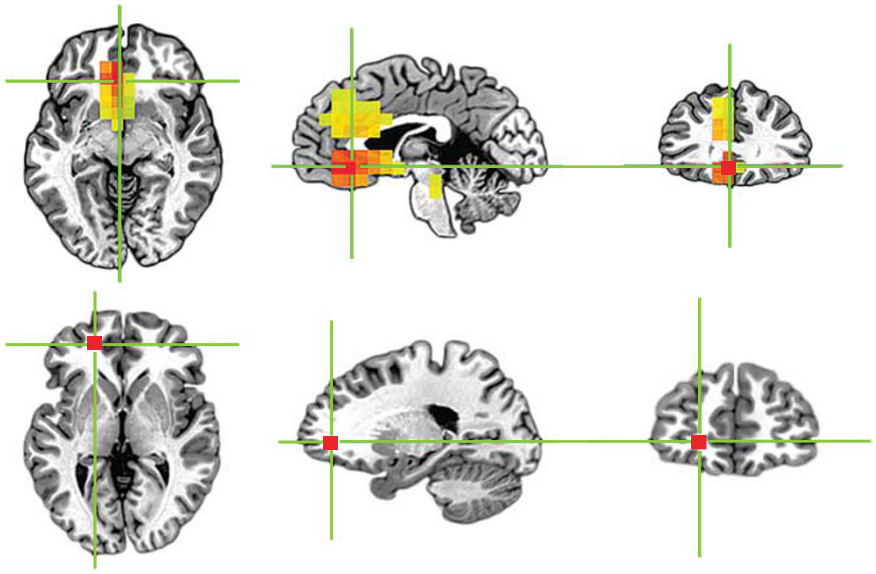

Figure 1. Group difference (top) between schizophrenics ($n = 15$) and normal control subjects ($n = 14$) for symbolic mutual information during rest with FDR threshold $q < 0.01$. The seed location is shown at the bottom. This is one example of hyperconnectivity in rostral prefrontal cortex.

Disorders Branch Sibling Study (Protocol 95-M-0150) at the NIH and supported by the NIMH Intramural Research Program. MEG was collected under Protocol 99-M-0172. SMI analysis was applied to resting MEG data from 15 proband schizophrenics (10 M/5 F, 20–45 years) and 14 normal control subjects (5 M/9 F, 19–36 years). The schizophrenic subjects all met the DSM-IV-TR criteria and were medicated to ~500 CPZ equivalents. Figure 1 illustrates a typical example of the network differences, showing characteristically higher values for SMI in schizophrenic than in normal controls, suggesting hyper-connectivity in rostral prefrontal cortex for short-range connections. Hypo-connectivity was also observed for long-range connections to lateral prefrontal cortical regions. The left prefrontal lobe location of this hyper-connectivity example is consistent with the literature implicating this area in the primary thought disorder, ambivalence, and thought-blocking characteristics of this patient group (Kuhn, 2004).

These preliminary findings are subject to refinement by incorporation of cofactors such as diagnostic scale, medication, and gender.The SMI measure of shared information is not dependent upon task-related activity and may be applicable to assessing psychiatric disorders and their response to psychopharmaceuticals.

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Copy Number Variation in Schizophrenia

Copy number variation contributes substantially to human evolution, normal phenotypic variation, and human disease (Malhotra and Sebat, 2012). To date, thousands of different genomic duplications and deletions, each spanning hundreds to millions of basepairs, have been mapped genome-wide, and collectively account for a significant fraction of human genetic variation. By reorganizing broad swaths of DNA, structural mutations potentially create new genes and regulatory motifs while also disrupting established genes.

Copy number variants (CNVs) tend to occur in regions rich in genes, segmental duplications, and mobile elements(Malhotra and Sebat, 2012). Some arise repeatedly as *de novo* mutations in genomic 'hotspots.' The genomic architecture of hotspots is typically characterized by large repeated segments that increase the risk for errors in replication, a process known as nonallelic homologous recombination. If a CNV is pathogenic, it may persist for only a few generations given negative selection. At the same time, in each generation many *de novo* CNVs are introduced given the highly repetitive nature of the human genome.

The importance of rare copy number mutations for neuropsychiatric disease is now well-established (Malhotra and Sebat, 2012). Our group was the first to demonstrate that individuals with

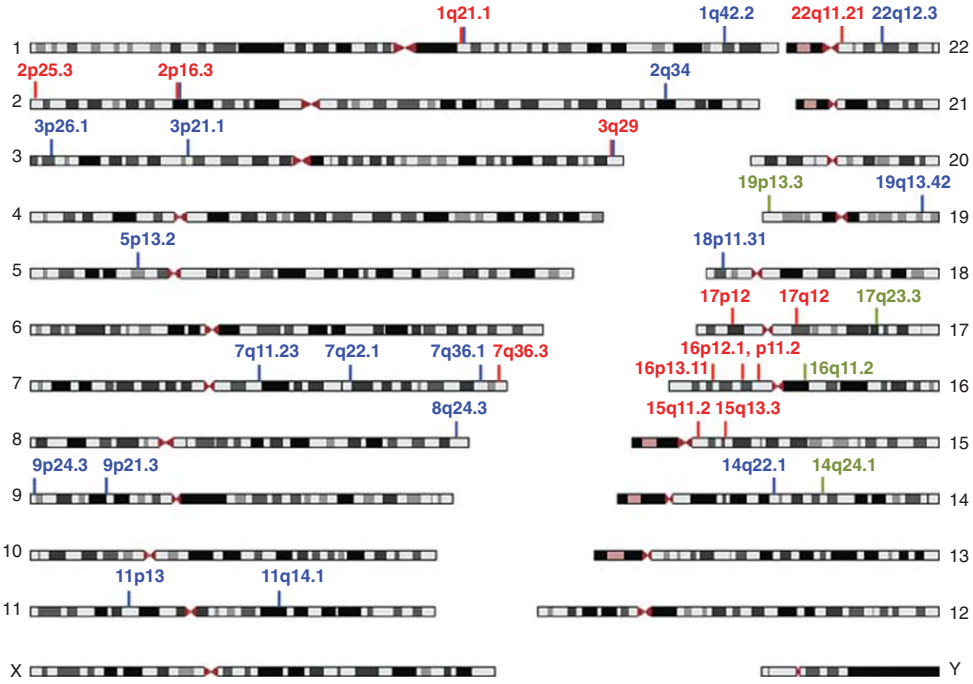

Figure 1. CNVs in schizophrenia. The figure depicts the cytogenetic locations of known genomic hotspots (Malhotra and Sebat, 2012) (red), case-specific CNVs (Walsh *et al*, 2008) (blue), and case-specific CNVs that created chimeric genes (Rippey *et al*, 2013) (green).

schizophrenia are significantly more likely than unaffected persons to harbor rare gene-impacting CNVs, with greater effect for patients with illness onset before age 18 years. Genes disrupted by CNVs in patients function disproportionately in signaling and neurodevelopmental processes, including neuregulin and glutamate pathways (Walsh *et al*, 2008). Subsequent research replicated and extended these findings. Most rare copy number mutations implicated in schizophrenia are unique, and many arose *de novo* or in recent generations. Others recur at genomic hotspots, including chromosomes 1q21.1, 3q29, 15q11.2, 15q13.3, 16p11.2, 16p12.1, 16p13.11, 17p12, 22q11.2, and the neuropeptide receptor VIPR2 (Malhotra and Sebat, 2012) (Figure 1).

One novel mechanism by which CNVs may cause schizophrenia is the creation of chimeric genes (Rippey *et al*, 2013). We recently found that persons with schizophrenia were more likely than controls to carry genomic duplications or deletions that resulted in a fusion gene. Each of the chimeric genes in patients differed from their parent genes in ways likely to be important to brain function, including differences in neuronal subcellular localization, levels of expression, and/or protein interactions. For example, one patient harbored a 150-kb deletion that resulted in a *MAP3K3–DDX42* fusion gene. The MAP3K3–DDX42 chimera produced a novel protein isoform that binds activated *MEK5* and likely acts as a dominant-negative inhibitor of *ERK5* signaling, a key pathway regulating neuronal differentiation and adult neurogenesis. By interfering with parent gene function, chimeras potentially disrupt critical brain processes important to schizophrenia.

Rare deletions and duplications, including in the same recurrent hotspot CNVs associated with schizophrenia, are also enriched in other neuropsychiatric conditions, including autism, intellectual disability, and idiopathic generalized epilepsy (Malhotra and Sebat, 2012). Many clinical laboratories now have assays that can detect hundreds of potentially pathogenic CNVs. Each disorder is associated with many different structural mutations. Each pathogenic CNV is associated with different phenotypes in different persons, including cognitive deficits in individuals characterized as 'unaffected' (Stefansson *et al*, 2014). Variable phenotypic expression of pathogenic events is likely mediated by a variety of different factors, including dose effects, epistasis, epigenetic mechanisms, and environmental exposures (McClellan and King, 2010).

Genes and loci disrupted by pathogenic CNVs provide a window to study key neurobiological mechanisms important to brain development and pathophysiology. For example, rare *de novo* CNVs in persons with schizophrenia were found to be enriched for genes that function within *N*-methyl-D-aspartate receptor postsynaptic signaling complexes (Kirov *et al*, 2012). Given the extreme genetic heterogeneity, such research is critical towards delineating specific genomically defined neurobiological subtypes within large clinically heterogeneous diagnostic groups, and ultimately towards developing biologically informed targeted treatments.

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Regulation of Extrasynaptic Glutamate Levels as a Pathophysiological Mechanism in Disorders of Motivation and Addiction

A growing body of evidence suggests that changes in glutamate transporter expression may be a factor that is common to many neuropsychiatric disorders. As an example, reduced glutamate reuptake capacity has been linked to a wide variety of conditions such as depression, schizophrenia, and addiction. Mathematical models suggest that under physiological conditions glutamate may diffuse and activate NMDA receptors within a radius of 0.5 μm from the release point (Tzingounis and Wadiche, 2007). Excitatory amino-acid transporters (EAATs) bind and transport glutamate, limiting spillover from synapses due to their dense perisynaptic expression primarily on astroglia. Thus, the spatial arrangement of glutamate synapses, their glutamate transporter buffering zones, and extrasynaptic glutamate receptors will determine the extent and effects of glutamate spillover (Tzingounis and Wadiche, 2007). Increased glutamate spillover may lead to a loss of input specificity, degrading the spatial precision of synaptic transmission. Decreased glutamate spillover, particularly in regions with high levels of physiologic spillover such as the hippocampus, could also disrupt plasticity by limiting spillover transmission. Disruption of glutamate reuptake with genetic models or pharmacological agents yields region- and mechanism-specific phenotypes. For example, the homozygous GLAST (called EAAT1 in the human) KO exhibits locomotor hyperactivity, social withdrawal, and abnormal acoustic startle—deficits analogous to the positive, negative, and cognitive symptoms observed in schizophrenia (Karlsson *et al*, 2009).

Several postmortem studies found changes in EAAT expression in schizophrenia consistent with diminished regional expression of astroglial (but not neuronal) glutamate transporter expression and activity (Shan *et al*, 2012). Expression of functional EAAT isoforms appears to be increased in neurons in schizophrenia; we have also found a change in the ultrastructural localization of EAAT2 protein, with an increase in the distance between asymmetric synapses and EAAT2 protein in the frontal cortex in schizophrenia (unpublished observations).

Recent work in a rodent model of heroin relapse provides strong evidence for glutamate spillover as a mechanism of disease. The surface expression and activity of GLT1 (rodent EAAT2) were decreased in the nucleus accumbens core in heroin-dependent rats (Shen *et al*, 2014). Reinstatement of heroin seeking was inhibited by ceftriaxone, a drug that increases GLT1 protein expression and activity. The authors used a change in the NMDA receptor excitatory postsynaptic current decay time as an index of synaptic glutamate spillover, to demonstrate increased decay in heroin- *vs* saline-yoked rats (Shen *et al*, 2014). The increase in decay mimicked the effects of glutamate transport inhibitors in the model, supporting the hypothesis that synaptic glutamate spillover has a central role in relapse and addiction.

The data in schizophrenia and heroin relapse are consistent with findings in depression, where decreased levels of glial transporter expression are reported in brain samples from mood disorder subjects and rodents exposed to chronic stress (Sanacora and Banasr, 2013). Treatment with drugs that increase GLT1 expression and function, including ceftriaxone and riluzole, has antidepressant-like effects in rodent models (Sanacora and Banasr, 2013).

We postulate that diminished perisynaptic glutamate buffering and reuptake may be a common pathophysiological mechanism in psychiatric illness, associated with a number of intermediate phenotypes, including positive (reward learning, reward valuation) and negative (fear, anxiety, loss) valence systems, cognition, arousal and socialization. The diverse biology of the glutamate transporter system, with cell- and splice-variant specific expression regulated by myriad paracrine factors, canonical signaling pathways, exosomal microRNAs, as well as pharmaceuticals such as ceftriaxone, makes it a high yield target that should be exploited for the development of new treatments for a wide array of psychiatric disorders (Lee and Pow, 2010).

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