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How Can Models Be Better Utilized to Enhance Outcome?

A Framework for Advancing the Use of Models in Schizophrenia

Kevin J. Mitchell, Patricio O'Donnell, Daniel Durstewitz, André A. Fenton, Jay A. Gingrich, Joshua A. Gordon, Wolfgang Kelsch, Bita Moghaddam, William A. Phillips, and Akira Sawa

Abstract

The heterogeneity of schizophrenia at the clinical and etiological levels presents a huge obstacle to understanding the biology of this disorder, or even knowing how to conceptualize it. This chapter discusses how animal, cellular, and computational models can be used to explore convergence at the intervening level of pathophysiology. It considers such models as experimental platforms to investigate specific neurobiological hypotheses, in particular to elucidate causal chains of pathogenic events, from initial molecular and cellular disruptions to eventual effects on neural networks and brain systems underlying specific symptom domains. The ultimate goal is to increase understanding of the neurobiological underpinnings of all aspects of the disorder (etiology, pathogenesis, pathophysiology, symptomatology) to a point where we can rationally identify new therapeutic targets or points of intervention to help break the deadlock in the development of treatments for this devastating disorder.

Introduction

What is the point of making an "animal model of schizophrenia"? What are we hoping to accomplish? Is it even possible? What is it that we are really trying to model?

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We propose that animal models are best considered as experimental reagents or platforms to investigate the neurobiological underpinnings of schizophrenia. This contrasts with the idea that animal models in some way recapitulate the disorder in its entirety or are mainly useful as a proxy for drug screening. Despite the commonly used shorthand, it is obviously not possible to generate an animal model of schizophrenia, given its etiological and phenomenological heterogeneity, and considering the uniquely human expression of so many of its symptoms. Moreover, if schizophrenia is an open construct, the boundaries and features of which are difficult to delimit even in humans, attempting to generate an animal that recapitulates the disorder as a whole is even more unrealistic.

The approach we propose is generally fairly agnostic about *face* and *predictive validity*, terms which have preoccupied the field for some time. Face validity means that the animal presents with some behavioral phenotypes that resemble particular human symptoms. Predictive validity refers to those phenotypes that can be reversed in the animal model using current antipsychotic medications. While such information is indeed very valuable and reinforces the notion that one is on the right track, face and predictive validity are not good exclusion criteria for saying whether an animal is really a "model of schizophrenia."

The expectation that a particular pathophysiological disturbance will manifest in an overtly similar way in animals and humans is not always justified. On the contrary, one might more reasonably expect a species-specific expression at the behavioral level. Manipulations that do not result in obvious face validity should thus not be rejected as irrelevant to understanding the disease. Similarly, limiting oneself to studying only those phenotypes that are responsive to current medications—especially using them to screen for drugs—inevitably becomes a circular exercise and may explain why no new drugs with novel mechanisms of action have been found using this approach (Carpenter and Koenig 2008; Abbott 2010).

We emphasize a different approach and propose that the term *animal model* be used to refer to an animal that has been manipulated in some way that is either known to be of etiological relevance to schizophrenia or that is thought to recapitulate a phenotype of relevance to some aspect of schizophrenia phenomenology. Different models may be useful for investigating etiology, pathogenesis, pathophysiology, or other aspects of the disease. As such, they represent discovery platforms to test specific hypotheses and elucidate the underlying biology.

In addition to the use of animal models, this research framework importantly includes human cellular models, such as neural cells derived from schizophrenia patient biopsies, for example, and computational models, which can be used to formally describe the interactions within and between levels of biological phenomena and to predict the effects of manipulations of various components. In this chapter, we present a conceptual framework for relating

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different levels of analysis of experimental models (genetic, molecular, cellular, circuits, systems, behavioral) and for encompassing the heterogeneity that is apparent at each level.

A Heuristic Framework for Schizophrenia Research

The clinical picture of schizophrenia is one of heterogeneity at the level of clinical symptoms (in terms of the particular profile of symptoms portrayed by any individual patient) as well as at the level of etiology, with a large number of distinct risk factors identified. Thus, to think of this heterogeneity while retaining the integrity of the central construct poses a major challenge. At the present time, the degree of heterogeneity at the intermediate level of pathogenic and pathophysiological mechanisms is largely unknown. Our working hypothesis is that there will be some reduction in heterogeneity at the level of pathophysiology, with convergence onto a smaller set of common mechanisms underlying various symptom domains. In this section, we consider how experimental models can be used to approach this question empirically.

Figure 13.1 presents a conceptual framework that encompasses these parameters in animals, based on a similar framework described for humans (see Corvin et al., this volume). A diversity of etiological risk factors (E_1–E_n) may

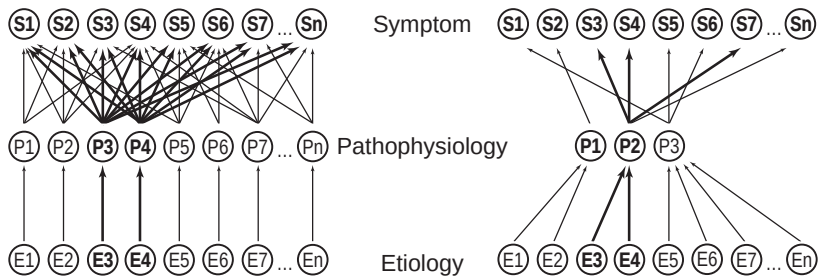

Figure 13.1 Etiology–Pathophysiology–Symptoms (E–P–S) framework. Two alternative scenarios are presented that relate the heterogeneous etiological factors associated with schizophrenia to the heterogeneous clinical symptoms (or behavioral phenotypes in an animal model). The scenario on the left depicts equivalent heterogeneity at the intervening level of pathophysiological mechanisms. Thus, pathogenesis arising from etiological factors E3 and E4 involves distinct pathophysiological mechanisms, P3 and P4. The alternative hypothesis is illustrated on the right, in which the degree of heterogeneity at the pathophysiological level is drastically lower, with phenotypic convergence onto a smaller set of common mechanisms which underlie diverse clinical symptoms. In this case, E3 and E4 induce a common pathophysiological mechanism. Note that the level of pathophysiology itself has multiple hierarchical levels (not shown), with possible convergence from various etiological factors at the level of biochemical pathways, cellular or developmental mechanisms, or emergent neural dynamics in microcircuits and extended brain systems.

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impact a range of molecular and cellular processes, leading to the emergence of a spectrum of pathophysiological phenotypes at the level of neural circuits and brain systems (P_1–P_n). These phenotypes may singly, or in combination, lead to the range of clinical symptom domains observed in patients or to the impairment in the animal equivalent of such systems (S_1–S_n). The question is whether there exists for each etiological factor a distinct and unique route of pathogenesis, or if there is instead some convergence onto a smaller set of pathophysiologies. Conceptualizing schizophrenia models within this Etiology–Pathophysiology–Symptoms (E–P–S) framework will be advantageous to elucidate neurobiological mechanisms of relevance to the disorder.

Epilepsy provides a useful exemplar to illustrate how such convergence can emerge (Figure 13.2). There are a large number of Mendelian conditions in which recurrent seizures are one of the clinical symptoms. The genes involved can be roughly subdivided based on the cellular level phenotypes observed or the protein function, including, for example, genes involved in proliferation or

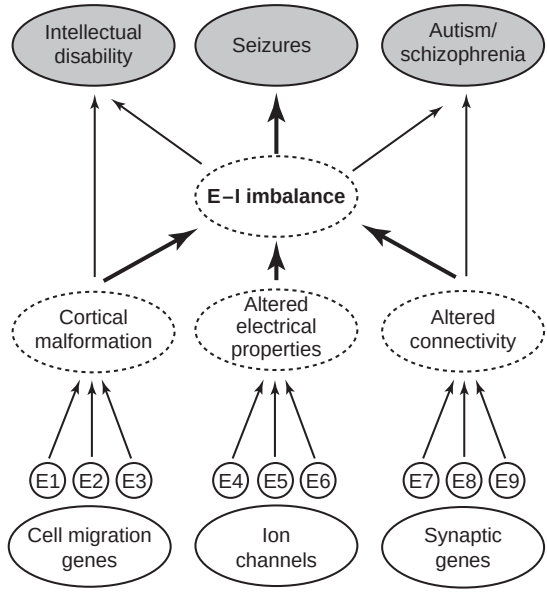

Figure 13.2 Epilepsy as an example of phenotypic convergence. Multiple strong genetic risk factors for epilepsy fall into several categories based on the functions of the encoded proteins (with the obvious potential existence of many more than are depicted). Mutations in genes within each of these groups may converge onto a distinct primary pathology affecting a particular cellular process, such as cortical morphogenesis, ionic flux, or synaptic connectivity. There may be further convergence in the downstream consequences of these changes, which may all lead to an alteration in the excitation-inhibition (E–I) balance in various parts of the brain and a predisposition to seizures. Depending on the pathophysiological mechanism and its penetrance, additional clinical symptoms may also emerge, including ones associated with intellectual disability, autism, schizophrenia, and other psychiatric conditions.

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cell migration, which can lead to cortical malformation when mutated, genes affecting synapse formation, metabolic genes, and genes encoding ion channels (Poduri and Lowenstein 2011; Greenberg and Subaran 2011). The heterogeneity of etiological factors can thus be reduced by defining gene function or direct phenotypic effect. Further reduction in heterogeneity is observed at the next level as each of these kinds of disturbance can result in a state of altered excitation-inhibition balance in some part of the brain, resulting in seizures. This is, of course, a superficial level of description—there are certainly distinct ways in which this balance can be disrupted—but it encapsulates a common theme: a type of common pathophysiology that can emerge from diverse primary insults affecting quite different cellular parameters (cytoarchitecture, synaptic connectivity, metabolic flux, or ion channel expression). At the symptoms level, there is also heterogeneity in the type and location of seizures and course of epilepsy. In addition, some genes that predispose to epilepsy also increase risk for other neuropsychiatric disorders, with a number of manifestations other than seizures (including autism, intellectual disability, and psychosis), emphasizing the point that none of these clinical categories is a closed construct.

Populating the E–P–S Framework

We can already begin to populate this framework for schizophrenia at various levels, based on information from diverse sources. At the etiological level, we now know of multiple strong genetic risk factors (Mitchell and Porteous 2011; Sullivan et al. 2012a), in addition to a number of loci with statistical evidence of association from genome-wide association studies (Sullivan et al. 2012a) and a multiplicity of environmental and experiential factors identified from epidemiology (Tandon et al. 2008; McGrath and Susser 2009).

Currently there are at least nine specific recurrent copy number variants for which there is compelling statistical evidence that they predispose to schizophrenia with relatively high penetrance, dramatically increasing risk compared to the general population (Sullivan et al. 2012a). Most of these, however, are also associated with other clinical outcomes, including autism spectrum disorder, epilepsy, and intellectual disability, adding another degree of heterogeneity to the E–P–S framework. Schizophrenia is thus just one possible endpoint caused by mutations in such genes. In addition to these, many other mutations have been identified where the statistical evidence for association with schizophrenia, in particular, is not yet compelling but where the aggregate evidence of some neuropsychiatric manifestation, including schizophrenia in some carriers, is quite strong (e.g., DISC1, SHANK2 and 3, CNTNAP2) (Mitchell 2011a). Regardless of how many cases of schizophrenia will eventually be shown to be associated with such mutations of strong effect, their

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identification provides an entry point to elucidate the underlying mechanisms experimentally.

As an example of the value of this approach, the identification of the genes underlying Mendelian forms of Alzheimer's disease, including APP, presenilin-1 and presenilin-2, opened an entire field of biological inquiry and ultimately revealed the involvement of these proteins much more generally in this disease (Bertram et al. 2010). We can hope for similar progress in schizophrenia research by following the strong leads we now have in hand. The recent identification of strong etiological risk factors provides an opportunity to follow a proven discovery path in schizophrenia research (Mitchell et al. 2011). It will be especially informative to compare the phenotypes in such models with those observed in well-characterized models generated by pharmacological, anatomical, or environmental manipulations. Such models have proven extremely informative in defining potential pathophysiological mechanisms and relating them to behavioral phenotypes (see O'Donnell, this volume).

At the level of pathophysiological mechanisms, there are also a number of good leads that can be included to help generate testable hypotheses. Pathophysiological mechanisms can be multilayered, with molecular phenotypes yielding synaptic and cellular alterations, which in turn drive circuitry and systems changes. At the molecular level, examples of leads include interleukin-6 and oxidative stress (Behrens and Sejnowski 2009) as well as NMDA receptors (Belforte et al. 2010). At the circuit level, leads include alterations in GABAergic interneurons (Gonzalez-Burgos et al. 2011; Lewis et al. 2005) and dopamine systems (Lisman et al. 2008; Howes and Kapur 2009; Grace 2010). Systems pathophysiological mechanisms currently studied include alterations in cortical or thalamocortical oscillations (Lisman 2012; Uhlhaas and Singer 2012) and hippocampal-prefrontal connectivity (Sigurdsson et al. 2010).

At the level of clinical symptoms and their behavioral correlates in animals, a range of well-established paradigms are available where phenotypes are consistently or at least repeatedly observed across various animal models, including genetic, pharmacological, developmental and others. These include behavioral traits, such as general hyperlocomotion, increased anxiety, and reduced social interactions, as well as task- or challenge-related phenotypes, such as working memory deficits, sensitivity to amphetamine, impaired prepulse inhibition, and others (van den Buuse 2010; Moore 2010; Young et al. 2010). Again, none of these is seen in all models nor should any of them be thought of as an exclusive criterion of the validity of any particular model. Some of them can be related quite directly to human traits, tasks, or psychological constructs, whereas for others a direct parallel is less obvious.

The E–P–S framework includes not just the specific factors at each level but also the known or putative relationships between factors at different levels. These represent the links in the causal chain (or network) from each etiological factor to the clinical manifestation. Any one of those putatively causal arrows represents a specific hypothesis that may be directly testable with the range of

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reagents and techniques we can now bring to bear in experimental neuroscience. Such hypotheses will be most precisely and tractably defined between adjacent levels of the framework, rather than stretching to test relationships across distant levels, where intervening, unknown complexities may exist.

Identifying Convergent Pathogenic Mechanisms

As stated above, a major goal in the experimental modeling of the effects of schizophrenia risk factors is to identify points and pathways of phenotypic convergence and possibly common pathophysiological states. The identification of such hubs would importantly provide new potential points of therapeutic intervention to reverse or compensate for a particular pathophysiological state that underlies one or more symptoms, or to prevent the emergence of such a state. A key component of such a research program is therefore to provide systematic comparison across multiple models in search of points of convergence at various levels.

Convergence may emerge in some cases at the level of primary cellular mechanisms mediated by the mutated genes. For example, several implicated genes, including *NRXN1* and *CNTNAP2*, play a role in cellular interactions at the synapse (Mitchell 2011a), which may mediate synapse formation and activity-dependent refinement. Members of the SHANK, DLG, DLGAP, and CNTN protein families may act in similar cellular processes, possibly even in the same biochemical pathways (Betancur et al. 2009; Ting et al. 2012). Mutation of other genes, such as DISC1 or CHRNA7, may also have an effect on synapse composition through different molecular pathways (Brandon and Sawa 2011; Lozada et al. 2012). Convergence on particular processes and pathways from analyses of multiple single-mutation models will also highlight potential molecular and cellular phenotypes to assess using human-derived cellular models, where oligogenic effects may be explored (see below).

In other cases, the primary molecular and cellular mechanisms may be very different, but there may be convergence at a higher level of the framework. For example, several models show alterations in gene expression and function of inhibitory interneurons in prefrontal cortex. These include mice expressing dominant-negative DISC1 (Hikida et al. 2007; Shen et al. 2008) as well as amphetamine-sensitized rats (Peleg-Raibstein and Feldon 2008) or rats with prenatal or neonatal manipulations that affect prefrontal cortical and hippocampal development, such as the antimitotic MAM or a neonatal hippocampal lesion (Lodge et al. 2009; O'Donnell 2011). Alterations in inhibitory neuron markers are one of the more consistently observed differences in postmortem studies of human patients and could represent homeostatic responses to reductions in pyramidal neuron activity (Gonzalez-Burgos et al. 2011).

Changes in dopaminergic signaling in striatum and cortex are also observed across many models (Lipina et al. 2010; van den Buuse 2010; Seeman 2011),

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paralleling consistent observations in human patients, including at prodromal stages (Howes and Kapur 2009; Howes et al. 2012a). Again, such changes could be induced secondarily through reactive mechanisms (Lisman et al. 2008; Grace 2010).

At an even higher level, changes in neural synchrony and oscillations are observed across several models. Neural dynamics at this scale are an emergent property of neuronal ensembles and may be affected by diverse insults. For example, a common pathophysiological state at the level of neuronal populations can emerge due to quite distinct effects at the single neuron level of various psychotomimetic drugs with different modes of action (Wood et al. 2012). Synchrony of neural oscillations may enable communication within and across regions that underlie various aspects of cognition, perception, and behavior. Defects in hippocampal-prefrontal cortex synchrony have been observed in animals modeling the 22q11 deletion (Sigurdsson et al. 2010), in animals that received a neonatal hippocampal lesion (Lee et al. 2012), and in animals subject to maternal immune activation *in utero* (Dickerson et al. 2010, 2012). Such changes correlate with defects in working memory and parallel observations in humans (Meyer-Lindenberg et al. 2001).

Although many details of the causal chains of events remain to be elucidated, these examples illustrate the kinds of explanation that might emerge within this framework and suggest specific and testable hypotheses at multiple levels. Importantly, more selective experimental manipulations in models present the opportunity to move beyond observational approaches and correlations to test causality directly across levels. For example, transgenic animals lacking a particular protein only at some stages or only in some cell types or regions provide tremendously powerful reagents to causally link specific cellular phenotypes to specific pathophysiological outcomes.

Sources of Phenotypic Variability

In considering the relationship between any genotype and an associated phenotype, it is important to consider not just the starting and ending positions, but also the developmental trajectory which connects them. This is especially relevant for the study of schizophrenia, where we know that phenotypic heterogeneity is high among carriers of the same mutation and even between monozygotic twins. How such variable expressivity might manifest in inbred mouse lines is an open question and an important one to keep in mind. Phenotypes may change on different genetic backgrounds, so a profile observed in one strain may not represent a ground truth.

The eventual phenotype may also be affected by environmental risk factors or experience and stress. Animal models provide a powerful platform to test for such effects, especially using animals that may have been sensitized by a "first hit," such as a predisposing genetic mutation (Oliver 2011). Incorporating

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possible interactions between genetic and environmental risk factors into animal modeling will be an important goal within this framework.

Another important source of variability may be far harder to control or study, however, and that is chance. The processes of neural development are incredibly complex, involving the activities of thousands of different molecular components. These processes are sensitive to what engineers call "noise": random thermal fluctuations at the molecular level which affect gene expression, protein interactions, and other molecular activities on a moment-to-moment basis (Eldar and Elowitz 2010). Such noise can affect the outcome of developmental processes, which can readily be observed at the neuroanatomical level as a probabilistic expression of cellular phenotypes across a population of cells (Raj and van Oudenaarden 2008). When this randomness is played out independently across the brain, it can lead to variation on a macro scale and variation in concomitant physiological and behavioral phenotypes (Mitchell 2007). For example, while the tendency to develop epilepsy is very strongly heritable, the precise type and anatomical focus of seizures are much less so (Corey et al. 2011). These parameters are far more affected by randomness in developmental outcome. One could certainly imagine how a similar scenario played out across other brain circuits could account for some of the variability in presentation in schizophrenia (Woolf 1997; Singh et al. 2004; Mitchell 2007). In animal studies, this variability could be a problem when phenotypes are compared across groups of animals. Alternatively, it could be leveraged by studying individual animals in greater detail, allowing correlation of the severity of defects across levels.

Pleiotropy and Cascading Effects

It is interesting to consider the possible relationships between different phenotypes observed in particular mutants. Co-occurrence of particular behavioral phenotypes could reflect

- a defect in a single underlying neural system on which they both rely,
- the independent expression of a single type of defect in multiple regions of the brain, or
- multiple mechanisms that are independently affected by mutation of the gene (mechanistic pleiotropy).

For example, an alteration in dopamine-mediated signal transduction can influence multiple cognitive functions, such as attention and working memory, stress reactivity, reward and motivational processing, and goal-directed movement (Howes and Kapur 2009; Stephan et al. 2009; Fletcher and Frith 2009).

It is also possible that a single type of defect arises in multiple parts of the brain, with diverse consequences. For example, we can hypothesize that a deficit in visual contour recognition observed in some patients may reflect an

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alteration in microcircuitry within the primary visual cortex (Butler and Javitt 2005). The identical microcircuit alteration in the prefrontal cortex, however, might alter synchrony of neural ensembles and also functional coherence with the hippocampus, thus causing a defect in working memory.

An alternative scenario is that a particular gene may be involved in quite different cellular processes in different contexts, including in tissues outside of the brain, and pleiotropic effects may arise through very different cellular mechanisms. DISC1 is a prominent example because it interacts with a wide range of proteins in various cellular processes (Soares et al. 2011). This kind of mechanistic pleiotropy is obviously even more likely when the effects of copy number variants are considered, where multiple genes are deleted or duplicated.

Many phenotypic effects will also be very indirect due to cascading effects of the primary cellular pathology. For example, alterations in cell migration or synapse formation will necessarily change future patterns of electrical activity, indirectly altering the activity-dependent refinement of circuitry that occurs at later stages and in other brain areas (Ben-Ari 2008; Ben-Ari and Spitzer 2010). This raises an important point when considering why it is that mutations in so many different genes may lead to quite similar and specific phenotypic outcomes. One possibility is that the convergence represents a property of the developing brain itself, in the way it reacts to a wide range of primary insults (Mitchell 2011b; Lisman 2012). It may not be the crime but rather the cover-up that does the damage.

For example, a lesion to the ventral hippocampus in early postnatal animals alters the development of cortical circuits, resulting in an excitation-inhibition balance that emerges during adolescence and a change in dopaminergic tone in the developing striatum and cortex (O'Donnell 2011). In turn, homeostatic synaptic mechanisms react to this change by altering the levels of dopamine receptors, which is thought to result in subcortical hyperdopaminergic state that may mimic aspects of psychosis. Such a "common pathway" may thus emerge as an active reaction to a range of insults, rather than the endpoint of a passive propagation of cascading effects.

Modeling the Time Course of Schizophrenia

An important and defining aspect of schizophrenia is the typical time course of the emergence and progression of the illness. Although subtle, quantitative differences in behavior can be seen early in life, most patients are typically without significant symptoms until early adolescence. At that time, typically between 12 and 18 years of age, a prodromal phase is often seen, characterized by a decline in social, cognitive, and educational performance. Progression to frank psychosis typically occurs in late adolescence to early adulthood. This time course is a unifying theme which cuts across much of the diagnostic and

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phenomenological heterogeneity of schizophrenia, pointing to the adolescent period as a crucial factor in the pathogenesis of schizophrenia.

There are a number of distinct and testable hypotheses to explain why schizophrenia typically manifests in adolescence:

1. It reflects a neurodegenerative process with a time course that coincides with adolescence.
2. Changes in some hormone levels directly induce symptoms in a vulnerable brain.
3. There is abnormal development of brain structures that are not fully online at early stages and which cause defects when integrated later.
4. Ongoing developmental processes in the adolescent brain are directly affected by the etiological factors and go awry at that time period.
5. Normal cellular processes of maturation reveal a latent circuit-level deficit due to initial differences.

Given the conservation of physiological changes during adolescence, animal models offer the means to distinguish these hypotheses. In particular, it is important to test whether these processes are aberrant in situations predisposing to schizophrenia and to examine the interaction between processes of maturation and primary phenotypic effects.

Adolescence is characterized by a host of coordinated changes in various structural and neurochemical parameters, including synaptic pruning, ongoing myelination, and changes in the expression of various neurotransmitter receptor subunits (Sturman and Moghaddam 2011). In humans, imaging data revealed that cortical thickness in the prefrontal cortex acquires adult profile late in adolescence (Shaw et al. 2006a), and cortical oscillations exhibit dramatic changes during this period (Uhlhaas et al. 2009). Furthermore, the activation of reward and cognitive systems also matures during adolescence (Galvan 2010; Casey et al. 2010). These processes appear largely conserved across mammalian species and may be driven by a diverse set of neurobiological phenomena which are also known to mature during adolescence. These include changes in the density of prefrontal dopamine innervation (Rosenberg and Lewis 1995) and dopamine receptors (Brenhouse et al. 2008), functional changes in the modulation of excitation-inhibition balance (Tseng and O'Donnell 2007) and processing of salient events by prefrontal cortex (Sturman et al. 2010) and striatum (Sturman and Moghaddam 2012). Thus, when using the E–P–S framework in schizophrenia models, it is critical to consider developmental aspects including those which take place during adolescence.

In addition to explaining the typical age of onset, longitudinal studies of animal models may be used to parallel studies of high-risk, prodromal, first-episode, and chronic schizophrenia patients. The development of powerful small-animal neuroimaging methods offers the means to follow the same individual animals over time with a technique that provides data directly comparable to that from human patients.

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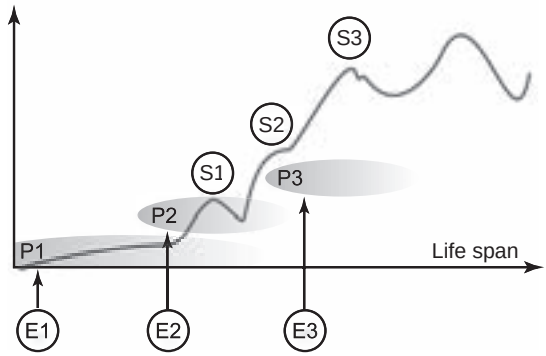

Figure 13.3 The E–P–S framework across the life span. Different etiological factors may come into action at different points in time (e.g., prenatal development, early postnatal critical periods, or during maturational processes of adolescence) and may give rise to interacting or independently acting pathophysiological mechanisms. Distinct pathophysiological sequelae may thus arise at different ages, with the subsequent emergence of clinical symptoms with a specific course. These distinct developmental trajectories, which lead to the emergence of pathophysiological states and behavioral symptoms, can be investigated discretely or collectively in accordingly designed animal models.

Using these approaches we may be able to map the timing of effects of different genes, the emergence of specific pathophysiological phenotypes, and the correlated emergence of behavioral phenotypes relevant to clinical domains (Figure 13.3). Thus, looking across the temporal domain offers another route to dissect the heterogeneity across levels.

Incorporating Computational Models into the Framework

Making sense of a framework that incorporates data across such disparate levels of analysis, from a large number of different models and experimental investigations, requires computational methods and can be greatly informed by computational theories. In particular, understanding the emergent properties of cells, synapses, microcircuits, or brain systems is essential to interpret how changes to specific components yield specific phenotypes. The study of neural dynamics offers a particularly promising tool (see Durstewitz and Seamans, this volume).

Dynamical properties of biophysical/biochemical systems, such as attractor states, oscillations or synchrony, arise from the nonlinear interactions among its many constituent components (e.g., molecules or cells), and may provide specific links between the neural “hardware” and the “software” level (cognition, behavior). For example, the activity of fast-spiking, parvalbumin-positive interneurons is known to drive the synchronous oscillations of local ensembles

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of pyramidal cells within the gamma frequency range (Cardin et al. 2009). These gamma oscillations and the associated synchrony in the spiking activity of neurons have, in turn, been linked to specific behavioral, cognitive, and perceptual functions by providing a basis for the neural coding of perceptual or mental objects (Uhlhaas and Singer 2012). Thus, through these dynamical mechanisms, genetic or molecular factors which interfere with the normal functioning of fast-spiking interneurons may lead to disorganization of cortical representations, and consequently to some of the symptoms observed in schizophrenia. Durstewitz and Seamans (this volume) describe another example; namely, how alterations in dopaminergic receptors may lead to changes in prefrontal cortical "attractor landscapes" with consequences for behavioral flexibility and information maintenance.

Thus, such a computational and neurodynamical framework may allow prediction of the effect of a mutation in some specific gene on neural dynamics at various scales, which in turn will have specific implications for behavioral and cognitive functions. It is important to note, however, that inferences in the reverse direction are much more difficult; given a particular behavioral difference, there will usually be a number of potential neurodynamical candidate mechanisms compatible with it. Even more limiting, a rather large variety of changes in one or more molecular components may have the same consequences for neural dynamics, making the backward inference from neural dynamics to underlying molecular cause extremely hard, if not impossible.

One current limitation for computational models is that many biologically important parameters or their statistical distributions may still be unknown or not sufficiently described. Filling in these blanks and using them to generate more hypotheses is thus an important research goal that will require an iterative and ongoing dialog between experimental and computational biologists. Ultimately, the detailed computational models of the systems involved will provide a powerful discovery engine for screening *in silico* through large areas of parameter space to identify potential molecular targets for therapeutic intervention.

The Promise of Human Cellular Models

A major difficulty in investigating the cellular correlates of a specific mutation in humans is that the cell types one is most interested in (neurons and glia) are inaccessible. A number of new technologies provide the means to derive neural lineage cells from human patients or carriers of specific mutations (Wilson and Sawa, this volume; Table 13.1). Many different molecular and cellular parameters of these cells can be characterized *in vitro*, including gene expression patterns, morphology, dendrite and axon extension, as well as synaptic connectivity. Derived neural cells can also be injected into animal brains to examine neuronal migration, synaptic integration, and other properties. Comparison of

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Table 13.1 Overview of current methods used to derive neurons from human tissue samples. Depending on the experimental question, the scale of the investigation, and other logistical considerations, different methods may be optimal under different circumstances.

Method	Advantages	Disadvantages
Derivation of iPS cells and differentiation into neural cells	• Can be expanded for any number of analyses • Protocols are improving; should allow more standardized, systematic analyses of the effects of different mutations or high-risk genotypes	• Expensive and time consuming
Direct conversion of fibroblasts to neural cells	• Is cheaper and faster	• Does not generate a permanent, expandable bank of stem cells
Olfactory epithelium biopsy to obtain neural cells	• Does not require genetic manipulation of the cells • Does not use differentiation protocols	• Only obtains olfactory neurons

these parameters across various primary mutations may cellreveal phenotypic convergence in some cases at the proximal level of particular biochemical pathways or cellular processes.

The ability to derive such neural cells will provide the unprecedented opportunity to examine the possible molecular and cellular pathology associated with specific patient genotypes in individuals who are also characterized at clinical, neuroimaging, psychological, and genetic levels. Such investigations will be even more powerful when derivations can be integrated with data from similar analyses in animals with the cognate primary genetic lesion.

In addition to characterizing and comparing the cellular effects of identified mutations, derived neural cells provide a platform for discovering and dissecting genetic etiology. Gene expression differences may highlight strongly deleterious mutations in cases where genome sequencing provides a long list of potentially causal candidates, for example. Perhaps more importantly, derived neural cells offer the means to assess the effects of a mutation in the context of the entire genotype of an individual. So far, all of the mutations identified as increasing risk of schizophrenia show incomplete penetrance and highly variable expressivity, manifesting in diverse ways across individual carriers. Genetic modifiers are very likely to have large effects on the phenotypic expression of any particular mutation but could act at very different levels. Comparing cellular phenotypes in cells derived from patients with a specific mutation versus healthy carriers of the same mutation could reveal genetic background effects at the level of a particular molecular or cellular phenotype. Alternatively, it might suggest that they come into play at a higher level, in how the system reacts to earlier developmental differences.

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There may also be many cases where multiple mutations are involved in pathogenesis (e.g., Girirajan et al. 2012). Though these may be difficult to identify and probably impossible to model directly in animals, the molecular pathways and cellular processes affected by such high-risk genotypes could still be investigated in cells derived from human patients.

Summary

Our ultimate goal is to increase understanding of the neurobiological underpinnings of all aspects of schizophrenia—etiology, pathogenesis, pathophysiology, and symptomatology—so that new therapeutic targets or points of intervention can be rationally identified to break the deadlock in treatment development for this devastating disorder. However, the heterogeneity of schizophrenia presents a huge obstacle.

To address this, we developed the Etiology–Pathophysiology–Symptoms framework as an overarching heuristic to guide experimental modeling of various aspects of schizophrenia. Rather than trying to overspecify criteria for validity of any particular preparation, experimental assay, or phenotype, the E–P–S framework embraces heterogeneity at etiological, pathophysiological, and clinical levels.

The point of generating models is not to recreate an entire disease state but to test specific hypotheses experimentally. In addition to well-characterized nongenetic models, the growing number of identified high-risk genetic lesions offers a proven discovery pathway to elucidate pathogenic mechanisms and provides explanatory links from molecular and cellular phenotypes to dysfunction of neural networks and brain systems underlying specific symptoms.

Given the very high degree of etiological heterogeneity, the identification of points or pathways of phenotypic convergence at the level of pathophysiology remains a major goal to be achieved. This will require systematic comparison across many different models and integration across levels of analysis. Fortunately, the tools to follow up on strong etiological entry points are now available, especially in terms of our capacity to analyze phenotypes at multiple levels in individuals, both in animals and humans.

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