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Special Report

The Judith Hoyer Lecture: Genes, pixels, patterns, and prevention

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1. Introduction

It is a singular honor to deliver this year's Judith Hoyer Lecture, a special occasion that heralds the opening of the Annual Meeting of the American Epilepsy Society. By its very creation, this lecture has quickly come to symbolize a vital partnership between the US Congress, the National Institute of Neurological Diseases and Stroke (NINDS), the Epilepsy Foundation, Citizens United for Research in Epilepsy, and the American Epilepsy Society to generate and deliver a national investment in scientific research that will lead to a meaningful improvement in the lives of people affected by epilepsy. Although the partnership is long-standing, the Hoyer Lecture now represents a perfect moment in each year to summarize our progress and reflect on the distance remaining to be traveled.

The road may be long, but at least we have a firm idea of where to go and ways to get there. We need the ability to discover the precise molecular basis for seizures in any individual with epilepsy, to pinpoint the actual brain networks affected, and to selectively reverse or even prevent the hyperexcitability in these cells while leaving others undisturbed. We also need to identify individuals at risk for epilepsy, and develop treatments that could prevent its appearance. These goals promise countless obstacles, some plainly visible from today's vantage point, and others well hidden—pitfalls and misinterpretations that will stymie even the surest scientist. Nevertheless, as we examine the clearly defined biological targets we have identified so far, and the increasing accuracy of the tools at our disposal, one fact is obvious: we now have unparalleled opportunities to take precise aim at the root causes of lifelong seizure disorders. Speaking for those in the audience today who are involved in both clinical and basic research, I believe there is an overwhelming sense that neuroscience is poised to deliver discoveries in epilepsy diagnosis and treatment that will alter the lives of tomorrow's children, and as NINDS Director Dr. Story Landis emphatically points out, it is essential to both communicate this encouraging message and to act on it.

From my own perspective, a symbolic barrier was broken by the Curing Epilepsy conference, held in Bethesda 5 years ago. First, it was carefully coordinated by the NINDS along with key members of community-based support groups working to promote epilepsy research. This surely marked the rebirth of a golden era in which we can move aggressively together to reduce the burden of epilepsy around the world by training young investigators and intensifying collaborative research programs at academic epilepsy centers. I consider this "postmodern" era of scientific discovery psychologically unlike the first, because I believe the conference produced a lasting change in the way neuroscientists need to think about epilepsy, namely, not only as a vehicle for understanding the puzzle of synaptic signaling within the brain, but as a condition that can and will be cured. There is not a basic scientist in the room who would not like to alleviate the burden of epilepsy, but only a few are encouraged to directly extrapolate their findings from the experimental disease model they study in the laboratory to real people with epilepsy. Instilling enough bravery among seasoned medical scientists (and their students) to repeatedly and deliberately use the word *cure* is no small accomplishment, and this milestone reflects the willingness, based on our current pace of discovery, to acknowledge that "translational" research approaches can now bring this about. I use this term in the accepted sense, that is, the ability to isolate a specific biological abnormality in a patient, study it in the laboratory, and then return to the patient with a therapeutic solution. Some might argue this has been the premise ever since Hans Berger documented the first synchronous electroencephalographic discharge in an epilepsy patient some 70 years ago, but it has seemed that until quite recently, the major

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emphasis among neuropathologists and synaptic physiologists has been to seek causes of epilepsy rather than cures, and the plight of neuropharmacologists, for lack of realistic experimental models, has been to rediscover the actions of existing drugs rather than design fundamentally new ones. The paradigm shift in basic research that is moving our field is now quite evident, and I would like to review some of the factors I think must be playing a role in bringing us to this remarkable tilting point, particularly within my field of epilepsy neurogenetics.

First, what of the challenges? Have they suddenly become any less daunting? Quite the contrary. For most, the term *curing epilepsy* means eliminating seizures; however, after speaking with a pediatric neurologist or the parent of a child with epilepsy one might say, "I wish seizures were the only problem." This is because seizures are a manifestation of not one, but one of a thousand possible disorders altering the excitability of the brain, and they are often accompanied by cognitive and behavioral problems that may precede the first seizure and remain once seizures are gone. In many fortunate patients, the seizures themselves appear to be the sole issue, but in others, they represent only the tip of a hidden pathological process that diminishes normal brain function in everyday life. Although we would like to think about achieving a cure in the largest sense, that is, reversing the underlying disorder rather than simply eliminating seizures, this would in many cases require anticipating a neurological disorder in a person before it becomes clinically apparent. So defining the exact cause and predicting the risk of epilepsy at the earliest time point in an asymptomatic individual remain major hurdles to surmount.

Second, we now understand that seizures themselves arise from many different molecular errors in early brain development, that repeated seizure episodes selectively alter the chemistry and structure of brain networks in ways that depend on the exact cause and age at onset, and that a single medicine effective for all patients has been elusive; indeed, nearly one-third of patients do not respond satisfactorily to any current therapy, despite the availability of newer drugs. Finding a universal medicine that will benefit all patients, particularly one that might suppress the onset of seizures before they arise, appears for the moment as unlikely for epilepsy as finding a single "magic bullet" to treat the many forms of human cancer.

Finally, as if diagnosis, prediction, and treatment were not difficult enough, comes the problem of global prevention. Epilepsy will never go away. Although we can work to minimize external hazards known to cause seizure disorders, we now believe a major fraction are due to heredity. Single-gene errors capable of altering brain excitability are continuously transmitted within families. Newly emerging genetic tests can warn of elevated risk for future generations within these pedigrees; however, even in otherwise healthy and unsuspecting families, novel mutations in these genes may still arise de novo in any newborn child. In addition, we are learning that there are an endless supply of less deleterious, but still risk-bearing gene variants carried in the general population, and a strong likelihood that these can and will combine to produce new cases of "sporadic" epilepsy in otherwise unaffected families. Fortunately, this common type of genetic risk is less likely to be passed on to a subsequent generation.

If the human problem remains as great, and we are learning that the biological causes are more complex than ever, what justifies the growing optimism for curing epilepsy? The answer lies squarely in the power of new research tools we have acquired from a wide range of scientific disciplines and now increasingly bring to the bedside.

2. Genes

Ten years ago, our understanding of the etiology of epilepsy was fragmentary. We recognized major categories of acquired brain pathology that result in seizure disorders, including trauma, infection, tumor, hypoxia, and hemorrhage, but did not (and still do not) understand why in some people they fail to do so. We also knew there were families with apparent Mendelian epilepsy displaying the inheritance of a single-gene error, but had few ideas of what cellular function the gene might regulate, how long it would take to identify one, and how many such genes there were to be found. Finally, and most mysterious of all, we knew that seizure disorders could also appear sporadically in the absence of any affected family member or obvious brain lesion, so-called "idiopathic" epilepsy. The details of this picture have changed dramatically. In the last decade, as a result of an extraordinary string of successes, a list of more than 80 genes linked to epilepsy has been assembled using positional cloning techniques in rare families and experimental neurogenetic strategies in genetically engineered laboratory mice. The families identified by clinician researchers provide human molecular geneticists with the DNA to map, and then isolate disease genes using advanced genetic databases that are just now reaching completion. We believe there are many more genes to be found, and can now rely on detailed knowledge of the human genome to accelerate the search. By following the translational paradigm, experimental neurogeneticists can then move the analysis of each epilepsy gene from the affected family to a "humanized" mouse model. Such mice serve an essential role in deciphering the pathogenesis of each particular form of epilepsy once it has been identified. This step employs the arcane tools of genetic engineering to recreate the exact mutant form of the gene and physically insert it (under a microscope) into cells that become the germ line of a mouse, from which a strain of epileptic mice can then be raised. Finally, molecular neurobiologists and cellular neurophysiologists examine the brain to localize the neuronal networks affected by the mutant gene and seek insight into ways to reverse the defect. Thanks to this approach, laboratories around the world now reproducibly analyze and interpret the exact sequence of cellular defects in the developing brain that lead to the human disorder,

and the genes themselves point to the targets for the next generation of new antiepileptic drugs. This translational process is truly "revolutionary," because with each turn of the cycle from human to mouse and back, a new gene is obtained for the diagnosis of epilepsy, and an exact recreation of the disease is performed in a mouse model that can serve as an ideal biological test system to discover a new medicine tailored to the mechanism of the family's disorder.

The hope for the success of this strategy must be tempered by the sobering realization that we are now confronting a very large number of different defective genes for epilepsy. How many of these can we hope to cure? Will each gene require a separate new drug? Although we are only in the earliest stage of this analysis, a look at the genes uncovered so far brings one potentially reassuring conclusion. Even though the 80 genes that have been linked to epilepsy so far encode proteins that are involved in strikingly different functions within cells, it seems that many converge on a smaller number of key signaling processes in the brain. One-third encode ion channels. Another third directly reduce synaptic inhibition in the brain. This functional clustering means that although there could be tens or even hundreds of gene mutations modifying individual steps within any one biochemical pathway, many are overlapping in their downstream effects, and like tributaries feeding a major river, only one or a few key sites are needed to modify the end result. If this principle of "pathway convergence" is correct, it implies a need for a few drugs that can modify activity within a final common pathway, rather than a drug for every gene that causes epilepsy.

Single-gene disorders are prized for representing the simplest case for "gene-forward" analysis of the brain; however, we now understand that these Mendelian epilepsies are relatively infrequent, and that the more common problem is one of complex inheritance, where multiple genes with modest effects combine to increase the risk of epilepsy. But which genes are these and how can they be found? Our group at Baylor College of Medicine considered this problem and realized that large-scale, high-throughput gene sequencing approaches could be applied to solve it. We began by selecting one of the categories of genes we knew to be a major cause of monogenic epilepsy, namely, ion channels, and developed an accelerated approach that leverages the enormous research investment in DNA sequencing power developed for the Human Genome Project. As there are a large number of genes encoding ion channels, more than 250, it made little sense to examine these one by one in each individual until a defect was found. Instead, we scaled the sequencing process to examine all of the candidate genes simultaneously, a strategy we term *parallel sequence profiling*.

This new approach looks at an individual's DNA and produces a profile of the variations in his or her ion channel genes that can be read much as a (very long) bar code, to see if we can recognize telltale patterns of variation within different types of epilepsy or according to their response to medication. This "genetic snapshot" of an individual's ion channels will then be used to construct a "risk table" to predict the likelihood of epilepsy and, potentially, to initiate preventative therapy. One hypothesis is that the number of ion channel variants is larger in patients with epilepsy than in unaffected individuals. Another possibility is that it is not the simple number, but the specific pattern of *channelopathy* (for that is the term describing ion channel defects that lead to neurological disorders) that is decisive. These alternatives are currently being examined with the support of the NINDS and the National Human Genome Research Institute and the formidable technical expertise of the Human Genome Sequencing Center at Baylor. The early results point to a significant insight into the problem. We are learning that individuals with sporadic epilepsy have not one or two, but frequent errors in many different ion channel genes. Some of these channels were not previously implicated as causes of epilepsy. We have also determined that individuals without epilepsy may also have alterations in ion channel genes, although apparently fewer, and not necessarily the exact same errors. The implications of these results suggest that the most common form of epilepsy may in fact be genetically quite complex, and provide direct evidence for the existence of multiple rare gene errors, each contributing a small fraction to the total risk of epilepsy.

This type of large-scale, translational genomic research, employing a multidisciplinary investigator group of clinical neurologists, neurogeneticists, molecular and cellular neurobiologists, and statistical geneticists, is the beginning of a highly collaborative, population-based approach to the molecular diagnosis and cure of sporadic epilepsy. Similar projects that expand the analysis of genetic variation in other categories of candidate genes, supplanted someday by the ability to quickly and inexpensively examine the individual's entire genome, will be the next important step in determining the genetic architecture of the epilepsies, and building a pharmacogenetic basis for gene-directed clinical therapy.

3. Pixels

Advances in the art of brain imaging, most notably the gain in speed in obtaining high-resolution images by increasing the magnetic field strength of MRI scanners, are providing extraordinary clarity in the investigation of seizure disorders. The latest and most powerful laboratory neuroimaging techniques define lesions in cortical and white matter structures at resolutions approaching the cellular level, and advanced computational algorithms sharply delineate areas, layers, and borders, permitting rapid measurement of the volume of any defined brain region. Another recent method, known as diffusion tensor imaging (DTI), now allows the tracing of similarly oriented neuronal fiber pathways within the brain. Connections delineated by DTI produce a picture of fiber tracts within the white matter never before seen in living brain, and provide the first dynamic insight into the evolution of

developmental brain disorders that alter axonal projections. It is impressive to juxtapose the fruit of this technology alongside its predecessor. In the 1940s, Professor Wendell Krieg used manual blunt dissection of the postmortem human brain to clearly reveal and then painstakingly draw fiber tracts in the brain. It was a labor of months to visualize how the wiring of the brain from a single individual was internally organized. Now these same axon fascicle trajectories can be imaged in a living patient in hours (Fig. 1). By refining our ability to identify changes that are invisible using standard imaging techniques, we increase the chances for early detection of neurological lesions leading to epilepsy. Coincidentally, the intrinsic beauty of these computer-generated images is wonderfully reminiscent of a painting drawn from a recent collection of work by artists with epilepsy (Fig. 2). The similarity of these images serves as a gentle reminder, as Congressman Hoyer mentioned, that the disorder we hope to understand at the molecular level has a human side, and that in recognizing the ingenuity of researchers, we must always maintain a focus on the creative abilities of people with epilepsy along with their disabilities.

These static images of brain anatomy with epilepsy are now being coupled with other imaging methods that assay the chemistry and activity of small regions within the intact brain. Brain lesions can now be localized by high-density, multielectrode array scalp recordings of electrical activity, and co-registered with noninvasive quantification of energy metabolism, neurotransmitter signaling molecules, and blood flow using SPECT, PET, and BOLD imaging techniques. These tools have been adapted for use in genetically engineered mouse models as well. Thanks to these noninvasive methods, we now have the ability to follow serial changes in the epileptic brain and generate a description of the natural history of dynamic changes in brain circuitry at every stage of the disorder.

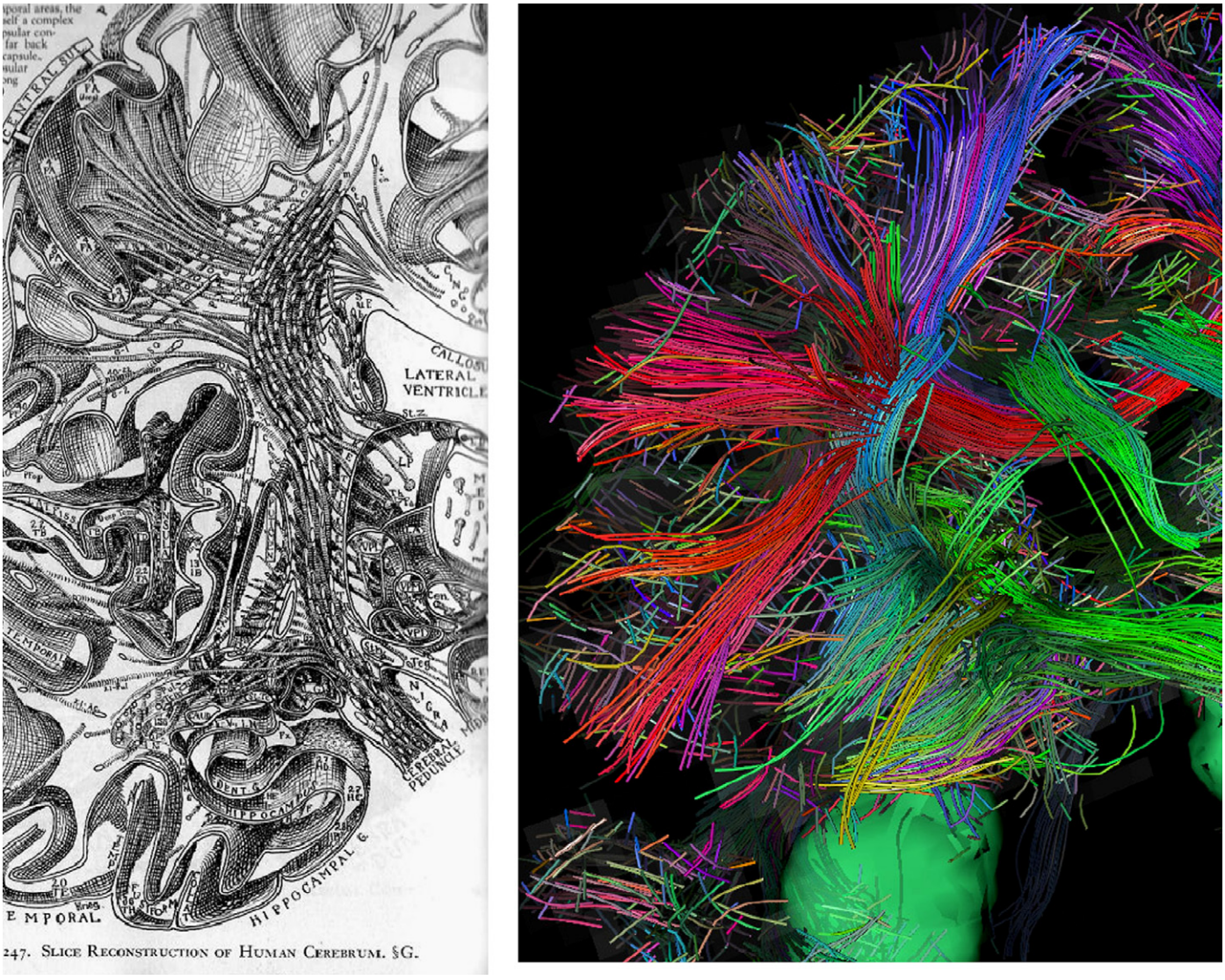

Fig. 1. Left: drawing of fiber tracts in the brain made on the basis of observations after manual blunt dissection. Reprinted from Krieg WJS. Functional neuroanatomy. Philadelphia: Blakiston; 1942. Right: tracing of brain neuronal fibers made using diffusion tensor imaging. Courtesy E. Grant (MGH).

Fig. 2. Painting by an artist with epilepsy that is similar to the computer-generated images produced by diffusion tensor imaging. Copyright © Patricia Bernard. All rights reserved. Reprinted from Schachter S, editor. Visions: artists living with epilepsy. San Diego: Academic Press; 2003.

4. Patterns

Pattern detection plays a pervasive role on the road to a cure for epilepsy. Beginning in the clinic and EEG laboratory, the observation and assignment of subtle commonalities among individuals with epilepsy have permitted major advances in medical management by categorizing epilepsy syndromes and seizure types. The construction of clinical syndromes has required decades of exhaustive information gathering by neurologists seeking to continually isolate and define subtypes of epilepsy.

In the epilepsy basic research laboratory, a new type of pattern analysis is underway, in this case the ability to discern coordinated changes in the activity levels of thousands of genes at a time, using the chip-based gene microarray. This tool for gauging the expression of genes in brain cells by measuring the levels of their mRNA transcripts has been so rapidly adopted in the last several years that the word itself has become a verb. By using a microchip to analyze brain tissue in mice (or in human epilepsy brain tissue removed at surgery), we are now able to perform experiments on essentially all 30,000 genes at once, rather than interrogating one gene at a time. Instead of histological stains that show whether a cell is dead or alive, or immunocytochemical techniques that show whether a specific protein is present, the expression of all gene products can be analyzed in a small brain region, or even in a single brain cell. This analysis can be performed at different stages in the development of epilepsy, and following treatment. Changes can be monitored from birth, or following trauma to the brain, to gain a full understanding of the process of epileptogenesis. When performed on a mouse brain that has been genetically engineered to replicate a human epilepsy, we come closer than ever before imagined to exploring the molecular origins of a human epileptogenic focus, and developing a timetable for new medicines tailored to correct or prevent this disease process.

5. Prevention

Preventing epilepsy means first of all identifying individuals who are at increased risk. Many external risk factors are known but, by themselves, are imperfect predictors of who will develop epilepsy. Assuming that some combination of historical and clinical data (including molecular neuroimaging techniques and biomarkers for the pre-epileptic state) can be assembled that might allow us to correctly select an individual at high risk, we are still left

with the challenge of discovering and validating neuroprotective treatments for a disorder that has yet to become manifest. What approaches can be developed to determine which of the manifold changes triggered by brain injury or the malfunction of the gene are indeed the cause of seizure disorders?

In many cases where epilepsy can be anticipated, for example in posttraumatic epilepsy, the prophylactic use of antiepileptic drugs has been found to be of little value in preventing the subsequent onset of seizures. One hope is that serial analysis of gene expression using gene microarrays as described above may guide us to new molecular targets and the development of effective drugs. This is a strategy currently being pursued with various animal models of epileptogenesis, and early results suggest that a distinct temporal pattern of molecular change precedes the onset of spontaneous seizures in distinct brain regions. Which changes cause epilepsy and which are those that might prevent it? A variety of these molecules have been genetically deleted from strains of mice, and the mice then subjected to repeated or prolonged seizures, and monitored to determine whether they will eventually display epilepsy. These studies reveal that certain genes enhance seizure susceptibility following injury, whereas others reduce it, leaving the clear implication that drugs designed to alter the activity of these genes could prevent or delay the development of epilepsy.

A second clinical scenario provides an illustration of a different research approach to prevention, namely, a newborn bearing a single gene mutation known to reliably produce disabling epilepsy. In this setting, the causative defect is known, and the most logical repair strategy is to compensate for the defective gene itself. If the mutation results in insufficient protein activity (a loss of function), the challenge is to augment gene expression in the deficient cells; if there is excessive gene activity, it must be reduced; and if the mutation changes the actual functional properties of the molecule, the disease gene must be silenced. The therapeutic approach requires genetic engineering strategies (that may one day be replaced by pharmacological agents with a similar mechanism) to mask the inherited defect, either by adding an additional copy of a missing gene to correct the biology of the cells or by silencing the defective copy. The latter technique involves creating a small piece of RNA that, when inserted into a cell, can precisely adhere to the crippled mutant gene product and prevent it from producing the deleterious form of the protein it normally encodes.

Until recently, the solution to these problems could be carried out only in isolated brain cells kept alive in culture dishes and, for various reasons, appeared unworkable in the intact brain. In recent studies, however, injections of genes directly into the brain that are carried into cells by pieces of viral DNA have been shown to produce persistent changes in neuronal behavior. Although only in its infancy, this technique is being applied to experimental mouse models of neurological diseases, including epilepsy. In a recent example, silencing the mRNA of a gene known to cause a specific form of human brain malformation leading to seizures was reproduced for the first time in an experimental model. It now seems plausible that similar strategies can be studied to reverse the effects of single-gene disorders, and thus the day has arrived that we can begin to determine in the laboratory which kinds of genetic epilepsy may be preventable by early intervention in the developing brain.

6. Looking forward

In summary, I believe we have reached a new level of optimism in our ability to envision curing epilepsy, not because the disorder is waning or the complexity has decreased, but rather because we now have access to powerful biotechnology unavailable even one decade ago that dramatically changes the way we can identify individuals at risk, isolate mechanisms and brain circuits that have gone awry, and develop new therapies based on realistic models of the many defects that underlie seizure disorders. If we learn to predict and minimize genetic risks by more informed counseling and treatment, minimize acquired damage to the brain, and find effective medicines that are easily tolerated, substantial reductions in the incidence and prevalence of seizures are on the horizon. The disorder cannot be eradicated, but the prospects for a cure in many individuals remain bright.

This vision is no mirage; however, it may remain maddeningly beyond reach unless we promote translational epilepsy research in the "second golden era" across broad interdisciplinary lines, and naturally, the priorities then depend on who is asked. Geneticists declare that to begin with, we need a cost-effective genetic "snapshot" of an individual's risk for developing epilepsy and response to medicines, and thus should invest heavily in increasing the speed and efficiency of analyzing large sets of genes in individuals with epilepsy. Only then can we be confident of the accuracy of our diagnosis and treatment plan.

What do neuroscientists think of this rosy prediction? They understand that genes are only the starting point. As biologists, they appreciate the plasticity of neuronal and glial networks in the developing brain, and the subtle changes in physiology that can lead to a cortical seizure at one moment and normal activity at the next. They analyze the intricate changes in neuronal firing, switches, and gates that regulate brain microcircuits. They are learning that genes alter neuronal activity and, also, that neuronal activity alters genes. They now understand that although seizures may damage the brain, neural hyperactivity can also induce new patterns of restorative gene activity and even the growth of new neurons in the brain. The same plasticity that may lead to a slow deterioration of brain function may be harnessed to prevent seizures or suppress them once they begin. What neurobiologists wish for are more powerful tools to visualize abnormal functional activity in brain networks to determine precisely which cells need to be modified in a particular individual, a timetable

of when they are most vulnerable, and better ways of selectively delivering a molecular treatment to these cells and no others.

Clinicians share a similar view. Having passed the point of establishing that an individual has epileptic seizures and no remedial medical or surgical options, they ask for new biomarkers to establish a precise molecular and cellular diagnosis, and higher-resolution electrodiagnostic and imaging tools to "stage" the changing biology of the lesion and follow treatment more effectively. They need new and better drugs to clinically manage gene expression in the brain, and need to match them to the right patient.

What do we need to reach this point? A renewed and well-balanced research investment, certainly. More genes, more pixels, more drugs, more care, more time for doctors to spend with their patients in search of a molecular diagnosis, more families willing to become involved in that research, and, most of all, more awareness among all of us that for the first time we now have tools and strategies to deliver cures for epilepsy. As Congressman Hoyer said, "The future is right now."