Li, Y., Cherry, A., Matteson, P.G., Paigen, B., Millonig, J.H. (2008). Quantitative trait loci affecting phenotypic variation in the vacuolated lens mouse mutant, a multigenic mouse model of neural tube defects. *Physiol Genomics,* 35(3), 296-304.

Article: Matteson, P.G., Desai, J., Korstanje, R., Lazar, G., Borsuk, T.E., Rollins, J., Kadambi, S., **Joseph J.**, Rahman, T., Wink, J., Benayed, R., Paigen, B., Millonig, J.H. (2008). The orphan G protein-coupled receptor, Gpr161, encodes the vacuolated lens locus and controls neurulation and lens development. *Proc Natl Acad Sci U S A,* 105(6), 2088-93.

Article: Kodirov, S.A., Takizawa, S., **Joseph, J.**, Kandel, E.R., Shumyatsky, G.P., Bolshakov, V.Y. (2006). Synaptically released zinc gates long-term potentiation in fear conditioning pathways. *Proc Natl Acad Sci U S A,* 103(41), 15218-23.

Article: Shumyatsky, G.P., Malleret, G., Shin, R.M., Takizawa, S., Tully, K., Tsvetkov, E., Zakharenko, S.S., **Joseph, J.**, Vronskaya, S., Yin, D., Schubart, U.K., Kandel, E.R., Bolshakov, V.Y. (2005). *stathmin*, a gene enriched in the amygdala, controls both learned and innate fear. *Cell,* 123(4), 697-709.