PRESIDENT'S REPORT

Research directions at Cold Spring Harbor Laboratory Cold Spring Harbor Laboratory follow an ever-changing landscape that is influenced by the intellect of our scientists, by the evolution of scientific problems that can be addressed by current or newly developed technologies, and by a dynamic institutional view that promotes collaborative research and a focus on specific problems that are of collective interest. The neuroscience program at the Laboratory is a prime example of how three different areas of research have morphed together to contribute to major new insights into cognition and cognitive disorders.

A fall 2011 paper published in the *Proceedings of the National Academy of Sciences* from the laboratories of Alea Mills and Michael Wigler included Internet links to three rather grainy time-lapse videos. Each is a few minutes in length and allows us to peer into the cages of several mice simultaneously as they go about their activities. These videos demonstrate something potentially significant about autism genetics. The story behind the research suggests something equally interesting about the way in which we do science at Cold Spring Harbor Laboratory and about the evolution of our neuroscience program.

Mills' team had worked for several years to breed mice with a specific genetic defect that mimics one seen in certain children with autism. Called a copy-number variation (CNV), the defect is a deleted region on chromosome 16, referred to as 16p11.2. Its occurrence in autism, reported by Mike Wigler in 2007, has since been characterized as one of the most common spontaneous genetic alterations, yet it only affects less than 1% of children who have been classified with autism spectrum disorder (ASD). But what is its biological significance? CNVs, which are missing or extra segments of DNA, sometimes occur in regions that encode our genes. The 16p11.2 region contains 27 genes. One of the questions that the Mills and Wigler teams wanted to answer was if a mouse is missing this segment of chromosome 16, or has an extra copy of it, is its behavior affected—and if so, how?

The team did indeed observe behavioral changes, most prominently in mice lacking one of two copies of the 16p11.2 region that are present in most people. A normal mouse—or person—should have two copies, one inherited from each parent. Importantly, the abnormal mouse behaviors seen in the videos resemble some behavioral abnormalities seen in children with autism. In one video clip, a normal mouse quickly learns to climb to the highest point in her cage, while her sibling, bred with the 16p11.2 deletion, is observed during the same time interval to make almost no progress in her climbing ability. Another of the videos shows a mouse with the "autism" deletion unable during an extended interval—unlike her normal sibling—to figure out how to descend from the ceiling of her cage. A third clip shows a mouse with the 16p11.2 deletion displaying repetitive, stereotypical behavior in climbing down from a raised platform, a behavior not observed in healthy mice.

These experiments and others that they have set in motion serve to illustrate the power of a three-pronged approach to neuroscience that we have taken at the Laboratory. The three prongs, broadly described, are human genetics, cognition, and connectivity. For some years, these have tended to run in parallel. The success in creating an autism mouse model is notable because it demonstrates how the three areas of concentration are complementary, mutually informing and enabling, and ultimately integrative.

Neuroscience is one of the most dynamic fields in biology today. It has come a long way since one of its antecedents, called biophysics, was a subject of research at Cold Spring Harbor in the decade before the Second World War. Our first advanced courses on the subject, which commenced in the early 1970s with critical support from the Sloan Foundation and advice from John Nicholls, Eric Kandel, David Van Essen, Reg Kelly, and others, coincided with the installation of teaching labs in the old "Animal House" research building, a 1912-built laboratory now named for Barbara McClintock.

Not until the late 1970s did the Laboratory hire its first staff neurobiologists, Birgit Zipser and Ron McKay, who were joined in 1980 by then University of California at San Francisco postdoc and

future Massachusetts Institute of Technology (MIT) president Susan Hockfield. Their collaborations soon generated insights into the development and organization of the mammalian nervous system. But, alas, these forays into the emerging field of what the 1983 CSHL Symposium called "Molecular Neurobiology" were not sustained because the Laboratory lacked year-round space for neuroscience experimentation. It was a missed opportunity, but one decisively addressed by Jim Watson and an ad hoc committee of the Board of Trustees. In 1985, they set the Laboratory on a course that would result in a full and lasting commitment to neuroscience and in the construction of a major state-of-the-art laboratory. Named for Arnold and Mabel Beckman, it would enable CSHL to initiate year-round neuroscience research in the Laboratory's centennial year, 1990.

The current era of neuroscience investigation at Cold Spring Harbor dates from this time, over 20 years ago. An early focus on the molecular basis of learning and memory led, in time, to studies pertaining to neural dysfunction in people—to memory loss and, separately, to neurofibromatosis (NF), an inherited illness in which tumors form in nerve tissue. These studies, initially focusing on the fruit fly *Drosophila*, enabled our new neuroscientists to include a genetic approach to understanding the brain, taking advantage of the rich genetics legacy and research at CSHL. Studies were also begun to address questions pertaining to synaptic development and function.

In 1996, we recognized that still more space was needed to accommodate our growing neuroscience research effort, and we began to plan construction of a new facility that would be devoted, in part, to new techniques of imaging neurons and their functions in living animals. The centerpiece of the Nancy and Edwin Marks Laboratory was the Advanced Neuroscience Imaging Facility, in which Karel Svoboda collaborated with Roberto Malinow and Zach Mainen to introduce a powerful new tool called two-photon excitation laser-scanning microscopy to CSHL and, through our advanced laboratory courses, to the rest of the neuroscience community. This technology enabled our scientists to obtain images of individual neurons and synapses during sensory processing in live animals, something not possible previously. By October 1999, when the Marks building was dedicated, our neuroscience program had begun to explore the brain and nervous system in the context of each of the themes that I have mentioned—human genetics, cognition, and connectivity. For example, Dimitri (Mitya) Chklovskii studied in the worm *Caenorhabditis elegans* the reconstructed wiring diagram of the 279 nonpharyngeal neurons, a subset of the worm's 302 neurons, to reveal principles about how neuronal circuits deviate from optimal wiring and connection solutions. However, with the departure of a number of our neuroscientists to the newly established Janelia Farm Research Campus of the Howard Hughes Medical Institute, and elsewhere, we shifted our focus to understanding cognition and cognitive dysfunction, linking human genetics to behavioral neuroscience in a program developed in part by Tony Zador.

Zador, with Adam Kepecs and former colleague Zach Mainen, introduced the study of cognition in rodents as a powerful system for understanding complex cortical events in the brain. Their studies enabled them to investigate how rodents, and ultimately humans, use external stimuli to make decisions and pay attention, and use working memory for cognitive tasks. Later joined by Anne Churchland and Florin Albeanu, they are investigating how external stimuli such as odors and sound work their way through the initial sensing regions of the brain to be coded in working and long-term memory that in turn influences behavior. Today, our neuroscientists—who have expanded their activities beyond Beckman and Marks to the new Hillside Laboratory buildings—are able to use sophisticated techniques called optogenetics, a method invented at Stanford University that enables individual neurons to be switched on and off with beams of colored laser light delivered painlessly via fiber-optic threads into the cortex of behaving rodents. Zador, Kepecs, and Albeanu, along with Stephen Shea and Glenn Turner, have mobilized this and other advanced recording technologies to explore how incoming sensory signals are processed in successively more complex layers of neural anatomy, from the sensory epithelia in the nose to the olfactory bulb and olfactory cortex in rodents, and analogous structures in fruit flies. Shea is studying the way in which the emotional salience of an experience—say, an encounter with a potential mate—is encoded, along that path leading from

olfactory receptors to the brain's olfactory cortex, where decisions are made based on processed signals. Some decisions, it is believed, are innate, whereas others, such as these, are forged in response to experience or environmental cues. Tony Zador, who heads our Swartz Center for Computational Neuroscience, established computational analysis of brain circuits as an important part of this work. Zador uses the rodent auditory system as his model in work that seeks to better understand how neural dysfunction can produce the kinds of deficits in social interaction and attention that we see in disorders such as autism and schizophrenia.

Bo Li's research places him at the nexus of behavior and another of the three major themes of neuroscience at the Laboratory: connectivity. When they study connectivity, our scientists broach the immense question of the mammalian brain's architecture and the relationship of structure to function. This can be studied at multiple levels of detail: from the structure of the synapses at which individual neurons communicate, to circuits and networks composed of ever-shifting ensembles of interconnected nerve cells, to entire regions of the brain in which specific kinds of tasks are handled by the neural network. In 2011, Bo Li and Fritz Henn collaborated to discover that neurons in a tiny area in the central brain called the lateral habenula (LHb) are hyperactive in depressed rodents. They found that dysfunction in this one area creates imbalances in other areas of the brain to which it sends neural projections. Understanding the nature of these interrelationships holds a potential key to new ways of treating depression, and, as a result of these studies, Fritz Henn has begun to translate these results into a clinical trial focusing on deep-brain stimulation in the LHb of patients with otherwise untreatable chronic depression.

Connectivity in its neuroscience context is the theme of many different CSHL studies at the cellular level. Linda Van Aelst, an expert on signal transduction, has complemented her work on brain cancer in children with research on how mutations in signaling molecules in the Ras and Rho families of proteins are implicated in various brain disorders. Linda has extensively studied functional implications of mutations of the *oligophrenin-1* gene in several neuropathologies, including X-chromosome-linked mental retardation, and has found that the gene is essential in activity-dependent maturation of synapses and the ability of an existing synapse to change, commonly called plasticity. Linda's interest in neurodevelopment is complemented by the work of Grigori Enikolopov and Alex Koulakov, who study the role played by stem cells during brain development as well as in the adult brain. In 2011, they found that each adult brain stem cell in mice is used only once to amplify into many neurons and hence is disposable, as opposed to stem cells in the blood or gut that self-renew many times during

their life span. Whether stem cells that have been expended can be enticed to revert to their former identity and be recruited back into the stem cell pool is a question that Enikolopov's team is now pursuing. Hiro Furukawa's research concerns another aspect of nerve cell biology, that of proteins that lie on the surface of individual nerve cells. He studies the structure of the immense multiunit protein that forms NMDARs (*N*-methyl-D-aspartate receptors) that control the strength of connections between neurons and thereby have a central role in learning and memory. Membrane receptors, the place where neurotransmitter molecules "dock" with nerve cells, are at the "front end" of cell-signaling networks and are of great interest as targets for drug discovery in diseases such as Alzheimer's and in cognitive disorders such as autism and depression. This year, Hiro's team discovered and mapped a new regulatory site in a class of NMDARs, progress that now opens the way to the development of a potentially new class of drugs to modulate the receptor.

By a very rough estimate, the human brain contains 100 billion neurons, but they are certainly not all the same cell type. Some are excitatory and activate circuits, and others are inhibitory and modulate neuronal networks. Josh Huang is studying a class of inhibitory neurons that are divided into about several dozen different types. He is interested in neural development and has invested much time and effort in creating a powerful new resource for the entire neuroscience community. His team has developed 20 different mouse lines, each of which is engineered to express markers of specific cell types. They recently demonstrated the exquisite specificity of this technology by imaging the main inhibitory nerve cell type in the mammalian brain, so-called GABA cells. In a fascinating series of images, Josh's team showed a particular subset of GABA cells as they moved from their place of birth in the mouse brain through various stages of the developmental process. We can see them migrate into the cortex and take up positions at which they forge connections with one another and with excitatory neurons. This type of plasticity underlies much of how our brain develops and responds to learning and memory of new tasks.

The way in which the brain is wired can be studied at many levels, from how individual neurons are connected to one another, to how groups of neurons form networks of cells that "compute" information, to how the small networks are connected to one another to form regions of the brain that are linked to recognizable tasks such as vision, motor control, and hearing. Finally, our brain is composed of large functional regions that communicate with one another and coordinate all sensory input and behavioral responses. Using funding from a National Institutes of Health TR-01 grant and funds from the Allen Institute for Brain Science, both awarded to advance potentially "transformational science," Tony Zador is developing a novel, high-throughput method to probe the connectivity of neural circuits at the level of individual neurons in mice—called the "connectome." He is collaborating with molecular biologist Greg Hannon to use very innovative technologies that allow molecular codes to be passed from one neuron to another in the brain, later capturing how the information is linked using powerful DNA sequencing technologies. The potential for understanding how networks of neurons are wired is enormous. Josh Dubnau, another TR-01 grant recipient, is approaching neural connections from an entirely different angle. He seeks to assemble a comprehensive store of information about how RNAs are translated into proteins needed for the assembly of synapses. He and his team are focusing initially on spatial and temporal control of translation in the context of fragile-X syndrome, an autism spectrum disorder in which the process goes awry.

On a different scale, two exciting imaging technologies have recently been developed at CSHL and are beginning to yield valuable data regarding brain structure and connectivity. Partha Mitra is progressing in an effort backed by a third TR-01 grant to map the mouse brain at what he defines as the "mesoscopic" level. Partha's project is focused on identifying the nature of the connections within and between different brain regions and eventually integrating the data with the Allen Brain Atlas, which has proven to be a valuable map of genes that are expressed in different regions of the mouse brain. Partha and his team have built a robotic laboratory that enables the analysis of many mouse brains and the storage of very large data sets for computational analysis.

An ultimate scale of understanding brain activity and connectivity is at the cellular level. Pavel Osten and his team have built an impressive automated system dedicated to systematic brain connectivity and functional studies. By automating and standardizing the process by which brain samples are divided into very thin sections and then imaged sequentially with precise spatial orientation by high-resolution microscopes, Pavel has opened the door to making whole-brain mapping at the cellular level more routine. In essence, each neuron in the brain has a gene that is turned on when the neuron has been recently active, expressing a green fluorescent protein (GFP) that can be imaged. It is possible to image all of the brain cells that "light up" after the mouse has a new experience or receives a drug that affects brain activity. This technology, along with the technology developed by Mitra, will enable an analysis of the connectivity and activity within the brains of mice bred with the autism 16p11.2 CNV. This application of brain imaging of mice with genetic alterations that mimic those in patients with Alzheimer's disease, autism, bipolar disorder, depression, and schizophrenia is one aspect of the "integration" of science that is characteristic of research at CSHL. It makes perfect sense, given a credible animal model of a brain disorder, to explore at the level of both structure and function how the brain in an abnormal phenotype differs from that in a healthy control. This should provide unique and, we hope, powerful insights into both disease causation and new therapeutic approaches.

The integration of connectivity and behavioral studies is only two-thirds of the story of integration. The final third concerns human genetics but in many ways is a key starting point for understanding how the brain functions. It is the part with the longest and richest history at Cold Spring Harbor—one that takes us back to our institutional roots and forward to current and future research. In what I consider a classic instance of research in the CSHL style, Alea Mills—a geneticist—became involved in an important neuroscience project because of a technique that she had previously invented called chromosome engineering. Some years ago, she demonstrated its utility in solving a decades-old mystery about a section of human chromosome 1 that is frequently deleted in neural, epithelial, and hematopoietic cancers.

It had long been clear that this region of the chromosome harbored an important tumor-suppressor gene. But the region was large and, like the 16p11.2 region in autism, was known to contain many genes. Which was the tumor suppressor? Mills and colleagues created mice with gains and losses of genomic regions that corresponded to the deleted region on chromosome 1. They confirmed that loss of the region predisposes to cancer, whereas gain of the region results in excessive tumor suppression. Ultimately, they identified *Chd5* as the tumor suppressor within the region and determined that the protein that it encoded was a remodeler of chromatin that regulates an extensive tumor-suppressive network. Thus, a technology developed to study cancer genetics made possible the engineering of the "autism mouse."

Another thread of current neuroscience research at CSHL can be traced to cancer research and goes back even further than chromosome engineering. During the 1980s, as Michael Wigler and his group studied the genetics of breast cancer, Mike became interested in comparing a woman's normal DNA to the DNA found in her tumor. In collaboration with former colleague Nikolai Lisitsyn, he invented a technique called representational difference analysis (RDA), followed by a second-generation method called representational oligonucleotide microarray analysis (ROMA) developed with Rob Lucito, to perform such comparisons. This evolved over several years into successive variants of the technique, including the recently developed ability to sequence the genome of a single cell, enabling comparisons to be made on a whole-genome scale. By collecting DNA from hundreds of women with breast cancer, Wigler and collaborators were able to build an extensive DNA database that, among other things, also enabled them to compare the DNA readouts of the noncancer, or normal, DNA from each woman. But unexpectedly, an important discovery followed that revised our notion of what normal means. Every supposedly normal sample contained a large number of DNA variations relative to the standard human reference genome. Each person was significantly different because they harbored gene CNVs. This led to the realization that CNVs were not a rare and exotic form of genome mutation but instead were present in every individual.

Wigler pursued an inspired hunch that some common genetic disorders—such as autism and schizophrenia—might be traceable to CNVs that occurred spontaneously, that is, not present in either parent of an affected individual. In subsequent work, this hypothesis was confirmed for both disorders. In autism especially, it became clear that spontaneous CNVs accounted for a large fraction of cases. With generous support from the Simons Foundation, a large sample of families with an autistic child was assembled. Analysis of the genomes of the affected child compared with that of the parents and sometimes an unaffected sibling enabled the identification of hundreds of regions in the human genome associated with autism. The surprising fact that we all have CNVs, coupled with the Wigler lab's invention of the technological means to identify them across the genome, was a major step along the path toward understanding the cause of the disorder and toward the eventual use of chromosome engineering to create a mouse model of autism.

Mike Wigler continues to explore the genetics of neuro-psychiatric disorders in humans. He and collaborators have also made a major contribution to describing the role of both spontaneous and inherited CNVs in schizophrenia. Another of our senior scientists, W. Richard McCombie, shares this interest in the human genetics of brain disorders. Dick heads the Laboratory's Stanley Institute for Cognitive Genomics and is sequencing patients with schizophrenia, bipolar disorder, and depression. The collaboration between Wigler and McCombie has enabled the genetic analysis of autism to proceed to identifying rare single-gene mutations.

The three paths of research in neuroscience—human genetics, connectivity, and the study of cognition—are rapidly converging. Furthermore, the Laboratory's recent investment in building a Quantitative Biology program is infusing our neuroscience program with a new source of intellectual vibrancy. In 2011, Ivan Iossifov, a CSHL Quantitative Biology Fellow, along with Michael Ronemus, Dan Levy, and Sarah Gilman in the Wigler lab, collaborated with Dennis Vitkup at Columbia University to undertake a novel quantitative study of CNVs in autism spectrum disorder. One of their published papers addressed a question that is now being asked in the Mills–Wigler collaboration about their autism mouse model: Which genes within a CNV of interest are actually involved in disease pathology?

Iossifov, Vitkup, and colleagues used a novel form of statistical analysis to identify the large biological network of genes affected by rare spontaneous CNVs in autism. They found that this network was strongly related to genes previously implicated in studies of autism and intellectual disability. And they confirmed that the genes in question relate primarily to the development of synapses as well as to the targeting of axonal fibers and the ability of young neurons to migrate. All are essential aspects of brain development. In more recent studies to be published in 2012, Iossifov, Ronemus, Levy, McCombie, Wigler, and collaborators have sequenced the genomes of families with a child with autism and have made some remarkable discoveries. First, the age of the father is a significant contributor to the occurrence of spontaneous gene mutations that are linked to autism. Second, a large percentage of the genes found to be causative of autism fall into a network of genes whose RNAs are regulated by fragile-X mental retardation protein (FMRP). Fragile-X syndrome is a disorder on the spectrum of autism disorders and is caused by a mutation in a single gene. By studying how the defect in the *FMRP* gene alters synaptic activity in the brain, Mark Bear and his colleagues at MIT have developed a drug that targets the neuronal mGluR5 protein that reverses the effect of the fragile-X syndrome mutation in mice. The drug is now in clinical trials in children with fragile-X syndrome. The fact that a disorder on the autism spectrum can be reversed is remarkable in itself, but the observations that genes that cause autism overlap with FMRP and fragile-X syndrome offer the possibility that more common forms of autism, or at least a subset of autism, might be treatable. This finding suggests how the fruitful integration of nominally distinct research approaches is providing major insight into how the brain functions and offers hope that some form of treatment may be on the horizon. I look forward to further substantial progress in our neuroscience research that is as exciting as it has been during the past 15 years.

Bruce Stillman, Ph.D., F.R.S.
President