From maps to mechanisms through neuroimaging of schizophrenia

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Functional and structural brain imaging has identified neural and neurotransmitter systems involved in schizophrenia and their link to cognitive and behavioural disturbances such as psychosis. Mapping such abnormalities in patients, however, cannot fully capture the strong neurodevelopmental component of schizophrenia that pre-dates manifest illness. A recent strategy to address this issue has been to focus on mechanisms of disease risk. Imaging genetics have made it possible to define neural systems that mediate heritable risk linked to candidate and genome-wide-supported common variants, and mechanisms for environmental risk and gene–environment interactions are emerging. Characterizing the neural risk architecture of schizophrenia provides a translational research strategy for future treatments.

One-hundred-and-two years after the term schizophrenia was first used, this disorder remains one of the most serious, disabling and baffling brain diseases. Modern imaging techniques (Fig. 1) have been very useful in mapping out networks in the brain that are affected in patients with schizophrenia. This has contributed to establishing schizophrenia research firmly in the broader neuroscience community, and has the potential to result in reduced stigma for patients and their families. However, a main hope of advancing the neuroscience of schizophrenia is that this will lead to new and better treatments, which are needed urgently. For this goal, mapping is not enough: convergent evidence shows that the disease process of schizophrenia long precedes manifest illness, and that abnormalities found in patients may reflect a complex and advanced condition that could be too late in the trajectory of the disease for guiding causal treatment or prevention, similar to being able to diagnose coronary artery disease only at the point of myocardial infarction. Can systems-level neuroscience help towards advancing the translational enterprise? Here, I propose ways in which this might be possible. After briefly recapitulating known functional, structural and network abnormalities in schizophrenia, I will discuss data indicating that neuroimaging is indeed useful in characterizing both genetic and environmental risk factors for schizophrenia that are likely to be causally related to the illness. I finish with some suggestions on how characterized mechanisms of illness risk can be effectively interfaced with traditional translational and drug-development processes.

Brain regions involved in schizophrenia

Structure

Extensive work studying the neuroanatomy of schizophrenia using imaging has shown clear abnormalities in at-risk subjects and both first-episode and chronic patients (Fig. 2). Overall grey matter, white matter and whole brain volume are decreased, whereas ventricular volume is increased. In the beginning of the disease, volumes are decreased in the hippocampus, thalamus, the left uncus/amygdala region, the bilateral insula and the anterior cingulate. In chronic schizophrenia, more extensive volume reductions are observed in the cortex, particularly in medial and left dorsolateral prefrontal cortex, but also in the left superior temporal gyrus. The magnitude of these alterations is mostly small to moderate, and there is considerable overlap between patient and control distributions. Hippocampal volume reductions are found in relatives of schizophrenia patients, indicating a heritable component, whereas the evidence for disease-related heritability for other cortical and subcortical features is more mixed. Volume increases in first-episode schizophrenia are restricted to parts of the putamen and spread in chronic schizophrenia throughout the dorsal striatum. These increases are not heritable and are probably a consequence of antipsychotic drug action. In addition to volume changes, abnormalities in cortical thickness, gyriﬁcation and subcortical shapes have been reported.

Microcircuits

Corresponding to these macroscopic alterations in the brain of schizophrenia patients are changes in local microcircuits (Fig. 3). In prefrontal cortex, pyramidal neurons—the main source of excitatory cortical–cortical neurotransmission—are reduced in size and packed more densely, indicating a reduction in axon terminals and dendritic spines that occupy

Figure 1 | Functional neuroimaging methods and their temporal and spatial resolution. Magnetoencephalography (MEG) and electroencephalography (EEG) image the electromagnetic effects of neuronal (assembly) action; their temporal resolution can be on the order of milliseconds whereas their spatial resolution tends to be less than that of fMRI, which images blood flow or oxygenation effects of neuronal activation, and PET, which uses radioisotopes to label molecules in the brain. fMRI and PET, in turn, are limited in their temporal resolution to several 100 ms (for fMRI) and minutes (for PET).
the space between neurons that may be a consequence of exuberant synaptic pruning during adolescence. Several interneuron populations in the prefrontal cortex are reduced, such as those containing parvalbumin, which show consistent signs of reduced GABAergic neural transmission, but also those expressing neuropeptides such as somatostatin or the cannabinoid receptor CB1. In the hippocampus, cell bodies of pyramidal neurons are smaller, dendritic spines are reduced, and there is inconsistent evidence of aberrantly located or clustered neurons in adjacent structures, especially the endorhinal cortex. In the thalamus, some studies indicate reductions in neuron number, especially in the mediodorsal nucleus and pulvinar. Taken together, these findings are in reasonable agreement with structural imaging results. They suggest abnormalities in local processing, especially in glutamatergic drive to GABAergic parvalbumin-containing interneurons and intracortical connectivity, but are also indicative of changes in long-range connectivity, including thalamic afferents. An important source of modulation in prefrontal cortex function is dopaminergic. The delicate balance between information maintenance and flexible adjustment of information that characterizes executive function depends critically on an optimal level of dopamine signalling which reaches prefrontal cortex from midbrain and ventral striatum; these prefrontal inputs appear to be reduced in schizophrenia.

Function
Cortical and subcortical information processing is functionally abnormal in both first-episode and chronic schizophrenia (Fig. 1). Most functional studies use an ‘activation paradigm’ in which a cognitive task is used to engage brain systems of interest, and the results are therefore usefully summarized under these cognitive domains, without suggesting a causal relationship between cognitive (sub)function, brain system and schizophrenia symptoms.

Executive function. Much attention has been focused on executive function (including working memory and selective attention), sub-serving flexible adaptation of behavioural patterns to external demands. Here, patients show quantitative abnormalities in dorsolateral prefrontal cortex (which have been linked to negative symptoms), rostral anterior cingulate and inferior parietal lobe. In dorsolateral prefrontal cortex, patients show relatively inefficient prefrontal activation under low cognitive load, indicative of decreased signal-to-noise ratio, and a decrease in activation when executive demands exceed capacity. There is evidence for compensatory activation in the ventrolateral prefrontal interneuron; purple, basket neuron; red, thalamic neuron; yellow, dopaminergic neuron in brainstem.
in patients, indicating a system that comes ‘online’ as the dorsolateral-prefrontal and anterior cingulate system starts to fail\textsuperscript{13}. Before manifest illness, vulnerability to psychosis has been associated with abnormal prefrontal activation at an intermediate level in high-risk populations\textsuperscript{14}.

**Episodic memory.** Episodic memory depends on interactions between the hippocampal formation and regions of the prefrontal cortex. In schizophrenia patients, dorsolateral prefrontal cortex activation is abnormally decreased\textsuperscript{15}. Less consistently\textsuperscript{14,15}, decreased activation has been found in the hippocampal formation, possibly because it is part of the ‘resting state network’ and therefore it is difficult to achieve a reliable baseline.

**Reward and salience.** In reward and salience processing, prediction errors are signalled by midbrain dopamine neurons projecting to the ventral striatum and dorsolateral prefrontal cortex. For perceptual salience tasks, increases of midbrain and ventral striatal signals have been demonstrated in schizophrenia\textsuperscript{26} and in high-risk subjects. Opposed to this, ventral striatal responses to reward seem to be reduced in schizophrenia in several\textsuperscript{17,18}, but not all, studies\textsuperscript{19} and correlate with negative symptoms\textsuperscript{19}.

**Emotional regulation.** In the domain of emotional regulation, activation of amygdala to emotional images seems to be consistently reduced in schizophrenia patients\textsuperscript{19}, but not relatives\textsuperscript{20}, whereas neutral face expressions may lead to greater limbic activation in schizophrenia patients and is correlated with flat affect\textsuperscript{21}. In a circuit between medial prefrontal cortex and amygdala, which is critical for the regulation of emotion processing, schizophrenia patients, but not healthy relatives, show reduced functional connectivity\textsuperscript{20}.

**Social cognition.** An area that has recently come under increased focus is social cognition. Neuroimaging has identified abnormalities in the medial prefrontal cortex, the temporoparietal junction and the amygdala in schizophrenia\textsuperscript{22}. Prefrontal abnormalities are consistent with the interpretation that patients ‘hyper-mentalize’ (that is, show prefrontal activation for stimuli that have no objective social or intentional content\textsuperscript{23}), a possible mechanism leading to delusions.

**Hallucinations.** One of the principal symptoms of schizophrenia is hallucinations, especially the perception of voices in the absence of external stimuli. Imaging results have demonstrated activity of auditory and speech processing cortices during hallucinatory experiences\textsuperscript{24}. These findings seem to correlate with the extent of functional and structural connectivity abnormalities to speech areas in the temporal lobe\textsuperscript{25}, lending support to the idea that dysconnectivity of this region is important.

**Connectivity**
Since Wernicke’s proposal at the end of the nineteenth century a disturbance of integrated activity has been viewed as fundamental for schizophrenia. Neuroimaging has been useful in defining abnormal circuits, especially with dorsolateral prefrontal cortex, and has shown that, rather than a uniform disruption or dysconnectivity, schizophrenia is characterized by ‘dysconnectivity’: functional interactions are altered in a regionally and functionally differentiated manner.

During working memory, dorsolateral prefrontal cortex connectivity is altered in patients with schizophrenia and subjects at risk\textsuperscript{26,27}. Interhemispheric prefrontal connectivity is reduced in patients and relatives, whereas a dysfunctional increase in the connectivity with the hippocampal formation (Fig. 4a) has been observed in chronic\textsuperscript{28} and first-episode psychosis, and in at-risk subjects\textsuperscript{29}. Data from resting-state networks indicate that dysfunctional increases of connectivity may be found within the extended limbic system in patients and subjects at risk\textsuperscript{27}. A similar lateral-neocortical versus temporal and extended limbic distinction is suggested by multivariate analyses\textsuperscript{30,31}. Recently, methods from topology have begun to be applied to brain networks\textsuperscript{32}. One conclusion emerging from this work is that the human brain has properties in common with other complex systems (such as the Internet) that support an efficient and robust transfer of information while keeping wiring between regions low\textsuperscript{32}. These ‘small world’ properties may be altered in schizophrenia\textsuperscript{33} and predict impaired cognitive performance. Abnormalities of adult brain network organization related to schizophrenia would be expected to follow aberrant early brain development, but we know little about the normal development of brain functional networks or how this might be perturbed pre-clinically in individuals at high risk. Further signatures of abnormal local processing and connectivity defined using AA) in ZNF804A (modified, with permission, from ref. 76), which again shows persistent coupling between prefrontal cortex and hippocampal formation, this time measured with fMRI during the n-back working memory task (b), and in electrophysiological measurements in a mouse model of high genetic risk (22q11DS) (modified, with permission, from ref. 84) (c). Error bars, standard error.

Figure 4 | A systems-level phenotype in patients relates to genetic risk and animal models. a–c. Abnormal prefrontal–hippocampal connectivity (measured as correlation of activity in PET across task conditions) during working memory (2-back) compared to a control task (0-back) in patients with schizophrenia and matched controls (modified, with permission, from ref. 28) (a), in carriers of the genome-wide significant genetic risk variant (genotype
functional magnetic resonance imaging (fMRI) and positron-emission tomography (PET) can be found with electrophysiological imaging (see Box 1).

Of these abnormal functional dorsolateral prefrontal cortex interactions, two systems deserve further discussion. The hippocampal formation provides input to the dorsolateral prefrontal cortex, and neonatal hippocampal formation lesions in animals induce prefrontal cortex abnormalities post-pubertally, indicating a causal role of the interaction between these two regions in schizophrenia. This fronto-hippocampal dysconnection hypothesis is also attractive as the hippocampal formation is selectively vulnerable to some early neurodevelopmental disturbances, such as obstetrical insults. Second, multiple parallel interactions between prefrontal cortex, thalamus and striatum form feedback loops critical for basic information processing; these feedback loops are disturbed in schizophrenia patients. This prefrontal-neostriatal system is modulated by midbrain dopaminergic neurons which project to cortex and striatum and are, in turn, regulated by prefrontal cortex efferents. This system is relevant for an understanding of acute psychosis.

A mechanistic account of acute psychosis
Abnormal dopaminergic neurotransmission is known to be important for psychosis because the effectiveness of antipsychotic drugs is directly related to dopamine D2 receptor blockade. Therefore, psychosis has been linked to a ‘hyperdopaminergic’ state, a hypothesis later modified to posit increases in striatum, while cortical dopamine was supposed to be reduced. Unambiguous evidence for this from neuroimaging is only provided for striatum, where patients with schizophrenia have elevated presynaptic dopamine synthesis and dopamine release and moderate increases of dopamine D2/3 receptor levels. Notably, elevated dopamine synthesis and release is also seen in subjects at risk for schizophrenia and in the prodromal stage, indicating that they are part of the risk architecture of the illness.

Although assays of dopamine D1 and D2/3 receptors in prefrontal cortex have remained incomplete, there is evidence that functional activation abnormalities in prefrontal cortex are tightly linked to striatal dopaminergic disinhibition in schizophrenia patients and high-risk subjects, indicating an abnormality of prefrontal regulation of the midbrain-striatal dopamine system. Because dopaminergic signals in midbrain and striatum are essential for the signalling of salience, abnormal subcortical dopamine release could lead to aberrant assignment of salience to non-salient events, providing a plausible account for the emergence of psychosis. Although functional imaging evidence for this model has been provided, it is indirect because measurements of midbrain dopamine release in relationship to psychosis in patients with schizophrenia have not yet been performed. Further work will also be necessary to clarify how other aspects of acute psychosis (such as hallucinations) are linked to dopamine dysregulation, as a salience account is unlikely to explain the entire spectrum of positive symptoms.

Why mapping is not enough
Despite these successes in delineating abnormalities in schizophrenia across the lifespan, such findings in manifestly ill patients can be related to numerous confounds other than illness status. For example, patients with schizophrenia often smoke, take a variety of medications, are in poorer somatic health, have different lifestyles and educational socioeconomic trajectories, may have been frequently hospitalized, and so on. Because it is almost never possible to control for these confounding measures, the true causal contribution of a systems-level finding obtained in this way is never certain. Even more importantly, as the evidence supporting the neurodevelopmental hypothesis of schizophrenia indicates, data gathered during the stage at which schizophrenia is currently defined capture a stage of the illness that may characterize the brain at too late a stage for intervention, and thus do not offer much in terms of finding new, and especially causal, treatments. Given the clear evidence from heritability studies that a large proportion of schizophrenia risk is related to genes, and a smaller, but still sizable, proportion is

BOX I
Oscillations and schizophrenia
Oscillations are important organizers of brain activity, plasticity and connectivity. They can be measured using electroencephalography (EEG) or magnetoencephalography (MEG). Oscillations in the gamma range are important for synchronized activity within local cortical networks. Essential for the generation of local gamma activity are parvalbumin-containing GABAergic interneurons under glutamatergic stimulation. Cognition requires that the results of local computations are globally integrated. Neural oscillations in the low (especially theta) ranges are critical for long-range connectivity because they engage larger areas and effectively modulate fast local oscillations, such as gamma oscillations. In hippocampus, highly synchronized theta frequency oscillations are observed which have been proposed to serve as a temporal organizer for cortex. This has recently been demonstrated in mouse, where hippocampal theta oscillations drive cortically generated gamma oscillations through phase locking. Importantly, NMDA (N-methyl-d-aspartate) antagonism can influence both local and long-range synchronization because NMDA receptors in superficial layers of cortex, the main recipients of long cortical connections, control local processing. Dopamine modulates these oscillations. In the prefrontal cortex in schizophrenia patients, reductions in the gamma and theta band have been observed at rest and during stimulus processing. Aspects of these features seem to be present in first-degree relatives of patients with schizophrenia, indicating a role in the risk architecture.

Temporal coordination of oscillatory activity is critical for experience-dependent plasticity and therefore in the maturation of cortical networks. For spike-timing-dependent plasticity to occur, a window of the order of tenths of a millisecond for the co-occurrence of pre- and postsynaptic spiking has been proposed, which can be achieved through co-stimulation of cortical neurons over the theta-cycle of the hippocampus. This opens the possibility that aberrant oscillations during critical periods can have an enduring effect on the shaping of cortical circuits beyond their immediate impact on local processing. Compromising both long-range coupling (through white matter tract maldevelopment or lesions) and local processing (for example, in interneurons) could have enduring effects on synaptic plasticity. Dopamine could have a modulating role in this process because intact mesocortical dopaminergic input is necessary for long-term potentiation to occur at hippocampal–prefrontal cortex synapses, reflecting dopamine-D2 receptor-mediated dopaminergic control over NMDA-receptor-dependent synaptic plasticity in prefrontal cortex, indicative of a ‘gating function’ of dopamine D2 receptors. A further link to the neurodevelopmental hypothesis is provided by the observation that long-range synchronization of the theta and gamma band undergoes profound changes during adolescence, when cortical–cortical connectivity continues to mature through myelination of long-range tracks. This indicates that the reduction of transmission delays between brain regions during adolescence, especially between hippocampus and prefrontal cortex, enables the kind of precise temporal coordination that is important for activity-dependent shaping of prefrontal circuits. Importantly, this emergence of long-range connectivity has been linked to maturation of cortical grey matter, indicating a causal sequence. Speculatively, in the context of the interaction of hippocampus and prefrontal cortex, a sequence of events seems credible in which hippocampal dysfunction leads to abnormal shaping of neurocortical circuits as soon as hippocampal–prefrontal connections become sufficiently stable during late adolescence. This deficit could even become progressive if experience-dependent plasticity continues throughout adult life.
related to environmental risk factors (as well as to the interaction between genes and environment), this indicates a strategy in which systems-level neuroscience is used to interrogate the neural effects of identified risk factors on the brain in an attempt to define a neural risk architecture of the illness. Because both common genetic and environmental risk factors affect healthy subjects as well as patients, such studies can often avoid the confounders associated with manifest illness and offer the hope to identify mechanisms that lie before the emergence of frank psychosis. Imaging genetics, which combines structural and functional imaging with genetic characterization of healthy participant samples, has shown itself to be a sensitive and specific method to define such mechanisms.

Recent genome-wide evidence indicates that many thousands of genetic variants explain a sizeable proportion of genetic risk for schizophrenia. This high complexity leads to challenges for imaging genetics, which uses the methods of genetic association with brain phenotypes. Just as one variant can have pleiotropic effects, so can several different genes influence the same neural pathways to risk. Results may be influenced by the genetic background. Although imaging genetics studies have provided evidence for both pleiotropy and epistasis, the problem of interacting genetic variants remains difficult. Although a variety of studies have investigated two or three variant interactions, few have been replicated so far and the underlying complexity is probably higher. This may be addressed by emerging multivariable methods that can deal with a large number of single nucleotide polymorphisms (SNPs) together with complex brain phenotypes, but these still need to be validated. Attention also needs to be paid to the heritability and reliability of the imaging paradigms.

As the ‘endophenotype’ concept predicts, the penetrance of genetic effects on the level of brain imaging is high: two meta-analyses of imaging genetics found effect sizes of 0.7–1.0, very considerably higher than what was found for association of the same variants with psychiatric diagnoses. In addition, imaging genetics has the critical advantage of mapping genetic effects across the brain, in many cases allowing researchers to tie in the large body of preclinical knowledge that specifies how a given neural system—affected by genetic variant—functions in healthy subjects and what molecular, cell biological and systems-level factors influence its development and neural processing.

Risk mechanisms in schizophrenia
Candidate genes
Because single common risk variants for schizophrenia only cause moderate increases in relative illness risk, it is not surprising that association evidence is often inconsistent, especially as the current definition of schizophrenia, which is based on patient introspection and clinical observation, is unlikely to correspond to one well-defined biological entity. Also, with regards to genetics, not enough functional variants are yet known in genes of interest, and functional genomics approaches are necessary to identify them, especially in very large genes such as neurelin 1 (NRG1). Nevertheless, several such variants have repeatedly found support in association studies and are backed up by meta-analysis, justifying their investigation through systems-level neuroscience techniques. Such systems-level findings, in turn, can serve as one approach to in vivo functional genomics that can aid the discovery of functional variants. In the following brief overview, I cannot cover the range of candidate genes explored using imaging genetics and schizophrenia. Therefore, I will provide three examples: catechol-O-methyltransferase (COMT), NRG1 and disrupted in schizophrenia 1 (DISC1).

COMT has been the most-studied gene in the imaging genetics literature. It encodes an enzyme that degrades catecholamines, including dopamine. COMT is particularly concentrated in the extrasympathetic spaces of the prefrontal cortex and hippocampus. Because prefrontal dopamine transporters are scarce, COMT is thought to have a key role in clearing dopamine in the prefrontal cortex. An evolutionarily recent functional SNP in COMT results in the amino acid substitution of valine (Val) with methionine (Met) at codon 158 (rs4680), leading to a significant decrease in enzymatic activity in the brain and lymphocytes of the Met allele, which therefore causes a higher level of prefrontal extracellular dopamine. The functional literature on the common rs4680 Val/Met polymorphism in COMT shows a highly consistent and large effect of rs4680 on prefrontal activation. Effects of rs4680 on brain structure are less consistent, possibly because they may differ in directionality between prefrontal cortex and hippocampus and show significant interactions with another putatively functional promoter region SNP. In multimodal neuroimaging, rs4680 modulated the functional interactions between midbrain dopamine synthesis and prefrontal function, mirroring post-mortem findings and indicating an entry point into the neural circuit for acute psychosis described above through this genetic risk variant.

NRG1 was first implicated in schizophrenia in an Icelandic sample. NRG1 and its receptor ERB4 have important functions during brain development through signalling axon guidance, progenitor cell proliferation and neural migration in cortex, and seem to have a special role in shaping the development of parvalbumin-containing GABAergic interneurons. Postnatally, NRG1 is implicated in activity-dependent plasticity at glutamatergic synapses, myelination and oligodendrocyte differentiation. Neuroimaging has uncovered possible functional and structural correlates of dysmaturation associated with genetic variants in this system. In high-risk individuals, carriers of a NRG1 risk SNP had an increased risk for psychosis, compromised activation in medial prefrontal and temporocingulate regions during a sentence completion task, as well as impaired prefrontal and middle temporal lobe activation during semantic fluency. Hippocampal volumes were smaller in carriers of a risk haplotype of NRG1 (ref. 62), and a risk SNP in the same region was associated in patients with larger ventricular volumes. That same SNP also associated with reduced structural connectivity in healthy controls studied with diffusion tensor imaging.

DISC1 was implicated by the discovery of a translocation disrupting the gene in a large Scottish pedigree with a high density of mental disorders. DISC1 is a multifunctional anchoring molecule that regulates different subcellular compartments, including at the synapse. It is involved in neural progenitor proliferation, differentiation and radial migration and dendritic arborization. In adult brain, DISC1 is highly expressed in hippocampus, where it has a key role in regulating adult neurogenesis.

Neuroimaging has identified the effects of genetic risk variants in DISC1 and prefrontal and hippocampal structure, function and interactions. A functional Ser704Cys polymorphism (Ser substituted for Cys at position 704) impacts on hippocampal structure and function, and prefrontal efficiency during verbal fluency. Hippocampal formation–dorsolateral prefrontal cortex functional connectivity was increased in risk allele carriers, an intermediate connectivity phenotype similar to that seen in overt disease (Fig. 4a). A common haplotype was associated with reduced grey matter in hippocampus and more prominently in prefrontal cortex.

It is interesting to consider possible molecular points of convergence between these risk candidate gene systems. It has been previously noted that multiple candidate genes have an impact on the plasticity of glutamatergic synapses. The recent evidence reviewed above extends this conclusion into the domain of early brain development. Both ERB4 and DISC1 are located in the postsynaptic density of glutamatergic synapses, where they co- localize with other susceptibility factors for schizophrenia and are exposed to varying levels of extra- neuronal dopamine regulated by COMT. Furthermore, activity- dependent synaptic pruning is likely to be mediated by all of these factors. Neuroimaging data are beginning to define functional interactions between these risk variants and the impact they have on prefrontal cortex activity and brain structure, validating these ideas from cellular
neuroscience on the systems level. It will be important to examine the neural circuits so defined in new animal models that carry several of these genetic risk variants, permitting an examination of their convergence on pre- and postnatal maturation and synaptic pruning, especially in adolescence.

**Genome-wide supported variants**

Despite their clear impact on imaging phenotypes, the usefulness of candidate genes for understanding schizophrenia is a subject of debate because their association with the categorical disease phenotype itself is inconsistent. Genome-wide association studies (GWAS) offer an alternative, hypothesis-free way to identify genetic variants associated with schizophrenia. This is especially welcome when treatment implications are considered, for which one needs to study factors clearly related to risk. Although GWAS will probably not provide all of the answers about the genetics of schizophrenia, any common variant that does survive the extreme amount of statistical thresholding that this method requires certainly merits study using intermediate imaging phenotypes. Of those variants, the one with the strongest support is zinc finger protein 804A (ZNF804A), encoding a protein of unknown, but possibly regulatory, function. Like many candidate gene variants, ZNF804A is pleiotropic on the level of psychiatric diagnoses, also being associated with bipolar disorder. In functional neuroimaging with a so-called ‘n-back’ working memory probe, healthy carriers of risk genotypes exhibited no changes in regional activity. However, they did exhibit pronounced gene-dosage-dependent alterations in functional connectivity, which was decreased from dorsolateral prefrontal cortex across hemispheres and increased with hippocampus (Fig. 4b), as described above for schizophrenia patients. Subsequent work has further implicated this variant in cognitive performance for executive cognition and episodic memory specifically, highlighting domains that are especially dependent on prefrontal–hippocampal interactions. Impaired white matter volume and integrity markers in carriers of this risk variant have been observed, as well as the inability to downregulate key parts of the mentalizing system in conjunction with impaired connectivity of this system to dorsolateral prefrontal cortex, indicating possible structural substrates and downstream functional activation effects of impaired prefrontal connectivity that mirror findings in patients. Interestingly, abnormally increased coupling of amygdala was also observed, a phenotype unlikely to be related to heritable risk for schizophrenia and therefore possibly related to risk for bipolar disorder, where similar findings in patients have been described. Another instructive variant is near calcium channel, voltage-dependent, L type, alpha 1C subunit (CACNA1C), first discovered in a GWAS for bipolar disorder but subsequently implicated in schizophrenia. Healthy carriers of this variant showed impaired hippocampal activation and connectivity during episodic memory, mirroring findings in overt schizophrenia, as well as abnormalities in subgenual cingulate and amygdala, highlighting a key regulatory system for emotion and affect implicated in affective disorders. Because bipolar disorder and schizophrenia share a large proportion of genetic risk, it is noteworthy that both CACNA1C and ZNF804A have an impact on circuits that support a pleiotropic effect on both disorders. Further work should study the remaining group of currently established SNPs with genome-wide significance for schizophrenia, including a variant upstream of neurogranin (NRGN) and a SNP in transcription factor 4 (TCF4), both probably involved in brain development, as well as a cluster in the major histocompatibility complex region on chromosome 6p22.1, which could indicate a gene by environment interaction system by implicating the immunological system in the pathogenesis of schizophrenia.

**Microdeletions**

As reviewed elsewhere, one important finding from GWAS is the increased occurrence of structural variations (microdeletions or microduplications) in schizophrenia, but possibly not with bipolar disorder. Of these, only 22q11, causing velocardiofacial or 22q11DS syndrome, was known previously. The new microdeletions are not solely associated with schizophrenia but also with other brain phenotypes such as mental disability, epilepsy and autism. Despite these pleiotropic phenotypical effects and their relative rarity, which makes them account for only a minority of disease risk, identifying and characterizing structural variations holds considerable potential because each of these are associated with significant risk, which exceeds that from common genetic variants.

Although none of the newly identified variants has been characterized on the systems level, previous work on the 22q11 deletion shows that a multimodal imaging approach is feasible and holds the promise to identify neural systems-level abnormalities associated with high genetic risk. Furthermore, as it seems likely that microdeletion risk cannot be explained by deletion or duplication of single genes, but rather interactions of genes jointly affected in their expression, imaging genetics can ask whether variants in such genes converge on neural systems implicated in schizophrenia. An example of this is the 22q11 microdeletion, which includes, besides COMT and several other candidate genes for schizophrenia, the proline oxidase gene (PRODH). A recent neuroimaging study showed that functional polymorphisms in PRODH associated with schizophrenia risk had an impact on prefrontal connectivity.

**Environmental risk mechanisms in human brain**

As reviewed elsewhere, convergent evidence supports an effect of environmental risk factors such as urban birth, prenatal stress, childhood trauma, migration and high expressed emotion. Although, as a group, these risk factors explain less risk than genes, individually they have an associated risk that far exceeds that of common genetic variants. The mechanisms underlying these environmental factors are largely unknown and are unlikely to be unitary. It has been proposed that one such mechanism may be social stress that plays out through activation of the hypothalamic–pituitary–adrenal axis and dopaminergic sensitization. One salient feature of social stress that has been hypothesized to underlie schizophrenia is social defeat, defined as a subordinate position or outsider status, especially if repeatedly experienced. Although direct epidemiological evidence for this hypothesis is missing, experimental studies provide a link to key neural systems features implicated in risk for schizophrenia. In animals, social defeat stress increases the firing rate of dopaminergic neurons in midbrain area and brain-derived neurotrophic factor (BDNF)-dependent activity in the ventral striatum. This indicates links to neural plasticity for which BDNF, a gene inconsistently associated with schizophrenia, is essential, and to the pathophysiology for psychosis outlined above. Although this specific finding has not been established in humans, acute psychosocial stress evokes striatal dopamine release, measured by PET. A further link to environmental risk factors is provided by the observation that in individuals with low maternal care, dopamine release was stronger, as in subjects with schizophrenia-associated personality characteristics.

Although there is indicative data linking social stress in general to subcortical dopamine systems, it remains a potentially fruitful, but currently almost unexplored, application of systems-level neuroscience to define mechanisms of specific environmental risk factors.

An early attempt in this direction has addressed the neural processing of stable and unstable social hierarchies. Social status strongly predicts well being, morbidity and survival. Patients with schizophrenia are strongly over-represented in the lower social strata. In a fMRI experiment, unstable hierarchies, which are associated with health risk, showed specific recruitment of, among others, amygdala and medial prefrontal cortex, identifying a key regulatory system for the processing of negative affect that has been associated with genetic risk factors for affective disorders and schizophrenia, for example in CACNA1C (ref. 81), and linking status processing to key theory-of-mind regions impaired in schizophrenia and carriers of risk genes in ZNF804A. It remains to be seen if amygdala and regulatory systems in the medial prefrontal cortex are also associated with other social risk factors such as migration and urbanicity.
Identifying such neuroenvironmental risk systems will also advance the field of gene–environment interactions on the systems level. For example, it has been observed that carriers of the rs4680 Val risk variant of COMT have higher risk for psychosis when exposed to cannabis92. Neuroimaging has begun to delineate a mechanism by showing that dopamine release in prefrontal cortex—measured indirectly via PET—by the psychotogenic component of cannabis, Δ9-tetrahydrocannabinol, is modulated by this COMT risk variant93. Further demonstration of gene–environment interactions will become feasible as genetic and environmental ‘main effect’ brain mechanisms become better defined and identified.

Systems-level strategies for translation in schizophrenia

The ultimate goal of defining risk mechanisms for schizophrenia preceding frank psychosis is prevention. For this, the tools described above and the dysfunctional circuits that they have defined will have to be applied in large cohorts longitudinally to define transition likelihoods and intervention points. Research of this kind is now underway (for example, in the European IMAGEN study). However, we cannot wait for these data to come in before acting, as translational research in schizophrenia is in urgent need of a conceptual redesign. There is no evidence that the excess mortality of schizophrenia has decreased in the preceding decades, and the number of mechanistically novel treatments for schizophrenia has been disappointingly low94. One reason for this poor performance of translational research in schizophrenia is the difficulty of applying the methods of modern drug discovery to a disorder whose pathophysiology was incompletely understood94. Although genetics is essential in this context, the identification of genetic variants by themselves, even if they are causative, does not mean that a viable drug target or treatment approach has been found, as the example of Huntington’s disease shows. Translation and drug development in psychiatry also face other bottlenecks such as a high degree of placebo responses, tolerability problems, regulatory issues and implementation of new therapies in clinical practice, which await solution.

A functional characterization of genetic (and environmental) risk is necessary to better identify translational entry points. For this, neuroimaging alone is not enough. Cellular models, and ideally access to neuronal tissue, are necessary to understand molecular and cell architectural changes in schizophrenia, clarify epigenetic mechanisms, and to develop molecular biomarkers. There is considerable potential in integrating across cellular and systems levels to develop multivariate biomarker panels. Animal models similarly need to be improved. However, systems-level neuroscience of risk mechanisms already provides some approaches that can help translation in several ways.

First, the characterization of molecular risk mechanisms provides a quantitative entry point for computational neuroscience approaches in translation. For example, it can be quantified relatively easily to what degree a given genetic variant impacts on the abundance or activity of the gene product; however, there is currently no principled way of inferring the systems-level consequences that such a change might have. An example that this approach is feasible is the application of the theory of dopamine modulation of prefrontal cortex computational dynamics to the differential effects of the rs4680 COMT variant95. A biophysically realistic computational model, which will map the global processing features discussed above together with enough detail on genetic effects in the synapse, would be useful to define and refine our understanding of precisely how risk mechanisms affect brain function and what neural computations are most vulnerable to them. This could potentially lead to a new generation of in silico psychopharmacology in which the effect of a drug with a given receptor-binding profile can be linked to a predicted systems-level response.

Second, understanding neural risk mechanisms can help in personalization of existing therapy. An example is again provided by the COMT rs4680 variant: the reduced prefrontal efficiency associated with rs4680 Val alleles predicts that subjects carrying this variant should preferentially profit from dopaminergic stimulation. This is in fact what has been observed for therapy with the COMT inhibitor tolcapone96, providing a proof of principle that an understanding of the neural effects of this variant through a combined imaging genetics approach can contribute to personalized proognitive therapy. Importantly, rs4680 also predicted prefrontal activation and working memory performance under antipsychotic therapy with olanzapine97.

Third, systems-level data can be helpful in designing a new generation of animal models. By definition, schizophrenia is a human-specific disease, because it affects human-specific faculties such as language. This does not mean that the pathophysiology of schizophrenia also needs to be human specific, but the question remains on how to optimally model relevant aspects of schizophrenia in animal models that are an essential requirement for drug discovery. By delineating neural systems properties that are consistently implicated in schizophrenia and ascertaining which behavioural features in a rodent are affected by them, a new generation of valid animal models can be designed. The molecular predictiveness of these models can be further enhanced by using genetically designed models to mimic a genetic risk variant associated with the disorder. This latter strategy will be the more promising as the amount of risk that can be attributed to the genetic risk factors increases. Therefore, modelling microdeletions could be especially fruitful. An impressive example is the recent discovery that mouse models for the schizophrenia-associated microdeletions 22q11 show abnormal hippocampal-prefrontal connectivity98 (Fig. 4c). Defining such systems-level features through animal neuroimaging and behavioural testing and relating them to behaviour will be essential in understanding what corresponds, in a rodent, to the human-specific symptoms of schizophrenia, an endeavour that may well lead to several definable sub syndromes that will by themselves constitute a useful development for drug discovery.

Finally, not all drug development is done with animal models. In fact, a useful entry point for systems-level neuroscience could be phase 1 studies, when new substances are first introduced into humans. At this point the question arises for which mental disorders, if any, that substance might be useful; the current ability to predict efficacy is poor99. Here systems-level neuroscience, especially neuroimaging, may make a contribution to proof of concept at an early stage by showing whether and to what degree new substances modify the relevant systems-level features, such as disturbed connectivity or neural oscillations in healthy humans. For this, it would not even be necessary (although it would certainly be advantageous) to have these systems-level features on the causal pathway to the disorder—as the example of striatal dopamine D2 blockade in currently available antipsychotics shows, there is no intrinsic necessity for an effective therapy to intervene in the causal pathway of the disorder, and progress may be made simply by using neuroimaging endpoints rather than traditional clinical endpoints99. Either way, the predictive value of this approach might be even further enhanced by stratifying healthy human subjects by common genetic risk factors that are related to risk for schizophrenia, such as the SNPs discussed above, which have been shown to bias neurocircuits also implicated in manifest disorder. In addition, many features of schizophrenia psychopathology can be transiently induced in humans; for example, it is possible to produce psychotic features or cognitive dysfunction using psychotomimetic drugs. This would constitute a revival and focusing of experimental medicine in psychiatry incorporating systems-level neuroscience in early drug trials. This concept, whose analogues have been extraordinarily fruitful in oncology and haematology, now awaits application to translation in psychiatry.


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