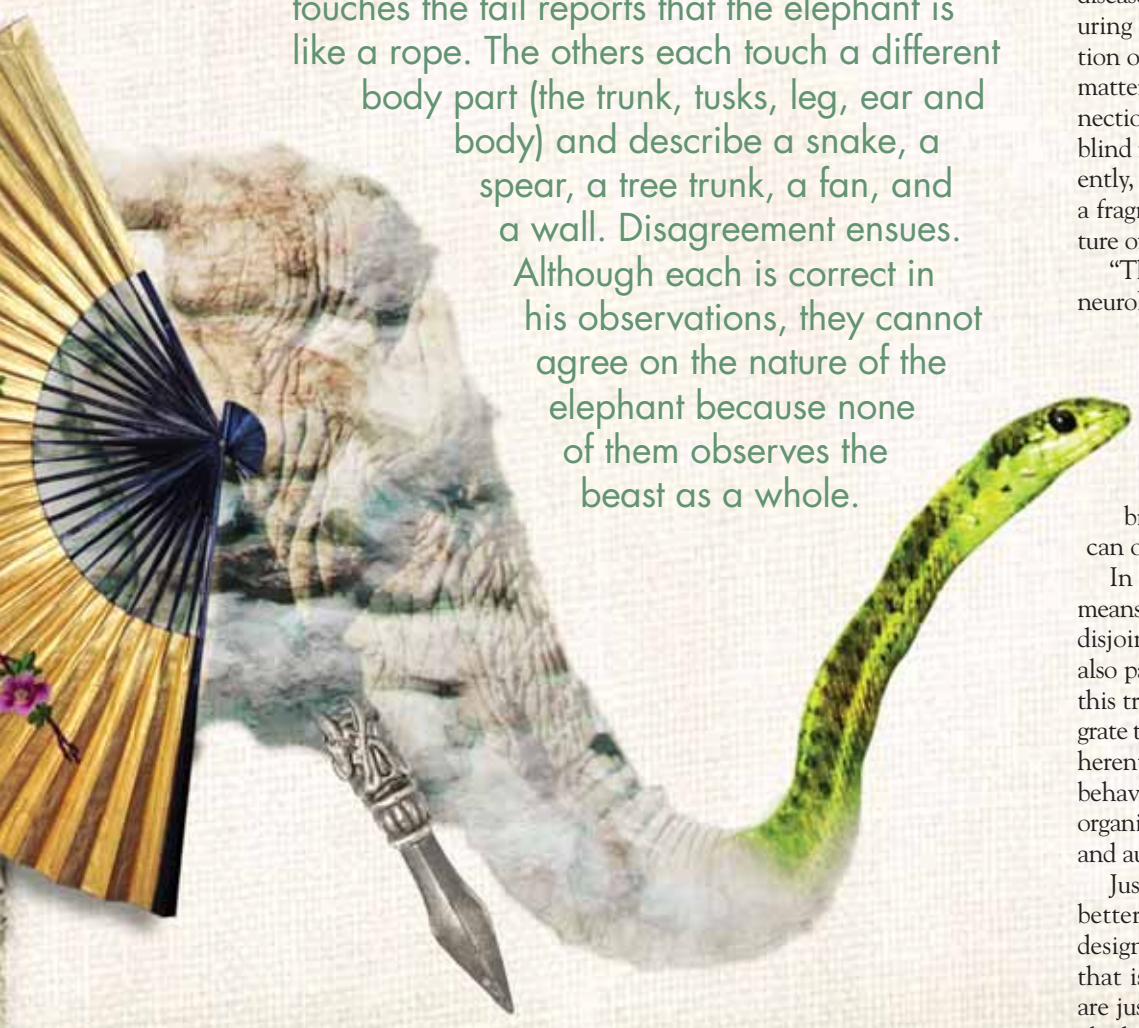


In an oft-cited story, six blind men each touch an elephant to describe its essential nature. The one who touches the tail reports that the elephant is like a rope. The others each touch a different body part (the trunk, tusks, leg, ear and body) and describe a snake, a spear, a tree trunk, a fan, and a wall. Disagreement ensues. Although each is correct in his observations, they cannot agree on the nature of the elephant because none of them observes the beast as a whole.

Integrating the FRAGMENTED MIND

Bringing the Whole Elephant into View

By Katharine Miller

The diverse researchers who study neurological diseases or psychiatric disorders such as schizophrenia are faced with a similar dilemma. They use multifarious approaches to understand the causes and effects of these diseases—sorting through the genome, measuring changes in the volume or concentration of gray matter, tracing the brain's white matter wiring, and spotting functional connections across brain regions. And just as the blind men each describe the elephant differently, so too do these various scientists report a fragmented and somewhat confusing picture of how mental illness affects the brain.

"The many approaches to understanding neurological disease and psychiatric disorders each offer a particular window into something that's gone awry," says **Arthur Toga, PhD**, professor at the Keck School of Medicine at the University of Southern California. But because all aspects of the brain work in concert, no single window can offer an integrated understanding.

In the case of schizophrenia, which means, quite literally, "fragmented mind," the disjointed nature of the research enterprise also parallels the disorder itself. People with this tragic mental illness don't seem to integrate their experiences of the world into a coherent thought process. As a result, they may behave in socially abnormal ways, have disorganized thoughts, and experience delusions and auditory hallucinations.

Just as their fragmented brains need to be better integrated, so too does the research designed to understand those brains. And that is now starting to happen. "Scientists are just beginning to join hands around the elephant," says **Olaf Sporns, PhD**, distin-

guished professor in the department of psychological and brain sciences at Indiana University, Bloomington. "They are collaborating more and also looking at the problem in all its complexity."

Some researchers are integrating structural information about the brain with genetic or functional data. Others tie genetics to phenotype or function. Still others are reaching for the whole enchilada using integrative systems analysis. While some pieces of the picture—such as environmental influences on the genome—remain out of focus, as the National Institutes of Health (NIH) and others are getting more interested in data mashing, progress is being made. "It's definitely the way to go," Toga says. "I think we'll see an accelerated pace of discovery because of it."

Genes and Schizophrenia

There's plenty of evidence that schizophrenia is highly heritable, yet no single genetic variant is the cause. And sample sizes have limited the productivity of genome wide association studies (GWAS). To address that problem, the Schizophrenia Working Group of the Psychiatric Genomics Consortium pulled together GWAS data from multiple institutions—amassing data for more than 36,000 cases and 113,000 controls. The study, published in *Nature* in July 2014, identified at least 108 genomic loci of significance. Many variants are located next to genes that operate in the brain or immune system—suggesting a possible link between the immune system and schizophrenia.

A separate study, also published in *Nature* in 2014, focused on identifying rare variants associated with schizophrenia by sequencing the exomes of 2,536 patients with schizophrenia and 2,543 unrelated controls. Individuals with schizophrenia had a significantly higher rate of rare disruptive mutations in protein-coding genes that were loosely suspected to play a role in schizophrenia. Moreover, disruptive mutations in 28 genes related to synaptic activity appeared in 9 cases versus none in controls; and disruptive mutations in 26 genes involved in calcium ion channels were found in 12 cases versus only one in controls.

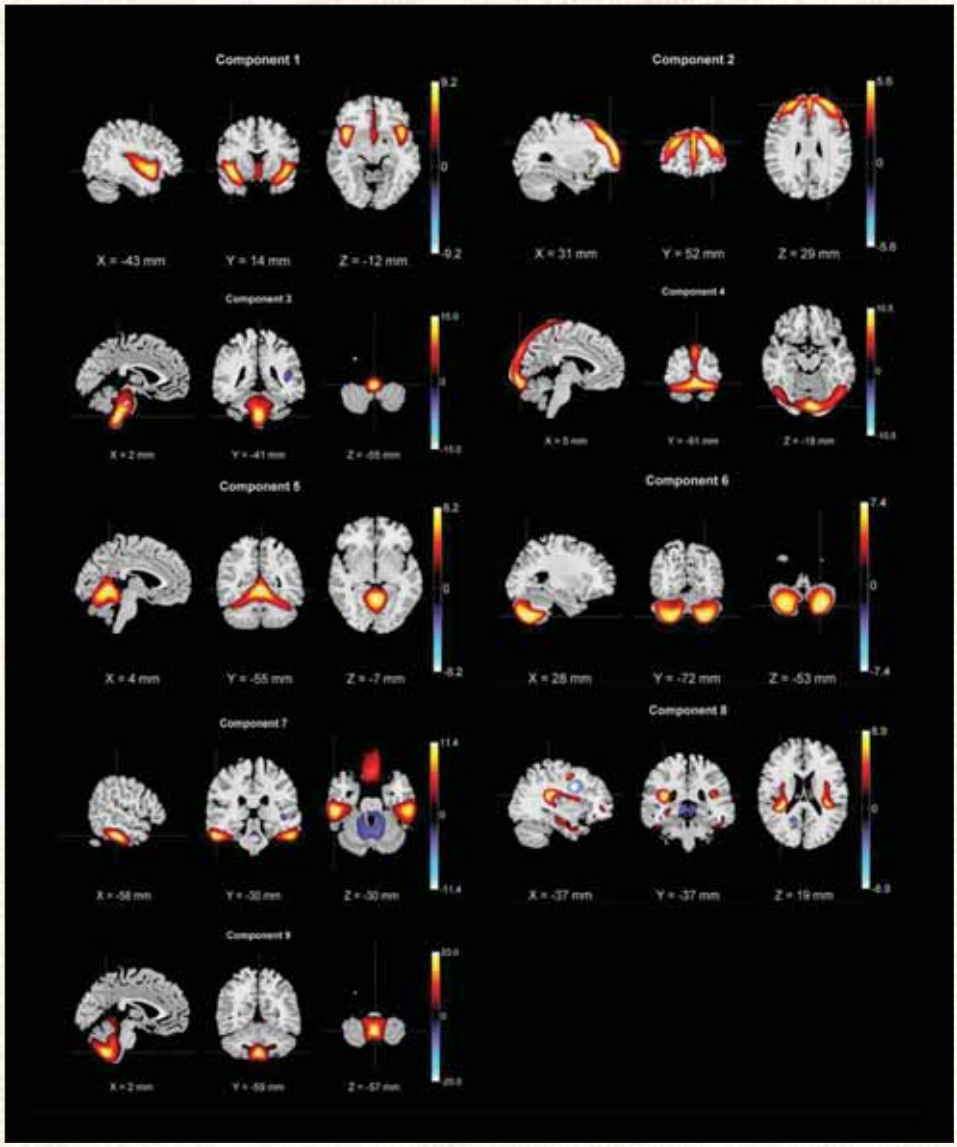
Genes in these two gene sets appear to explain about one percent of schizophrenia cases. "It's consistent with the idea that there are many rare variants scattered throughout the genome, some of which probably confer risk for schizophrenia," says **Benjamin Neale, PhD**, assistant professor in the Analytic and Translational Genetics Unit at Massachusetts General Hospital, and an associated researcher at the Broad Institute.

Schizophrenia and the Thinking Brain:

A Matter of Gray Matter

Schizophrenia patients often experience disorganized thinking, hallucinations, and problems with attention, memory and language. And that in turn suggests a problem with the brain's gray matter. "The gray matter is where the good stuff happens," says **Jessica Turner, PhD**, associate professor of psychology and neuroscience at Georgia State University. "It's where the synapses are—where the cells fire. Without gray matter you can't think."

In imaging studies, researchers have long observed gray matter abnormalities in the brain structures of schizophrenic patients. Some such studies look at gray matter volume—the amount of gray matter inside the borders of particular brain structures—and some look at gray matter concentration—the density of gray matter judged by characteristics of the voxels. Most look for clusters of voxels that differ from healthy controls (univariate approaches) while a few have begun looking for patterns of variation



Turner's mega-analysis identified nine different spatial patterns (components) where gray matter concentrations in schizophrenia patients differed significantly from controls. Here, the nine patterns are shown in order from most significant to least. For the first seven components, patients had less gray matter than controls. Note that the spatial patterns were not defined by a brain atlas but rather revealed by the analysis of voxels. Red areas represent voxels where the differences were more highly statistically significant (z score > 2.5). Reprinted with permission from CN Gupta, VD Calhoun et al., Patterns of Gray Matter Abnormalities in Schizophrenia Based on an International Mega-analysis,* Schizophrenia Bulletin *(2014) doi: 10.1093/schbul/sbu177.

among those clusters (using multivariate approaches). Schizophrenia researchers also struggle with sample sizes that may be insufficient to reach statistical significance.

Seeking to generate reliable, replicable results, Turner and her colleagues set out to conduct an international mega-analysis of gray matter concentration using a multivariate approach and a large dataset. They gathered together MRI images from eight prior studies, including scans of 936 healthy controls and 784 people diagnosed with schizophrenia. The method they used—called parallel independent components analysis (ICA)—lets the data speak for itself, revealing spatial patterns that co-occur in patients compared to healthy controls. And the data did speak, revealing nine "components" or spatial patterns of interest. One pattern in particular caught the researchers' attention: reduced gray matter concentration in three areas (superior temporal gyrus, inferior frontal gyrus and insula) of the brains of schizophrenic patients. And the pattern was highly replicable. "You can find this over and over again in chronic schizophrenia," Turner says. "You can put it in the bank."

Is there potential for similar mega-analyses to reveal patterns in other mental illnesses? "Oh my goodness, yes!" says Turner. But she also points to the ENIGMA consortium as a model for future work. Rather than a mega-analysis, which brings all the data to one lab, ENIGMA leaves the data where it is and sends software scripts to participating investigators whose results are then combined. "ENIGMA gets more power out of collaboration and cooperation than we could out of doing our own little studies," Turner says.

For example, in 2008, the dynamic wave of gray matter loss that occurs as schizophrenia develops was revealed in a collaborative effort by 40 labs around the world led by **Paul Thompson, PhD**, professor in the Keck School of Medicine at the University of Southern California and director of the ENIGMA Consortium. In work published in 2008, Thompson and his colleagues also used time-lapse imaging to study the effects of various schizophrenia medications on the brain over the course of a year. Remarkably, they found one medication, olanzapine, that seemed to reduce gray matter loss compared with others.

Gray Matter and Genetics

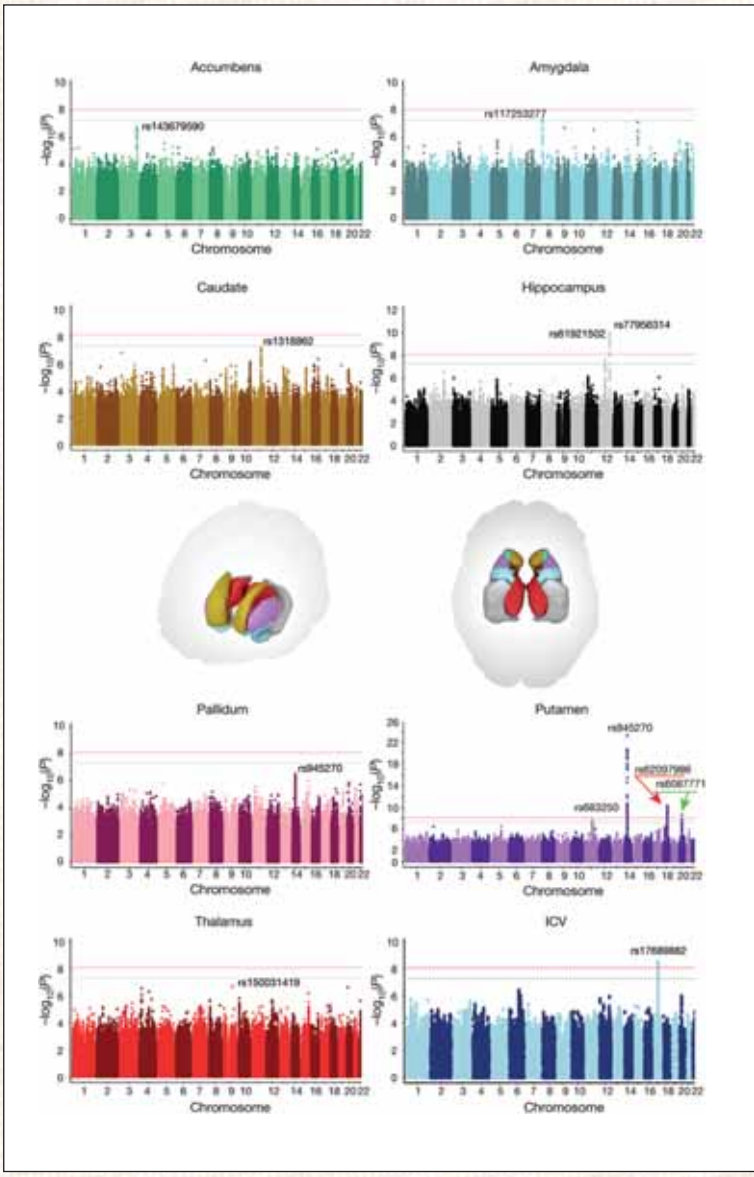
Turner and her colleagues are now combining imaging and genetics approaches to schizophrenia to see if there's a relationship between the pattern of gray matter loss they observed in their mega-analysis and genetics. "Let's see if there's a relationship between this imaging pattern and cases/controls in GWAS," she says. An earlier project with a smaller number of subjects suggested the pattern of loss in schizophrenic patients is heritable. The results of Turner's team's genetics work are due out soon.

ENIGMA, which stands for Enhancing NeuroImaging Genetics through Meta-Analysis, is also driving forward with the emerging field of neuroimaging genomics. The idea is to use brain images to screen the genome for common variants that might affect the brain. To date, such approaches have identified genes linked to such things as brain or hippocampal size as well as a few genes linked to Alzheimer's Disease.

These early studies suggest, however, that neuroimaging sample sizes will need to be quite large if they are to avoid the problem of false positives—genes that seem linked to imaging features in a particular sample but cannot be replicated in other samples. This concern has led to the ENIGMA Consortium's efforts to combine images from many labs.

To date, the ENIGMA Consortium's Schizophrenia and Bipolar Working Groups have been focused on extracting meaningful information from neuroimages. But with support from the NIH's Big Data to Knowledge (BD2K) program, they hope to soon publish work that ties these results to genetics.

In a *Nature* paper published in January 2015, the ENIGMA network showed the potential promise of such work. That study found eight common variants (SNPs) that consistently predict the size of structures on brain MRI scans from over 30,000 people from 33 countries worldwide. Although non-genetic factors are clearly important, a tantalizing question is whether these genetic variants that correlate with brain structure also correlate with risk for brain diseases. For example, those eight genetic hotspots seem to affect the size of several brain regions implicated in schizophrenia, and some of them appear to affect risk for Alzheimer's disease, Parkinson's disease, and obsessive-compulsive disorder (OCD). An ongoing partnership between the Psychiatric Genomics Consortium and ENIGMA is comparing the two groups' find-



Recent neuroimaging genetics research by ENIGMA identified genetic variants (SNPs) associated with volume differences in various parts of the brain. These Manhattan plots are colored with a scheme to match the corresponding structure in the central diagram. Two different measures of genome-wide significance are shown with a gray dotted line ($P = 5 \times 10^{-8}$) and a red dotted line ($P = 7.1 \times 10^{-9}$). The most significant SNP within an associated locus is labeled. Reprinted with permission from Macmillan Publishers, Ltd., DP Hibar et al., Common genetic variants influence human subcortical brain structures,* Nature *(2015). doi:10.1038/nature14101.

ings to see if risk genes for schizophrenia might exert their affect by influencing the composition and integrity of the brain.

Schizophrenia and the Networked Brain:

A Matter of White Matter

According to a separate theory, differences in the brain's wiring could increase vulnerability to schizophrenia. In the brain, wiring means white matter—the bundles of axons that connect distant regions of the brain to one another.

Researchers can create wiring diagrams of the brain by tracing the diffusion of water along neuronal bundles, a method known as diffusion tensor imaging (DTI). And researchers like Sporns can then analyze these static images as networks. This approach has revealed some interesting things. For example, Sporns and his colleagues have found that highly connected parts of the human brain are also highly connected to each other, a characteristic called a "rich club." And, intriguingly, in schizophrenic patients, the connections between the members of the rich club are somewhat impaired while connections among less highly connected nodes are not.

Sporns thinks the rich club nature of the structural connectome is key to the brain's ability to function coherently. Our brains are constantly interacting with our environment and integrating information from many sources—our senses, memories, muscles, skills, internal physical states—to make sense of the world and guide our behavior in an integrated fashion. "Rich club, with its distributed pattern of highly connected hub nodes is analogous to a highway system for accomplishing this integrative task," Sporns says. But if a pathological mechanism weakens or disturbs that rich club connectivity, there's a penalty that is expressed in brain disorders such as schizophrenia, he proposes.

Bringing Functional Networks to Structural Networks

Having uncovered the brain's rich club structural network, Sporns decided to explore the relationship between static anatomical networks and functional networks that are much more dynamic, with changes on the scale of seconds or faster.

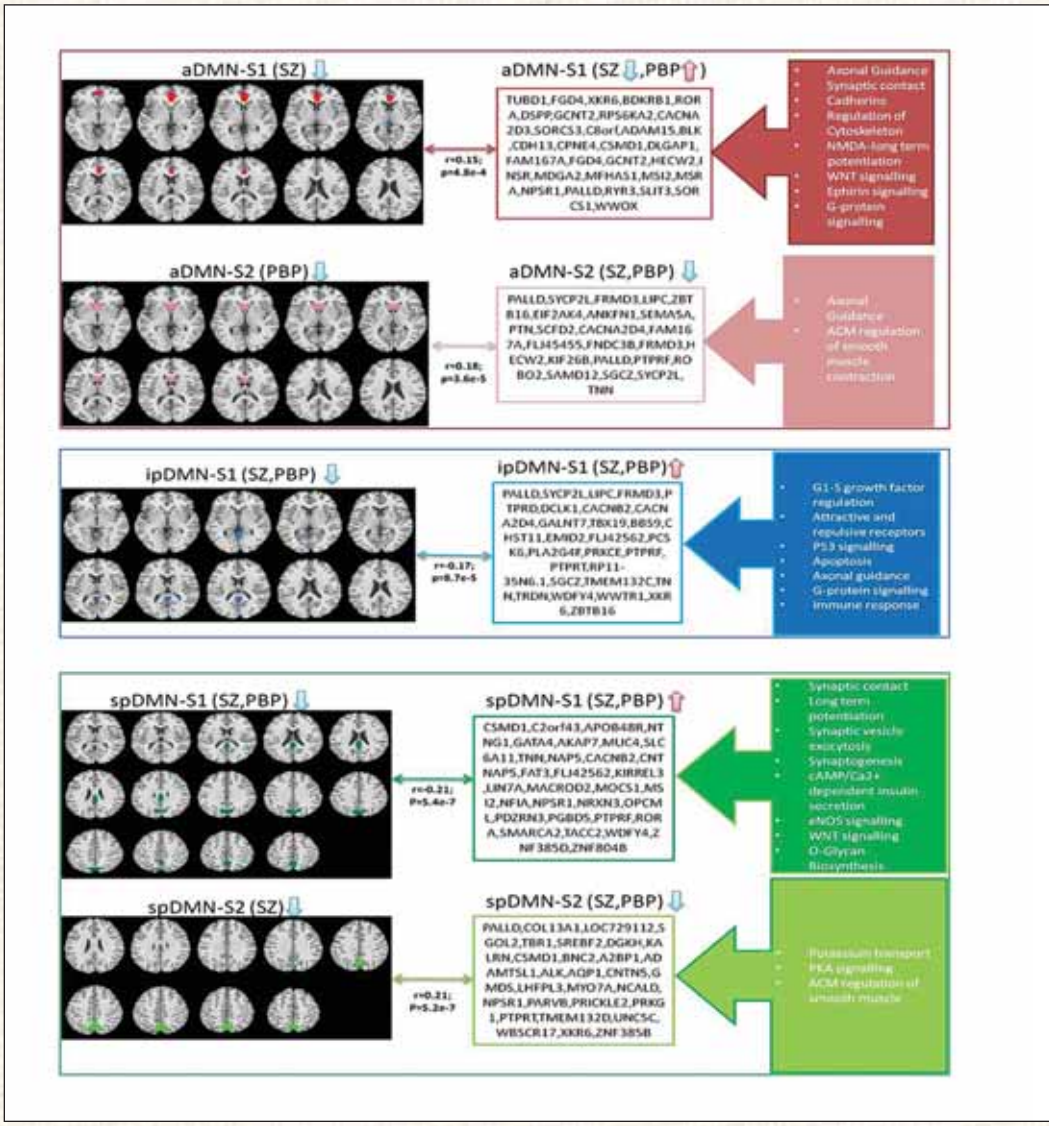
When brain researchers talk about function, they usually mean either how electrical activity changes among electrodes placed in the brain during an electro-encephalogram (EEG); or how blood flow in the brain changes over time (while at rest or doing a specific activity) as measured using functional magnetic resonance imaging (fMRI). "These methods produce a time series of neural activity using electrodes or voxels," Sporns says. "There's no movie that directly shows how neurons send messages to each other." So when researchers talk about functional connectivity in the brain, they are referring to activation patterns cross-correlated among different brain regions.

Just as network approaches can help researchers understand the structural connectome, so too can they reveal interesting features of the functional network. Previous work had shown that the functional networks of schizophrenia patients had reduced global communication capacity. Could that be due to reductions in rich club density? To find out, Sporns and his colleagues looked at structural and functional connectivity in the same patient population. And they found an increased coupling between the two types of connectivity in schizophrenia patients. That is, functional interactions were more directly related to the brain's anatomical connectivity in patients than in controls—possibly indicating less flexible or dynamic brain function in patients.

"Network approaches have given us a way of looking at schizophrenia from a different vantage point than we're used to," Sporns says. "And they've given us some hypotheses that we can now go out and test."

The Chronectome: Dynamic Connectivity

In a functional MRI, various parts of the brain light up together or separately in patterns that change through time. In the past, most fMRI studies have evaluated differences between patients and controls by essentially averaging these patterns during a particular activity and time period. These averages do reveal differences between people with and without schizophrenia. "But two brain regions might be highly correlated



in the first few seconds and decline in correlation immediately," says **Vince Calhoun, PhD**, executive science officer at the Mind Research Network and distinguished professor of electrical and computer engineering at the University of New Mexico. "If you just look at average connectivity, you'll miss the change."

So Calhoun and his colleagues decided to scan people in a resting state to specifically look at whether connectivity dynamics themselves might reveal patterns of brain function that differ across people with schizophrenia or bipolar disorder and healthy controls. The work, discussed in *Neuron* in November 2014, found five states (correlations among specific regions) that exist routinely in both cases and controls, but when they looked at the "dwell time"—the percent of time spent in each state—the schizophrenia patients occupied two particular states much longer than the controls. "You do learn some interesting things from the averages," Calhoun says. "But when you unpack it and look at what goes into that average, you actually learn a lot more."

Since Calhoun's first dynamic connectivity paper was published in 2009, the approach has "kind of exploded," he says. "It reinforces the value in looking at the data that way."

Integrating Genetics and Phenotypes

Standard GWAS can only point to individual variants that are associated with a mental disorder, says **Igor Zwir, PhD**, postdoctoral scholar working with **Robert Cloninger, PhD**, at Washington University in St. Louis. And many of the findings are weak and inconsistent. In any event, Zwir says, "Individual genes basically do not cause mental disorder. Genes act in concert as well as with the environment."

Moreover, because schizophrenia is actually a spectrum of disorders that varies widely in severity and covers a whole range of symptoms from positive (such as delusions, disordered thoughts) to negative (such as lack of interest in others, inability to feel pleasure or act spontaneously), Zwir notes, the gene clusters that interact to produce different sets of symptoms may be different as well.

So Zwir and his colleagues decided to take a self-organizing approach to a large set of GWAS data for about 4,000 schizophrenia patients whose symptoms and their severity were also well documented and a similar number of healthy controls. Without any presumption as to which gene mutations (SNPs) would co-occur, they partitioned the patients based on shared sets of SNPs. They did the same thing for phenotypes—allowing the data to cluster people without any presumption as to which traits go together.

Next, they optimized the relationships between the clusters of SNPs and the clusters of traits. And for each association they calculated the risk. "If there's a 90 percent risk, then that association includes 90 percent cases and 10 percent controls," Zwir says. Ultimately, the clustering honed in on eight sets of SNPs with associated phenotypes.

When Zwir and his team reported these results in September 2014 in the *American Journal of Psychiatry*, the press coverage was intense, with many publications declaring the existence of eight subtypes of schizophrenia. The researchers received calls from *Time*, *Newsweek* and even Anderson Cooper. "Why did we have this press?" Zwir asks. "It's because people need to relate genetics with disease and it isn't often done."

At the same time, some members of the research community criticized the group's methodology. Without weighing in on the details of that debate, Turner commented that no matter how one feels about the statistical details, "This is a very rich, very reasonable approach, and the findings made sense."

Moreover, because of the Zwir paper, Turner is now trying to apply similar methods to imaging data. But it's difficult to get phenotypic data that's properly standardized, she says. "When you try to break it down and look at what exactly the symptoms are and how bad the hallucinations are or how bad the reality disorganization or cognitive deficit, they are not well quantified. Different people use different scales."

Undeterred by the critics, Zwir and his colleagues have recently applied the same clustering algorithm to see whether schizophrenia phenotypes cluster with different patterns of white matter loss (using DTI) in schizophrenia. Though their sample size was relatively small (47 patients and 36 healthy controls) they found at least three distinct clusters of symptoms and white matter patterns—one pattern associated with bizarre behavior; another with prominent delusions; and a third with negative symptoms, including disorganized speech. The work was recently submitted for publication.

Integrating Genetics and Function

In another effort to integrate different perspectives, Calhoun worked on a project to find genetic patterns that coincide with brain functional network patterns in a group of subjects that included patients with schizophrenia and bipolar disorder as well as their healthy family members and healthy but unrelated controls.

The work, under the leadership of **Godfrey Pearlson, MD**, at Yale, used a large array of genetic information (single nucleotide polymorphisms) and focused on the default-mode network (DMN) of the brain. "It tends to be more active when you're not focused externally," Calhoun says. This network typically shows reduced functional connectivity in people with schizophrenia and bipolar disorder. Using parallel independent component analysis, an approach developed by Calhoun's group, the team was able to find genetic patterns and DMN subnetwork patterns that co-occur in a group of subjects. "This gives us a richer set of features that we can pull out of the data without starting from a region of interest," Calhoun says. The group then went further, and sought to understand the possible molecular underpinnings of the genes identified in the study. The results, published in

(Opposite page) Calhoun and his colleagues parsed a dataset of functional scans and genetic information for schizophrenia and bipolar patients using a method Calhoun's lab developed called parallel independent component analysis. The technique revealed five subnetworks of the brain's default mode network (the part that's focused internally) and their associated genetic components, represented here by the top 10 genes as well as genes that feature more than three SNPs within each network. The blocks at right list significantly enriched ontology terms within each genetic cluster. Arrows pointing up and down indicate whether the loading coefficient for that particular feature (fMRI or gene) was significantly higher or lower for patients or controls. Reprinted with permission from SA Meda et al., Multivariate analysis reveals genetic associations of the resting default mode network in psychotic bipolar disorder and schizophrenia,* PNAS *vol. 111:19 (2014).

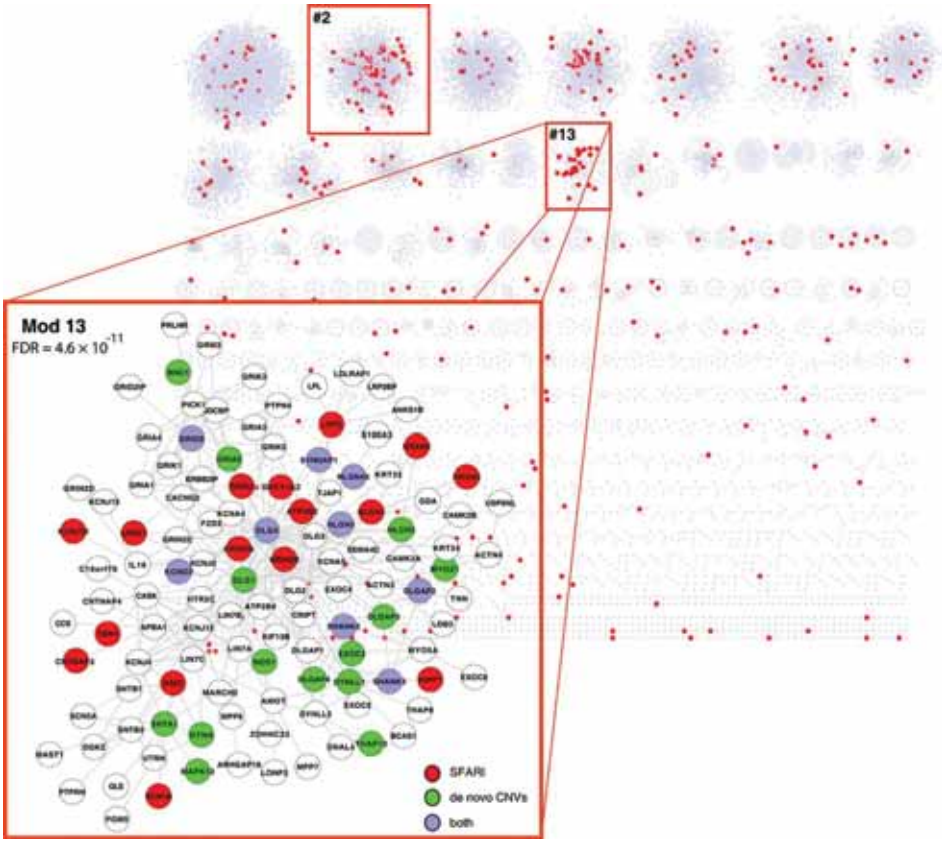
PNAS in April of 2014, pointed to mechanisms that had been previously implicated in schizophrenia and bipolar disorder as well as several novel mechanisms.

Integrative Systems Analysis

Michael Snyder, PhD, professor of genetics at Stanford, has long been in the business of data integration. Since the 1980s he has been working with combinations of data including genetics, genomics, transcriptomics and metabolomics. "We're pretty comfortable working across these areas and integrating lots of different information," he says.

Snyder and his colleagues took the entire human protein interaction network (the "interactome") and mapped its organization at an intermediate scale. "If you imagine all the proteins are the world, the map we set up is kind of like at the state level, where groups of proteins are working together," he says. They then took known genes for autism spectrum disorder (ASD) and mapped them onto these clusters. "Two modules screamed out at us," Snyder says. "But especially one. Autism kids have a high chance of mutations in our module."

Snyder's team didn't stop there. They used whole genome sequencing to look at 25 kids with autism and found they were



Snyder and his colleagues identified genetic modules (#2 and #13 above) in the human protein interactome that are enriched for autism–associated genes (in red). The topological modules are physical clusters on the protein interaction network where member genes intensively interact with each other but sparsely interact with non–member genes on the network. The zoom–in view of module #13 is colorized to show known autism genes (red) and genes affected by autism spectrum disorder–associated* de novo *copy-number variations (green). Genes annotated by both were in blue. Reprinted from J Li, M Shi, Z Ma, S Zhao, et al., Integrated systems analysis reveals a molecular network underlying autism spectrum disorders,* Molecular Systems Biology, *10 (12) 2014.

For diseases like schizophrenia and other psychoses, such a combination could be quite powerful, Snyder says. He bases that assessment on recent work his team did in the area of autism.

enriched for mutations of genes in the module. They then looked at what the module does using the Allen Brain Atlas, and found that half are expressed in most neurons but half of them are primarily expressed in the corpus callosum. The importance of the corpus callosum in ASD was also confirmed in mouse models.

"I think this kind of analysis will be very fruitful when applied to other areas," Snyder says.

Data Fusion: Bringing Multiple Imaging Approaches Together

Calhoun is very interested in pulling together multiple types of imaging to see what they can show us about the brain. Often, researchers integrate imaging data by overlaying one image on another. However, such approaches will not necessarily recognize if a change in one area of the brain correlates with a change in another part of the brain.

Calhoun favors a different approach he calls data fusion, in which each method informs the other without any assumptions about which information is more important. "We don't, at the beginning, make a critical assumption that might lead us down a wrong path," he says. "My approach is to move the simplification step to the end."

For example, at the IEEE Engineering in Medicine and Biology Society Annual International Conference in 2014, Calhoun and his colleagues presented a data fusion approach to combine three types of imaging data—functional MRI, EEG and structural MRI (white and gray matter volumes)—in a study comparing schizophrenia patients and controls. They found that the combined data was more predictive of schizophrenia status than any single imaging modality alone.

Treating the Elephant

As researchers begin to grasp hands around the elephant, they may start to find connections that explain how genetics and the protein interactome play into gray matter loss and reduced rich club network structure across the full range of psychotic phenotypes.

Ultimately, such an integrative approach could lead to better diagnosis and treatment options for the millions who suffer from schizophrenia or other psychoses.

Turner looks forward to the day when a brain scan and genetic test can help physicians steer people toward appropriate treatments based on a molecular understanding of what's going on; or allow early interventions in young people, to prevent gray matter loss before it can get started. To get there, many perspectives on the psychotic brain will have to collectively tell one story. □