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The Revolution Has Begun

Steven E. Hyman and Karoly Nikolich

The results of this Forum give reason to hope that new therapies for nervous system disorders are not only possible but also realistic. This optimism, visible in the vigorous discussions captured in this book, is grounded both in an acute understanding of the fundamental challenges that exist and the synergies that grew out of the process. Much remains to be accomplished if promising ideas, solutions, and paradigms are to be realized in academia and industry. Thus given the importance of innovative ideas and experimentation, we have no desire to reach premature closure on precise approaches to either neurodevelopmental or neurodegenerative disorders. There was, however, broad agreement that genetics and a greater emphasis on human biology offer essential springboards for effective new approaches to pathophysiology and therapies.

After decades of stagnation marked, inter alia, by all too many disappointing failures in costly, late-stage clinical trials, what is the basis for our optimism? Central to this is the remarkable advent of new tools and technologies during the last decade which are truly revolutionizing our ability to study the nervous system in unprecedented ways (Hyman 2012; Pankevich et al. 2014). The resulting sense of promise that we perceive comes with the recognition that a revolution in translational neuroscience has already begun. Our use of the term "revolution" is not accidental: the participants of the Forum agreed that for brain disorders, successful discovery and development of disease-modifying therapies require most certainly a revolution, not merely incremental progress realized through past approaches. We (the attendees and coauthors of this volume) are already participating, to a great degree, in this much needed transformation; this Forum provided valuable space to reflect on what has happened and to discuss, and occasionally argue, about what needs to be done to accelerate progress. Those from industry made clear that they understood the high prevalence of many brain disorders, the vast unmet medical needs, and the enormous global disease burden associated with these diseases. Equally, however, they stressed that unless the new biology discussed at the Forum produced more than incremental advances, it would be difficult to justify significant investments in brain and nervous system disorders when other areas of research (e.g., cancer) were awash in targets, had clear paths to target

validation, and possessed biomarkers to facilitate clinical trials. In addition, we also recognized that lessons from other therapeutic areas must be adopted by translational neuroscience if the vast challenges ahead are to be met.

Why has progress been so slow? If we are truly at the beginning of a successful revolution, and not merely involved in another false start, it is critical to reach an understanding of what has gone wrong in recent decades and to identify important obstacles that remain:

1. Phenotyping and disease classification pose major challenges, given the dearth of biomarkers. Some disease definitions (e.g., those in the DSM classification of mental disorders) should have been considered heuristics or "early drafts," yet were permitted to become reified, thus forming a procrustean bed that damaged both translational and clinical investigation (Hyman 2010).
2. Common forms of neurodevelopmental and neurodegenerative disorders are greatly influenced by genetics, but their genetic architecture has proven far more complex than initially suspected, thus dooming older approaches such as candidate genes and linkage analyses. It is critical for the field, as a whole, to embrace successful unbiased genome-scale approaches to genetics.
3. The human brain is generally inviolable in life. Although indirect approaches (e.g., electroencephalography and diverse noninvasive imaging modalities) have contributed much to studies of disease, they do not readily yield molecular or pathophysiological information. Only recently have new ligands for positron emission tomography, along with measures of cerebrospinal fluid, made it possible to stratify patients with dementia for clinical trials (Jack and Holtzman 2013). Given the agreed centrality of human biology, these observations were seen to argue for attention on two fronts: the ability (a) to engineer diverse human neurons and glia *in vitro* and even to model circuits *in vitro* and (b) to advance studies of patients with, for example, new phenotyping methods inspired by genetic findings.
4. For many pathophysiological studies, brain scientists must rely on postmortem tissue, with all its challenges. This stands in marked contrast to oncology where, for instance, excisional biopsies provide living tissue for both clinical purposes and scientific study. This suggests that postmortem studies of brain should be supplemented with *in vitro* studies of human neurons, while recognizing that these approaches are likely to have complementary limitations.
5. Brain disorders are generally not cell autonomous, adding an additional layer of complexity in terms of the need to study synapses and circuits.
6. Animal models have often proven problematic for both neurodevelopmental and neurodegenerative disorders because of their evolutionary distance from humans. Many cell types and circuits in the human brain

that are involved in neurodevelopmental disorders (e.g., in prefrontal cortex) are evolutionarily quite recent and differ markedly from ubiquitous rodent models. Attention to evolution will be critical in thinking about animal models in the future, as will the recognition that the questions posed for animal models are important but limited.

From intense discussions of the foregoing issues, a conceptual roadmap emerged to hasten the hoped-for revolution in pathophysiology and therapeutics (see Figure 14.1). This roadmap outlines an iterative approach, alluded to throughout this volume, aimed at grounding target identification, target validation, and clinical trials on solid scientific foundations based on known mechanisms of disease. As noted, given the emergence of powerful new tools and technologies over the last decade, a well-validated pathophysiology has become possible.

What are the tools and technologies that make a revolution possible? Participants at the Forum agreed that genetics offers foundational advances for such a revolution (McCarroll and Hyman 2013). For neuropsychiatric disorders such as schizophrenia, autism, and bipolar disorder, which lack a distinctive, analyzable neuropathology, genetic insights may prove to be the only opportunity we have to gain insight into pathophysiology. Even for Alzheimer disease, in which amyloid plaques and neurofibrillary tangles have yielded important biochemical clues, genetics is helping to identify critical processes involved in pathophysiology (Lambert et al. 2013).

The discovery of common genome-wide significant loci associated with disease as well as rare variants that influence pathogenesis would not have been possible without a technological revolution, which was arguably the most important product of the Human Genome Project. This yielded inexpensive microarrays to identify common variation, efficient and inexpensive DNA sequencing, and powerful new computational tools. It is staggering to realize that the cost of DNA sequencing has declined over the last decade by approximately one millionfold. Realization of the potential of these technologies has also led to significant changes in how science is organized: given the need for large sample sizes, extensive and durable global coalitions have been formed to investigate human genetics.

One frequently discussed concern regarding the promise of genetics has been the failure to devise effective therapies for monogenic diseases of the nervous system, such as Huntington disease and familial forms of amyotrophic lateral sclerosis, even decades after the genes were discovered. The multiple alleles of small effect that confer risk for common non-Mendelian disorders, such as schizophrenia, most cases of autism, and nonfamilial neurodegenerative disorders, should pose a far greater challenge. No one at the Forum argued that it would be simple to exploit genetic information arising from polygenic disorders, yet as noted, there was significant optimism for the intermediate and long term. Lessons derived from older attempts to develop therapies for

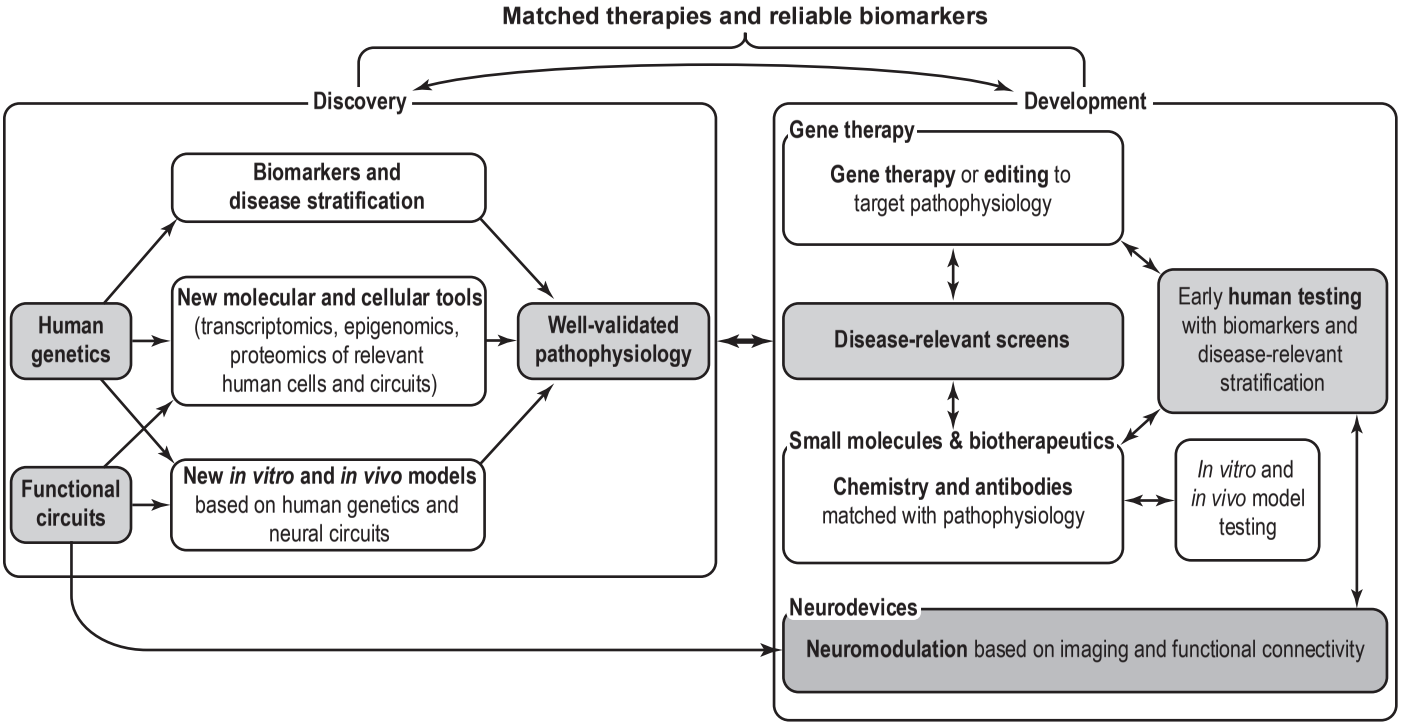

Figure 14.1 Conceptual roadmap outlining new approaches to target identification, target validation, and clinical trials grounded in human genetics and human biology and thus putatively on mechanisms of disease.

monogenic disorders will be valuable for all genetically grounded efforts in nervous system therapeutics. Specifically, there was recognition of the special difficulties posed by toxic gain of function mutations, such as those underlying Huntington disease and some forms of amyotrophic lateral sclerosis, and at present greater likelihood of being able to silence such genes in the nervous system, albeit with significant challenges remaining for delivery of the therapeutic agent. It was also noted that limitations of mouse models are increasingly being appreciated, thus it is likely that animal models will be used to answer questions for which they will be informative rather than misleading. In addition, the advent of stem cell technologies and other approaches to reprogramming cells into neurons should benefit translational neuroscience with respect to both monogenic and polygenic disorders. For example, screens using stem-cell derived motor neurons have been used successfully for a monogenic form of amyotrophic lateral sclerosis (e.g., Yang et al. 2013).

For polygenic brain disorders, it will clearly be challenging to put genetic information to work in the service of pathophysiology and therapies; however, in principle, there is a feasible path. This matter received attention from Forum participants who work on both neurodevelopmental and neurodegenerative disorders, but was a central focus for the former, given the lack of alternative sources of molecular clues. Geneticists at the Forum addressed the widespread fallacy that alleles of small effect could have little biological utility. Whatever its penetrance, any allele shown to have genome-wide significance for an association implicates the gene within which it is a variant in the pathophysiological process—or if the association is to a regulatory sequence, the gene (or genes) to which it is linked. Genes, in turn, implicate pathways or protein complexes in disease processes. Once the directionality of the risk alleles are determined and the relevant genes placed among protein interactors, potential therapeutics can be targeted to increase or decrease, as appropriate, the activity of that pathway or protein complex (McCarroll and Hyman 2013). What matters is not the effect size of the allele that points to the relevant gene, and thus the relevant pathway; what matters is that the association with disease be established with certainty. Far too much effort has been wasted chasing down biological effects that ultimately proved irrelevant because they were inferred from "candidate gene approaches" that were underpowered or otherwise poorly designed.

Beyond the need for continuing the discovery of risk-associated loci, most of which still need fine mapping, and identification of risk-associated variants through DNA sequencing, additional molecular information must still be gleaned if genetics results are to prove fully useful. Unlike metabolism, cardiovascular disease, or cancer in which critical biochemical pathways are well described, risk-associated genes identified in studies of schizophrenia and autism implicate protein complexes that require better definition in relevant cell types, such as the postsynaptic specialization of excitatory neurons. The computational interactomes that do exist and the proteomic results derived from highly

mixed cell populations will have to be supplanted by more refined proteomic experiments, which might benefit from comparing results from postmortem brain with results from *in vitro* preparations in which large numbers of identical cells of a single neural type can be purified.

As risk alleles, risk genes, and risk pathways are identified, they can be studied in dissociated human neurons and glia *in vitro*, in small circuits reconstituted from dispersed cells, and in brain organoids (Lancaster et al. 2013). However, most nervous system disorders involve the dysfunction of specific neural circuits. Important new technologies have started to provide exceptional insights into short- and long-range circuits, pathways of the brain. The human and mouse connectome projects have reached major milestones and have provided first blueprints of the brain's major connections at both small and large scales (Smith et al. 2013). Optogenetics (Boyden et al. 2005) has opened up precise new ways of mapping functional circuitry in neural or other excitable cells. Using light-activated ion channels, it has become possible to activate and inhibit, with unprecedented precision, specific neural circuits in live, free-moving animals involved in thought, learning, memory, emotions, and disease-associated behaviors (Asrican et al. 2013; Steinberg et al. 2014). Optogenetics has offered insights into how specific circuits determine behaviors, from which high precision models have been developed to examine a range of behaviors and behavioral perturbations (Deisseroth 2014).

A powerful approach is offered by the combination of genetics and optogenetics. Animal models can be developed based on genetic mutations identified in patients. Such models offer a unique opportunity to identify, and subsequently modify, circuits that underlie behavioral abnormalities. These studies allow targets in neuronal circuits to be identified which, when manipulated, can repair the dysfunction. The resulting knowledge offers a new basis for translation: the malfunctioning circuits in the rodent models can be tested in human brains using imaging and various stimulation techniques. Furthermore, single cell genomics in specific disease-associated circuits allows high granularity for candidate genes that encode targets capable of modulation by small molecule drugs (Riley et al. 2014). Such approaches may ultimately allow drug discovery and development to become robust and reliable. The path toward effective therapeutics appears to be based on specific brain circuits that mediate symptoms of brain disease. Optogenetics has become routinely applied to study circuits involved in anxiety, anhedonia, feeding disorders, learning and memory, as well as many others.

In addition, deep brain stimulation, best developed for movement disorders (Benabid et al. 2009) but also under investigation for depression and obsessive-compulsive disorder, has opened up new opportunities to activate or inhibit certain neural circuits in patients that can be further studied with neuroimaging and other technologies (Ostergard and Miller 2014).

Attention was also given to how a new, well-validated pathophysiology could be translated into effective therapies. This will involve improvements

in chemistry to address targets previously thought impossible or exceptionally difficult to "drug" as well as continued improvements in getting both small molecules and antibodies across the blood-brain barrier. Gene therapies have become, after a long period of gestation, increasingly safe, and thus may provide additional therapeutic tools.

Commensurate to the scientific enquiry inherent in our conceptual roadmap (Figure 14.1), academic institutions, foundations, biotech and pharmaceutical companies, and regulators need to work together to deliver candidate treatments more rapidly to patients. In addition, improved organization is needed in how basic and translational science is conducted if there is to be a true renaissance in developing therapeutics for nervous system disorders.

In preparing for the Forum, the program advisory committee recognized that translational neuroscience was at a critical juncture—one that could lead down two paths: extinction or revolution. The unique challenges and numerous failures to treat complex and often slowly developing diseases of pathological neural circuits have been disheartening. Yet, profound progress has been made, through discoveries and techniques, to better our understanding of the underlying pathophysiological mechanisms of nervous system diseases. The revolution has indeed begun, and the results of this Forum give reason to hope that new therapies for nervous system disorders are both realistic and possible.