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REVIEW

Identifying novel interventional strategies for psychiatric disorders: integrating genomics, 'enviromics' and gene–environment interactions in valid preclinical models

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Psychiatric disorders affect a substantial proportion of the population worldwide. This high prevalence, combined with the chronicity of the disorders and the major social and economic impacts, creates a significant burden. As a result, an important priority is the development of novel and effective interventional strategies for reducing incidence rates and improving outcomes. This review explores the progress that has been made to date in establishing valid animal models of psychiatric disorders, while beginning to unravel the complex factors that may be contributing to the limitations of current methodological approaches. We propose some approaches for optimizing the validity of animal models and developing effective interventions. We use schizophrenia and autism spectrum disorders as examples of disorders for which development of valid preclinical models, and fully effective therapeutics, have proven particularly challenging. However, the conclusions have relevance to various other psychiatric conditions, including depression, anxiety and bipolar disorders. We address the key aspects of construct, face and predictive validity in animal models, incorporating genetic and environmental factors. Our understanding of psychiatric disorders is accelerating exponentially, revealing extraordinary levels of genetic complexity, heterogeneity and pleiotropy. The environmental factors contributing to individual, and multiple, disorders also exhibit breathtaking complexity, requiring systematic analysis to experimentally explore the environmental mediators and modulators which constitute the 'envirome' of each psychiatric disorder. Ultimately, genetic and environmental factors need to be integrated via animal models incorporating the spatiotemporal complexity of gene–environment interactions and experience-dependent plasticity, thus better recapitulating the dynamic nature of brain development, function and dysfunction.

LINKED ARTICLES

This article is part of a themed section on Animal Models in Psychiatry Research. To view the other articles in this section visit http://dx.doi.org/10.1111/bph.2014.171.issue-20

Abbreviations

ASD, autism spectrum disorder; ADHD, attention-deficit hyperactivity disorder; BDNF, brain-derived neurotrophic factor; CNV, copy number variant; DISC1, disrupted-in-schizophrenia 1; DREADD, designer receptor exclusively activated by designer drug; DSM-5, Diagnostic and Statistical Manual of Mental Disorders, 5th Edition; EE, environmental enrichment; G × E, gene–environment interaction; NLGN3, neuroligin 3

Introduction

Psychiatric disorders encompass a major, and growing, burden internationally, with prevalence estimates ranging from 12% (Turkey) to greater than 40% in the USA (WHO International Consortium in Psychiatric Epidemiology, 2000). The unmet need of identifying effective prevention and treatment strategies is enormous and requires sophisticated approaches to understand the pathogenesis of each disorder along with rational design of therapeutic interventions. In the quest to identify causative factors and pinpoint treatment targets, aberrant biological phenomena are characterized in human subjects using a broad range of techniques including epidemiology, genomics, imaging, *post-mortem* biochemical analyses, and peripheral biomarkers. This has resulted in the identification of genetic and environmental risk factors, as well as biomarkers, for each disorder. Subsequently, research programmes often turn to animal models to examine the influence of these factors in neural function and dysfunction, in a controlled, methodical manner. These models are used to try to pin down the mechanisms underlying the production of the 'symptomatology', and novel interventions are aimed at treating the precise disruption. The most successful interventions can then be taken into clinical trials, where they are evaluated in a test group. This patient group is identified by diagnosis, rather than symptomatology (an important caveat in disorders such as schizophrenia), and commonly multiple treatment strategies will have been attempted previously, but with limited success. The outcome measures reported are often restricted to the primary symptomatology (e.g. the positive symptoms in schizophrenia), and the novel compound must surpass the performance of the existing compounds on this measure, in order to move forward into the clinical environment. However, the success rate in developing successful treatments in this manner is low and, sadly, there are very few examples of new treatment strategies that have progressed through this 'rational drug design' schema and returned positive outcomes (DiMasi *et al.*, 2010; Burrows and Hannan, 2013b). Understanding why this approach is failing to identify effective therapeutics could help to restructure our approaches and our expectations. How can we optimize preclinical models to maximize the chances of clinical success? In what ways can we use these preclinical models to inform our strategic approach for the development of therapeutic interventions?

From 'unknown unknowns' to 'known unknowns': psychiatry, neuropsychiatry and the changing landscape of diagnosis and treatment

A major challenge in neuropsychiatry is unravelling the staggering complexity of multiple genetic determinants interacting with poorly understood environmental factors to give rise to clinically diverse phenotypes. The standard in psychiatric genetics has been the common disease-common variant hypothesis that risk for disorders will be found in a combination of common alleles, each conferring modest risk (State and Levitt, 2011). This approach has led to the identification of many candidate genes associated with various psychiatric conditions, including schizophrenia (Ross *et al.*, 2006; Allen *et al.*, 2008; Ng *et al.*, 2009; Jia *et al.*, 2010), autism spectrum disorders (ASDs) (Basu *et al.*, 2009; Pinto *et al.*, 2010; Neale *et al.*, 2012) and attention-deficit hyperactivity disorder (ADHD) (Gizer *et al.*, 2009; Williams *et al.*, 2010). While there is considerable aetiological heterogeneity between and within these disorders, recent data show an overlap of a number of the identified genetic risk factors. For example, genetic variants associated with schizophrenia have also been identified to increase risk for bipolar disorder (Purcell *et al.*, 2009), ASD (Sebat *et al.*, 2007; Weiss *et al.*, 2009) and ADHD (Williams *et al.*, 2010).

Taking a different approach, the contribution of rare genetic variations has received considerable attention, driven by advances in next generation sequencing that have increased the feasibility of identifying low-frequency alleles on a genome-wide scale (Sebat *et al.*, 2009). An achievement in the study of rare variations in psychiatric disorders has been the emergence of copy number variation (CNV) analyses, demonstrating an increase in *de novo* CNVs in cases versus controls (Sebat *et al.*, 2007). However, the assumption that rare, functionally deleterious disease alleles of large effect show a direct correspondence with a disorder has been challenged (Kumar *et al.*, 2008; Weiss *et al.*, 2008), inferring that simple models are not likely to be sufficient to unravel the complexity underlying disorders such as schizophrenia. The complex heterogeneous phenotype of these disorders cannot be sufficiently explained by paradigms focusing on the initial genetic insult but may need to include environmental factors, epigenetic mechanisms, epistatic and stochastic events. Addressing this, whole-genome sequencing coupled

with computational approaches that integrate information at genomic, transcriptomic and proteomic levels have recently helped to construct hypotheses on processes likely to underpin these disorders (Parikshak *et al.*, 2013; Cristino *et al.*, 2014). 'Missing heritability' in psychiatric disorders may be at least partly explained by unexplored genetic variants, including tandem repeat polymorphisms, as well as gene–environment interactions (G × E) (Hannan, 2010; Svrakic *et al.*, 2013). Evidence from model organisms suggests that some genes are more susceptible to modulation by the environment and that this may confer evolutionary advantages (Burrows and Hannan, 2013a). Developmental trajectories can be derailed by the absence or presence of deleterious genes and adverse environments. Resultant phenotypes reflect the brain's ability to buffer combinations of these deleterious gene mutations and/or environmental stressors (McGrath *et al.*, 2011). The human brain may be particularly susceptible to this due to the relatively rapid evolution of brain structure and function and the marked changes in population size, admixture and environment in the past few thousand years. Decades of epidemiological studies have highlighted the importance of environmental contributions to the risk of developing neuropsychiatric disorders and exposure to these adverse environments may divert brain development away from its normally canalized neurodevelopmental trajectory and towards disease-susceptible decanalized brain maturation (McGrath *et al.*, 2011). Several environmental risk factors for schizophrenia have been identified including season of birth, vitamin D deficiency, urbanicity or population density, and maternal viral infections (Cannon and Clarke, 2005; Brown, 2006; Patterson, 2007; McGrath *et al.*, 2010). In addition, during adolescence, stress and cannabis abuse have been identified to negatively impact on an individual's risk of developing schizophrenia (van Os *et al.*, 2002; Cannon and Clarke, 2005; Henquet *et al.*, 2008; van Winkel *et al.*, 2008). Incorporating G × E in future research designs may provide further insights into the biological mechanisms underlying psychiatric disorders.

Thus, while substantial progress has been made in identifying combinations of genes that increase risk for psychiatric illness, and environmental insults that also correlate with the likelihood of developing a psychiatric condition, characterizing the biological bases of these syndromes has proven more difficult. Key factors underlying this difficulty may include the heterogeneity within disorders, significant co-morbidities found across disorders, and the spatiotemporal complexity of neurobiological aetiologies.

Although a detailed description of clinical practice and medical policy is beyond the scope of this review, diagnostic practice and variability is of critical relevance to preclinical science and the elucidation of the mechanisms underlying the diverse symptoms that cumulatively form the diagnosis. Recent changes in the Diagnostic and Statistical Manual of Mental Disorders (DSM) illustrate the importance of better defining both the clinical aspects and the biological bases underlying the disorders in order to better 'maintain' those currently suffering, but also for the development of intervention strategies that reduce the morbidity and mortality of psychiatric illness. In this context, a commonly raised issue, important to optimization of preclinical research and clinical trials, including our rethinking of animal models, is the heterogeneity of patient groups. This most often refers to heterogeneity of symptomatology, with syndromes requiring only a subset of a broad range of clinical symptoms, translating to a patient population comprising individuals within the same diagnostic group, but potentially demonstrating distinct symptoms. Moreover, even the individual symptoms may present quite idiosyncratically across different patients. A detailed description of the individual's spectrum of disease, in combination with powerful statistical analyses, could lead to a better understanding of how these subgroups segregate, and which molecular, neuroanatomical and pathophysiological modalities can be attributed to producing the deficits. The feasibility of incorporating the staggering complexity that underlies psychiatric disorders into research designs has been discussed by Insel *et al.* (2010) with the recent launch of the Research Domain Criteria project. The aim of this project is to describe the 'component parts' of psychiatric dysregulation by understanding difficulties in terms of cognitive, neural and genetic differences (Cuthbert and Insel, 2013), supporting research that moves beyond descriptive syndromes in psychiatry, and towards a nosology informed by disease cause. For example, an ASD genetic study might include siblings and parents with broader autism spectrum phenotype rather than restricting the cohort to those meeting strict ASD criteria. Furthermore, a focus on co-morbid symptoms may provide further insights into the biological mechanisms underlying psychiatric disorders.

There is substantial comorbidity of psychiatric conditions with neurological, medical and other psychiatric disorders. For example, depression is commonly diagnosed in association with cardiovascular disease, epilepsy, post-traumatic stress disorder and Huntington's disease (Hryvniak and Rosse, 1989; Kanner *et al.*, 2012; Morris *et al.*, 2012; Du *et al.*, 2013; Hare *et al.*, 2013), among others. Co-morbidity has several consequences including complicating the dissection of the biological mechanisms responsible for the distinct disorders; however, these co-morbidities may also provide a way for accessing information on these complex disorders. This has been elegantly shown recently, by Blair and colleagues, who demonstrated an association between Mendelian disorders and other complex conditions, subsequently showing that the Mendelian variants are probably contributing to disease risk for a range of complex diseases, providing a 'unique insight into the aetiology of complex diseases' (Blair *et al.*, 2013). Furthermore, a recent genome-wide analysis aimed at finding a signal in the noise, led to the identification of risk loci with shared effects on five major psychiatric disorders (Cross-Disorder Group of the Psychiatric Genomics Consortium, Genetic Risk Outcome of Psychosis (GROUP) Consortium, 2013). In addition to the demonstration that common variants with low penetrance cumulatively contribute a significant proportion of risk for these disorders, there have also been compelling studies demonstrating that rare penetrant genetic events, such as CNVs within defined regions, contribute significant risk. Interestingly, the instability of the genome appears to have increased relatively recently, in evolutionary terms, suggesting that the risk for disease may be intricately paired (co-morbid) with improved cognitive capacity and more recently evolved brain functions (Nithianantharajah *et al.*, 2013). Thus, divergent genetic events may disrupt brain development and maturation,

ultimately producing symptomatically indistinguishable disorders, at least by current clinical practice.

However, as a counter argument to the significance of the genetic findings to date, and allowing for increasing sensitivity of genetic tools to the sheer number of genetic permutations, the search for genetic causes of complex polygenic diseases is fraught with difficulties. This has been discussed recently by Sullivan (2013), who illustrated the lack of conclusive evidence for the causal involvement of a prominent 'schizophrenia gene' – disrupted-in-schizophrenia 1 (*DISC1*). Sullivan argues that the genetic evidence for *DISC1* as a schizophrenia susceptibility gene is not strong, despite interesting biological roles. Indeed the example of *DISC1* genetics in schizophrenia may apply more broadly to psychiatric illness and associated findings emerging from international consortia, positing a combination of common and rare genetic disruptions underlying psychiatric illness more generally. Placing these recent studies in the context of the current research programmes and findings that have emerged in independent lines of research within specific disease contexts, the heterogeneity and complexity of psychiatric genetics is likely to reflect the population variability and intricacies of the underlying neurobiology.

In parallel with these genetic discoveries, research into many psychiatric illnesses has evolved from a focus on neuromodulators such as 5-HT (in depression and anxiety) (Leonardo and Hen, 2006) and dopamine (in schizophrenia, ADHD and addiction) (Langer *et al.*, 1981; Castellanos, 1997; Baik, 2013) to a focus on neurotransmitters, with glutamatergic hypotheses having been proposed in the context of schizophrenia, addiction and more recently depression (Reissner and Kalivas, 2010; Javitt *et al.*, 2012; Krystal *et al.*, 2013). Of course, changes in a single neuromodulator or neurotransmitter will not explain the complexity of a given psychiatric disorder. Systems and computational neuroscience approaches will be required to bring together the complexity of genetic, transcriptomic and proteomic changes with cellular and network abnormalities across multiple psychiatric disorders. Thus, these findings can be used to integrate many streams of research, helping to establish the conceptual foundations upon which we may use existing information to inform the pathogenesis of diverse psychiatric disorders. A major remaining challenge is how to bring together these diverse research programmes in order to inform the larger picture of mental illness.

The discovery of this 'psychiatric pleiotropy' may also provide a critical piece of evidence for the mechanisms by which environmental influences are reflected in the clinical phenotypes. If different combinations of overlapping genetic disruptions and polymorphisms underlie the various disorders, then environmental factors (at differing time points during development) and associated G × Es may help explain how these common disruptions manifest differently.

As discussed earlier, the genetic elements thought to contribute to risk for developing schizophrenia are notable for their heterogeneity, pleiotropy, and in some cases, instability. These regions are thus also prime targets for additional expressional control driven by G × Es. One of the mechanisms by which this control is achieved is via epigenetic regulation of the genome. Studies investigating these phenomena have identified bidirectional control of gene expression by epigenetics in an apparently heritable manner, with negative environmental factors modulating gene expression to produce biological outcomes (Zhang *et al.*, 2010), while positive environmental interactions (Branchi *et al.*, 2011), as well as epigenome by drug interactions (Dong *et al.*, 2009), are capable of restoring functional capacity to epigenetic architecture.

However, despite these significant advances, it can be argued that we still know very little about the biological bases for psychiatric disorders and how the distinct symptomatologies emerge and evolve over the course of each illness. These advances in psychiatric research, particularly in the realm of genomics, have given us a greater appreciation of how little we know about the aetiology and pathophysiology contributing to brain dysfunction in these diverse disorders. We can use this 'known unknown', to guide future investment into biological research, and to bring together converging research programmes. How does this inform intervention strategies and animal models?

Animal models of psychiatric symptoms: current status quo

Animal models generally seek to attain multiple levels of validity, with reference to the human disorder; construct validity, face validity and predictive validity (Burrows *et al.*, 2011). With diagnosis of illness in human subjects already being fraught with difficulty, it is clear that modelling these diseases in animals is not a simple task. Moreover, the high verbal dependence of diagnosis in humans further complicates an already difficult situation. As a result, researchers have typically satisfied themselves with reaching one level of validity or resorting, rather than modelling a subset of disease-specific symptoms, and endophenotypes that parse the disorder into particular components, which are genetically dissociable.

Animal models are generally rated based upon the presence of a subset of behaviours widely referred to within the context of a distinct psychiatric disorder. Importantly, this assessment has often been developed and validated, driven by our early knowledge of the disease. While progress in elucidating the mechanisms' underlying disorders has made slow, but steady progress, this has not always been well translated into the optimization of animal models. This ultimately means that most current approaches only extend our ability to treat deficits that have already been well characterized, without incorporating novel evidence, but rather 'reinventing the wheel'. This problem is especially prevalent in the depression and schizophrenia fields.

While re-evaluating the current state of animal models, we should incorporate our increased understanding of the human disorder. An important contributor to the complexity of this issue is the nature of psychiatric symptoms, as distortions of healthy humans affect cognition, hampering progress into understanding psychiatric disorders. Interestingly, the primary class of symptoms in psychiatric diseases can be the most difficult to model: positive symptoms in schizophrenia, depression, the complex breakdown in social behaviour in autism. Tests are often designed for one aspect of the

phenotype, but in the absence of a good alternative, the use of these tests expands to fill a purpose for which they were not intended.

'Genetic construct validity' of animal models

The gold standard for developing a mouse model of a human disorder has been to identify the aetiology, be it a genetic disruption (such as the tandem repeat expansion of the *huntingtin* gene in Huntington's disease) or environmental insult (such as traumatic brain injury), reproduce it in an animal model, and explore the pathology in order to develop novel treatment strategies. However, for most psychiatric disorders, we lack definitive insights into the pathogenesis. What does one do in the absence of a clear aetiological picture? For a while, the complexity of the aetiological bases for psychiatric disorders has been apparent. Twin studies have demonstrated clear heritability (Sullivan *et al.*, 2003; Posthuma and Polderman, 2013), albeit polygenic heritability with variable penetrance. Within this context, an important consideration in developing animal models with good construct validity has to do with the nature of disease-associated genetic variants. There is questionable feasibility of modelling common variants of small effect due to the fact that they do not often lead to gene and/or protein changes and there is debate regarding the ability to model small genetic changes in mouse orthologues of human genes (Kvajo *et al.*, 2012). Instead, the alternative approach is to approximate the predicted effects of the mutation through constitutive or conditional gene knockout models, rather than reproducing the original mutation. While a number of mouse models have been created to investigate truncation and point mutations in the DISC1 protein, a well-researched candidate gene for schizophrenia and affective disorders with a range of functions relating to neurodevelopment, only one has utilized the DISC1 mutation found in the Scottish family (Koike *et al.*, 2006). This transgenic mouse showed comparable spatial and temporal expression patterns of the mutant allele. A similar approach was used to explore the neuroligin 3 (NLGN3) R451C point mutation identified in human patients with ASD (Jamain *et al.*, 2003). The $NLGN3^{R451C}$ mouse has been shown to display social impairment and altered synaptic function (Tabuchi *et al.*, 2007; Foldy *et al.*, 2013), phenotypes not seen in the NLGN3 knockout mouse, indicating a gain-of-function that cannot be modelled with traditional approaches. These examples illustrate the need for attention to detail when generating new animal models, so as to approach, and ultimately achieve, optimal genetic construct validity.

'Environmental construct validity' of animal models

In addition to the presumed genetic factors contributing to the disorder, environmental factors have also been shown to contribute significantly to mental illness. However, compared with the large-scale genetic approaches to genome-wide association studies, whole exome/genome sequencing and associated meta-analyses and bioinformatics, a sophisticated understanding of environmental factors, and associated G × Es, is sadly lacking. We propose that what is needed is a complementary 'enviromics' approach, in which the 'environe' contributing to the pathogenesis and progression of each disorder, and multiple disorders ('environmental pleiotropy'), is systematically catalogued and computationally analysed. Just as molecular biological technology and bioinformatics are revolutionizing the genomics, transcriptomics and proteomics fields, there is increasing capacity to collect and systematically analyse large collections of environmental and epidemiological data, to form the basis of enviromics. At the nexus of genomics and enviromics sit G × Es, which may involve dynamic perturbations of brain development and function via evolved biological mechanisms such as decanalization (McGrath *et al.*, 2011; Burrows and Hannan, 2013a).

Focusing on schizophrenia, epidemiological studies have shown that heritable factors contribute only around 50% of the risk, as illustrated by monozygotic twin studies (Kessler, 1980). Despite this widely known statistic, animal models incorporating environmental components have lagged behind uniquely genetic or pharmacological constructs. While a recent increase in 'environmental models' is observable (further details later), it remains the case that combining genetic and environmental factors in a valid model is a rarity.

In determining the nature of the environmental influences best used to assess such questions, epidemiological data contribute important information. Two widely investigated models of environmental influences include early immune activation, and vitamin D deficiency (Kesby *et al.*, 2006; Meyer, 2014). These models are based upon the findings that maternal infection and low vitamin D levels during critical developmental periods appear to be risk factors for developing specific psychiatric disorders, including psychosis. Models of perinatal stress have also emerged; however, their reproducibility and robustness is currently a limiting factor (Henn and Vollmayr, 2005).

The term 'two hit' model has been used extensively in recent years to describe models of multiple 'hits' during development and adulthood. However, the complexity and pleiotropy of psychiatric disorders discussed in this paper suggest that most genetic and environmental factors do not constitute spatially or temporally discrete 'hits', but rather more subtly divert brain development and function from its 'normal' trajectory as a result of complex bidirectional interplays between multiple genetic variants and environmental mediators and modulators.

System approaches in animal models

One way to approach the modelling of psychiatric disorders is to start with hypotheses based on clinical data, and pursue specific molecular or cellular systems, allowing that hypothesis to be systematically tested. For example, as the field of

schizophrenia research has shifted from a predominant focus upon the dopaminergic system, to glutamatergic and other systems, so too have the animal models evolved. With glutamate as the main excitatory neurotransmitter in the brain there are many ways in which a glutamatergic pathology can be achieved, for example disrupting glutamate receptors (for receptor nomenclature see Alexander *et al.*, 2013), synaptic cohesion, and/or the excitation–inhibition balance in these disorders (Belforte *et al.*, 2010; Nithianantharajah *et al.*, 2013). These approaches have been extended to pharmacological models, such as NMDA receptor blockade producing paradoxical outcomes (Abi-Saab *et al.*, 1998), and the methylazoxymethanol developmental disruption model producing abnormalities in parvalbumin-positive neurons (Lodge *et al.*, 2009). These models have provided insight into developmental neuroscience and the fine balance evident in neuronal maturation and synaptic function. Moreover they have been able to provide causal links, in some cases, between a molecular or cellular pathology to behavioural disruptions. But have they guided the development of new therapeutic interventions?

Another important consideration is the timing of the intervention. Evidence from clinical trials suggests that the patient population targeted, in addition to the stage of pathogenesis, may play a key role in the individual's response to select pharmaceutical compounds. For example, a recent study from Wunderink *et al.* (2013) suggested that remitted first-episode psychosis patients with dose reduction/discontinuation of antipsychotics showed superior long-term (7 year) recovery rates. The findings described not only underline the need for effective treatments, as opposed to maintenance drugs, but also illustrate the diverse temporal profiles of these disorders, and highlight the need to take this into account when developing drugs and choosing patients for clinical trials.

An additional limitation to animal modelling has been the failure to take into account the spatial, and especially temporal, validity of research models. Mapping the progress of these diseases from prodromal states to full-blown syndromes is critical in order to appropriately target treatment strategies. This is elegantly illustrated by recent findings describing the progressive disruption in the glutamatergic system across the timeline of the disease. The authors (Schobel *et al.*, 2013) describe spreading dysfunction in the hippocampus of human patients as they transition from prodrome to psychosis, and follow up by reproducing the pathogenic development in mice. This approach then facilitates investigation of the pathophysiology and biological mechanisms at the specific time points, using the model to delve further into the pathology (Schobel *et al.*, 2013). Furthermore, this approach captures a unique insight into the disease implicating glutamatergic dysfunction as a driver in psychosis, although it is well established that psychotic patients also possess dopaminergic dysfunction (Laruelle and Abi-Dargham, 1999). Moreover, an overwhelming majority of antipsychotics target dopamine receptors, suggesting that, regardless of the origin of the disruption, it spreads beyond the hippocampus and beyond glutamate, encompassing broader systems, and highlighting the importance of factoring these aspects into treatment strategies.

'Freerange mice' and G × Es: how I learned to stop worrying and love complexity

While consensus appears to support highly complex mechanisms involving interplay between genetic, epigenetic and environmental factors, as instrumental in the pathogenesis of psychiatric disease, there is yet to be systematic translation of this seemingly irrefutable knowledge into comparably complex animal models. While we have been trained to reduce the complexity and variables within an experiment, this historical necessity is now limiting our ability to create disease models with sufficient complexity to adequately model the disease state, and may be resulting in models that are too simplistic to accurately provide predictive validity for therapeutic targets.

So, how do we use our knowledge of G × E interactions and the progressively developing nature of these psychiatric disorders to design better models, and thus identify adequate and effective treatment strategies? The vast majority of animal model studies are conducted using 'standard housing' conditions, which constitute a form of sensorimotor deprivation that does not approximate average human levels of environmental stimulation. One approach, when deciding which candidate therapeutic interventions to advance to clinical trials, is to perform a secondary screen under conditions of environmental enrichment (EE), so that only those candidates that continue to show significant therapeutic effects using EE housing of the animal models are translated to the clinic (Nithianantharajah and Hannan, 2006; Burrows and Hannan, 2013b). While there seems to be a general reluctance to introduce the level of natural variability that can be found in response to environmental manipulations, there is good evidence to suggest that this is achievable and important in working our way towards unveiling the mechanisms underlying the behaviours, and revealing putative treatment targets.

Moreover, the evident co-morbidity and symptom overlap within and across psychiatric disorders should be embraced. Rather than dismissing a model as being too disrupted, displaying a breadth of phenotype that extends beyond the textbook definition of a single disorder, this complexity and phenotype overlap may be more closely modelling the reality of the human syndrome, providing valuable information as to a common cause for psychiatric illness, in line with the emerging genetic findings. Utilizing these models as a starting point, it would then be of benefit for identifying the precise biological mechanisms underlying the disparate phenotypes, unifying the emerging human findings with the strength of animal research (i.e. the ability to precisely dissect mechanism). Importantly, the technology to achieve this dissection is now available, including optogenetics, designer receptor exclusively activated by designer drug (DREADD) systems, and finer control of gene expression in rodents using systems such as floxed genes, facilitating regionally defined analyses and tetracycline operators that confer temporal control to the experimenter. Optogenetics is now a widely available technology, and has accelerated our understanding of the precise cell populations underlying phenotypes that may be said to

relate to psychiatric symptomatology. The main criticism with this approach has been the nature of the stimulation (which is set by the user and may not reflect an electrophysiological signature with *in vivo* relevance). This criticism, however, can be addressed by combining this elegant and flexible technique with either genetically engineered receptors, which again will inform the role of a specific cell type (as determined by the promoter under which the DREADD receptors, for example, will be expressed), or with a flexible genetic construct that allows the user to turn the expression on or off in a temporally and regionally selective manner. The latter allows us to model the effect of altered gene expression during precise developmental and adult windows. Regardless of whether the altered expression is caused by mutation of a single nucleotide or repetitive DNA sequence, or by epigenetic mechanisms perhaps precipitated by an environmental insult, such animal models can be used to assess how altering expression in this temporally-restricted manner can set the developmental trajectory off course, ultimately resulting in divergent dysfunctions.

Together these new approaches and models allow for more refined analysis of gene and cell function. One disadvantage of these more sophisticated models has been the associated costs, both in time and resources. This may be set to change, with new technologies for genome editing greatly accelerating the process. Clustered regularly interspaced short palindromic repeats–Cas-based technology uses an adaptive response identified in bacteria, which allows them to rapidly modify their immune response, thus avoiding lethal infection. This unique ability to direct nuclease activity and edit the genome provides a useful strategy for rapidly generating complex animal models with multiple genetic mutations or polymorphisms. Understanding neural development and adult brain function, at this level, will no doubt require collaborative efforts and a willingness to invest greater resources in more refined questions. However, it may be the approach that delivers the most definitive answers.

Another interesting line of convergent evidence is the importance of experience-dependent plasticity in maintaining healthy brain function. Decreases in signs of cellular plasticity have been observed in human *post-mortem* tissues of psychiatric groups (Roberts *et al.*, 1996; Black *et al.*, 2004; Stockmeier *et al.*, 2004). Moreover this has been shown to align with animal models where adult neurogenesis has been shown to play a critical role in anxiety (Kheirbek *et al.*, 2012; Mendez-David *et al.*, 2014) and depression (Boldrini *et al.*, 2012), as well as altered dendritic complexity and changes in brain-derived neurotrophic factor (BDNF), a neurotrophic factor known to be involved in neural plasticity and critical periods of brain development. Indeed, some current pharmacotherapies have been shown to achieve at least part of their effects by targeting these systems (Boldrini *et al.*, 2012; Mendez-David *et al.*, 2014). For example, re-opening critical periods of plasticity with selective 5-HT re-uptake inhibitor, antidepressant drugs (Karpova *et al.*, 2009) or increasing basal cellular plasticity (with drugs that target BDNF), in combination with environmental approaches such as cognitive behavioural therapy, may provide significant relief of symptoms and allow for rewiring of the disrupted neural circuitry.

Conclusions

In summary, genetic, pharmacological and environmental disruptions are most often evaluated within highly constrained systems. Preclinical models often involve genetically identical animals, inbred and maintained in barren, but reproducible housing, with limited social engagement; the models are relatively removed from the reality of human experience. Moreover, the very nature of the scientific method, where researchers go to great lengths to minimize variability on all but one independent measure, maximizing the potential for reproducibility and identification of causation, eliminates the natural variability required to reproduce the diversity of phenotypes seen in humans, and thus the suitability of novel treatment approaches for the syndrome. As a result, research programmes are unable to put to use all the information at our disposal, namely that psychiatric disorders arise as a result of complex interactions of multiple genetic and environmental factors, varying in nature, and resulting in diverse symptomatologies. Moreover, the times at which experimental evaluations occur in animal models, usually early on following presentation of the behavioural phenotypes, fails to capture the more temporally evolved illness often being assessed in clinical trials, and patient populations. Perhaps the approaches currently being assessed in most preclinical models would better qualify as early intervention strategies, rather than longer-term candidate treatments for the fully expressed disorder.

With various intellectual and technical paradigm shifts currently influencing the research landscape, how can we best get these programmes back on track, and make more significant inroads into improving key indicators and quality (as well as quantity) of life for the sufferers of psychiatric disorders? The challenge of developing new preventative and therapeutic interventions requires a multifaceted approach, targeted to elucidate the risk factors, the progression of the disorders, as well as clarifying the biology of both the acute and chronic disease states.

Recent developments are prompting a re-evaluation of preclinical research into psychiatric disorders. Many pharmaceutical companies have drastically cut investment into the development of novel therapeutic compounds for psychiatric illness, and multiple failed clinical trials of what appeared to be promising compounds should prompt the research community to reconsider the approaches being put into practice. Moreover, the recent release of the DSM 5th Edition (DSM-5) has created an active forum for open discussion between medical practitioners and basic psychiatric researchers. Conflicting demands of diagnosis for individual treatment and insurance purposes, versus more detailed description of disorders as clusters of symptoms, need to meet in a complementary manner. Ultimately, psychiatric researchers will need to integrate both approaches within their own models to elucidate the biological mechanisms underlying the manifestation of each endophenotype, symptom cluster and psychiatric disorder.

Our knowledge of the genetics of psychiatric disorders is advancing at an exponential pace, revealing breathtaking complexity, heterogeneity and pleiotropy. The use of specific gene mutations and polymorphisms in animal models of psychiatric disorders struggles to keep pace, and a more

sophisticated approach to genetic construct validity is urgently required. At the same time, we need to be cognizant of how genomes and enviromes combine via G × Es, thus integrating environmental construct validity into animal models. As genetic and environmental construct validity is optimized, face validity should follow and can be calibrated with the latest clinical biomarkers at molecular, cellular, cognitive and behavioural levels. Finally, predictive validity has been hampered by the limitations of current therapeutic interventions, and it is hoped that new drug and non-drug treatments can be fed back into animal models to further hone validity.

The challenges associated with developing accurate and valid animal models of psychiatric disorders cannot be underestimated. Nevertheless, we have, for the first time, promising genetic and environmental candidates that can be systematically explored in new preclinical models. It will require concerted international efforts in which the limitations of current models are confronted and the complex aetiologies of psychiatric disorders are accurately reflected in sophisticated new models, via dynamic bidirectional exchanges between basic and clinical research.

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Conflict of interest

The authors declare that they have no conflicts of interest in the writing of this review.

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