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How Can Animal Models Be Better Utilized?

Patricio O'Donnell

Abstract

Although animal models of schizophrenia have been around for some time and new ones are proposed regularly, their usefulness is still questioned. Many current concepts on schizophrenia pathophysiology have been driven by animal research, yet when these concepts were translated into novel therapeutics, the results have been less than promising. This chapter reviews many of these models and new concepts, and argues that the problem has been that animal models were not used enough in preparation to clinical trials. Furthermore, a great deal of animal work has been directed to establishing their validity—a misguided and far from useful effort. Validity concepts are outdated and not adequate for research relevant to a disorder for which its etiology and pathophysiology are unknown. Models need to be appreciated based on their usefulness: for a disease without a clear pathophysiology, animal models are essential tools to test specific hypotheses about neurobiological and behavioral outcomes of manipulations that produce pathophysiological conditions. Novel targets should only be translated into clinical efforts after comprehensive work in animal models has been conducted to allow establishing mechanisms of action, biomarkers to identify optimal populations to be targeted, and even whether those targets are better thought of as adjuvants or sole treatments. Recognizing what animal models can and cannot achieve will go a long way in benefiting schizophrenia research.

Introduction

Modeling complex psychiatric disorders such as schizophrenia in animals is certainly a challenge. In fact, it could be argued that reproducing this uniquely human disease in animals, and particularly in rodents, is an almost impossible task. However, many different animal models have been proposed and studied over the past few decades. With the advent of genetic models, this field has grown further and new models are proposed almost every month. Schizophrenia research has gained important insight from animal work, and many pathophysiological scenarios previously proposed for this disorder have

either been reinforced or dismissed based on animal model studies. For example, the early emphasis on the dopamine hypothesis gave way to the current focus on excitation-inhibition balance, glutamate receptors, and GABA interneurons in cortical circuits. Novel pharmacological approaches have been sought based on animal model work. Unfortunately, these new treatments have failed to provide conclusive results, and some of them are now on the verge of being dismissed. Did we miss the mark? Are animal models misinforming the field, and are we on a wild goose chase? Or is it still too early to jump from animal work to novel therapeutics? Here I will argue that animal models will be extremely important in driving the field forward, but we need to drastically change the manner used to conceptualize them.

Can We Truly Model Schizophrenia in a Rodent?

Perhaps the primary problem with the current use of animal models in schizophrenia research is that we took the concept of modeling disease from the neurology realm. In that field, models are used for their ability to reproduce the disease in animals. For example, Parkinson's disease has several powerful animal models (e.g., 6-hydroxy dopamine in rats, MPTP in mice and monkeys) that reproduce the critical pathophysiology: loss of dopamine cells. These models have contributed to a better understanding of the timing of dopamine loss and its consequences, the role of oxidative stress, and other cellular damaging processes, etc. In Huntington's disease, several different mouse and rat transgenic models with poly CAG repeats in the *huntingtin* gene reproduce a genetic change strongly associated with disease etiology. In both cases, animal models were designed with the goal of very closely reproducing the disease. In these and other areas of medicine, three litmus tests of validity for animal models were developed:

1. face validity, or the ability to reproduce manifestations of the disorder;
2. construct validity, or the fidelity in reproducing disease etiology or pathophysiology; and
3. predictive validity, or the ability to show beneficial effects of drugs that work in the human condition.

While these validity criteria have been the boon of neurology research, they are the bane of psychiatric research. If we as a field believe we can reproduce schizophrenia in a rodent, we are deluded (pun absolutely intended). How can we talk about construct validity for a disease for which we do not know the etiology and have little clues about pathophysiology? Most confusing, why do we emphasize predictive validity when we try to assess aspects of the disease that are not treated well by current medications? We have obtained droves of information with the existing proposed models, and novel models are constantly being added to address genetic, environmental, and developmental factors. It is

due time to leave behind the neurology legacy and think about animal models in the frame of psychiatric disorders that need work to elucidate their neurobiological mechanisms. Specifically, we can use animal models as reagents to test defined hypotheses about risk factors or possible pathophysiological scenarios. We can use them to assess what kind of biological processes can be related to the deleterious impact of certain gene variations, environmental insults, or developmental anomalies with the goal of gaining a better understanding of clinically relevant biomarkers. We can test the neurobiological underpinnings of endophenotypes observed in patients (imaging, neurophysiology, and even postmortem) with manipulations that generate specific cellular, synaptic, or circuit alterations in animals and assess whether they yield similar imaging, physiological, or behavioral alterations. All these efforts will be most productive, however, if we do not kid ourselves into thinking that the models reproduce a disease as complex as schizophrenia. Thus, if we move beyond the limiting concept of validity, we can use animals to test hypotheses efficiently in a manner that can help us accept or reject ideas about schizophrenia etiology and pathophysiology, which can then be advanced to human studies.

Despite heated arguments about their validity, many different animal models have indeed provided important insight on possible mechanisms that may contribute to the disease. Several models have been proposed to address environmental, developmental, and genetic factors, as well as the role of specific transmitter systems and brain regions. Experimentalists have been conducting research that provided useful information all along while conceptualizing their research in a house of cards framework of validity. There is no perfect model, and if we are able to escape the validity trap, we can learn something from practically every model proposed. Below I will review some of these models, addressing their usefulness and ability to test schizophrenia-related hypotheses.

Pharmacological Models

Noncompeting NMDA receptor antagonists have been extensively used, and they have provided critical information that led to the formulation of a currently popular hypothesis on schizophrenia pathophysiology: cortical disinhibition. Agents such as phencyclidine (PCP), ketamine, or MK-801 have been used in several species, including humans, to study mechanisms associated with the psychotomimetic effect of PCP that was initially reported in the 1950s (Luby et al. 1959). Although there has been an argument regarding whether acute or chronic NMDA blockade is the more "valid" model, studies with either single dose or repeated treatment have provided data indicating that NMDA blockade results in enhanced glutamate levels in the cortex (Moghaddam et al. 1997), increased pyramidal cell firing, and decreased interneuron firing (Homayoun and Moghaddam 2007). As imaging data have been reinterpreted in the 2000s

to qualify the old "hypofrontality" functional concept as the result of a higher level of baseline activity and reduced capacity in prefrontal networks (Callicott et al. 2000), the notion that a psychotomimetic agent such as NMDA antagonist would cause disinhibition seemed to fit well. Therefore, the view that cortical disinhibition may be responsible for cognitive deficits in schizophrenia was driven by animal model work. This is an example of fruitful use of an animal model; of course, now we need to move beyond the initial observations and pose specific hypotheses addressing mechanisms that could result in such a disinhibited state. For example, open questions include whether NMDA receptors in cortical inhibitory interneurons are primarily targeted by NMDA antagonist, causing increase pyramidal cell firing, whether the excitation-inhibition imbalance is the result of a larger network effect instead of selective effects on inhibitory interneurons, and whether cortical disinhibition can be causal to cognitive or other behavioral deficits. All these are testable hypotheses. Only with a better understanding of cellular and synaptic mechanisms yielding a disinhibited cortex will we be able to design better therapeutic tools. In addition, the NMDA antagonist findings have been frequently interpreted as indicating there is something wrong with NMDA receptors in schizophrenia. However, obtaining schizophrenia-related outcomes with a pharmacological blockade of NMDA receptors does not necessarily mean that NMDA receptors are impaired in the disease; reducing function in the receptor population targeted by these antagonists may have a downstream effect that could reproduce schizophrenia pathophysiology without requiring abnormal NMDA receptors in the disease. NMDA antagonist models have been extremely useful, regardless of their validity, and the data obtained with them have driven the field to establish new hypotheses. An example of the leads that NMDA antagonists have opened is the role of immune activation and oxidative stress in vulnerable neuronal populations, as parvalbumin (PV) interneurons are altered by NMDA antagonists in a manner that requires interleukin-6 and oxidative stress (Behrens et al. 2008). The field is now ripe to challenge those hypotheses with further experiments and, in doing so, we may gain insight about neurobiological processes that could play a role in schizophrenia.

Developmental Models

Although NMDA antagonists have provided support for several current concepts regarding the pathophysiology of schizophrenia, these models lack a developmental component. It is now commonly accepted that schizophrenia is a developmental disorder in which a combination of predisposing gene variations and environmental factors may alter neural circuits with a protracted developmental trajectory (Waddington 1993; Pantelis et al. 2005). Although there are cognitive deficits prior to diagnosis, full-fledge symptoms do not appear until late adolescence. This could be due to either delayed deleterious

effect of a persistent condition that eventually produces enough changes to alter behavior, or alterations put into evidence late in development by the protracted maturation of cortical circuits. Animal models, again irrespective of how well they fit validity criteria, can be used to test these possibilities. Several models are being used in which a perinatal manipulation is introduced so that behavioral, neurochemical, anatomical, and electrophysiological anomalies emerge during adolescence. The two most extensively used models are the antimitotic methylazoxymethanol acetate (MAM) during gestational day 17 in rats and the neonatal ventral hippocampal lesion (NVHL).

The NVHL model and its variations (intrahippocampal injection of tetrodotoxin, TTX, or lipopolysaccharide) is widely used and, with near 150 publications over the past several years, it is probably the most extensively explored (Tseng et al. 2009; O'Donnell 2012). This model was developed in the early 1990s to test the hypothesis that an altered early postnatal developmental trajectory in a brain region linked to schizophrenia (the hippocampus) results in behavioral anomalies with a delayed onset (Lipska et al. 1992). This is another example of a useful model that provided data beyond simple validation, yielded important information about prefrontal cortical synaptic processes, and added a developmental perspective to the disinhibition hypothesis. At the time the model was generated, the notion that schizophrenia is a developmental disorder had been proposed, but the only evidence available was from postmortem studies which showed altered cytoarchitecture (Kovelman and Scheibel 1984). As those human findings could not be replicated, the neurodevelopmental hypothesis of schizophrenia required testing to affirm its plausibility. Lipska and Weinberger decided to explore the impact of neonatal lesions of the ventral hippocampus and other brain regions on adult behavior as a way to assess whether early alterations could result in deficit with an adult or adolescent onset. The ventral hippocampus in rats was chosen because this region corresponds to the anterior hippocampus in primates, and the early postnatal period was selected for the lesion because it corresponds to the third trimester of pregnancy in terms of brain development. A narrow window was identified in which a lesion would yield adult rats with several behavioral anomalies: postnatal day (PD) 6–8. Adult rats with a NVHL show hyperlocomotion, exaggerated response to stress and stimulants, prepulse inhibition deficits, loss of social interactions, and a variety of cognitive deficits including poor working memory, set-shifting deficits, and reversal-learning deficits, and most of these deficits are only fully observed in adult, not preadolescent, animals (Swerdlow et al. 2001; Brady et al. 2010; McDannald et al. 2011). Furthermore, there have been reports of altered prefrontal cortical circuit physiology, also with adolescent onset. In particular, prefrontal cortical fast-spiking PV-positive interneurons fail to acquire the periadolescent changes in modulation by dopamine (Tseng et al. 2008), rendering adult prefrontal circuits in a state of disinhibition. Indeed, cortical disinhibition can be evidenced in excessive firing of pyramidal neurons during epochs in a choice task that correspond to decision

making and high levels of dopamine cell firing, as well as in the loss of beta oscillations during those epochs (Gruber et al. 2010). This is a remarkable convergence with what was previously identified with NMDA antagonists, but is now a consequence of an early developmental manipulation. Again, this is another example of a good use of a model, with a manipulation designed not to produce a disease state but to test a specific hypothesis about pathophysiological processes. In recent years, a great deal of effort was placed on assessing cognitive phenomena in the NVHL model, with the goal of determining whether cognitive constructs altered in schizophrenia show deficits in the model as well and, if so, whether novel therapeutic ideas could be beneficial in these animals as a way to test these new approaches in a diseased brain. Thus, a model that has been frequently sidelined (despite being extensively studied) because of the perceived lack of validity due to the "lesion" aspect has been extremely useful in demonstrating that early developmental perturbations can indeed yield late onset behavioral deficits; it has also reproduced cognitive deficits that can be linked to phenomena observed in schizophrenia. This model has serious shortcomings in terms of validity (a lesion is not normally part of schizophrenia), but it has nonetheless been extremely useful. Indeed, the model should not be interpreted as reproducing hippocampal pathology in the disease; its consequences are most likely due to the impact of altering hippocampal function influence on the development of downstream structures such as the prefrontal cortex (PFC). The NVHL model has provided important information on cellular and systems elements that contribute to adult cognitive deficits and is providing interesting data on the potential role of immune activation and oxidative stress in interneuron deficits (O'Donnell et al. 2011). This model may be useful in addressing such open questions as whether cortical fast-spiking interneurons are an early factor that, when affected, drives altered excitation-inhibition balance; whether cellular processes (including, but not limited to immune activation and/or oxidative stress) are responsible for the behavioral deficits; and whether other interneuron types may be affected and give rise to the deficits. Currently the NVHL model is also used to screen for efficacy of novel compounds targeted to improve cognition in schizophrenia. Thus, in spite of validity shortcomings, this and other models have been useful for testing hypotheses and gaining insight.

Another valuable developmental model is the administration of the antimitotic MAM at gestational day 17 in rats. For decades, the administration of MAM at early gestational dates was used to study cortical development; in the 2000s, a slightly later date of administration proved to cause delayed onset of behavioral deficits similar to those observed with the NVHL model (Flagstad et al. 2004). The impetus for the MAM model was to test whether a developmental manipulation that did not entail an explicit lesion could produce the emergence of schizophrenia-related anomalies in adolescence and early adulthood. Although it could be argued that by avoiding a lesion, a "shotgun" approach of impaired microtubule function in the entire brain was introduced,

data indicate that the deficits seem prominent in the hippocampus and PFC regions, suggesting a degree of selectivity on the impact of the MAM treatment (Moore et al. 2006). As in the NVHL model, here we have a developmental manipulation designed to test the impact of early deficits on adult behaviors. Adult offspring of MAM-treated dams exhibit hyperlocomotion, enhanced reactivity to stress, prepulse inhibition deficits, loss of PV immunostaining, loss of high-frequency oscillations, and cognitive deficits (Flagstad et al. 2004; Gourevitch et al. 2004; Moore et al. 2006; Penschuck et al. 2006; Lodge et al. 2009). This model is also extremely useful in providing the opportunity to link early developmental deficits with adult dysfunction in dopamine systems. The ventral hippocampus is critical in driving the activity of subcortical dopamine projections, and the altered VH function induced in adult rats by the gestational MAM treatment results in excessive activity in subcortical dopamine systems (Gill et al. 2011). This model is also used for drug screening, and it is another example of clever experimental design to address the possible contribution of biological processes to altered functions that may be relevant to schizophrenia. Although the MAM model has validity issues, it has proven extremely useful in testing specific hypotheses and has provided insight regarding possible pathophysiological processes and their behavioral consequences.

Environmental Models

Several models have been developed to test the possible impact of environmental factors hypothesized to play a role in schizophrenia. Epidemiological data indicate a strong association between schizophrenia and maternal or perinatal infection or parasitic disease. It has been hypothesized then that immune activation during early development may yield altered brain circuitry that could be relevant to schizophrenia (Brown 2006). Several animal models were designed to test this hypothesis, including gestational administration of the viral particle poly I:C or the bacterial endotoxin lipopolysaccharide (LPS). Adult offspring of treated dams express a variety of behavioral deficits such as reduced prepulse inhibition, altered latent inhibition, and several other indicators of cognitive function (Zuckerman et al. 2003; Meyer et al. 2006). Furthermore, recent work with these models reveals loss of PV immunostaining in prefrontal cortical regions (Meyer et al., unpublished data), providing a remarkable convergence in key pathophysiological observations with several other models. Immune activation has strong epidemiological support, and testing its impact in animals may reproduce a causal or predisposing factor (Meyer and Feldon 2012). However, beyond the real or perceived validity of these models, their usefulness resides in their ability to test specific hypothesis about the neurobiological impact of a factor with strong contribution to the disease. Unveiling the cellular and systems neuroscience aspects these manipulations produce will

certainly advance our understanding of the neurobiological processes likely to be affected in schizophrenia.

Other environmental factors proposed to play a role in the disease have also been modeled in animals, including vitamin D deficiency, pre- or postnatal stress, gestational hypoxia. Although less studied, these models are no less important. If we accept that we do not need to validate models in terms of disease reproducibility and that models are useful tools to test specific questions about consequences of possible pathophysiological scenarios, then all environmental-based models have an important role to play.

Genetic Models

Perhaps the group of animal models that has grown most rapidly is the cluster of genetic manipulations possibly associated with schizophrenia. Although schizophrenia is a disorder with a clear genetic predisposition, the role of genes is complex. Although the common view involves interactions among multiple gene variants, each contributing a very small risk, and environmental factors, recent work has identified a few genetic modifications with high penetrance. These include chromosome deletions such as the 22q11 and other copy number variants (CNVs). As schizophrenia-predisposing gene variations continue to be identified, mouse models expressing such variations are developed. This is a long list that cannot be addressed in its totality. Examples of single-gene mutations with suspected link to the disease include dysbindin (for which knockout mice exist), DISC1 (for which several manipulations also have been used in animals), and neuregulin. Unfortunately, a great deal of effort has been placed on proving the validity of these models. Even if we admit that individual genes may contribute only a small fraction of the risk, studying the neurobiological processes triggered by altering the ERB4 gene or the DISC1 gene is extremely useful. By gaining such basic understanding, we can then link these genes with cellular activity, brain circuit function, and animal behavior in a manner that can illuminate about factors that can be affected in the disease.

Several genetic manipulations have recently been used to test specific hypotheses about the impact of a specific gene variation on neurobiological processes. For example, among the diverse genetic variations that confer risk for major psychiatric disorders stands a truncated DISC1 gene. A Scottish family with a chromosome translocation in which 70% of its members present with schizophrenia or bipolar disorder permitted the DISC1 gene to be identified as one of the truncated genes in the translocation (Millar et al. 2000). Interestingly, the protein encoded by the DISC1 gene proved critical for NMDA synapse development and cortical interneuron function. A mouse overexpressing a truncated DISC1 gene, which acts as a dominant negative, produces several behavioral, neurochemical, and electrophysiological changes that emerge in the adult animal and are shared by several other animal models (Hikida et al.

2007). Another genetic model was produced to test the hypothesis that deficits in NMDA receptors in cortical inhibitory GABA interneurons can selectively produce abnormal behaviors and schizophrenia-relevant endophenotypes. The obligatory NR1 subunit of NMDA receptors was knocked out of PV interneurons in the cortex, resulting in loss of high-frequency oscillations, reduced prepulse inhibition, and altered cognitive functions (Belforte et al. 2010). Again, this is a hypothesis-testing use of an animal model that is not constrained by lack of validity. One could argue that there is no loss of NMDA receptors in schizophrenia, but these mice have been critical to show the impact of altered interneuron function on a number of schizophrenia-relevant phenomena, thereby proving useful to test hypotheses about loss of PV interneuron function.

Finally, there is strong impetus in testing mouse models that recapitulate rare, highly penetrant gene variations. Mice with a microdeletion in chromosome 22 (22q11), similar to what in humans produces a high incidence of schizophrenia, have shown altered PFC–hippocampal synchrony (Sigurdsson et al. 2010), thus providing a link between a gene variation with strong association with the disease and a relevant pathophysiological construct. Several open questions can be addressed with the diverse genetic models available today, such as why mutations can in so many different genes lead to a common pathophysiology or whether there are convergent biochemical/cellular pathways or developmental processes affected by different genetic manipulations. These are examples of possibilities in which the hypothesis-based use of animal models can help move the field forward.

Biomarkers and Endophenotypes

Perhaps the best use of animal models is to test hypotheses related to biological processes that can underlie schizophrenia endophenotypes and ultimately to help identify biomarkers that can be associated with endophenotypes and pathophysiological conditions. For example, the currently popular notion that cortical disinhibition is critical for cognitive deficits requires extensive animal work to be translated in more efficacious treatments. As inhibitory interneuron deficits may be a central tenet of the disinhibition scenario, animal models which test the impact of altered interneurons will be extremely useful in determining a variety of outcomes that can be related to schizophrenia phenomena. Many reports have emerged over recent years of altered cortical oscillations in diverse models that affect cortical interneurons, thus opening the door to establishing clinical neurophysiological readouts of interneuron deficits. More, however, remains to be done. Although there are animal studies using EEG and auditory evoked potentials in a manner similar to what is used in schizophrenia patients, these studies typically employ intracerebral or subdural electrodes. The signal obtained with these electrodes is clearly stronger but may differ greatly from the scalp recordings used in humans. A more human-like

recording strategy (i.e., outside of the skull) is required for EEG and related signals to become more easily translatable. Such an approach would allow animal work to unveil neurobiological processes related to human neurophysiological signals and to understand processes that can alter them.

Another theme that is gaining ground in schizophrenia research is the possible role of immune activation, inflammation, and oxidative stress in the disorder. This is an area in which animal model work will be extremely important. Pending questions include whether inflammation and oxidative stress can be expressed selectively in interneurons, providing a link to the disinhibition hypothesis and perhaps information regarding mechanisms that can yield disinhibition. In addition, we need to establish whether inflammation and oxidative stress can yield cognitive and behavioral anomalies. By testing these questions in animal models and learning about biological processes associated with these variables, we may gain information regarding human biomarkers and how they relate to endophenotypes.

A theme that the current research with animal models should incorporate is the role of dopamine. Most recent animal model work has concentrated on cortical GABA and glutamate. Although these are clearly important players with a critical role in cognition, the link between dopamine and positive symptoms cannot be discounted. It is essential that dopamine systems gain more prominence in animal model work. With the emergence of the disinhibition hypothesis, the dopamine hypothesis seems to have taken a backseat. There are many open questions that need to be answered to obtain a better integrative view of GABA, glutamate, and dopamine systems. Can dopamine alterations emerge as a consequence of cortical disinhibition or are they unrelated? Does dopamine play a role in putting disinhibition into evidence? Animal testing of positive symptoms is problematic; arguably, they cannot be reproduced in a rodent. However, if we focus less on the validity of the models and more about using manipulations to test hypotheses related to the role of dopamine in behavior, we may be able to obtain information that can subsequently be used to guide human studies.

Animal Models and Novel Therapeutics

Ultimately, animal modeling should be at the service of novel medication development. Although a large number of targets (e.g., GABAergic, cholinergic, glutamatergic) have been identified using the models described above, we have so far failed to identify useful targets. For example, as cortical disinhibition hypothesis gained support with animal work, it was reasonable to consider developing new compounds that targeted a disinhibited cortex. Although there were some promising leads, such as the initial report of a metabotropic glutamate agonist mGluR2/3 having similar efficacy as olanzapine (Patil et al. 2007), others failed (e.g., Buchanan et al. 2011). Furthermore, subsequent studies with

the mGlu2/3 receptor did not provide conclusive data. Many factors played a role in this process: some trials were underpowered or showed a high placebo effect, patients were not selected according to specific biomarkers, etc. As these efforts are costly, some are now cautioning against the use of animal information to drive human trials. I argue that these efforts were conducted too early, with little biological information other than a hypothesis developed based on an array of data. More work needs to be done in animal models to determine whether reducing excess glutamate or increasing GABA-A tone does restore excitation-inhibition balance and, if they do, what are the optimal tools (mGluR agonists vs. allosteric modulators; what GABA-A receptor selectivity works). Furthermore, schizophrenia is a heterogeneous disease; it is more likely part of a continuum of neurobiological processes which spans across other related psychiatric disorders. We need to embrace heterogeneity in animal studies and design hypotheses to illuminate how biological processes (and potential treatment targets) can cause diverse sets of clinical outcomes. On one hand, disinhibition may be a feature of bipolar disorder and autism; on the other, there may be a subset of schizophrenia patients in which cortical disinhibition is a prominent feature and others in which it is not. The same goes for dopamine alterations or any proposed pathophysiological scenario.The field, therefore, needs to identify biomarkers that can be associated with pathophysiological conditions. If we are able to determine EEG signals, evoked potentials, imaging alterations or cognitive tests that have a strong correlation with disinhibition in animal models, we can then use those markers to select patients for trials based on biology. Finally, when considering the cognitive realm, it is possible that any benefit of novel agents may be offset by the deleterious impact of traditional antipsychotics on cognition if the trials were designed with the new drugs as adjuvants. Animal models could be useful in determining whether differing effects can be expected from isolated or adjuvant administration of a particular novel compound. Animal models need to be used differently and more extensively before moving on to the next generation of treatment. This would permit trials to use the most likely to succeed targets, schedules, and patient population.

Conclusion

Animal models of psychiatric disorders are important, and it is crucial that we have a diverse set of tools to test biological processes relevant to these disorders. If we knew what the pathophysiological processes in schizophrenia were, we would only need one or a few models to reproduce it. But we don't. Therefore, we need to avoid the pressure of having the "most valid" model and instead use the models to explore specific hypotheses about the contribution of different factors, from genes to the environment. Animal models can also be better employed to seek correlates of neurobiological processes with readouts

that are similar to human biomarkers. To achieve this, we need to replace the notion of validity with usefulness. A useful model would allow us to test the impact of factors that are hypothesized to play a role in the etiology or pathophysiology of schizophrenia. A useful model would also allow us to study neurobiological processes that are affected by any suspected factor without being limited by a perceived lack of validity. If we recognize that schizophrenia cannot be reproduced in animals, then we are free to use animal manipulations to explore biological processes that can have relevance to schizophrenia as well as other psychiatric disorders. The affected neurobiological processes in these studies could then be tested in patients using imaging or other techniques. By de-emphasizing the validation aspect of a model (i.e., the need to mimic the human disease), we can move the field forward by using animal manipulations to test hypotheses about the roles of genes, development, neurotransmitters, or environmental factors.

Why are clinical trials on novel compounds that were designed on the basis on animal data not working? Briefly, concepts developed with animal work were taken to the clinic too early. All of the attempts based on the disinhibition hypothesis were doomed to fail because they were predicated on small pieces of evidence; we did not have a complete understanding about the mechanisms that were yielding to the disinhibition observed in different models. More work with models is needed, for example, to test whether cortical disinhibition is indeed responsible for cognitive deficits and to elucidate the cellular and/or systems mechanisms that may yield disinhibition or any other pathophysiological construct following developmental, genetic, or environmental manipulations. Only with that information will we be able to understand the neurobiological mechanisms of stimulating mGluR receptors or enhancing GABA-A receptor activity. However, we should not throw out the baby with the bathwater. As our gaps in knowledge are being filled, novel compounds can still be tested on those models in which a pathophysiological state relevant to their targets is present. The pharmaceutical industry needs to invest more heavily in testing compounds in animal manipulations which model the pathophysiology intended for the new agent and, most importantly, which identify biomarkers that can be associated with a positive effect of these agents. Only then can a sufficiently powered clinical trial be conclusive in accepting or rejecting a particular target.

How, then, can animal models be better utilized? The answer is simple: by using them to test specific hypotheses related to the flow of etiological/risk factors from pathophysiological processes to behavior and clinical manifestations.

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