

# Bridging the Gap between Patients and Models

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## Abstract

Classically, research into human disease tends to be done in a top-down or bottom-up manner, starting from symptoms or genes, respectively. While bottom-up approaches may work well in oncology, and might advance understanding of monogenic neuropsychiatric diseases, successful application for complex, multifactorial disorders is more difficult and has resulted in many translational failures. This chapter investigates the existing obstacles and explores options to overcome them. Complex diseases need to be dissected into measureable, manageable factors and investigated in a comparable, compatible assembly of model systems to test hypotheses, concepts, and ultimately drug candidates or other therapeutic interventions. While some of these factors might best be investigated top down, a bottom-up approach might be more effective for others. Both approaches may only be successful up to a specific point. Thus, the two must be linked and a bidirectional approach pursued. Inclusion of patients is essential as are behavioral readouts, since disease-associated dysfunctions or symptoms are often behavioral in nature. To connect models and humans, behavioral readouts need ideally to be linked to evolutionary conserved neural substrates. Some anchor points already exist and new promising ones, such as induced pluripotent stem cells (iPSCs), are emerging. Recent developments may speed up translation of research into clinical applications (e.g., faster drug screens in a patient-specific manner). When positioning different models, it is important to characterize their predictive power diligently, to emphasize their scientific rigor, and to not overstate their application potential. Finally, to effect faster transition from research to clinical applications, organizational structures are needed to foster interdisciplinary research and collaborations between academia and industry. A “third space” concept is proposed to conduct early proof of principle studies (Phase 0 and I). To increase the success rate in clinical development so as to provide actual benefit for patients, proactive interaction is needed between all organizational entities involved in drug development and therapeutic discovery (e.g., academia, guid-

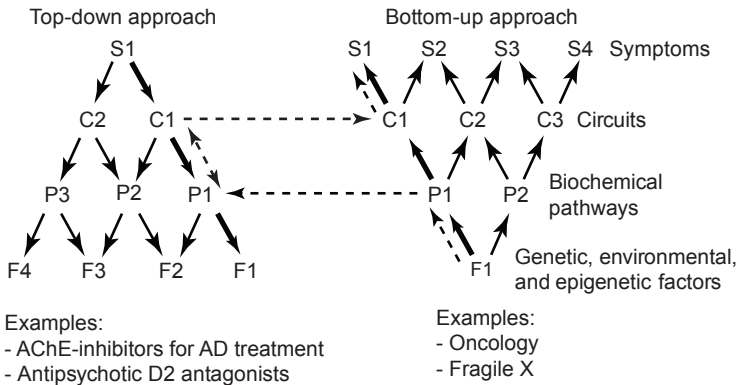
ance agencies, biotech, device and pharmaceutical companies, regulatory agencies, and funding agencies).

## Learning from the Past: Unidirectional Approaches

To outline discovery strategies clearly and to systematize arguments, we categorized research processes into bottom-up and top-down approaches (for a definition of terms used in this chapter, see Appendix 13.1). The bottom-up approach starts with genes, whereas top-down procedures begin with a syndrome or phenotype. The two streams likely overlap at the level of molecular pathology, although in some instances overlap at other levels is also possible. Depending on whether pathological changes are found from analyses of brain tissue or predicted and found from genetic studies, these approaches should be considered top-down or bottom-up, respectively (Figure 13.1).

### Top-Down Approaches

The top-down approach takes some features or attributes of a disease or disorder from a detailed phenotypic analysis and uses these either to predict possible drug targets and new therapeutic concepts or to test the efficacy of a drug discovered by serendipity. These disease/disorder phenotypes should be



**Figure 13.1** Top-down versus bottom-up approaches in research. Top-down approaches begin at the level of symptoms (the usual starting point is one symptom, S), and move down to genetic or environmental factors (F). Bottom-up approaches work their way up from a genetic factor to identify the corresponding symptom. Naturally, the approaches have the most likelihood of success at the level one step away from the starting point: circuits (C) for top-down approaches and biochemical pathways (P) for bottom-up approaches. The most difficult step is the gap between biochemical pathways and circuits. Here, knowledge derived from basic research can serve as a bridge. Broken lines indicate a desirable research approach to combine the fields; see Appendix 13.2 for further discussion in regard to Parkinson disease.

investigated in detail at the level of molecular or cellular pathology as well as neural system/circuit dysfunction, cognition and behavior, and epidemiological factors. The top-down approach has worked quite well in diseases characterized by a dominant symptom and relatively direct delineation to underlying pathways and pathology, such as Parkinson disease (PD). For PD, motor dysfunction could be associated with degeneration of the nigrostriatal dopamine (DA) pathways, which appeared sufficient to explain much of the movement deficits of this neurodegenerative disease. Therefore, it made sense to attempt replacement of depleted DA as a major therapeutic concept; hence the introduction of L-DOPA therapy and DA receptor agonist medications. However, cognitive deficits in PD patients, which have been attributed to other, extrastriatal pathology, were found to be largely independent of DA, and are thus not treatable with DA replacement (Appendix 13.2). For a detailed review of a historic perspective as well as contemporary top-down models for aspects of schizophrenia, see Appendices 13.3 and 13.4.

Applying modern analysis methodologies to top-down approaches today may start with molecular profiling of patient biospecimens—molecular substrates from patients' surrogate tissues (e.g., cerebrospinal fluid or olfactory cells obtained through quick and safe nasal biopsy). This allows a set of targets to be identified directly in humans. Many neurodegenerative disorders occur as a result of the combination of genetic and environmental factors. Unbiased proteomic and/or candidate molecular approaches can be applied to identify targets of interest. In contrast to genetic sequences, which contribute to the identification of targets associated with trait change, the molecular analysis of biospecimens provides the opportunity to identify both trait- and state-associated targets (see Appendix 13.1). This approach is considered to be the most effective when applied during early disease stages or even with subjects in the prodromal stage. Molecular profiling can also be combined with molecular imaging, such as positron emission tomography (PET) and magnetic resonance spectroscopy (MRS). One of the most promising examples is the identification of potentially predictive markers for Alzheimer disease (AD), such as pathological changes in amyloid PET imaging and alteration of A $\beta$ 42 and phosphotau in cerebrospinal fluid (Lista et al. 2014; Blennow et al. 2012).

### **Bottom-Up Approaches**

To date, oncology provides some of the best examples of success for the bottom-up approach. Specific subtypes of cancer are identified by their genetic mutations and corresponding antibodies or other antagonists are generated to treat them. As a generalization, all cancers share a common phenotype: excessive proliferation. However, the drivers of the phenotype (e.g., mutated kinases) vary from one cancer subtype to another. This means that there are two broad classes of potential treatments: standard chemotherapeutics which block cell proliferation in a relatively nonspecific manner or act on tumor-specific

pathways that regulate proliferation. In one of the initial examples of this approach, mutations of the HER2 gene were identified as causative genes in some forms of breast cancer. Trastuzumab (Herceptin) is an anti-HER2 antibody developed to treat this specific condition (Sliwkowski and Mellman 2013). Several further antibodies followed the same paradigm, and the cancer field is progressing rapidly with this approach. Companion diagnostics are developed in parallel to the development of therapeutics. Continuing progress with this approach is expected to result in highly individualized but highly effective treatment of cancers.

In contrast to the oncology field, there have been no clearly positive examples for the bottom-up approach in the area of neurologic and psychiatric diseases. For instance, despite the discovery of a dominant disease gene in Huntington disease two decades ago, no effective therapy has thus far been developed (Ross et al. 2014). The most advanced example is provided by fragile X syndrome and the link to mGluR5 receptors. Fragile X syndrome, a monogenic cause of autism for which the identification of an expansion of CGG repeats in FRM1 gene leading to altered FRM1 functions (mRNA transport), helped identify mGluR5 as a potential therapeutic target (mGluR5 antagonists can rescue some of the defects resulting from FRM1 deficiency in knockout mice; see Appendix 13.5). Although this identified a promising therapeutic concept, translation to human disease has thus far been unsuccessful, illustrating the challenge of bridging model and human systems.

### **Summary of Unidirectional Approaches**

Although unidirectional approaches have proven successful in disease areas such as oncology and a few promising examples have emerged for diseases of the nervous system, the majority of CNS disorders are characterized by their complexity and multifactorial etiology. Diseases such as schizophrenia, AD, or bipolar disorder are syndromes composed of multiple symptoms, which imply the contribution of several genes to different degrees, including complex interactions between these genes and environmental factors. The combined implication of genetic and nongenetic (epigenetic) factors is one of the major obstacles in the understanding of these diseases. For example, schizophrenia may involve up to 10% of all known genes, and the different forms of the disease may implicate different genes or genetic loci in distinct patients or families. Further, environmental factors (e.g., early life stress or nutritional deficiencies) are known to play a contributory role. Thus, schizophrenia is not only multigenic but also highly diverse genetically, involving epigenetic factors. A unidirectional approach might not be best suited for tackling such complex diseases. In comparison to cancers, the genetics appear to be more complex, with each disease having multiple drivers. In addition, neurological and neuropsychiatric diseases are associated with an unknown number of phenotypes.

Understanding how to divide each disease optimally into discrete therapeutic categories would represent a significant advance in the field.

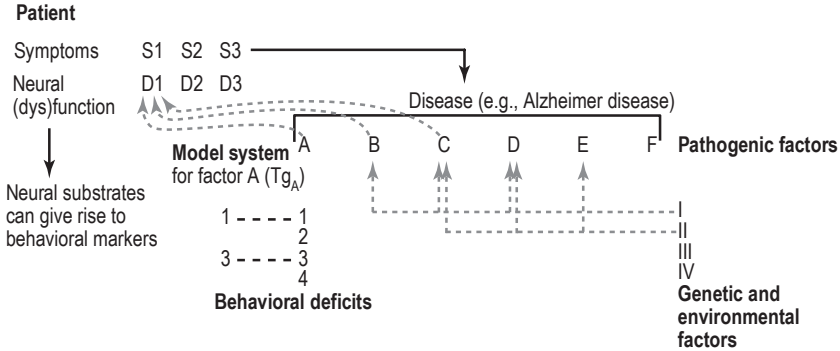
### **The Bidirectional Proposition**

It seems clear that neither a strict bottom-up nor a top-down strategy alone suffices to approach complex neuropsychiatric disorders. Instead, both approaches are equally important to pursue in parallel and interactive ways. While the ultimate goal of both approaches is identical, on the short term both differ principally in their output to new therapeutic concepts. Top-down approaches are closer to patients and their symptoms; thus, they tend to lead to symptomatic rather than curative treatment concepts. On the other hand, understanding the genetic basis of syndromes or disease groups might not immediately lead to an understanding of how symptoms are produced, but even without such knowledge, genetics may guide the discovery path toward curative rather than symptomatic treatments. An integration of genetic and epigenetic factors will be essential in this respect.

One might consider, for instance, generating animal models with mutations or copy number variations similar to those identified in patients, alone or together, and in combination with an environmental manipulation. One could also consider using induced pluripotent stem cells (iPSCs). Nonetheless, open questions and limitations remain: the type of cells to use (those directly involved in the pathology are not accessible), variability of cultures, no recapitulation of environmental factors, and associated epigenetic processes. One could employ biospecimens (e.g., cerebrospinal fluid, olfactory cells, or blood) to identify transcriptomic and/or proteomic alterations associated with the disease. In addition, functional (e.g., symptomatic) readouts remain essential to the ultimate demonstration of clinical efficacy. These examples illustrate the need for a bidirectional approach and highlight the limitations of a strict separation between bottom-up and top-down approaches.

### **Deconstruction of Neural Diseases in Measurable Manifestations**

Neural diseases are complex constructs, and patients manifest variable combinations of symptoms and disabilities. Thus, neural disorders have to be deconstructed into measurable manifestations (symptoms, behaviors, disabilities, or functional disturbances) that can be mapped onto specific neural substrates or neural dysfunctions (Figure 13.2). Importantly, each given symptom may map onto more than one disease, and the combinations of symptoms in a given disease may vary significantly. This can give rise to phenotypic variability even though the neural substrate, or the causative gene or genes, may be the same. Thus, better results may be exacted if we aim to treat specific behavioral or cognitive alterations and their underlying neuronal substrates, rather than



**Figure 13.2** Mapping manifestations of complex polygenic human neural diseases to models. A patient might show symptoms (S1, S2, S3), and associated with each symptom is one neural dysfunction (D1, D2, D3). Together the symptoms build the disease. The disease is associated with several pathogenic factors (A–F), and each pathogenic factor results potentially in behavioral deficits (1–4). Model systems (like a transgenic mouse) might show only part of the behavioral deficits (here, 1 and 3), even though they fully express the pathogenic factor. Pathogenic factors result from the interplay between mutated genes and particular environmental factors (I–IV). One gene can be associated with several pathogenic factors, and one pathogenic factor can be dependent on more than one of the disease genes. Finally, each pathogenic factor can contribute to more than one neural dysfunction, and each neural dysfunction can be associated with more than one pathogenic factor.

address disease disabilities or symptoms in a holistic manner. The complexity of pathogenic factors in polygenic diseases leads to a multiplicative space of factors which, if modeled completely, would lead to an exploding number of possible combinations. Principal component analysis and related statistical techniques, such as discriminant cluster analysis, can potentially be used on large data sets to determine the interrelationships between, for example, disease/disorder behavioral/cognitive/physiological symptoms, pathologies, and neural circuit/network dysfunctions to determine their interrelationships and how many orthogonal (i.e., distinct) clusters or factors (i.e., phenotypes) make up the disorder.

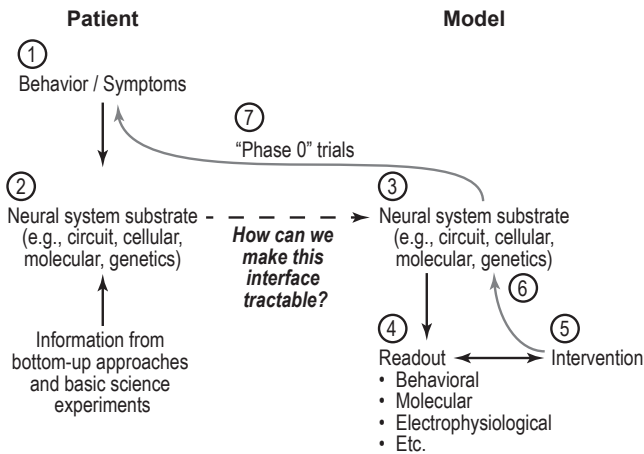
### Evolutionary Conservation as a Bridging Point between Human and Model Systems

Neural dysfunctions can serve as targets for the development of therapeutic concepts and inform models. This conceptualization is consistent with the new recommendations for clinical trials from the U.S. National Institutes of Mental Health (NIMH) which emphasize the need to have patient-centric outcomes (e.g., improvement of quality of life or suppression of a given symptom or

disability), while demonstrating the link and specificity of the tested intervention with a specified neural target or underlying neural dysfunction. Neural dysfunctions can be measured using a variety of methods, including neuroimaging as well as neuropsychological, neurophysiological, and neurochemical techniques. Importantly, the introduction of methods into the process of therapeutic concept discovery will facilitate the rigorous testing of interventions in humans and so enable the bridge from humans to models, whether whole animal, cellular, or computational.

The expectation is that neural substrates and dysfunctions will map better from human to model systems than symptoms or manifestations of disease. The mapping of neural substrates from humans to a suitable model will enable the identification of the pathogenic factor or factors that account for a given dysfunction. In experimental model systems, it is then possible to move from the pathogenic factors to the responsible genes or gene products.

The bridge from humans to models is bidirectional (Figure 13.3). Eventually, after a therapeutic concept is identified, translation from the model to the human



**Figure 13.3** Process of bridging animal models to patients and back. (1) Symptoms are identified in patients. (2) A neural system substrate associated with this symptom is then identified. Importantly, this can only be done based on information from previous basic science experiments and disease-oriented, bottom-up research. (3) An evolutionarily conserved neural system substrate is identified in the best-suited model system, again informed by basic research. (4) An appropriate readout is chosen. (5) This readout is used to evaluate the success of the intervention, which ultimately works on the neural system substrate (6). (7) Findings should then be translated to humans in a proof of principle manner using identical or at least similar readouts. Results obtained in humans should then be back-translated to the model for further refinement. The critical step is the interface between patient and model system.

and back will be necessary. We argue that an experimental medicine approach is critical here, as it permits rigorous testing of candidate therapeutic concepts before embarking on traditional clinical trial phases. This approach would assess the ability of the hypothesized mechanism of action of the therapeutic concept to alter the neural substrate and normalize the neural dysfunction that could lead to an improvement in symptoms or disability. As formulated by the NIMH<sup>1</sup>: “Rather than testing the intervention in a traditional efficacy outcome trial, the experimental medicine trial involves objective measures of target engagement and effects on brain function as initial evaluation points.” Such approaches may include dose finding studies and a comparison of different methods of intervention delivery. The results of such experimental medicine assessments ought to represent an early “Go/No-Go” decision point; demonstration that a given therapeutic concept adequately engages and modifies the neural dysfunction is necessary to ask whether it can affect clinical symptoms.

The selection of inappropriate neural dysfunctions and their use as the bridge between human and model systems, along with the lack of experimental medicine approaches, may account for past failures to translate therapeutic concepts to humans and achieve greater success in clinical neurotherapeutics. The suitable point to bridge human and model systems is at the level of evolutionary conservation. Three points are of relevance here:

1. The level of conservation may vary with different model systems.
2. Conservation will likely decline in the following order: gene, gene product, molecular mechanism, cellular function, neural circuit, behavior, symptom.
3. A model will obviously be progressively closer to the symptoms of human disease in the opposite order: behavior, neural circuit, cellular function, molecular mechanism, gene product, gene. This emphasizes the importance of behavioral measurements.

### **The Importance of Behavior for Investigating Mental Disorders**

While the approach of evolutionary conservation may suggest the importance of the neural system substrate over behavior, and thus imply that behavioral measures are less important, there are good arguments to include behavioral studies in this process, especially when there are no overt pathological hallmarks of a disease available:

1. Drugs generated using the bottom-up approach for most (if not all) diseases/disorders of the nervous system will eventually have to be tested

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<sup>1</sup> <http://www.nimh.nih.gov/funding/opportunities-announcements/clinical-trials-foas/nimh-clinical-trials-funding-opportunity-announcements-applicant-faqs.shtml> (accessed March 3, 2015)



in clinical trials. Here, the main outcome measures, however crude, are overtly behavioral (or cognitive) in nature and are generally based on clinical measures of behavior, including functional outcome.

2. Clinical trials can be improved by combining better-defined behavioral measures with measures of the integrity of neural circuitry (e.g., through neuroimaging as well as electroencephalography and magnetoencephalography) to be able to link the neural changes to specific symptoms (i.e., behavioral changes).
3. To understand how treatment impacts a mental disorder—for example, depression—sensitive measures are needed to assess the degree to which a patient is suffering from (a) undue bias to think negatively, (b) a diminished hedonic response that derives from a primitive approach/reward or reinforcement system, or (c) both. These measures, then, need to be related to the specific neural circuitry deficits (from which different biomarkers may emerge). Currently, our understanding of neural circuitry functions is not sufficiently advanced to use reverse inference logic. For example, the hypothesis “this circuit is damaged so it *must* be a reward problem” must be tested and updated continually. Further, in many cases it is necessary to “challenge” the neural system behaviorally in order to identify a circuit dysfunction, such as in provocation studies in obsessive-compulsive disorders or relapse cues in addiction, including attempts at remediation.
4. To enhance the predictive power of animal models and tests, these need to be related to the measures of Phase I and III clinical studies. Thus, where a “reward system” deficit is invoked, there should be an animal model or test equivalent, ideally behavioral as well as neural (see previous point). Even if it were not necessary to use the behavioral measure, it would greatly increase the confidence in interpreting any neural change as having an important functional consequence, thus also raising the predictive power of Phase I and II outcomes for Phase III.
5. Automated behavioral measures can be made to be very reliable and with small error variance, suitable for detecting the effects of drugs or genetic manipulations, even when clear equivalents to humans (e.g., for human language) do not exist.
6. Many examples of complex behavior can be reduced to evolutionary building blocks of the more sophisticated functions in humans, such as goal-directed behavior, working memory, spatial attention (for an example of cholinergic treatment of AD, see Appendix 13.6), and performance of clinical neuropsychological tasks such as the Wisconsin Card Sorting Test (a test of cognitive flexibility sensitive to damage or dysfunction of the human frontal lobes). The latter can be reduced to tests of discrimination learning and can be used in parallel in humans, including patients, and animals including monkeys, rats and mice (see Robbins, this volume). To optimize the tests across these species,

different sensory modalities may have to be employed; that is, different visual features for primates, smell and touch for rodents. However, the basic structure of the behavioral tasks, and hence the functions governing cognitive flexibility, are similar if not identical (e.g., reversal learning depends on the orbitofrontal cortex). Moreover, for some tasks, such as the stop signal reaction time (SSRT) test, there may be comparable pharmacology across species (see Robbins, this volume). These examples provide considerable confidence that the same type of function, mediated by the same type of circuit, is being measured across species.

7. Finally, one can begin to relate (by experiment) basic processes of learning discovered in animals (to humans), and aberrations of these processes (to patients) in a way that may relate to their symptoms and help explain them. An example of this (logically “triangulating”) approach is the prediction error deficits in causal learning (i.e., how events can be predicted or expected) that relate to functioning of frontostriatal circuits and can be mapped using electrophysiological or neuroimaging methods (e.g., the BOLD response using functional MRI). NMDA receptor antagonists, such as ketamine, impair learning by disrupting these prediction errors, and the degree of disruption in humans predicts perceptual impairments which contribute to delusions in human volunteers. Similar disruptions are observed in first episode patients in schizophrenia. Given that the NMDA receptor antagonist similarly impairs the readout of prediction errors during learning in experimental animals, these disruptions in animals can, in principle, act as “biomarkers” of more complex delusional phenomena in humans.

### Summary of Bidirectional Proposition

Translation from patients to models, or from a model to the patient, should never be approached solely in a top-down or bottom-up fashion, but rather always through a bidirectional approach. This requires us to identify the point(s) of evolutionary conservation, be it on the level of neural substrate or (in certain cases) behavior. Fortunately, much work has been done and published in this realm, often coming from basic research approaches, once again emphasizing the importance of this research line. To allow easier access to this information, communication and efforts between researchers must be enhanced, especially between clinicians and basic scientists, but also between academia, industry, and governmental and other agencies. (This point is taken up below, where a “third space” is proposed to accomplish this goal.) Finally, instead of attempting to cover the complete disease spectrum all at once, a clear focus on specific, tractable aspects of disease comparable between models and species will help accelerate the process.

## **Choosing the Right Model System and Evaluating Predictive Power**

According to our idea of evolutionary conservation, models should faithfully reflect a given neural substrate or dysfunction. A neural substrate may be a neural circuit, a cellular element, a molecular target, a gene, or a gene product. It is important to recognize, however, that one or more pathogenic factors may be relevant for a given neural substrate/dysfunction. The experimentally measured model readout should be a faithful reflection of the impact of a tested intervention onto the neural substrate. Ideally, the most proximal marker of the neural substrate (e.g., circuit activity, cellular firing pattern, molecular or gene expression) offers robust and faithful metrics. Thus, if a given therapeutic concept engages the neural substrate in the same manner in both the model and the human, one could argue that a sufficient level of predictability will be achieved. Nevertheless, behavior should not be neglected as it can be extremely important and helpful in linking the neural substrate to an overtly measurable malfunction, ultimately bridging proximal efficacy measures from early clinical trials into Phase III.

### **Rodent Model**

Although animal models are sometimes said to have “failed” in predicting drug efficacy, closer examination shows that these “failures” have not necessarily been failures; instead, they stem from false prior expectations, overstatements, and inappropriate extrapolation or overgeneralization of findings. For example, a positive drug effect in a simple screen of passive avoidance learning in a mouse or rat may be taken as optimistic evidence of a general cognitive enhancement, even though the effect in such a test may arise simply from an effect on a special form of emotional learning. Care must be taken when extrapolating to human memory. On the other hand, drug failure may have been due to factors other than misinterpretation of the animal data, including a poorly designed clinical trial that did not include measures to track preclinical translation. Back-translation from such trials is, in practice, rarely employed to check the validity of the animal model objectively. Overall, the predictive power of a rodent model should be assessed in the context of the entire clinical development cascade, taking reference to related tests in humans that use similar readouts and target neural substrates of evolutionary conservation. These are rarely conducted in Phase III but rather much earlier to obtain proof of mechanism or principle. Thus, the predictive power of such tests in early human trials needs to be aligned to those used in Phase III. An informative story about cholinergic influence on AD and the predictive power of rodent models is given in Appendix 13.6.

## Transgenic Mice

The etiology of neurodegenerative and neuropsychiatric disorders is complex and multifactorial. Using experimental animal models has been more helpful in dissecting than simulating this complexity. Such models have provided a large number of useful insights into the pathogenic activities of individual factors suspected of contributing to the development of these or other neurological conditions.

For example, overexpressing familial AD-linked human amyloid precursor proteins (hAPPs) in neurons elevates amyloid beta ( $A\beta$ ) levels in brain and is sufficient to cause a range of AD-like disease manifestations in these models, including impairments in learning and memory, behavioral alterations, synaptodendritic rarefaction, synaptic and neural network dysfunction, formation of amyloid plaques, neuritic dystrophy, astrocytosis, microgliosis, and vasculopathy (LaFerla and Green 2012). Several molecular alterations first observed in such models have been subsequently identified in humans with AD; for example, depletions of calbindin in dentate granule cells and of specific voltage-gated sodium channel subunits in parvalbumin-positive GABAergic interneurons in the neocortex (Verret et al. 2012; Vossel et al. 2013).

Because several other factors likely contribute to the pathogenesis of AD, including apolipoprotein (apo) E4, tau,  $\alpha$ -synuclein, inflammatory mediators, and vascular alterations, it is not surprising that hAPP transgenic mice do not replicate all aspects of this complex human condition (LaFerla and Green 2012). Similarly, it is unreasonable to expect that testing drugs in such models will be sufficient to predict their efficacy in the much more complicated human condition. Notwithstanding, some therapeutic interventions have extrapolated quite well from hAPP mice to patients with AD or amnesic mild cognitive impairments, including the removal of amyloid plaques by anti- $A\beta$  antibodies and the reversal of abnormal network activity by the antiepileptic drug levetiracetam (Vossel et al. 2013).

Compound experimental models combining different genetic and/or pharmacological manipulations can be used to simulate part of the complexity observed in the human conditions. In the AD field, such models have revealed interesting co-pathogenic interactions between  $A\beta$  and tau,  $A\beta$  and apoE4, apoE4 and tau, and  $A\beta$  and  $\alpha$ -synuclein. Naturally, species barriers must always be kept in mind and make it critical to validate results obtained in animal models in human subjects. Whether iPSC-derived cell culture models can form a useful bridge in this process and whether they may be able to predict therapeutic efficacies more reliably than animal models remains to be determined.

To arrive at a therapeutic concept, it is important to quantify the predictive power of a given experimental state. For transgenic mice, increased rigor in preclinical trials that test the robustness of an effect plays a pivotal role. It should become standard (even if expensive) to include more independent

biological cohorts and different age groups. Further, the species comparison can give confidence in a result.

Although the phenotype of a given transgenic mouse model may indicate large overlap to the human disease, careful consideration of the best strategy to generate and analyze these mouse models remains key. Although AD mice carrying triple mutations in APP, PS, and tau replicate most of the pathological features of the disease, they might not be perfectly relevant: the introduced genetic lesions do not appear together in patients; the transgenic, mutated tau is not even associated with familial AD but rather with another form of dementia, namely frontotemporal lobe dementia (Hutton et al. 1998). In addition, artificial promoters are introduced into each gene, and thus the interactions between gene products may not be captured appropriately. In regard to assays, protein disposition, synaptic function (slice), and cognitive functions (*in vivo*), and their interactions need to be studied. Finally, the contribution of environmental factors and their interaction with genetic factors need to be integrated into the models to recapitulate fully the etiopathology of the disease.

### Nonhuman Primates

According to the idea of evolutionary conservation, models should faithfully reflect a given neural substrate/dysfunction. Due to their evolutionary similarities, nonhuman primates are more closely related to humans than other animals, thus leading to the greatest overlap in neural substrates and behavior. Nonhuman primates are, therefore, a prime (in some cases the only) model for brain structures and functions which are specific for primates.

One explicit example is the connection between motor cortex and spinal cord, which is uniquely organized in primates. In rodents, there are no direct connections between corticospinal neurons and the cervical motor neurons that innervate forelimb muscles—brainstem pathways and spinal interneurons relay cortical input to motor neurons. In nonhuman primates and humans, direct corticospinal connections with motor neurons have evolved, together with an increase in the size and number of the corticospinal fibers. This development correlates with an improvement in dexterity, particularly in the ability to perform finger-thumb precision grip (Lemon 2008; Courtine et al. 2007). This is reflected in the field of neurodegeneration or neuroregeneration, where nerve cell regrowth studies in monkeys have led to key discoveries that were ultimately channeled into a new treatment concept. In human adults, the neurite outgrowth inhibitor (“Nogo”) protein prevents the healing of damaged nerves following a spinal cord injury (Schwab 2004). Experiments on monkeys have demonstrated that antibodies developed to counter “Nogo” following spinal cord injuries led to significant functional improvements through nerve growth (Freund et al. 2006). Currently, “Nogo” antibodies are being used in clinical studies on patients with spinal cord injuries. Another example from the neurodegenerative realm is the MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine)

primate model used to decode the pathology of PD and to develop therapies (Capitanio and Emborg 2008). Nonhuman primates exposed to MPTP develop symptoms similar to PD, whereas rodents show a less specific response to MPTP. Studies on MPTP-treated nonhuman primates have enabled detailed investigation into the pathological consequences of PD, and thus a range of therapeutic approaches have become possible, such as the targeted administration of DA agonists and use of deep brain stimulation (Jenner 2003). Specifically, deep brain stimulation is a new and effective procedure for treating patients with movement disorders that would have been barely conceivable without research on primates (Rosin et al. 2011). Further, investigations on nonhuman primates are important for research of aging because the closer genetic relationship to humans produces a highly similar aging phenotype (Roth et al. 2004).

In highly cognitive or social contexts that rely on prefrontal cortex structures that are unique in primates, the nonhuman primate model is irreplaceable. Here, nonhuman primates allow for deeper insights into the neural substrates underlying these higher-order brain functions (Watson and Platt 2012). Many neuropsychiatric diseases (e.g., schizophrenia or attention-deficit/hyperactivity disorder) are accompanied by brain dysfunction in the frontal lobe (Robbins and Arnsten 2009; Nelson and Winslow 2009). Here, research on rhesus monkeys has been decisively important for decoding the mode of operation of the frontal lobe, which is specific to primates (Castner et al. 2000). A significant driving power for developing drug-free treatment for psychiatric diseases is expected from this research branch (Goldman-Rakic et al. 2004).

While transgenic nonhuman primates are an exciting prospect (Niu et al. 2014; Sasaki et al. 2009), there are also other more established techniques for correlative and causal investigation of the primate brain. Electrophysiological characterization of brain functions and neural correlates of behavior, as well as electrical and optogenetic manipulations for causal probing of the same, promise to widen our knowledge substantially (Wang et al. 2012; Diester et al. 2011). In particular, optogenetic techniques that allow for axon-specific manipulations could help disentangle complex pathways, thus elucidating the role of subcircuits for a specific behavior. When electrical stimulation is employed, we cannot be certain that only a local area or one single pathway is affected. Optogenetic manipulations, by contrast, allow for this very precise stimulation. By stimulating or inhibiting axons, while measuring simultaneously the impact on neural activity as well as on behavior, it is now possible to measure the impact of a pathway very specifically (Deisseroth 2014). Such a deeper understanding of the neural underpinnings of an observed behavior or symptom will also allow evolutionary conserved bridging points to be identified. Further, noninvasive brain stimulation approaches that can be applied to animal models, as well as to humans, offer the appeal of potentially serving as consistent biomarkers in models and patients. For example, transcranial magnetic stimulation can be used in such a manner (Freitas et al. 2013; Fox

et al. 2012; Shafi et al. 2012; McClintock et al. 2011). Research on nonhuman primates is subject to justified ethical concerns about working with highly differentiated and sensitive animals. There is consensus that experimentation with these species needs to be restricted to areas where lower animal species provide insufficient information, and that they need to follow highest standards of animal welfare.

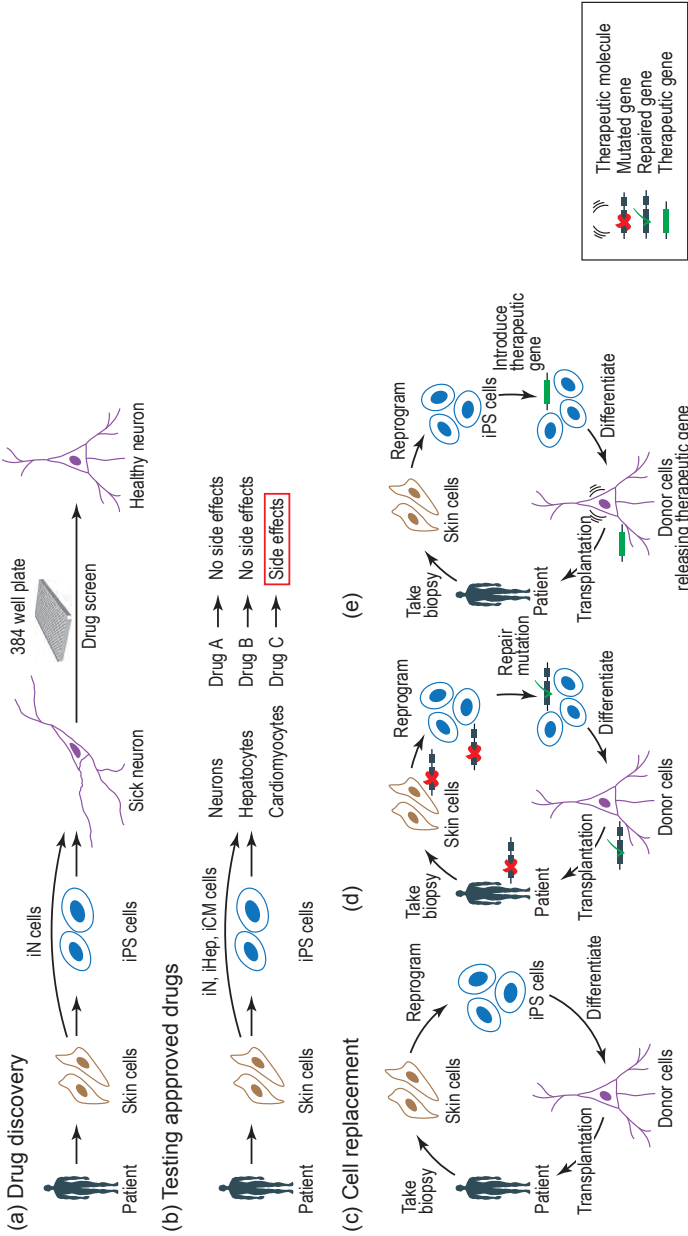
## **Induced Pluripotent Stem Cells and Induced Neuronal Cells**

### *Current Possibilities and Limitations of Stem Cell Technologies*

There is tremendous excitement about recent progress in cellular reprogramming and stem cell biology. Skin fibroblasts can be converted into iPSCs or induced neuronal cells (Vierbuchen et al. 2010; Mitsui et al. 2003). iPSCs can be differentiated in a second step into neurons, glia, and, in principle, any other cell type of the body. Fibroblasts can also be directly converted into various cell lineages, such as cardiomyocytes, hepatocytes, oligodendrocytes, and neural progenitor cells (Graf 2011; Vierbuchen and Wernig 2011). From a disease modeling point of view, these reprogramming methods are attractive because for the first time disease-relevant cell types (e.g., functional neurons) can be generated from the patients' own cells containing the patients' authentic genetic material—a “dream” for disease modelers since such a complete genetic representation cannot be accomplished in model organisms given the vastly different genomic organization in other species. This is particularly relevant, given the complex polygenetic basis of the major neuropsychiatric and neurodegenerative diseases. For these reasons, the community is extremely excited about the potentially transformative new cell models that are on the horizon to enrich our current portfolio of disease models.

There are several principal applications of these reprogramming techniques. In the context of neurological and psychiatric diseases, patient-specific neurons could be used (a) to divide each disorder into phenotypic categories (such as reduced number of glutamatergic synapses), which is vitally important since many genes may alter the same “druggable” process; (b) to establish disease-relevant screens that identify the best therapeutic classes for each phenotypic category; (c) to test each therapeutic candidate across large numbers of patient neurons; and (d) to choose patients most likely to benefit from specific drugs for clinical testing and, eventually, for treatment. Finally, a few relevant iPSC-derived cell types, such as cardiomyocytes and hepatocytes, could be pre-screened from hundreds of patients for toxic side effects. Another principle application of this technology is cell transplantation, where the idea is to interfere positively with disease processes (Figure 13.4).

As with every nascent technology, there are currently important limitations to this methodology. First, at the moment, protocols exist that claim similarities to cortical, spinal motor, and midbrain DA neurons, but they typically have



**Figure 13.4** Three putative applications of induced pluripotent stem (iPS) cells. (a) Drug discovery: Neurons with disease phenotypes are used in screens to identify effective drug candidates. (b) Testing approved (or investigational new) drugs: Prior to clinical administration, tissue and cell types of primary patient-specific neurons, cardiomyocytes, and hepatocytes are used to find the safest and most effective drugs for each individual patient. (c) Cell replacement: Patient-specific cells can be transplanted to replace missing neurons, correct genetic defects, or provide a local source of growth or neurotrophic factors. Induced neuronal (iN) cells; inner cell mass (iCM) cells; induction of functional hepatocyte-like (iHep) cells.



low yields and represent mixtures of various undefined cells (Ming et al. 2011). Given the possibility that specific pathological defects might occur only at specific types of neurons or synapses, it will be important to have good control over these parameters. Differentiation protocols will have to be devised to generate pure or at least defined combinations of specific neuronal and glial subtypes. Second, a general issue in the iPSC field is that differentiated progeny are typically immature. This is true for neurons, glia, cardiomyocytes, hematopoietic cells, and hepatocytes (Ming et al. 2011; Sahakian and Jaenisch 2009). This is not surprising, since human fetal development takes nine months, as opposed to three weeks for a rodent. It will have to be determined what time frame can be tolerated for feasible disease models. The maturation state of brain cells is a concern because many neurodegenerative diseases occur in the adult brain and late in life. Therefore, even the production of fully mature cells might not be sufficient; we may need to produce “aged” cells. An extremely interesting approach to accomplish this goal is to introduce genes known to cause premature aging. It seems that at least certain cell biological effects typical of aging can be recapitulated in this fashion (Miller et al. 2013a). Unfortunately, however, the maturation problem has not been overcome by this approach, despite the observation of cell biological aging effects. Finally, human cells cultured *in vitro* cannot readily reconstitute complex neural circuitry as seen in the brain. This may require a three-dimensional organization of the cultured cells; thus the cultured iPSC-derived neuron system is more equivalent to dissociated brain cultures and is fundamentally different from the standard brain slice preparation performed in rodent or in rare epilepsy-associated human brain resections. Still, promising progress has been made in the generation of three-dimensional retina and corticoid spheres, which take advantage of the remarkable ability of embryonic stem cells and iPSCs to self-assemble (Kadoshima et al. 2013; Lancaster et al. 2013; Nakano et al. 2012). At this time, early developmental processes can be recapitulated in such organoid cultures, but the lack of vascularization imposes size limitations and thus may restrict the degree of maturation. We note, however, that even the most advanced human cellular and organoid model will be restricted to issues between gene and neural circuit. The connection between circuits and behavior is not accessible by a culture system, although new avenues are under discussion on how to overcome this hurdle (see next section).

There are already a couple of examples in which iPSCs have shown their immense potential, suggesting that they can play a role in disease modeling (see Rubin, this volume). For example, in spinal muscular atrophy (SMA), a genetic motor neuron disease with early onset, several papers have shown that iPSC-derived motor neurons carrying the SMA mutation can recapitulate many of the known disease processes. In addition, experiments using these cells have predicted other aspects of disease pathology that await verification in SMA patients. Another example is amyotrophic lateral sclerosis, a motor neuron disease with late onset and many subcategories (genetic and idiopathic). *In vitro*

drug testing is only rarely carried out using motor neurons. One mouse model is available that highly overexpresses a mutated *SOD1* gene. These mice might capture only ~2% of the patient population. Thus, not surprisingly, drug tests in mice have not been predictive of clinical responses in patients. So, instead of generating more transgenic mice which again might capture only a small fraction of patients, iPSCs might offer a valuable alternative. Since they can be generated from large numbers of patients with various backgrounds, therapeutic concepts and drug candidates could be pretested in these highly individualized cultures before going into clinic.

All therapeutics need to be effective *and* safe. An important aspect of iPSC-based disease modeling is that it permits the generation, in a patient-specific way, of multiple cell types. For example, for evaluating motor neuron disease drug candidates, three cell types might be particularly relevant: motor neurons (for efficacy), cardiac myocytes, and hepatocytes (for safety).

To evaluate any new strategy using iPSCs, screens with already known drugs and careful observations of the effects are necessary. In the future, iPSCs might be used as an important add-on to more traditional approaches. They could play a role at the very start of drug discovery efforts and/or they could be valuable in stratifying compounds for clinical testing. They could also play a vital role in choosing patients most likely to benefit from particular treatments (see Figure 13.4 for three putative applications of iPSCs).

Current findings with iPSCs are exciting, and we want to point out that handling large numbers of iPSCs is possible. However, it will be difficult to capture entirely environmental factors in this system. In this case, the use of surrogate tissues to identify more homogeneous patient populations is useful. For example, olfactory cells from the neural epithelium, obtained through quick and safe biopsies, maintain a reasonable extent of neural molecular profiles.

#### *New Horizons for Disease Modeling: Mouse-Human Brain Chimeras*

As discussed, currently used (typically mouse) models of human disease traits offer benefits as well as important shortcomings. Some scientists might provocatively state that mouse models have essentially no predictive value to test drug efficacy in humans. While everyone recognizes that any model is naturally imperfect, certain aspects of disease have been well represented by these models and, indeed, yielded predictive power, such as the specific effects of cholinergic treatments (see Appendix 13.6). In principle, the utilization of human cells is obviously preferable. Thus a logical step is the utilization of human iPSCs or induced neuronal cell systems. However, phenotypic analysis is limited *in vitro*. Obviously, for circuit function and behavioral studies, intact brains and organisms are required. Moreover, there are important cell biological limitations with these cells, most notably cell type specification and functional maturation. Current *in vitro* technology can, at best, supplement mouse

models but it cannot replace them (Figure 13.4). Thus, there is an urgent need to develop *in vivo* models utilizing human cells.

A general solution would be to generate mouse-human brain chimeras consisting of mouse and patient-derived brain cells. In that setting, essentially all shortcomings discussed would be addressed: (a) patient-derived brain cells could be studied *in vivo*; (b) the brain represents the ideal environment for neuronal maturation replacing long-term cultures; (c) regular participation in embryonic development would ensure the generation of authentic neuronal and glial subtypes with appropriate projections into other brain areas.

This could be achieved in several ways. First, one could take advantage of the pluripotent nature of iPSCs. Mouse iPSCs introduced into a blastocyst will participate in normal embryonic development, yielding chimeric mice made up of host blastocyst-derived and iPSC-derived cells. By analogy, human pluripotent cells could similarly participate in embryonic development, resulting in organs that contain both mouse and human cells. Preliminary attempts at this approach appear promising (Gafni et al. 2013). Second, human neuroectodermal cells derived from human iPS cells could be introduced into the early developing mouse brain. Third, transplantation of glial-restricted precursor cells into the neonatal mouse brain would generate chimeric mouse brains containing mostly human glia and mouse neurons (Han et al. 2013b). Naturally, such experiments would have to be executed under close ethical supervision.

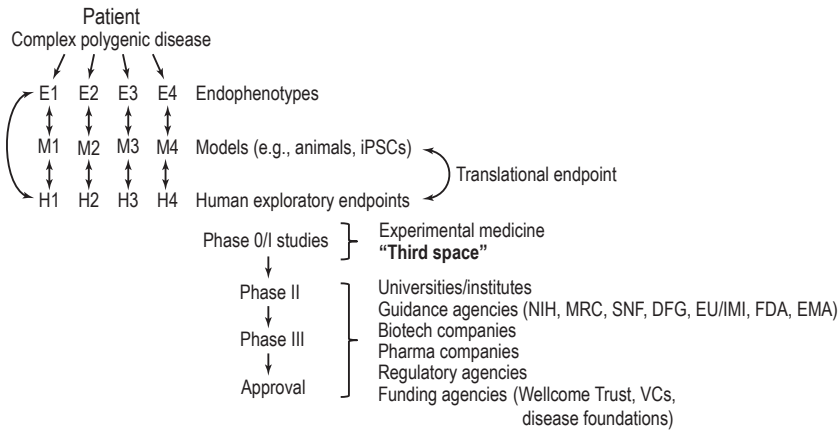
### Summary of Model Systems

Any model is successful if it does not pretend to be a model of the “disease” but rather of a given pathogenic factor. Models need to reflect, as faithfully as possible, the human neural substrate that best characterizes the symptoms of disease caused by the pathogenic factor being scrutinized.

### Increasing Translational Success

Over the past decade, rapid progress in basic neuroscience has brought many enthusiastic claims of advances in effective drugs and other therapies to treat neurologic and psychiatric diseases. Commensurate with this has been a large number of significant failures in Phase III studies. As a result, the neuroscience field has obtained a reputation for being unpredictable, ultra-risky, and extremely expensive. There is an impression of a prevailing culture of easy promises from basic scientists; careless moves from Phase II to Phase III, in spite of marginal Phase II data; and a lack of adequate “probability of success” predictions. For these reasons, many biopharmaceutical companies have shifted efforts away from neuroscience toward oncology and immunology.

The translation of data from models to humans, and the development of therapeutics to approval, is a complex and regulated process—one that involves many diverse organizations and players. Figure 13.5 attempts to summarize



**Figure 13.5** Translational sciences and therapeutics development: process and participating organizations. Treatment approaches that emerge from bottom-up and top-down approaches are typically validated at the preclinical level in various animal and *in vitro* models. The models (M) reflect specific endophenotypes (E) of a complex polygenic disease. Exploratory clinical endpoints (H) corresponding to the outcome measures greatly facilitate early clinical development and detection of efficacy in humans. Optimal translational endpoints are identical in humans and models and are measured with the same or closely related technologies. The general area of “experimental medicine” exceeds traditional departmental structures, which compromises translational efforts. Clinical development (in particular, Phase II and III studies) involves multiple organizational structures from academia, government, industry, charities, and financing. These efforts are often poorly aligned and integrated. NIH: National Institute of Health (U.S.); MRC: Medical Research Council (U.K.); SNF: Schweizer Nationalfonds (Switzerland); DFG: Deutsche Forschungsgemeinschaft (Germany); IMI: Innovative Medicines Initiative (EU); FDA: Food and Drug Administration (U.S.); EMA: European Medicines Agency; VC: venture capital.

the process as well as the involved organizational entities for the subsequent discussion.

### A Dedicated Home for Translational Sciences: The “Third Space” Concept

Academic scientists have become increasingly engaged in translational biology and medicine. Once promising neural or molecular targets are discovered, many academic laboratories strive to reach a point where they have (a) chemical entities or interventions that reliably engage the target; (b) useful assays to document, characterize, and measure the engagement; and (c) enough target validation data to be able to transfer the technology to a biotechnology or

pharmaceutical company. Indeed, both government funders and patient groups encourage academic scientists to pursue these goals and even provide some of the necessary resources for different stages of the process (e.g., the National Institutes of Health, the National Center for Advancing Translational Science, the Michael J. Fox Foundation, and The Wellcome Trust).

Despite this support, however, most academic centers lack the necessary infrastructure (e.g., well-curated, accessible chemical libraries or engineering laboratories for prototyping) to realize effective early stage discovery and develop novel therapeutic concepts. In addition, most academic biologists and neuroscientists do not know how to conceptualize the various steps in the process, how to set up and conduct screens at the appropriate scale, or how to judge whether the process (e.g., chemistry or device development) is proceeding well. Trained personnel (e.g., medicinal chemists, engineers, project managers) are often lacking, as is a broad base of necessary expertise inherent in translational work. Importantly, the expertise needed for “experimental medicine” is not contained within traditional departmental structures of academia; thus, as attempts are made to conduct translational efforts, problems arise all too easily. An effective infrastructure needs to be guided by individuals with industry experience—people who are able to work closely with the academic experts championing the project.

Efforts to create such an infrastructure and develop the necessary expertise within academia are underway. One example is the “Reactor” program in Harvard Catalyst.<sup>2</sup> Launched in 2013, Reactor aims (a) to develop innovative technologies and methodologies and assist their clinical implementation, and (b) to bridge the chasm between discovery of basic biomedical observations and their clinical applications through the provision of resources, mentoring, and expertise. Reactor convenes cross-disciplinary, cross-institutional teams and communities, providing support to expedite clinical and translational research. It facilitates skill development, team building, mentoring, funding, and identifies external resources to assist investigators and teams in the translation of clinical discoveries. Reactor’s ultimate goal is to leverage these discoveries to impact patient care through the development, testing, and adoption of novel prevention strategies, diagnostics, therapeutics, and biomarkers. Other similar organizational experiments are underway. In all of these efforts, the ability to address and respond to all challenges and obstacles, both those that are known and those which will emerge, will be of paramount importance.

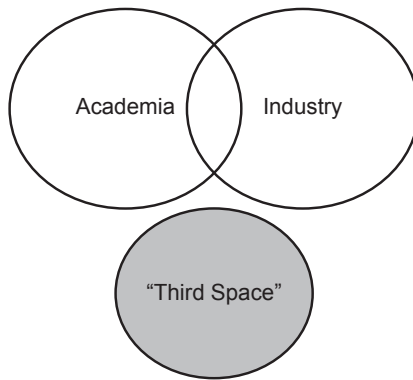
There are serious and hard obstacles to overcome if a therapeutics facility is to be created within academia. Extensive intellectual and technical platforms are needed and diverse expertise (ranging from intellectual leadership to business development officers, to chemists, engineers, etc.) must be secured. Moreover, there is an inherent conflict between intellectual property and business considerations: results may not be publishable within expected

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<sup>2</sup> <http://catalyst.harvard.edu/programs/reactor/> (accessed March 3, 2015)

academic time horizons. Here, arrangements reached by universities engaged in classified research (e.g., MIT's Lincoln Laboratories,<sup>3</sup> Draper Laboratory,<sup>4</sup> and the Applied Physics Laboratory at Johns Hopkins University<sup>5</sup>) can offer guidance.

Instead of anchoring a therapeutics facility within academia, controlled by a single university and a single company, we propose that a separate, dedicated home be created to optimize translational science. External to the structures inherent in academia and industry, this "third space" (Figure 13.6) would provide a fertile environment for experts from both fields. Staffed by scientists (and not graduate students or post docs interested in academic careers), this "third space" would be dedicated to early phase human experimental trials and the identification of human endophenotypes for translation. One could envisage the development of a "third space" by a regional consortium of universities and hospitals, which would share in its governance and funding as well as benefit from its pooled expertise. Critical issues to establish at the onset include intellectual property rules, review mechanisms to judge effectiveness,



"Third Space" for experimental medicine

- Staff scientists (no graduate students)
- Early phase human experimental trials
- Identification of human endophenotypes for translation
- Philanthropy to launch
- Financial sustainability will be a challenge

**Figure 13.6** The "third space" is a separate, dedicated area external to academia and industry that brings experts from both fields together on neutral ground. Staffed by scientists (not graduate students or post docs interested in an academic career), early phase human experimental trials would be conducted and human endophenotypes identified for translation. Charitable organizations could help in its launch, but long-term financial sustainability would require careful planning.

<sup>3</sup> <https://www.ll.mit.edu/> (accessed March 3, 2015)

<sup>4</sup> <http://www.draper.com/> (accessed March 3, 2015)

<sup>5</sup> <http://www.jhuapl.edu/> (accessed March 3, 2015)

and realistic business plans. Charitable organizations could help in its launch, and partial support could be provided by agencies such as the National Center for Advancing Translational Science. Careful thought and planning would be necessary, however, to ensure long-term financial sustainability.

### **Late-Stage Clinical Studies toward Approval and Patient Benefit**

Beyond translational medicine, bridging models and Phase 0/Phase I clinical studies, advancement of a drug candidate into Phase II, and Phase III clinical studies necessitates the involvement of biotechnology and pharmaceutical companies, regulatory authorities—the U.S. Food and Drug Administration (FDA), the European Medicines Agency (EMA)—as well as funding agencies and investor groups. There is no generally accepted format for these interactions. There are positive examples of precompetitive efforts between academic groups and pharmaceutical companies, the Alzheimer Disease Neuroimaging Initiative being the most visible one. Obviously, negative examples of such interactions exist as well, often caused by an inappropriate level of bureaucracy, leading to ineffective collaborations. Small biotech companies and associated venture capital groups play an important role in drug development. Typically, they operate outside of the established frameworks of university–pharmaceutical company interactions, interacting in a more informal networking way with universities, pharmaceutical companies, and regulatory authorities. In recent years, charitable organizations and advocacy groups for specific diseases have become important contributors as providers of information, contacts, and funding. The development of effective therapeutics would benefit from closer integration of and communication among all involved parties. To this end, we offer specific recommendations, in the following section, to improve the success in translational biology and medicine, and to assist in subsequent development toward approval and actual benefits to the patients.

### **Specific Recommendations**

#### *Collaborations between Scientific and Clinical Researchers from Different Fields*

The gap between scientific and clinical researchers from different fields is growing due to external pressure. Fragmentation of institutions and of communities (e.g., epilepsy, stroke) limit communication; thus failures are repeated across individual fields. Here, we need to draw lessons from cancer research where, although a breakdown is made into cancer types, cancer is perceived as one big research topic because all cancer types share one important property: excessive proliferation. In neuroscience, diseases are discussed separately, and, as a result, the public perceives research fields into these diseases as unrelated and individually small. In oncology, the situation is simpler, due to a

single driving factor, increased proliferation, even if there are specific drivers for individual types (e.g., in bone cancer). In neurology, by contrast, the single readout is different: we lack an equivalent to “proliferation” as the one uniting factor. In psychiatry, a major obstacle is the lack of subjective biomarkers corresponding to disease and symptom. Thus, major efforts on biomarker identification in psychiatric disorders are expected. Since manifestations of patients with psychiatric disorders change, careful distinction of state and trait markers is required in biomarker exploration studies. Although still preliminary, change in C reactive protein in manic symptoms may provide a good example of a possible state marker (Dickerson et al. 2007).

To overcome this issue, the National Institutes of Health have initiated a network that is not tied to a specific disease, to enable better learning between diseases (only Phase II trials are included to ensure better communication and make studies comparable). In Germany, at the *Forschungszentrum für neurodegenerative Erkrankungen* (German Center for Neurodegenerative Diseases), clinicians and scientists jointly conduct translational research. More national research hospitals need to be established so that science does not compete with patient care.

### *Collaborations between Academia and Industry*

It would be beneficial to have institutions which support academia–industry collaborations that are capable of addressing all neural diseases instead of many specialized ones. Within such institutes, academia and industry would work together, and it would be possible to carry out Phase 0 trials (as was done in cancer research). Early human efficacy studies need to be done in genetically identifiable populations. Tailor-made fits between academic and industry scientists might be best suited for such successful collaborations. Interactions between academia and industry are often hampered by business development and intellectual property concerns, as well as a lack of understanding by universities of how to negotiate with industry. One possible solution would be to create an entity aimed at assisting effective communication. To this end, a translational group has been established at the National Institute of Health that combines management and consulting experts. Another solution might be to establish a mobile translational unit, staffed with personnel from industry, chemistry consultancy, and lawyers. When scientists find a good target, they could obtain advice from the mobile unit on how best to move forward. The European Innovative Medicines Initiative aims to improve the development of innovative treatments. Launched under the European Commission’s Sixth Framework Program for Research, it assembles relevant stakeholders such as academia, biotech companies, regulators, patient organizations, etc., and is led by the European Federation of Pharmaceutical Industries and Associations. Specific objectives include overcoming the many, significant hurdles inherent in drug development (e.g., the difficulty in predicting safety and efficacy of



potential drug candidates). Overarching strategic objectives are approached by formulating specific calls for proposals to form public-private partnership consortia in research in Europe; EU funding is provided to academic partners and is matched by equal in-kind contributions from participating industrial partners. As such, the Innovative Medicines Initiative provides a suitable platform that facilitates and promotes interactions and collaborations between academia and industry on joint work concerning pre-competitive aspects relevant to drug discovery utilizing mutual capabilities of partners. Although there are still obstacles to overcome, such as reducing bureaucracy, and the fact that the performance of individual consortia highly depends on the partners' willingness to subordinate their specific interests to joint objectives, the initiative may eventually prove useful in introducing a more cooperative spirit between academia and industry.

#### *Collaborations between Multiple Industry Partners*

A good working example for pre-competitive collaboration between pharmaceutical companies is the Dominantly Inherited Alzheimer Network, which includes ten participating industry partners. In such a consortium, it is very important for all partners to focus on one goal and build milestones based on objective measures. Successful pre-competitive collaborations are conceived to focus on target finding and validation, biomarker and target-engagement measurements, rather than drug development.

Industrial collaborations typically involve profitable, large pharmaceutical companies and exclude small biotech companies and related experts. Biotech companies and associated venture capital investors constitute an innovative, highly talented, and informal group. They operate largely through social networks and, in the United States, are particularly strong in the Boston and San Francisco Bay areas. Venture capital groups are often driven by experienced physician-scientists, a group of people that could contribute more significantly in the overall search for effective treatments.

Biotech companies have been highly effective in taking discoveries at universities to the application level, often in otherwise ignored diseases. Typically they bring a new concept to the level of Phase I and Phase II studies. If successful, the companies are often absorbed by large pharmaceutical companies which then take the treatment to approval. In neuroscience, examples include AC-Immune (antibodies for AD), Rinat Neuroscience (anti-NGF antibodies for pain), Avid Radiopharmaceuticals (PET imaging agent for plaques in AD), and EnVivo Pharmaceuticals (nAChR agonist to treat cognitive deficits in neurodegenerative and psychiatric conditions). It seems worthwhile to attempt to integrate biotech and venture capital groups more effectively into the discussions and interactions between universities, governmental agencies, and large pharmaceutical companies. Small biotech companies typically do not have the funds to buy into pre-competitive pharmaceutical efforts. A separation

between profitable and non-profitable companies may be an approach to solve this problem.

In this context, several foundations should be mentioned: the SMA Foundation, the CHDI Foundation, the Michael J. Fox Foundation, the Simons Foundation, and the Accelerate Brain Cancer Foundation (ABC<sup>2</sup>). These foundations have become effective contributors, supporters, and funding sources for specific disease areas. For example, the SMA Foundation, together with academics, biotech and pharmaceutical companies, has made a major contribution to the promotion of credible therapies to treat motor neuron degeneration; ABC<sup>2</sup> multiplied the number of clinical trials for brain tumor and, similarly, other foundations have boosted discoveries and therapeutic approaches for a variety of brain diseases.

Over the next decade, we envision an increasing number of collaborations between pharmaceutical companies, biotech companies, academia, investors, foundations, as well as private sources to promote high-quality science-based therapies.

### *Translational Approaches in Psychiatry Usually Fail: What Can Be Done?*

Several reasons have contributed to the frequent failures observed in translational approaches taken in psychiatry. It is very difficult to motivate psychiatrists to take part in a translational research track, in contrast to the situation in neurology. Most of the psychiatry resident programs in the United States do not include training in scientific investigation, and offer only limited exposure to basic study design (in particular, hypothesis-driven research design), statistical analysis, and interpretation of results. The current environment of reduced funding for scientific pursuits as well as the increased demand for revenues generated from clinical responsibilities in psychiatry, places time and financial constraints on young clinicians, often discouraging a long-term commitment to research. Meanwhile, basic science researchers who wish to study psychiatric illness are limited in opportunities to learn about the complex and heterogeneous manifestations and courses of psychiatric diseases. While basic scientists approach neurological diseases in a correct manner by utilizing molecular pathological hallmarks in the pathology, there are no objective markers available in psychiatric disorders as yet. It is difficult to gain insight into psychiatric disorders without direct patient exposure; knowledge from textbooks and journals can mislead basic scientists as they build working hypotheses to study psychiatric disorders.

The “dual gap” in training of early clinicians and young basic science researchers has hampered progress in translational psychiatry. In response, Johns Hopkins Medicine recently initiated a workshop entitled “Mind the Gap,” creating a forum in which young researchers and resident physicians discuss research topics directly raised from clinical questions. This workshop proved beneficial for all groups. Shared research interests voiced by basic scientists

and clinician-scientists benefit from the guidance and resources provided by senior faculty members (mentors) (Posporelis et al. 2014).

More emphasis should be placed on training and mentoring psychiatrists so as to provide clinical scientists a broader and deeper knowledge in neuropsychiatry. This could help bridge the somewhat arbitrary separation between neurology and psychiatry. Neurological disorders frequently display psychiatric manifestations, such as depression and psychosis. Studying these manifestations is crucial to patient care management as well as to a better understanding of disease mechanisms (Robinson et al. 1984; Cooper and Ovsiew 2013; Schwarz et al. 2012). There are some promising attempts to fill this need, but more initiatives are needed. For example, the Sidney R. Baer Jr. Foundation Fellowship in Clinical Neurosciences is a joint effort between the neurology and psychiatry departments at Beth Israel Deaconess Medical Center and Harvard Medical School. Designed as a three-year fellowship, it includes both clinical and research mentoring and training. Fellows are matched with two clinical mentors (one from the psychiatry faculty, one from the neurology faculty at Harvard Medical School) as well as two research mentors (one an active clinical/translational scientist, one a basic researcher). Seed funding and appropriate mentoring and support is provided so that a research project can be developed during the fellowship. This provides solid clinical foundation in clinical neurosciences as well as training across the research-clinical divide.

### *Increasing Interactions with Regulatory Agencies*

Regulatory agencies are often seen as impediments and excessively conservative. Positive examples, however, prove that this is generally not true. Very often, the cautious and critical reactions on the part of the regulatory agencies reflect the necessary focus on safety issues, but lack familiarity with the science behind the new drug development programs. It is thus beneficial to include regulatory agencies as early as possible into the discussion. In the United States, pre-investigational new drug meetings provide a format for such early discussions, and the European Medicines Agency has comparable mechanisms to obtain early feedback. Invitations to conferences on translational medicine extended to staff from regulatory agencies will help provide early familiarity with emerging new approaches.

The recent discussions on regulatory paths to approval for drugs in AD and PD illustrate this interactive approach. In particular, the development of PET-imaging agents for the amyloid pathology in AD provides the most prominent example for successful implementation of the proposed approach. Direct discussions between academic groups, biopharmaceutical companies, and the Food and Drug Administration led to a novel and generally accepted path for the development of PET radiopharmaceuticals to visualize pathological deposits in neurodegenerative disease. The approach was then summarized in a highly visible paper published by FDA officials (Yang et al. 2012a).

*Improving the Transition between Phase II and Phase III*

While previously discussed approaches and employment of appropriate models target primarily an improved success rate in the transition from Phase I to Phase II, drug development for nervous system disorders still confronts a high attrition rate in Phase III. Over recent years, success rates have declined rather than improved (Arrowsmith 2011). Sufficiently large patient populations are required to exact the regulatory endpoints implicit in Phase III trials, thus necessitating a major financial investment from drug companies. However, the high failure rate experienced late in the value chain imposes a major commercial risk for most corporations. Rigid application of translational models and experimental medicine approaches, using appropriate surrogate markers, may enable a front-loaded testing of more therapeutic concepts, thus leading to faster failing of candidates and a more rigid review of Phase II data, followed by a more diligent selection of principles being promoted to Phase III.

One important improvement for the transition between Phase II and Phase III trials would be to create well-characterized patient groups. This general, important challenge impacts both translational research and clinical neuroscience. Although the creation of patient data bases with specific quantitative measures is time-consuming, expensive, and not directly supporting academic advancement, it is imperative if we are to enable effective patient-based research. A “third space” concept, similar to the notions developed above, is to establish and maintain patient repositories. Such efforts could be part of experimental clinical neurosciences initiatives and might be jointly funded by governmental agencies, industry consortia, and foundations. In addition, academic health centers and many national health care systems are currently investing substantial funds to establish electronic medical records; these could be further developed to allow semantic query functions and enable patient selection and even randomization in research. Agreement on basic assessment tools and the establishment of suitable test batteries, careful validation, and quality control will facilitate success in late-stage clinical trials.

**Summary of Recommendations to Increase Translational and Development Success**

Due to significant failures in late-stage drug development and unfulfilled promises, drug discovery and development for neurologic and psychiatric diseases is considered extremely risky, and many biopharmaceutical companies have shifted their efforts away from these disorders. Recommendations to ameliorate the situation include various organizational and behavioral measures. In particular, we propose the creation of “third space” organizational structures to unite academic and industrial groups involved in experimental biology and medical research. Furthermore, to increase the success rate in clinical development and provide actual benefit for patients, we propose that proactive interactions be

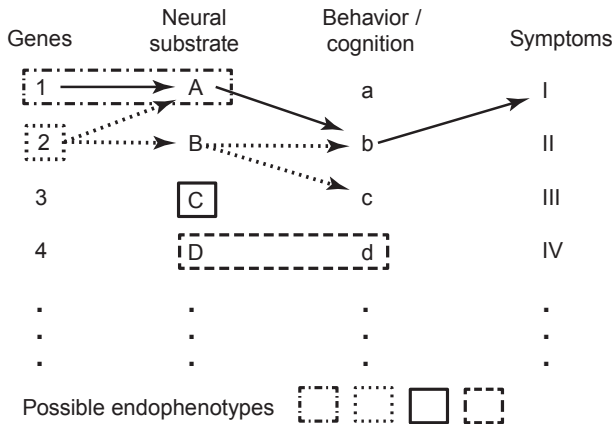
supported between all organizational entities involved in drug development and therapeutic discovery (e.g., academia, guidance agencies, biotech, device and pharma companies, regulatory agencies, and funding agencies).

### Appendix 13.1

**Biomarker:** a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention (definition created by the National Institutes of Health Biomarkers Definitions Working Group). Biomarkers that fulfill specific criteria can be endophenotypes.

**Bottom-up research:** research approach that starts from genetic or environmental factors.

**Endophenotype:** term used to parse symptoms into more stable phenotypes with a clear genetic connection. In psychiatric genetics, an endophenotype is used to bridge the gap between high-level symptom presentation and low-level genetic variability (“intermediate phenotype”). Endophenotypes are a subclass of biomarkers. For a biomarker to qualify as an endophenotype, the following criteria must be fulfilled: (a) the endophenotype must be associated with illness in the population; (b) it precedes clinical symptoms; (c) it is primarily state-independent (i.e., it manifests in an individual whether or not illness is active); (d) within families, endophenotype and illness co-segregate, and endophenotypes can be manifested on various levels (see Figure 13.7). In summary, endophenotypes can provide better links to genetics, purer samples of patients for clinical trials, and early markers of vulnerability to disease or disorder.



**Figure 13.7** Visualization of the different versions of endophenotypes. An endophenotype can either be an altered gene, an altered neural substrate, an altered behavior, or combinations of the different options. Importantly, an endophenotype precedes clinical symptoms.

**Phenotype:** composite of an organism's observable characteristics or traits, such as its morphology, development, biochemical or physiological properties, and behavior. A phenotype results from the expression of an organism's genes as well as the influence of environmental factors and the interactions between the two.

**Phase 0 trials:** Exploratory "first-in-human" studies to test activity in a small number of subjects.

**Phase I trials:** Establish safety, dose range, and adverse effects in small groups of subjects.

**Phase II trials:** Establish efficacy of the drug, usually against placebo with larger groups of subjects.

**Phase III trials:** Confirmation of safety and efficacy in the to-be-treated patient population with large groups of subjects.

**Prodromal stage:** early stage or symptoms of disease before characteristic symptoms appear.

**State marker:** a state marker reflects the status of clinical manifestations in patients.

**Top-down research:** research approach that starts from syndrome or behavioral phenotype.

**Trait marker:** A trait marker represents the properties of the behavioral and biological processes that play an antecedent, possibly causal, role in the pathophysiology of the psychiatric disorder.

## Appendix 13.2 Top-Down and Bottom-Up Approaches to Parkinson Disease

Current understanding of PD, the existing therapy, and the ongoing efforts for improved therapy reflect fruitful bridging between top-down and bottom-up approaches. Neuropathological and neurochemical investigations of *post mortem* brain tissue revealed significant degeneration of the ascending dopaminergic system originating in the midbrain. These discoveries, made approximately sixty years ago, led directly to DA replacement therapy and the approval of the first drug treatment, L-DOPA, the precursor of DA in combination with an inhibitor of metabolism outside of the brain. This combination drug and various direct agonists on DA receptors are the mainstay of current Parkinson therapy (Carlsson 2002).

Lewy bodies, pathological inclusions in the brains of PD patients, were first described one hundred years ago, long before the recognition of the dopaminergic deficit. Little progress in understanding was made until about fifteen years ago, when a mutation causing familial PD was found to be located in the gene coding for  $\alpha$ -synuclein, a protein aggregated in the Lewy bodies (Polymeropoulos 2000). The discovery of several other disease genes followed, which allowed investigators to create hypothetical pathological

pathways integrating the various genetic discoveries. Furthermore, several strains of transgenic animals carrying various single or combined disease genes have been created. Initially, even though some of the mice exhibited Lewy body-like deposits in the brain, none of them showed a dopaminergic neuron deficit, which would be expected in an advanced animal model of PD (Bezard and Przedborski 2011). More recently, however, strains of transgenic mice with dopaminergic neuron deficits have emerged, thus providing a bridge to the original neuropathological discoveries.

The bridge between bottom-up and top-down approaches has brought integrated hypotheses of disease mechanisms, involving several identified disease genes and advanced transgenic animal models, which exhibit dopaminergic neuron degeneration as well as Lewy body pathology. This progress supports the ongoing enthusiasm in the field; in particular, that optimal drug targets can be identified and that potential drugs and treatments can be tested in animals with high predictive power.

### **Appendix 13.3 Historic Perspective on Top-Down Models for Aspects of Schizophrenia**

In schizophrenia, the disorder phenotype is even more complex, with positive symptoms (e.g., hallucination, delusions), negative symptoms (e.g., apathy, social withdrawal), and cognitive deficits (e.g., in working memory). Models were driven by the need to understand the initial, serendipitous discovery of the antipsychotic effects of chlorpromazine and the subsequent motivation to introduce more efficacious compounds with fewer side effects. These led to the hypothesis that a DA overactivity state in schizophrenia is, at least partly, responsible for the positive symptoms (e.g., hallucinations and delusions) of the disorder, and the isolation of a specific DA receptor (D2) responsible for the antipsychotic effects. This was consistent with the phenomenon of amphetamine paranoid psychosis often found after bingeing on high doses of stimulant drugs, such as amphetamines. Initially, new drugs, such as the butyrophenones, were synthesized and screened against amphetamine-induced hyperactivity or stereotyped behavior in rats treated with amphetamines, which increases synaptic levels of DA in such brain regions as the nucleus accumbens and the dorsal striatum (i.e., caudate putamen). Amphetamine locomotor hyperactivity has been shown to be associated with the nucleus accumbens, whereas amphetamine-induced stereotyped behavior is linked with DA-dependent functions of the caudate putamen, suggesting abnormalities of DA regulation in those areas, broadly shown later to be correct (e.g., Howes et al. 2009). The predictive power of these pharmacological assays was excellent, although they were subsequently superseded by actual measures relating clinical potency to measures of D2 DA receptor binding. However, although the D2 antipsychotics were actually one of the major achievements in this area over the last fifty years, they

clearly fail to treat both of the other two major facets of schizophrenia effectively—the negative symptoms (encompassing apathy and social withdrawal) and cognitive deficits—which in group terms are quite broad. However, this masks underlying heterogeneity in terms of a predominance of memory impairments, often associated with the hippocampal malfunction or “executive” impairments associated with prefrontal cortex dysfunction. In fact, one prominent monkey study by Castner et al. (2004) showed that chronic treatment of rhesus monkeys with the D2 antipsychotic haloperidol produced an apparent downregulation of the D1 receptor and a significant impairment on a spatial working memory task that was remediated by treatment with a D1 DA receptor agonist. Clozapine (an atypical neuroleptic with many additional modes of action besides the D2 receptor antagonism that is presumably responsible for its antipsychotic effect) emerged after an intense program of drug development, which included animal model studies to differentiate its mode of action from that of the typical antipsychotic compounds. However, its action on negative symptoms and cognitive deficits is generally regarded as weak.

Investigators thus began to query the genesis of the DA overactivity state, which through PET studies has recently been shown definitively to occur in the caudate nucleus, even in the unmedicated and prodromal state prior to first episode schizophrenia (Howes et al. 2009). An early view (Weinberger 1987; Murray and Lewis 1987) was the neurodevelopmental hypothesis, which suggested an early cortical (including hippocampus) deficit that led to (a) dysregulation of midbrain DA cells and (b) ancillary effects (e.g., impaired cognitive function). This early developmental cortical dysregulation hypothetically included changes in glutamate receptors (e.g., NMDA), leading to a glutamatergic hypothesis of schizophrenia fueled by evidence that certain NMDA antagonists, such as ketamine and phencyclidine, in humans produced a characteristic psychosis not dissimilar to that observed in schizophrenia. This led to an entirely different strategy of administering drugs affecting glutamatergic mechanisms, including the mGlu2/3 receptors (the Lilly mGluR2/3 agonist pomaglutam; Patil et al. 2007), which has not held up on subsequent clinical trials.

This approach has also been used to generate appropriate animal models based on acute or chronic treatment with glutamatergic antagonists, such as the NMDA receptor antagonist phencyclidine or (less successfully) ketamine (Moghaddam 2013). Whether the glutamate hypothesis eventually bears fruit is unknown; however, the mGluR2/3 agonist might work better early in the course of schizophrenia, when MRS measures of glutamate indicate in many patients a hyperglutamatergic state in the cortex, which presumably would be diminished through treatment with an mGluR2/3 agonist. Even if the drug does not work in chronic schizophrenia, it may protect first-episode patients from progressing to a chronic state by preventing or reducing initial episodes of schizophrenia.



### Appendix 13.4 A Brief Survey of Contemporary Top-Down Models in Schizophrenia

Given the evidence that schizophrenia is a neurodevelopmental disorder (although there is also evidence for a progressive decline in many patients after initial psychotic episodes), the animal model approach has been to manipulate brain development in such a way that it mimics some of the main features of schizophrenia. Various means have been used to achieve this, inspired by early studies on schizophrenia which show enlarged lateral ventricles, indicating possible loss of brain tissue. The latter has been confirmed through structural scanning to include loss of brain volume, especially in the temporal lobe, and changes in the hippocampus. The other main anatomical changes in schizophrenia include a loss of cortical parvalbumin-containing interneurons or Chandelier cells as well as cortical thinning and GAD67 protein changes in the prefrontal cortex (Moore et al. 2006).

Many of these changes as well as dysregulation of midbrain DA neuron activity can be simulated by affecting the hippocampus during development. Neonatal lesioning of the hippocampus (Lipska and Weinberger 2000) produces many of these changes as well as interesting behavioral effects of possible relevance, including impairments in cognitive function (e.g., spatial working memory) and social interaction. Indeed, it is possible that this early, self-imposed social isolation itself produces some of the neural changes, given the role of social play in neural plasticity and brain development, as would the effects of isolation rearing, which by itself can lead to several relevant impairments including upregulation of midbrain DA system function (Hall et al. 1998).

Another top-down model is the MAM-E17 model (Moore et al. 2006). MAM is a mitotic neurotoxin which essentially retards neural development. When administered to pregnant dams at the E17 stage, its effects are limited to forebrain structures such as the hippocampus (which is especially affected) and neocortex. The brains are smaller, as is the hippocampus itself, presumably as a consequence of cell death and rearrangement during maldevelopment. There are also changes in the prefrontal cortex and an upregulation of the mesolimbic DA system projecting to the nucleus accumbens. Animals have some cognitive deficits (e.g., associated with hippocampus and prefrontal cortex). Reversal learning in MAM-treated rats (also known to be impaired in schizophrenia) is rescued by an mGluR5 PAM, consistent with the loss of mGluR5 receptors in the prefrontal cortex (Gastambide et al. 2012).

The earlier model based on neonatal hippocampal lesions reproduces behavior that often looks similar to that of the MAM17 model (Lipska and Weinberger 2000). An interesting aspect of the neonatal lesion model, possibly analogous to early schizophrenia, is that its initial symptoms are social (self-isolation); these may exacerbate development of additional symptoms by depriving the animal of social play and hence possible hippocampal-cortical connectivity.

Environmental factors and their modeling: Early on, it was recognized that the etiopathogenesis of schizophrenia involves environmental factors. While genome-wide association studies have revealed many genetic risk variants (22 loci with up to 8,300 independent single nucleotide polymorphisms, copy number variants; Ripke et al. 2013; Kirov et al. 2014) with low effect, twin studies have highlighted a missing heritability (Sullivan et al. 2003). Further, detrimental environmental conditions such as physical and psychosocial abuse, parental loss during early childhood, chronic social stress and social defeat, history of maternal infection during pregnancy, stress due to birth complications, and urbanicity or cannabis use in adolescence are recognized as critical risk factors for the disease.

Based on these observations, environmental models have been developed in experimental animals. Models of maternal inflammation induced by polyI:C injection during gestation in rat or mice have reproduced some of the negative and cognitive symptoms (Kneeland and Fatemi 2013). Isolation rearing of rat pups after weaning impairs working memory and sensorimotor gating in the prepulse inhibition (PPI) of the acoustic startle response, as observed in schizophrenic patients, in adulthood (Weiss et al. 2001). Consistent with the DA hypothesis of schizophrenia, isolation rearing also alters burst firing of DA neurons in the ventral tegmental area, DA neurotransmission in PFC and nucleus accumbens, and increases DA release and receptor sensitivity in the accumbens and striatum (Hall et al. 1998). Further, several models have combined manipulations in double-hit paradigms. In rat, neonatal MK801 or phencyclidine injection and subsequent rearing in social isolation from weaning on impair PPI response (Lim et al. 2012; Gaskin et al. 2014). In mice, the combination of prenatal inflammation by poly I:C and postnatal chronic mild stress recapitulates PPI deficits and increases behavioral hypersensitivity to amphetamine (Giovanoli et al. 2013).

Comparison of genetic models with pharmacological and environmental models is useful even if each of these models has limitations. Genetic models are partial, an MAM model may be superior for modeling frontal hippocampal interactions, while the neonatal lesion may be the best model for DA upregulation. Environmental models are complex. Overall, there are no convincing models of cognitive deficits or of how cannabis use in adolescence may lead to symptoms. Other environmental factors such as birth complications and urbanicity are difficult, if at all possible, to model.

### Appendix 13.5 Background Information to Fragile X

In the case of fragile X syndrome, a monogenic cause of autism, CGG repeat expansion leads to methylation and transcriptional silencing of the *FMRI* gene (Verkerk et al. 1991), which encodes the fragile X mental retardation protein. Although this gene is homologous to the mouse *FMRI* gene, transcriptional

silencing does not occur with the introduction of CGG repeat expansion. Thus initial mouse models attempted to recapitulate the human mutation by knocking out the *FMRI* gene (Dutch-Belgian Fragile X Consortium 1994).

Because synaptic plasticity is the foundation of most theories of learning and memory in the brain, a turning point for the field came in 2002, when studies showed that protein synthesis-dependent, mGluR5-mediated, long-term depression in the hippocampus was *exaggerated* in the *FMRI* knockout mice (Huber et al. 2002). Recognition of a number of parallels between phenotypic features of the disease and predicted or known consequences of (over)activation of GpI mGluRs, led to the “the mGluR theory of fragile X” (Bear et al. 2004), which was formally tested by establishing *FMRI* knockout phenotypes relevant to the disorder, and examining these in the context of mGluR5 knockdown (Dölen et al. 2007).

Because this manipulation corrected phenotypes in a number of brain regions at the biochemical, synaptic, circuit, and behavioral levels, these results led to the first speculation that autism might be a “synapsopathy” (Dölen and Bear 2009)—a term coined to distinguish fragile X syndrome from neuropsychiatric disorders such as AD and PD, where the primary pathophysiology appeared to be localized to a particular brain region (Bear et al. 2008). The therapeutic potential of mGluR5 was subsequently further validated by pharmacologic manipulations across development that could address concerns of pathophysiological hysteresis; these have suggested that even late intervention can correct behavioral phenotypes in mice (Chen et al. 2014b; Pop et al. 2014; Michalon et al. 2014; Gantois et al. 2013).

Despite these advances, failure to appreciate the difference between how the mutation is achieved in animal models and human patients have contributed to misalignment of preclinical to clinical studies. Specifically, in a recent Phase III trial, when fragile X patients were treated as a homogenous group, mGluR5-modifying drugs failed to show significant therapeutic effect. However, transcriptional silencing in human patients is not digital; instead, the size of the repeat expansion is inversely correlated to expression of the fragile X mental retardation protein, which is directly correlated to autistic symptoms. Furthermore, when data were stratified by repeat expansion size, patients with the most severe disease presentation showed significant improvement.

### **Appendix 13.6 Historical Perspective for the Cholinergic Hypothesis of Alzheimer Disease**

An interesting historical perspective is provided by the cholinergic story in AD (Everitt and Robbins 1997). Early pathological work established a correlation between intellectual deficit and cholinergic markers in the neocortex of patients with the disease. Preclinical studies sought to investigate the causal role of these deficits using pharmacological approaches in humans (e.g., testing

effects on cognition of the anticholinergic drug scopolamine) and in experimental animals using lesions of varying selectivity of the cholinergic neurons that had been shown to degenerate quite early in AD. Several studies converged to show relatively consistent deficits in attention type functions in rats (and also monkeys) with such lesions, with less obvious effects on memory and many aspects of learning. These deficits were remediated by procholinergic drugs, including acetylcholine esterase inhibitors and nicotine. A parallel clinical trial of an acetylcholine esterase inhibitor, which had overall significant clinical efficacy, only showed improvement in the same attentional paradigm configured for patients—but not in concurrent tests of memory—in parallel with changes on a clinical scale that highlighted enhanced alertness (Sahakian et al. 1993). Therefore, these translational data from the animal model to the patients' data more or less correspond to the current clinical judgment that acetylcholinesterase inhibitors are very far from being a drug to “cure” all of the symptoms of AD, but may have a mild beneficial effect in certain restricted domains of functioning. Two subsequent observations are relevant: (a) the same behavioral effects of acetylcholinesterase inhibitors have recently been shown in the “triple transgenic” mouse model of AD (Romberg et al. 2011) and (b) this drug treatment may well prove to be more efficacious in neurodegenerative diseases such as Lewy body dementia and PD dementia (Emre et al. 2004), where there is evidence of often greater cholinergic deficit than in AD.