Convergence in neuropsychiatric research

Labs in different neuropsychiatry subfields don't always see eye to eye, but convergent approaches help them join forces to study these difficult conditions.

Vivien Marx

Schizophrenia, autism spectrum disorder (ASD), bipolar disorder and attention deficit/hyperactivity disorder (ADHD) are deeply challenging neuropsychiatric conditions[1]. It disheartens scientists and patients that in vitro assays, genomic analysis, animal models and human brain imaging have yet to deliver definitive insights about them. "Hypotheses are easy and coming up with proof is hard," says Patrick Sullivan, who directs psychiatric genomics at the University of North Carolina at Chapel Hill, and is also on the Karolinska Institute faculty. Research consortia bridge disciplinary divides, and a renewed focus on convergence aims to integrate insight and data from disparate assay modalities. Convergent neuroscience, as framed in requests for applications from the US National Institutes of Mental Health, is about establishing causal and probabilistic links across contiguous analytical levels, from genes, molecules and cells, to circuits and behavior.

But there are turf issues, for example between genomics labs and those focusing on brain scans. Cross-disciplinary consortia promise to brook divides, but too many researchers tell a colleague only "what they think you need to know, which creates silos and loses much of the benefits of cross-disciplinary collaboration, and reduces the opportunities to learn from each other," says a scientist who wished to not be named. Over time, such behavior will retreat. "The cool thing, I guess, is that we actually are now getting a suite of technologies that are up to the task that we need," says Sullivan, the principal investigator of the Psychiatric Genomics Consortium (PGC), which includes more than 800 scientists from over 40 countries. He's also part of PsychENCODE[2], a consortium focused on regulatory elements in neuropsychiatric disorders.

Genome-wide-association studies (GWAS) show loci and gene variants that matter in neuropsychiatric disorders, says University of California San Francisco (UCSF) researcher, Martin Kampmann. The catalog of risk variants has "been really exploding, especially for these complicated diseases, over the last five years." ASD researchers can now study a substantial number of validated risk genes or variants. Next is to go "from this cataloging of things to a mechanistic insight," which can hopefully pave therapeutic paths. He and others explore the variant(s) that shape a disorder, as well as where and when it originates in human brain development.

Scientists are working on convergent ways to study the underpinnings of autism spectrum disorder and schizophrenia, among others. But there are turf issues. And genetics cannot, on its own, deliver all the answers. Credit: DrAfter123/DigitalVision Vectors/Getty

Convergence

Neuropsychiatric research is definitely moving toward convergence, says stem cell researcher Oliver Brüstle, of the University of Bonn. To him, it means complementing findings about brain architecture with information about genomic variants and developmental changes. Some labs might only look at phenotype but neglect genotype. "I think we really do need both," he says. "Convergence, to me, has always been about convergence of genetic variation on pathways," says Trey Ideker, of the University of California San Diego. The heterogeneity of neuropsychiatric disorders and the role of many genes can converge onto a "commonality" of protein complexes and pathways. GWAS have pointed labs toward protein-coding regions but, in neuropsychiatric disorders, "it's pointed us at regulatory variants," says Sullivan. It's one of many research aspects that can benefit from convergent approaches that, to him, help to fill out a picture, such as a single-nucleotide polymorphism (SNP) indicating a gene of importance in a genomic dataset. Convergence, says Radboud University Medical Center molecular psychiatrist Barbara Franke, flows information from one investigational level to the next, so knowledge can travel from molecules to cells to circuits. To do this, collaboration across disciplines is "what is really, really needed," she says. "And that is where we are, as a field." She is part of PGC and co-founded ENIGMA, a research consortium devoted to genetics and imaging, such as MRI and diffusion tensor imaging (DTI) related to brain disorders. Convergent neuroscience is not new, she says, but it's taking on new importance given the need for collective progress in neuropsychiatric research.

Imaging genetics

Labs hunt for signs of how risk genes or variants might affect brain structure or activity in brain scans of people with neuropsychiatric afflictions. They parse MRI scans to see how gray matter volume might change in ADHD, and measure cortical thickness and the surface area that stretches across the gyri — the hills and valleys of the brain's walnut-like shape. Among other aspects, Franke and others believe that ADHD appears to originate during development, it affects neurite outgrowth and projection, and is shaped by factors involved in synaptic transmission[3]. Perhaps, says Franke, dopamine neurotransmission, an "old" candidate, also plays a role.

The amassed genetic and transcriptomic information from projects such as PsychENCODE indicate that ADHD appears to involve many genes, each with a small effect. Hints about ADHD can come from imaging genetics research, in which molecular information is computationally combined with structural information.

"Hypotheses are easy, coming up with proof is hard," says Patrick Sullivan.

Credit: Ulf Sirborn

Neuropsychiatric disorders do not generate easy-to-discern brain lesions. "They're very subtle phenotypes that are quite hard to measure accurately," says Sullivan. To tease out statistically significant visible signals, researchers scale up cohorts. To look at one gene variant in one brain region, a lab needs at least 500 people, which is still a low number, says Franke. Finding 10,000 people with schizophrenia for a study is hard, trying to harmonize their brain-imaging phenotypes, "damn, that's tough," says Sullivan.

Franke sees promise in advances such as "voxel-based GWAS", in which the brain is segmented into 3D-pixels. "It's a lot of computation" to, for example, segment the brain into two million voxels and test around ten million genetic associations in each, she says. But such analysis can bring labs closer to a more precise understanding of these disorders. At Radboud's imaging center, DNA is collected from people taking part in studies. "For that reason, we were one of the first to have a big cohort to look at," she says. It takes scalable model systems to explore implicated pathways and processes, to find the probable involved cell types and brain regions, she says. Rat and mouse studies can be too costly and time-consuming, but unconventional models — fruit flies or zebrafish — can let labs measure and manipulate at scale. "This will help us understand the dynamics of the system that we don't get from the bioinformatics studies," she says. To these data, labs can add information about people. After all, many facets of neuropsychiatric disorders cannot be shown in model organisms.

"A true bottleneck in our field right now is advancing the utility of diverse experimental systems," in which to interrogate the function and dysfunction of selected genes and pathways, and to develop criteria for how to appropriately match the lab's questions with model systems, says Steve Hyman, who directs the Stanley Center for Psychiatric Research at the Broad Institute of MIT and Harvard. Some will ideally be human cellular systems, perhaps 2D or 3D, as in organoids, but for regulatory loci "informative patterns of gene expression depend not only on species but cell type, which for the nervous system is daunting." Work in model organisms will be necessary, especially if the genes in question influence circuit development, cognition or behavior. "In doing so, it is critical to be clear-eyed about what is conserved from the chosen species to humans," he says.

Cortical thickness and changes in brain surface play a role in neuropsychiatric disorders, "but the links from genes to cortical thickness to disease, that's still a huge leap to make," says Franke. "We are now getting to the point where we have enough power to robustly examine the impact of genetic variants that influence psychiatric disorders on the gross morphology of the brain," says Sarah Medland, a researcher in psychiatric genetics at QIMR Berghofer Medical Research Institute; she also co-founded ENIGMA and co-chairs its GWAS working group.

In the cortex, GWAS reveal more genome-wide significant variants and higher SNP-derived heritabilities for surface area traits than cortical thickness traits, she says. "This could mean that the effects of common variants on cortical thickness are smaller than those we see on surface area, or that the relative contributions of common variants as compared to other types of genetic variation are smaller." While such findings, in part, reflect the studies' statistical power, they may also suggest that some of the reported effects of neuropsychiatric disorders on cortical thickness may be a consequence of the disorder rather than a cause. ENIGMA brings together researchers from around the world to collectively answer questions and conduct well-powered and robust analyses, says

Convergence flows information across levels of investigation, from molecules to cells to circuits, says Barbara Franke.

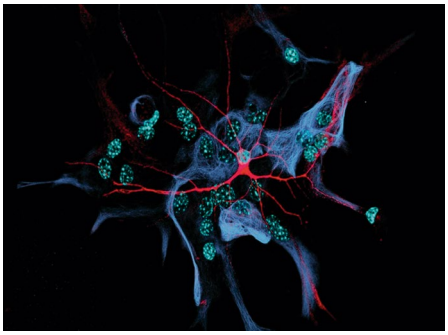
Credit: Milette Raats

Medland. Given how separately the genomics and brain imaging communities have evolved and grown, they can seem to be different planets, says Franke, but "I think the planets are moving closer." Induced pluripotent stem cells (iPSCs) are part of the needed scalable systems, she says. Labs can compare patient cells with those from non-afflicted people, and generate a variety of specifically altered neurons to see cell function effects.

Single stem cells

Stem cells differentiated into neurons in five experienced labs might differ subtly from one another, says Brüstle. The neurons might vary in terms of differentiation stages or subtype, perhaps due to passaging techniques, media or differing cell densities in a dish. To avoid such confounders, he and colleagues developed a reductionist system. "The ultimate simplicity is to have a single neuron talking to itself," says Brüstle. Neurons typically communicate with other neurons; when cultured as single cells they create synapses with themselves. Such autaptic systems, widely used in neuroscience, had not, to his knowledge, been combined with stem cells.

Along with colleagues at VU University Amsterdam and the Max Planck Institute of Experimental Medicine, his team combined an autaptic system with controlled differentiation of iPSCs into neurons grown on microdots of glia[4,5]. The labs joined up in the European consortium CoSyn, the European Consortium Comorbidity and Synapse Biology in Clinically Overlapping Psychiatric Disorders. "We wanted to marry, essentially, the different expertises" says Brüstle — his lab's experience with stem cells and his colleagues' experience with electrophysiology and rodent neurons in autaptic systems. To translate the autaptic system for a stem-cell scenario, he says, "we had to sift through a whole series of challenges," including some "boring, tedious" experiments. They teased out maturation times, media types and the best choice of astrocytes to co-culture with neurons, to build a scalable system for culturing hundreds, potentially thousands, of autaptic systems that deliver results with statistical power.



When cultured alone, a neuron (red) will 'talk' to itself. When coupled with stem cell techniques, this is a way to model neuropsychiatric disorders. Credit: A. Shaib, JS Rhee, MPI Exp. Medicine

The researchers quantitatively assessed synapse function and physiology to assure, for example, the neurons were comparable to ones derived and cultured the classic way. Using transcription factors, the team accelerated and coordinated differentiation of excitatory or inhibitory neurons, as experimentalists might need. They introduced genes into a safe harbor locus. This system, says Brüstle, diminishes the variability and noise that labs encounter in dishes with many communicating neurons and when population-based electrical activity is measured with multi-electrode arrays. A lone, autaptic neuron 'talking' to itself is not typically found in the brain, but it is a standardized way to study synaptic plasticity, function and dysfunction. Pathology can sometimes be the sum of several effects. "There's no way you can model complex cognitive functions in a dish," he says, neither in 2D nor 3D systems. But such assays might reveal dysfunction at pre-or post-synaptic connection points that matter in these disorders.

Brüstle says the neuropsychiatry community is increasingly open to such approaches. Labs can use patient cells and work with specific neuronal subtypes such as excitatory, inhibitory or dopaminergic neurons. With stem-cell-based disease modeling, labs model variants and 'repair' them using, for example, gene-editing to insert mutations and assess phenotypic changes. "All this comes with strings attached," he says. Multiple rounds of manipulation can make the system fragile. How to relate such assays to the human brain's circuits of millions of communicating neurons is unclear, he says. Even organoids are limited reflections of typical brain architecture. But they deliver higher resolution for studying how mutations affect synaptic transmission. It's insight that has to be complemented by other assays.

CRISPR-screens

When cells from patients and people not afflicted by a disorder are used to generate iPSCs, they will differ from one another partly because of differing genetic backgrounds, says UCSF's Kampmann. But amid the noise sits valuable information, especially about neuropsychiatric disorders. He is using CRISPR-based screens to model gene expression changes in neuropsychiatric disease. Using iPSCs, he and his team control and manipulate gene expression in different ways: they use CRISPRi — a gene interference approach to knock-down genes and explore genetic loss-of-function — and gene activation with CRISPRa. The team is tweaking the system to enable, for example, inducible CRISPRi to initiate gene perturbation after stem cells have completely differentiated to neurons, which helps with interpreting phenotypes. Kampmann and his team have built a high-throughput, automated imaging-platform to, for example, track effects of gene interference or activation on neuron differentiation, or to measure neurite-branching in developing neurons[6]. They characterize phenotypes of single cells from patients and non-afflicted people, and address the finding that some variants associated with neuropsychiatric disease don't necessarily change the amino acid sequence of encoded proteins but do change gene expression. "We can take all of these disease candidates and actually change the expression in a controlled way, and ask what are the changes we observe along this lab model of human brain development," says Kampmann. He is part of the Psychiatric Cell Map Initiative (PCMI)[7] that includes scientists at University of California San Francisco, Berkeley and San Diego (UCSD) and aims to map physical and genetic networks in neuropsychiatric disorders.

"We're trying to understand all the components in the cell that go awry or can go awry in these psychiatric diseases like autism and schizophrenia," says Ideker, who's part of the PCMI. To do so, they develop experimental and computational technologies to map biological systems, in particular to map genes and proteins to pathways. Genetic variation across patients is wide, which is why Ideker, Nevan Krogan from UCSF/Gladstone Institutes, and others, hope to develop methods that can be applied across projects, including The Cancer Cell Map Initiative and the Host Pathogen Mapping Initiative. Not only is there work to do, he says, "we are hiring." Ideker and his team apply deep-learning techniques including his lab's DCell[8] for genotype-to-phenotype translation. They used it to predict yeast growth and now want to adapt it to analyze datasets of genetic mutations and variants as well as proteomic measurements; including disrupted protein–protein interactions revealed by UCSF's Ruth Huttenhain and Krogan. The PCMI aims to map that pattern onto pathways indicative of risk, such as for schizophrenia, and integrate it with knowledge about cells and tissues. Such mapping may one day play a clinical role. "That's the grand challenge that precision medicine is going after and, importantly, systems biology is going after," says Ideker.

Single-cell to architecture

In autism labs, some findings about disease-associated changes in gene expression involve deeper cortical layer neurons, which project out of the cortex to, for example, the thalamus, brain stem and spinal cord. Other labs see more evidence for involvement of upper cortical layer neurons, which typically connect to other cortical regions, says Arnold Kriegstein, a UCSF developmental neuroscientist. Now, there is perhaps a way to reconcile these disparate findings. As an early adopter of single-nucleus RNA sequencing (snRNA-seq), the lab analyzed post-mortem brain tissue of people with autism between 4 and 22 years old, who had died of other causes[9]. In their brains, transcriptional changes were apparent in deeper cortical layer neurons, which act as inter-cerebral connectors. Changes were also apparent in cortico-cortical projection neurons in upper cortex layers.

The identified cells have functions in common, says Kriegstein. For iPSC-based disease models, says Dmitriy Velmeshev, a postdoctoral fellow in the Kriegstein lab, this is a potentially helpful finding. But it's still hard to generate fine-tuned subtypes of neurons in a dish. This tuning can depend on cells' intrinsic properties, connectivity and surroundings. He chose snRNA-seq as a better way to look at cell-type specific changes in ASD than fluorescence-activated sorting of cortical cells or gene co-expression network analysis. Given how sensitive snRNA-seq is, he needed to modify existing protocols and address sample quality. Brain banks indicate sample quality with RNA integrity numbers (RIN), but many samples had degraded far from their originally measured RIN and were excluded.

To study autism, some groups analyze what might go awry early in development, says Kriegstein. He and his team chose a different tack: they looked at the brains of people with ASD symptoms. The cortex changes over a person's lifetime. The neurogenesis and neuronal migration of early development, "it's all history by the time you present later in life," says Kriegstein. In his view, snRNA-seq offers "huge potential" to gain insight into a range of different genetic diseases. It would be informative to look at

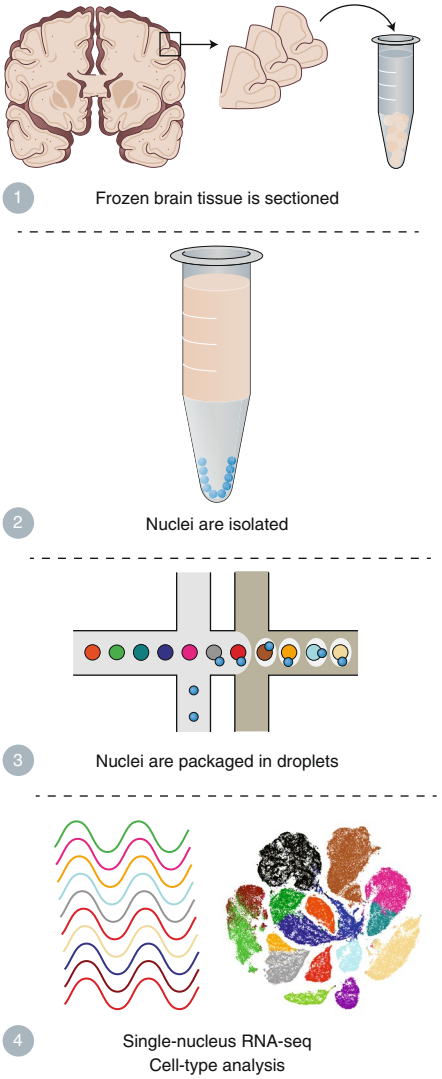
both DNA and RNA in those cells, he says. Labs are working on such a possibility but, "I don't think there's technology yet." Looking at both RNA and DNA might help with dissecting sample heterogeneity and help to classify them better in terms of genetics and cell types, says Velmeshev. This could support convergent analysis to see, for example, if symptoms have similar underpinnings. Many genetic findings in neurodevelopmental disorders such as ASD, he says, stem from rare mutations identified in families, such as the Simons Simplex Collection with samples from families with one ASD-afflicted child.

Bigger cohorts will be helpful, says Velmeshev. Despite exome sequencing, they were unable to tease out molecular subtypes. Also, in autism, some individuals have mosaicism in their bodies' cells, with different mutations and changes that can occur later in life. The lab has begun studying Dup15q syndrome, which leads to a form of autism and is due to duplication of part of chromosome 15. It causes epilepsy and developmental delay. Even in this genetically defined syndrome, the disorder can be quite heterogeneous, he says. Their findings in ASD, says Kriegstein, hint at where to look next, such as the upper cortical layer neurons and synapses between cortical regions. Their approach could be applied in ASD to explore possible involvement of the cerebellum and striatum, a subcortical region with connections to the cortex. "There's quite a lot that could be done with this technology." His lab is not affiliated with consortia "but we're hoping to be in the not too distant future."

A tall order

'Madness', "has been part and parcel of humanity for a long time," says Sullivan. It's long been observed to run in families, he says, a fact that a play first performed in the late 1800s could allude to. In Henrik Ibsen's 'Ghosts', Oswald tells his mother about his illness and recounts his doctor saying: "There has been something worm-eaten in you from your birth. …The sins of the fathers are visited upon the children." Viewed as scandalous by many, the play is about family predicaments. Oswald is 'mad', probably suffering from neuro-syphilis, which became a treatable infectious disease, says Sullivan. But not all forms of 'madness,' such as schizophrenia, have successful treatments.

Schizophrenia can cause constant, debilitating hallucinations in some, mild symptoms in others, or sometimes disappear after just a few episodes. Every generation of researchers has leveraged their best technologies to battle schizophrenia, says Sullivan: linkage analysis, candidate-gene analysis or small-scale GWAS. The results have not been secure or replicable, he says. "Largely, we've actually confused ourselves."The genetic signal in schizophrenia is subtle. Elevated risk in families does not automatically mean that many family members will be afflicted. "There's going to be hundreds of genes involved in schizophrenia, that's what data are indicating right now," he says. Progress in schizophrenia-research is slow. Early genetic linkage studies, such as those on Huntington's disease, or candidate gene research in Alzheimer's disease, found a high degree of association with certain genotypes, says Sullivan. The research community sensed they might readily find genes responsible for conditions such as schizophrenia, which has not proven true, he says. Sullivan was involved in a one of the first GWAS projects on major depression. They had around 1,700 cases, now considered unacceptably low. Current depression studies have over 100,000 cases, which is needed given the involvement of so many genetic loci, and to deliver statistically significant results. "We've learned what's been required," says Sullivan. It led to consortia such as the PGC, in which members join resources — case studies, genetic data and tools — to study around ten different psychiatric disorders, including schizophrenia, ASD and depression. "We're essentially a mega-analysis consortium," he says.



Using single-nucleus RNA sequencing, researchers explored cell-type-specific changes associated with autism. Credit: D. Velmeshev, Kriegstein lab UCSF

In training scientists, Sullivan knows they cannot have expertise in all approaches brought to bear in neuropsychiatric research: imaging, organoids, stem cells, genomics and proteomic analysis, and statistics. If he tried to operate a mass spectrometry machine, "I'd probably break it," he says. But his sense of the difference between good and poor data can guide team science. Putting new technologies to work on neuropsychiatric disorders will let labs look at the next level of complexity and shine light on realistic cell-based models, simple circuits and organoids. "Maybe that will be enough to learn some key lessons, maybe it won't," he says. "But this is way more interesting and orders of magnitude more realistic modeling than we've ever had before," says Sullivan, "And with the genetic data, we actually know where to look."

Scientists in bioinformatics and genetics are clustering information, but "learning each other's language is really difficult," says Franke, yet it's an important quest. Patient organizations tell her how urgently they need to convince others, including some physicians, that neuropsychiatric disorders such as ADHD are "not just something that lives in our minds; this is a real disorder," she says. Genetic insight is advancing disease research, now that molecular knowledge helps generate a more fine-grained view, says Brüstle. "The same thing," he says, "is happening and will happen in the context of neurodegenerative and neuropsychiatric disorders." ❐

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