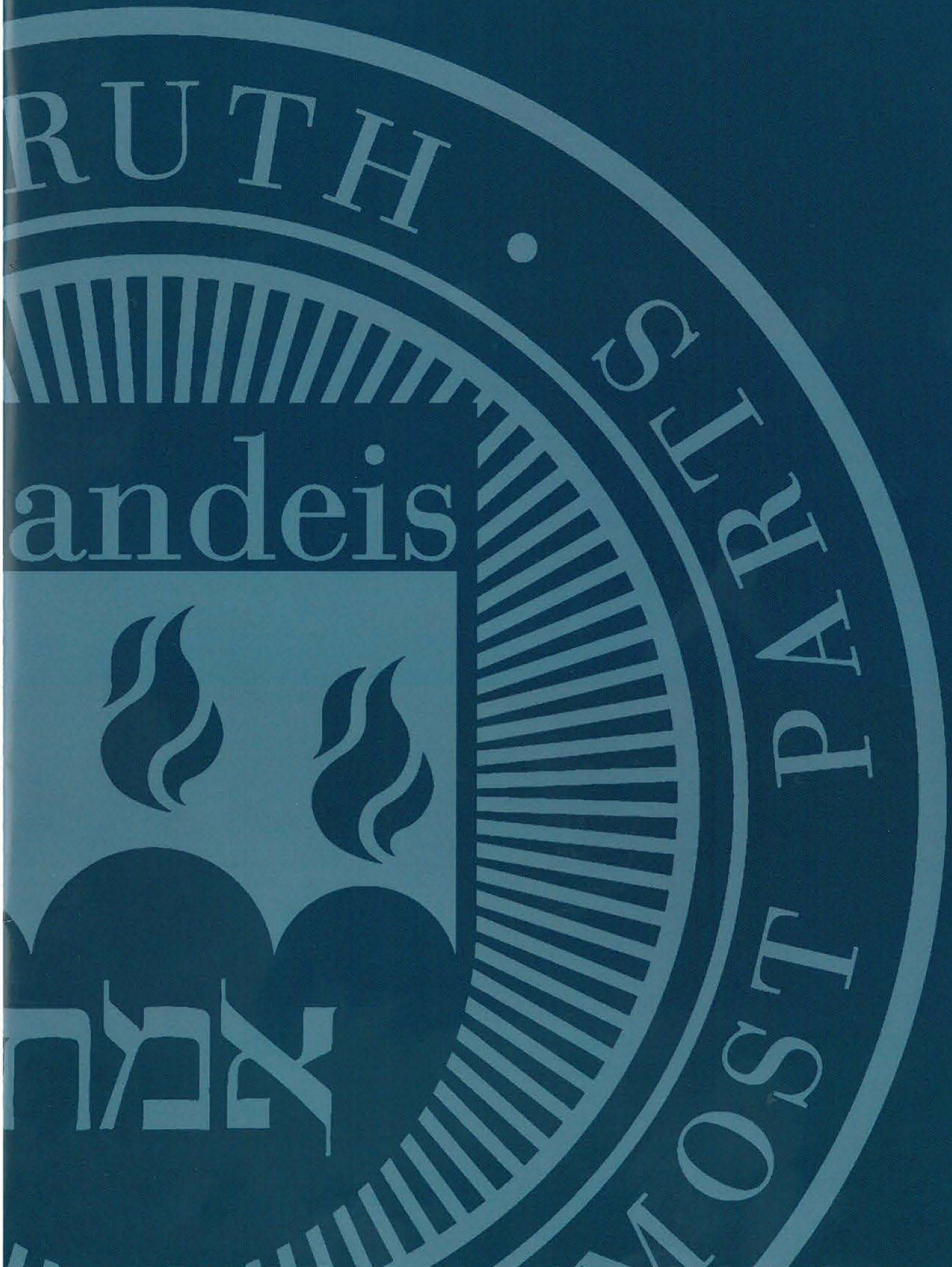
Brandeis University

Benjamin and Mae Volen National Center for Complex Systems

The M.R. Bauer Foundation Colloquium Series, Annual Scientific Retreat and Distinguished Lecturer Series

August 2011



The M.R. Bauer Foundation Colloquium Series, Annual Scientific Retreat and Distinguished Guest Lecturer Series 2010–2011 Summary

Brandeis University

Benjamin and Mae Volen National Center for Complex Systems

Table of Contents

The 2010–2011 M.R. Bauer Foundation Colloquium Series, Annual Scientific Retreat and Distinguished Guest Lecturer Series

Introduction

The Volen National Center for Complex Systems is devoted to the science of the mind. In keeping with the vastness of the theme, the Volen Center brings together scientists from a multitude of disciplines — biology and chemistry, computer science and physics, psychology and cognitive science — who apply diverse methodologies and research programs to a shared objective: to enlarge our knowledge and deepen our understanding of mental functioning in all its forms. For more than 15 years, the M.R. Bauer Foundation has generously supported this robust intellectual community. The impact of that support is evident in these proceedings of the M.R. Bauer Colloquium Series, Distinguished Guest Lecturer Series and Scientific Retreat.

The range of topics encompassed in these proceedings reflects the richness of the field.

- Mental functioning depends on complex circuitry in the brain, and a number of presentations took up questions concerning these neural circuits. A recurring theme in these talks is plasticity and development: what determines the specific form that the neural circuitry acquires, and what enhances or impedes this development?

- Underlying modern neuroscience are new technologies that make it possible to observe and analyze the functioning of the brain. How these technologies can best be correlated and combined is the theme of a number of presentations.

- Memory and learning is another rich topic. As a number of presentations illustrate, the science of the mind brings an experimental approach to perennial questions about how we learn and how we retain what we have learned.

- Sense modalities are another important area of exploration. Neuroscience has taught us that sensation relies not only on sensory intake through sense organs, but also on sensory processing in the brain. The complexities entailed in this are addressed in presentations on vision and hearing.

All of the talks summarized here are vivid illustrations of the scientific spirit guiding these investigations. In one sense, inquiring into the nature of sensation and movement, habit and action, and learning and memory is not new — these have been integral to efforts over the centuries to understand human and animal life. What is new is the application of scientific methods and tools to this inquiry. The study of these topics is now governed by the discipline of the laboratory and the demands of evidence.

The research findings presented here are on the frontiers of science. This research may eventually lead to practical applications, with concrete consequences for human well-being. But its significance extends beyond any practical implications, enriching our understanding of the human — both what we share with other creatures and what is singular to our species. The science of the mind is, ultimately, the pursuit of human self-understanding.

We at the Volen Center remain deeply grateful for the steadfast support of the M.R. Bauer Foundation, which makes possible the visits and seminars presented here. These lectures and colloquia keep the Volen Center at the center of current work in neuroscience. Through these proceedings, we are pleased to share these riches with our colleagues across the country.

Arthur Wingfield, D.Phil.
Nancy Lurie Marks Professor of Neuroscience and
Director, Volen National Center for Complex Systems

The M.R. Bauer Colloquium Series Summaries

Introduction

The idea that "form follows function" was prominently articulated by Louis Sullivan in 1896. One of the premier architects of his time, Sullivan was faced with the immense challenge of balancing land limitations and industrial needs with the aesthetically pleasing architectural forms of the past several centuries. A pragmatist at heart, Sullivan quickly realized that in a world of growing economic concerns, building design would need to rely on functional value, not just on structural beauty. Thus, the skyscraper was born. Later, the industrial designers of the 1930s such as Normal Bel Geddes, Raymond Lowey and Richard Buckminster Fuller ushered in the "streamlined decade" bringing the form-follows-function principle to airflow designs for cars, locomotives and ocean liners — some realized and others only imagined.

Today the relationship between form and function remains in many modern objects, from clothing to automobiles to corrective eyewear. By contrast, the human brain, arguably one of the most complex and intricately designed constructions ever observed, tends to follow a different mantra. Neuronal systems, unlike Sullivan's famous skyscraper or Lowey's streamlined locomotive designs, derive function from their form, not the other way around. Indeed, even small alterations in a neuron's form can have large effects on its function and on the function of the surrounding neural networks, often with debilitating consequences.

As we explore the form and function relationship through the lens of the 2010–2011 M.R. Bauer Colloquium series, we have arranged the summaries of the presentations of our six colloquium speakers in terms of physical scale. In moving from the small signaling molecules of brain cells, known as neurotransmitters, to the mechanisms that govern learning and memory, it will become apparent that, at any scale, form and function are intrinsically bound. Especially notable is the variety of animal and systems models represented in these presentations, as each reveals important information about neural structures and their development. We have added a brief introduction in italics before each of the more technical presentation summaries. Our goal in adding these short introductions is to place each of the presentations in their broader context of this year's theme: the relationship between structure and function in the human and animal brain.

Nicholas Spitzer, Ph.D.
Institute for Brain and Mind
University of California, San Diego
(October 4, 2010)

Activity-Dependent Transmitter Specification: Novel Plasticity

Dr. Nicholas Spitzer's research examines the fundamental question of how the brain cells, or neurons, "decide" which neurotransmitters to release. It is well known that different neurotransmitters, the small molecules that neurons use to communicate, are capable of eliciting different responses. Dr. Spitzer's lab has shown that there is a complex set of genetic programs that neurons use to select which type of neurotransmitter to use and therefore how they communicate with other neurons in a given circuit. These are the basic building blocks of neural signaling that eventually give rise to behavior. Dr. Spitzer shows how these principles are revealed in the intriguing phenomenon of camouflage coloration in amphibian larvae.

Brain cells within neural circuits signal each other largely through the release of neurotransmitters and the activation of their receptors at synapses. Ever since Otto Loewi and Henry Dale discovered the chemical synapse, it has been thought that these transmitters are fixed characteristics of neuronal identity. The specification of neurotransmitters and receptors is a fundamental developmental process, critical for the establishment of functional connections at synapses. With a vast number of neurons, specifying the appropriate neurotransmitter for each one is a challenging task. Moreover, since neurons can receive thousands of different synaptic inputs, matching presynaptic neurotransmitters with appropriate postsynaptic neurotransmitter receptors is a daunting undertaking. The problem is even further complicated by the existence of the large number of neurotransmitters and neurotransmitter receptors.

Genetic programs are essential for the differentiation of different neuronal phenotypes and have been shown to establish default transmitter phenotypes. However, perturbations of calcium-dependent electrical activity in the embryonic spinal cord increase or decrease the number of neurons expressing the neurotransmitters glutamate, GABA, acetylcholine and glycine by as much as 50 percent. Additionally, it has been found that altering light exposure, which changes the sensory input to the circuit controlling adaptation of skin pigmentation to background, changes the number of neurons expressing dopamine in the brain of amphibian larvae in a circuit-specific and activity-dependent manner. Neurons newly expressing dopamine then regulate changes in camouflage coloration in response to illumination. Thus, physiological activity alters the numbers of behaviorally relevant amine-transmitter-expressing neurons in the brain at postembryonic stages of development. The results may be pertinent to changes in cognitive states that are regulated by biogenic amines.

The Spitzer lab has identified several molecular mechanisms that link endogenous calcium spike activity with intrinsic genetic pathways to specify neurotransmitter choice in the embryonic amphibian nervous system. In the brain of these model organisms, activity modulates specification of serotonergic neurons by regulating expression of the Lmx1b transcription factor. Activity acts downstream of Nkx2.2, but upstream of Lmx1b, leading to regulation of the serotonergic phenotype. Changes in the number of serotonergic neurons change larval swimming behavior. These results link activity-dependent regulation of a transcription factor to transmitter specification and altered behavior. In the spinal cord, early activity modulates transcription of the GABAergic/glutamatergic selector gene *tlx3*. Calcium signals through phosphorylation of the cJun transcription factor, which in turn binds to a cAMP response element (CRE) site in the *tlx3* promoter. Binding with this CRE site modulates transcription, thus regulating the neurotransmitter phenotype. This mechanism provides a basis for early activity to regulate genetic pathways at critical decision points, switching the phenotype of developing neurons.

Karl Kandler, Ph.D.
University of Pittsburgh
School of Medicine
(October 18, 2010)

Tuning Up Inhibition

Moving away from the specification of neurotransmitters reveals another striking structure and function relationship. How neurons decide which connections to form and break has important implications for effective brain function. For example, during mammalian development, neuronal connections form in the portions of the brain responsible for sound localization. How these neurons connect to one another serves as the structural basis for hearing. Dr. Kandler's presentation to the Volen Center explored the molecular mechanisms through which these processes occur. As one of his tools Dr. Kandler makes use of "knockout" mice — animals in which a specific gene has been deleted.

Accurate hearing depends on precisely organized and fine-tuned neuronal connectivity in the brain. During development, this organization emerges gradually through mechanisms that are still poorly understood. To gain more insight into these mechanisms, Dr. Kandler's lab has been investigating the processes that are involved in the establishment of a precisely organized inhibitory pathway in the mammalian primary sound localization system.

The Kandler lab has demonstrated that an inhibitory pathway, the glycinergic projection from the medial nucleus of the trapezoid body (MNTB) to the lateral superior olive (LSO), is refined by the silencing of most initial connections and the strengthening of maintained connections. Interestingly, this reorganization occurs while MNTB-LSO synapses not only release glycine, but also co-release GABA and even glutamate. Glutamate released from MNTB terminals can activate postsynaptic NMDA receptors, which significantly contribute to local calcium responses in aspiny LSO neurons. To test whether this transient glutamate co-release is important for the refinement of the MNTB-LSO pathway, the Kandler lab has investigated the development of the MNTB-LSO pathway in mice missing the vesicular glutamate transporter 3 (VGLUT3) gene. These mice demonstrate a lack of glutamate release from MNTB terminals, while glutamate release from other terminals in the LSO is undisturbed. These results demonstrate a significant impairment in the refinement of the MNTB-LSO pathway in VGLUT3 knockout mice, indicating that glutamate co-release is a novel and important synaptic mechanism in the refinement of the inhibitory MNTB-LSO pathway. Due to the wide expression of VGLUT3 in a variety of other non-glutamatergic circuits, glutamate co-release may also be involved in the plasticity of non-glutamatergic synapses elsewhere in the brain.

Karel Svoboda, Ph.D.
Howard Hughes Medical Institute
Janelia Farm Research Campus
(May 2, 2011)

The Cortical Circuits Underlying Object Localization

From the structure of connectivity we move to the level of neuronal circuits. Certainly the most complex of these circuits can be found in the cerebral cortex, the evolutionarily "new" brain in human and animal species. Understanding cortical circuitry offers an answer to some of the most intriguing questions in neuroscience. It is the cerebral cortex that carries our higher-level learning, memory, consciousness and cognition. In his presentation Dr. Svoboda explored one of these questions: how cortical circuits transform sensory input into a behavioral response.

Dr. Svoboda's work focuses on the cerebral cortex, which is the largest part of the mammalian brain. Neocortical circuits within this structure are remarkably similar across functional areas and species and have been shown to play a role in most flexible behaviors. The Svoboda lab attempts to understand the principles that organize neocortical circuits and to decipher how they process information and guide behavior by focusing on the circuitry underlying whisker-dependent somatosensation in mammals.

Mice and other rodents use their whiskers to recognize and determine the locations of objects. Dr. Svoboda's lab has developed quantitative psychophysical methods for tracking the motor strategies and sensory inputs used by mice in whisker-based object localization. At the same time, members of his lab can record from specific neuronal populations using electrophysiological and imaging methods within mapped neural circuits. These measurements have provided hypotheses regarding how activity in specific neurons might drive behavior. In particular, in layer four of the barrel cortex (areas of the rodent brain responsive to single bristles), object location might be coded by the number or timing of action potentials. Experiments utilizing precisely timed photostimulation have shown that mice judge object location via the number of action potentials and disregard precise spike times, two elements underlying this unique behavior

Barbara E. Jones, Ph.D.
Montreal Neurological Institute
McGill University
Montreal, Quebec, Canada
(November 1, 2010)

Neural Systems Controlling Sleep-Wake States

Structure is not only found in terms of neurotransmitters, connections and circuits but also in the more abstract patterns to which these components give rise. The structure or form of activity across the brain is often seen to follow an oscillatory pattern, commonly referred to as brain waves. Importantly, the frequency of these patterns seems to change across differing behavioral contexts. Perhaps the most striking example of behavioral changes stemming from brain wave patterns is seen when examining the difference between the sleep and awake states. For this reason, we were delighted to hear from Dr. Barbara Jones, whose lab examines how these patterns are modulated by distinct groups of neurons.

All mammals and birds display three distinct states: waking, slow wave sleep (SWS) and rapid eye movement (REM) sleep. These three states are distinguished by electroencephalographic (EEG) and electromyographic (EMG) imaging techniques, as well as behavioral characteristics. Waking is characterized by high-frequency (gamma) EEG activity together with high postural muscle tone on the EMG, while slow wave sleep is characterized by low-frequency (delta) EEG activity, along with reduced muscle tone. REM sleep, paradoxically, can be characterized by high frequency (gamma) EEG activity (like waking) together with postural muscle atonia, typifying deep sleep. Different neural cell groups that contain distinct neurotransmitters and project to different targets that influence EEG or EMG can play different roles in these states. Discovering their discharge profiles across the sleep-wake cycle can reveal the way in which they generate or modulate sleep-wake states and their polygraphic features.

By applying juxtacellular recording and labeling of neurons in naturally sleeping-waking rats, the Jones Lab has been able to determine the discharge profile of several different identified neuronal populations across the sleep-waking cycle in association with EEG and EMG activity. In the basal forebrain, cholinergic neurons discharge in association with fast (gamma) EEG activity during both waking and REM sleep, and thus in association with cortical activation, independent of muscle tone. Glutamatergic and GABAergic neurons are also present in the same region and are differentiated according to their discharge profiles and thus their putative roles. Four functional sets or pairs of glutamatergic, with cholinergic and GABAergic neurons, could be distinguished: (1) the W/PS group, which includes cholinergic neurons and discharges with cortical activation during waking states and REM sleep; (2) the slow wave sleep group, which discharges with cortical slow wave activity during slow wave sleep; (3) the REM group, which discharges with muscle atonia during REM sleep; and (4) the waking group, which discharges with muscle tone and behavioral arousal during waking. These sets or pairs of excitatory/inhibitory neurons can function in an oscillatory manner through balanced excitation and inhibition. Through projections to the cerebral cortex, the waking/REM sleep and slow wave sleep groups modulate EEG activity across the sleep-wake cycle through projections to the cerebral cortex, while the REM sleep and waking groups are able to modulate EMG activity across the sleep-wake cycle via projections that influence the brainstem and spinal cord. Through interconnections between these circuits, different cell groups can oscillate to generate alternating EEG and EMG activities that characterize the three sleep-wake states. Other neuromodulatory systems can then further influence these circuit-level dynamics, and thus influence the resulting behavior.

John Krakauer, Ph.D.
Center for Functional Recovery After Brain Injury,
The Johns Hopkins Hospital
(December 6, 2010)

Rethinking Motor Adaptation and Motor Skill

The precisely executed axel jump of the champion figure skater, the rapidly moving fast ball and the well-timed interception in football are all athletic maneuvers requiring near perfect execution of form to attain a result. In many ways, the sports arena greatly exemplifies the relationship between form and function. How motor skills are acquired, and when lost, potentially relearned, was the subject of Dr. John Krakauer's talk when he visited Brandeis for our M.R. Bauer Colloquium series.

Society often seems to reward motor skill above everything else, at least judging by the salaries of professional athletes. Conversely, loss of motor function caused by neurological injury and disease carries immense cost to individuals and society. Both attainment of high levels of skill, and the rehabilitation of lost skills, depend on motor learning, which makes the study of motor learning of great scientific and practical interest.

Adaptation is a process by which systematic errors introduced by perturbations are reduced by updating a forward model to close prediction errors. Motor adaptation is dependent upon a brain structure known as the cerebellum and has been shown to be both an implicit and often reward-indifferent behavior. Through the combined use of a battery of techniques, including quantitative motion analysis, structural and functional imaging, non-invasive brain stimulation and robotics, Dr. Krakauer's work argues that other learning processes are involved in motor learning in addition to adaptation. These processes include: (1) use-dependent plasticity, which leads to the directional biases often observed in motor skills following adaptation, and (2) operant reinforcement-biases, which are often seen when adaptation is associated with successful error reduction. Work from the Krakauer lab suggests that this operant reinforcement is the process that underlies not only the ability to learn but also to relearn or unlearn a given motor skill. By generating models of how motor commands are translated into sensory consequences, the Krakauer lab hopes to better understand ways in which consistent and coordinated movements are produced in the face of environmental variability and changes in one's internal state.

Barbara Knowlton, Ph.D.
Department of Psychology
University of California, Los Angeles
(March 28, 2011)

Habit Learning in Humans and Animal Models

Will Durant (1885–1981) wrote that, "We are what we repeatedly do. Excellence, then, is not an act, but a habit." The essence of this statement is implicitly represented in the work of many modern day neuroscientists. In her presentation, Dr. Knowlton described work showing that habit memories have very unique properties with respect to other types of memory, and that the formation of these memories can be localized to a particular structure in the brain.

Over the last several decades, neuroscientists have learned that the brain does not treat memories, in the broadest sense, as a single format. Rather, our brains produce different types of memories that are acquired, coded and stored in different manners. For example, the way we remember the names of our friends and family is rather distinct from how we remember to drive a car or ride a bicycle. Our capacity to remember names and events is referred to as declarative memory, while our ability to ride a bicycle has been classified as a procedural memory. Furthermore, the division between procedural and declarative memories is not merely categorical, it is supported by the evidence that impairing or removing different parts of the brain may affect declarative but not procedural memory, and vice versa. These compartmental differences between types of memory raise interesting questions about how we store information about the world. The Knowlton lab is interested in a particular type of memory known as habit learning.

During the course of her presentation, Dr. Knowlton argued that much of our ongoing behavior is based on habits that allow us to use our attentional resources for other tasks. Habits, as we all know, are learned, and so must be stored somewhere in the brain. The key to understanding this process lies in converging data from humans and animal models indicating that habits depend on a brain structure known as the striatum. Dr. Knowlton discussed the notion that habit memories have distinct behavioral properties. Her results suggested that learning under distraction is likely to result in habit memories that are less flexible than memories learned under full attention. On the other hand, patients with disorders of striatum, including Parkinson's disease, may have difficulty with habit learning, and thus must effortfully and consciously retrieve information that would otherwise be retrieved automatically as a habit.

The M.R. Bauer Distinguished Lecturer Series Summaries 2010–2011

Introduction

Every year the Volen center is honored to receive two week-long visitors through the M.R. Bauer Distinguished Lecturer Series. This year our distinguished lecturers were Dr. Kristen Harris from the University of Texas at Austin and Dr. Claude Desplan from New York University. Both of our honorees spent the week speaking to small and large groups on interesting aspects of their research. The weeklong format is very special, as it allows the M.R. Bauer Distinguished Lecturers the time to visit our laboratories and engage with graduate students, postdoctoral fellows and often undergraduates working in our labs. These meetings are unhurried opportunities to pass their knowledge — and scientific wisdom — to these young scientists.

Kristen M. Harris, Ph.D.
Professor of Neurobiology
Fellow, Center for Learning and Memory
University of Texas at Austin
(Week of September 20, 2010)

Structural Substrate of Synaptic Scaling During LTP

In many ways, the work of Dr. Kristen Harris epitomizes this year's theme of the structure-function relationship. In her formal public lecture, one of the features of our week-long visitors, she highlighted how structural changes in dendritic spines, the small protrusions through which neurons receive information across synapses, will change over time in response to incoming signals. Work from the Harris lab has shown that dendritic spines will change their shape in response to different stimuli, thus altering their function.

The Harris Lab is interested in changes in synaptic structure that accompany and support learning and memory. Her lab studies long-term potentiation (LTP) in the developing and mature hippocampus, because LTP has many of the physiological characteristics that are expected to occur during learning and memory in the brain. The lab's working hypothesis is that structural synaptic plasticity serves to modify synapses in the creation of new memories, which competes with homeostatic mechanisms that serve to prevent saturation of synaptic strength and neuropathology. To probe this issue, Dr. Harris has focused on dendritic spines, which are the major postsynaptic targets of excitatory axons throughout the brain. Using cutting-edge imaging techniques, members of the Harris lab can measure dendritic spines and their presynaptic axonal partners using 3D reconstruction approaches. Use of these techniques has shown that neighboring spines can operate as independent compartments, and that dendritic spines can also share resources along a dendritic segment, which may serve to regulate the total amount of synaptic input that can be supported by a dendrite.

In addition to her formal public lecture, Dr. Harris also gave more focused talks to smaller audiences and led a neuroscience class for upper-division undergraduates and first-year graduate students. In her formal talk, titled "Structural substrate of synaptic scaling during LTP along mature hippocampal dendrites," she presented research demonstrating that LTP induced by a naturalistic pattern of stimulation results in several ultrastructural changes with time following the induction of LTP in hippocampal brain slices from mature rats. First she described in detail the new methods her laboratory has developed: (1) to rapidly fix brain slices to preserve synaptic and dendritic structure; (2) to provide unbiased sampling of the dendritic population that underwent LTP; and (3) to ensure that alteration in synapse number and size that occurs during slice preparation does not obscure the experimental effects.

Dr. Harris then went on to show new results that offered evidence for synaptogenesis through elevation in shaft and stubby spines, as well as filopodia, during the first 30 minutes following LTP. Later, by two hours after induction of LTP, small spines and their synapses were eliminated, while those that remained were significantly enlarged relative to spine synapses receiving only control stimulation. Presynaptic axonal boutons associated with the small spines were also eliminated. Only the spines with enlarged synapses contained polyribosomes, suggesting that local protein synthesis was needed to sustain the synapse enlargement. Furthermore, the presynaptic axonal boutons associated with synapses that were enlarged during LTP had fewer docked and reserve pool vesicles, suggesting that more vesicles were released, but that machinery needed to replenish them at the enlarged synapses had not yet been recruited to the presynaptic boutons by two hours after induction of LTP. Dr. Harris next described how synapse elimination was perfectly counterbalanced by synapse enlargement, providing strong evidence for structural synaptic scaling following synaptic plasticity. The Harris lab is extending these studies into the intact brain of behaving animals, and preliminary work from the lab suggests that LTP at the medial perforant pathway results in the sharing of resources even beyond the region of synaptic activation (and thus affecting pathways elsewhere along the dendritic arbor).

In a second lecture, Dr. Harris described the process of synaptogenesis and the formation of dendritic spines during development in the hippocampus using their methods of 3D reconstruction from serial electron microscopy at different ages. Animals are not born with dendritic spines, but instead have virtually spine-free dendrites that develop filopodia and shaft synapses during the first postnatal week. By about postnatal day 12, the first mature dendritic spines are beginning to form, and as animals mature more spines emerge. Dr. Harris linked the ontogeny of post-tetanic potentiation (PTP), which lasts only a few seconds, to the beginning of synaptogenesis. Short-term potentiation (STP), lasting about two hours, then takes over at postnatal day 10, before spines have formed. Enduring LTP lasting longer than three hours was linked to the formation of dendritic spines. Dr. Harris presented a new induction paradigm that will allow researchers to test whether stimulation that induces enduring LTP after postnatal day 12 can be used to shift from STP to enduring LTP when given twice. This new paradigm should provide new insights into how experience shapes and prepares dendrites and their synapses for experiences as the animals mature and face new challenges in their environments.

Claude Desplan, D.Sc., Ph.D.

Professor of Biology
New York University
(Week of March 21, 2011)

Color Vision in *Drosophila*

Our capacity for vision is one we often take for granted (at least until something goes wrong). In order for us to view the world, a remarkably large number of circuits and mechanisms must work in concert to construct the perception of a visual environment. Dr. Claude Desplan studies vision in the fruit fly, Drosophila melanogaster, *whose compound eyes contain hundreds of retina-like structures known as ommatidia. These ommatidia contain several types of neurons, which act in a manner similar to the rods and cones that are integral for human vision. Dr. Desplan is interested in how these different types of neurons arise in the ommatidia of the fruit fly and how the signals from these cells are interpreted and integrated by the fly brain.*

The fruit fly *Drosophila* uses a "compound eye" for visual functions. It is made up of 800 unit eyes called ommatidia that form an image with 800 pixels. Each ommatidium contains eight photoreceptors. Six of the photoreceptors (called R1-R6) are like the human rods; they are involved in motion detection and are identical in all ommatidia. The remaining two photoreceptor types (R7 and R8) play a major role in color vision and differ in different parts of the retina: *p* ommatidia contain an R7 photoreceptor that is UV-sensitive, while *p* R8 photoreceptors are blue-sensitive. The *y* ommatidia have an R7 that is sensitive to a different UV light wavelength and an R8 that is green-sensitive. The *p* and *y* subsets are distributed stochastically throughout the retina in a 30:70 ratio. Comparison between R7 and R8, and between *p* and *y* ommatidia, allows flies to discriminate between colors, with *p* ommatidia involved in the detection of short wavelengths and *y* ommatidia for longer wavelengths. In the dorsal rim area of the retina (DRA), ommatidia serve to measure the vector of light polarization for navigation on cloudy days. A fourth subset located in the dorsal third of the eye serves to detect the orientation of the sun for navigation on sunny days.

Both of Dr. Desplan's talks focused on the cascade of genes that specify the different subsets of photoreceptors through a series of fate restrictions and how this cascade is modified to define the various regions of the retina in *Drosophila*. The gene *homothorax*, for example, is required for the formation of DRA ommatidia, while another gene called *spineless* is expressed in a stochastic manner in a subset of R7 cells (*y* R7). These molecular mechanisms allow for the specification of the whole retina by specifying the *y* choice in R7 and allowing R7 to instruct R8 of its choice. Finally, *Iro*C genes determine the region where ommatidia detect the orientation of the sun and co-express Rh3 and Rh4 in R7 of the *y* subtype.

Processing of color information occurs in the medulla part of the optic lobes that receives input from R7 and R8. The medulla is formed by about 40,000 neurons surrounding a neuropil where photoreceptors and medulla neurons interconnect. Associated with each set of R7/R8 projections, there are about 800 "columns," which are the functional units in the medulla. Dr. Desplan described his work addressing how more than 70 cell types are specified in the medulla and connect to photoreceptors in a retinotopic manner to process color information. This information is then sent to higher brain centers in the lobula complex and central brain to mediate color behavior. The function of each of these neuronal subtypes was addressed by silencing small populations of medulla neurons and testing the consequence for color discrimination. For this purpose, the Desplan lab utilizes a flight simulation paradigm, where the fly is trained to associate color with a reward or punishment before being tested in the absence of the reward. Further insight into the mechanisms underlying color discrimination will hopefully shed light on the fundamental processes likely to be shared by all visual systems, from flies to mammals.

Volen National Center for Complex Systems Scientific Retreat 2011

On April 25, 2011, the Volen National Center for Complex Systems held its annual scientific retreat. This year the event took place at the Warren Conference Center, located in rural Ashland, Mass. This location served as an ideal venue for the exchange of scientific ideas between members of the Brandeis neuroscience community. Of special note, it allowed students and faculty alike to take a break from their daily activities, broaden their perspectives and enjoy the company of their colleagues. It is through these retreats that new collaborations among Volen Center faculty members often arise.

During the course of the retreat, four Volen Center researchers (professors Stephen Van Hooser, Avital Rodal, Pengyu Hong and David DeRosier) shared their work by giving talks to the full Brandeis neuroscience community in attendance. As will become evident in the following summaries, these presentations described cutting-edge imaging techniques and what they tell us about form and function. We were delighted to have as our keynote speaker Dr. Aniruddha Das from Columbia University, who discussed his research on regional blood flow in the brain (brain hemodynamics) and what this tells us about mental function. Finally, and no less important, the retreat served as a forum for graduate students to share their work in a poster session where they received valuable feedback from peers and faculty.

Monday, April 25, 2011

9:00–10:00 a.m.
Arrival and breakfast

10:05–10:15 a.m.
Arthur Wingfield, D.Phil.
Nancy Lurie Marks Professor of Neuroscience and
Director, Volen National Center for Complex Systems
Brandeis University

10:15–11:15 a.m.
Aniruddha Das, Ph.D.
Associate Professor of Neuroscience and Psychiatry
Columbia University
"Brain Hemodynamics: So Much More Than Just a Measure of Local Neural Firing"

11:15 a.m.–noon
Stephen Van Hooser, Ph.D.
Assistant Professor of Biology and Volen National Center for Complex Systems
Brandeis University
"Choosing Which Way to Go: the Development of Direction Selectivity in Visual Cortex"

Noon–1:00 p.m.
Lunch

1:00–2:00 p.m.
Student poster session

2:00–2:45 p.m.
Avital Rodal, Ph.D.
Assistant Professor of Biology and Volen National Center for Complex Systems
Brandeis University
"Synaptic Growth: Signals on the Move"

2:45–3:30 p.m.
Pengyu Hong, Ph.D.
Assistant Professor of Computer Science and Volen National Center for Complex Systems
Brandeis University
"High-Content Neuronal Screening"

3:30–4:15 p.m.
David DeRosier, Ph.D.
Professor of Biology, Emeritus, Abraham S. and Gertrude Burg Chair of Life Sciences
and Volen National Center for Complex Systems
Brandeis University
"Cryo-PALMing the Synapse"

4:15–5:15 p.m.
Mingling, coffee and sweets

Aniruddha Das, Ph.D.
Associate Professor of Neuroscience and Psychiatry
Columbia University

Brain Hemodynamics: So Much More Than Just a Measure of Local Neural Firing

For the brain to function it needs oxygen. To support this need there exists a complex circulatory system that provides the appropriate levels of oxygenation according to changing energy needs to follow regional brain activity. For this reason the relative level of blood flow to different brain regions can serve as a correlate and measure of local activity within the brain, and it serves as the basis for blood oxygen level dependent (BOLD) functional magnetic resonance imaging (fMRI). Dr. Das' work has demonstrated that changes in blood flow are not merely a passive response to increased brain activity, but rather, may actually be involved in the anticipation of activity changes. Dr. Das' work has begun to shed light on how dynamic structural changes in the circulatory system assist neuronal function in an anticipatory fashion.

Brain imaging — such as functional magnetic resonance imaging (fMRI) — is based not on measuring neural activity but rather on brain hemodynamics, which refer to local changes in blood volume, blood flow and oxygenation. Despite the explosive current growth in the use of brain imaging, the relation between the hemodynamic imaging signal and local neural activity is still poorly understood. It is typically assumed that the imaging signal is uniformly a consequence of local neural responses. Specifically, the signal is assumed to arise when increased neural activity consumes oxygen in the blood locally and triggers a local inflow of fresh oxygenated blood. However, this assumption is based on studies in anesthetized animals. Very little is known about the links between the imaging signal and neural activity in alert animals engaged in behavioral tasks, an experimental situation that better represents the typical imaging study in humans.

Dr. Aniruddha Das' lab addresses this issue by recording neural signals through the use of implanted electrodes, while simultaneously imaging with fMRI in the brains of alert monkeys while they perform a visual discrimination task. Their studies have revealed a complex relationship between brain hemodynamics and neural activity. This complexity is evident at two levels. First, the Das lab has found that when the animals are engaged in a systematic visual task, the visual region V1 hemodynamic signal contains a strong task-related component in addition to the visually evoked response. The task-related component is a novel anticipatory signal, independent of visual input, that dilates local arteries and brings in fresh blood in time for expected visual trials. Notably, this signal is not predicted by local neural spiking, in sharp contrast to the trial-related signal that is predicted to better than 95 percent accuracy by spiking. Next, researchers in the Das lab found that even the visually evoked hemodynamic signal is likely not driven by deoxygenation in the blood as is often believed. On the contrary, visual stimulation first triggers a rapid increase in blood volume leading to an increase rather than a decrease of blood oxygenation. This stimulus-triggered blood volume increase presumably reflects an immediate dilation of local blood vessels, occurring in parallel with the increased neural activity, so as to bring additional blood to the cortex before the increased demand can lead to deoxygenation of the blood.

The Das lab is currently pursuing the consequences of this finding, both in its implications for interpreting brain images and the insights it may offer into brain processing. For brain imaging, they showed that the trial-related signal adds linearly with the stimulus-evoked signal in periodic tasks. The trial-related signal can be large, grossly distorting the net signal. However, subtracting a "blank trial" baseline leaves just the stimulus-evoked component that is robustly correlated with local spiking. The nature of the trial-related signal and its relevance to brain processing is still an open question, however. The signal is strongest when the animal is fully engaged in the task, and it changes character dramatically when the animal commits an error. However, unlike visuospatial attention, the signal is not spatially localized. It is correlated with fluctuations in heart rate and pupil dilation and thus likely reflects an autonomic process, possibly mediated by neuromodulatory input to V1. However, it is not global arousal: it is modality specific, being present in V1 only for visual and not auditory tasks. The goal of understanding these issues and exploring the significance of this novel signal forms the core of the Das research program.

Stephen Van Hooser, Ph.D.
Assistant Professor
Brandeis University

Choosing Which Way to Go: the Development of Direction Preferences in Primary Visual Cortex

At this year's Volen retreat we were pleased to hear from Dr. Stephen Van Hooser, who is one of our more recent additions to the Brandeis neuroscience faculty. Dr. Van Hooser's work focuses on a particular class of neurons within the visual cortex that have the property of responding only to features in the visual field that are moving in a particular direction. Dr. Van Hooser's work has demonstrated that this neuronal characteristic is by no means an intrinsic property, but rather a preference that arises as a result of visual experience.

The proper development of brain circuits critically depends on interactions between processes that are specified genetically and those that require sensory experience. The Van Hooser lab studies these interactions in the ferret visual cortex. At the time of eye opening, neurons in the ferret visual cortex will selectively respond to bars of light or edges that have a particular orientation. However, at this stage there is no selectivity in the direction of motion, a property that arises later as the animal matures. Dr. Van Hooser's question is how directional selectivity arises in visual cortex.

In his presentation, Dr. Van Hooser presented evidence that visual experience is required for the development of direction selectivity. This is evidenced by the fact that animals raised in the dark and not receiving visual stimulation will fail to acquire direction selectivity. Furthermore, exposing animals to a motion-training stimulus causes a more rapid emergence of direction selectivity in visually naive animals. Using advanced microscopy, Dr. Van Hooser and colleagues monitored how the response properties of individual cells were altered by this training stimulus. The direction preference that neurons would eventually acquire could be predicted based upon small initial biases that were present at the time of eye opening. This indicates that processes independent of experience play a role in determining a cell's future direction preference. However, these initial biases are not immutable, as exposure to a single direction of motion causes a majority of neurons to develop a preference for the trained stimulus direction. Finally, Dr. Van Hooser presented preliminary evidence indicating that cells in the layer of cortex that receives the primary projection from the retina, via a structure called the lateral geniculate nucleus, may acquire direction selectivity before cells in other layers. Future experiments will continue to uncover the circuit mechanisms underlying the development of direction selectivity in cortical circuits.

Avital Rodal, Ph.D.
Assistant Professor
Brandeis University

Synaptic Growth: Signals on the Move

Dr. Avital Rodal, the newest member of the Brandeis neuroscience faculty, examines how neurons set up elaborate branches that are tailored to send and receive electrical signals over large distances and through complex networks of connections. These connections undergo dynamic structural and functional changes in response to external growth cues, which provides the basis for synaptic plasticity during both development and adulthood. The Rodal lab is focused on understanding how these growth cues are interpreted and manipulated within the cell and how their misregulation contributes to neurological disease.

Growth factors are received by membrane-bound cell surface receptors. Afterwards, the cell surface receptors are transported into the cell along with the pieces of membrane they are bound to. The membrane-associated receptors are then shuttled from one compartment to another. Interestingly enough, these growth factor receptors change their signaling properties depending on the compartment in which they are located. Therefore, the degree to which growth factor signaling occurs can be modulated based upon the rate at which these receptors move between compartments. These cellular events play a prominent role in the disease-related defects of Alzheimer's disease (AD) and Amyotrophic Lateral Sclerosis (ALS) and may also serve as a potential point of intervention for these diseases. The challenge is to understand how networks of hundreds of interacting membrane-deforming proteins, which are responsible for moving these receptors, are able to control cargo traffic. The Rodal lab seeks to understand how these proteins might themselves be regulated.

One powerful method to probe the cellular function of complex networks of interacting proteins is to visualize them in action using high-resolution microscopy wherein proteins of interest are fluorescently labeled. The Rodal lab is able to take advantage of recent advances in fluorescence microscopy that have opened new windows into cellular events within complex tissues. These advances have increased the speed and resolution at which rapid cellular dynamics can be recorded and analyzed. Dr. Rodal's group has used these new techniques to devise time-resolved trafficking assays for imaging membrane traffic in fruit fly neurons. The advantages of using the fruit fly system are that the components of the membrane traffic machinery are highly similar to their human counterparts, and that fruit flies can be genetically manipulated to express fluorescently tagged proteins allowing researchers to follow the dynamics of their protein of interest.

Dr. Rodal is also using the synapses that form between motor neurons and muscles (neuromuscular junctions) as a model. Using this system, the Rodal lab has shown that dynamic interactions between subcellular membrane compartments lead to the transfer of growth factor signaling receptor cargo from one compartment to another. The Rodal lab has also found that this transfer results in a reduction of receptor signaling activity and a concomitant reduction in neuronal growth. As a next step in these studies, the lab is developing new methods to image receptor traffic in more complex neuronal arbors in the central nervous system, as well as in fruit fly models of aging and neurodegenerative disease. By exploring the connection between disease states and growth-signaling pathways, the Rodal lab is uncovering specific changes in membrane traffic that occur during normal development and disease and that are important for neuronal growth and survival.

Pengyu Hong, Ph.D.
Assistant Professor
Brandeis University

High-Content Neuronal Screening

With estimates for the number of neurons in the human brain falling in the range of billions, and the number of connections they form being orders of magnitude larger, understanding the interactions operating within the nervous system is a Herculean task. For this reason computational methods of analysis are becoming increasingly important. Dr. Hong described his work in which he is developing methods for sorting through massive amounts of imaging data taken from neuronal cultures in order to extract information about how the morphology of these cells changes over time in response to varying conditions.

High-content neuronal screening has recently become a powerful high-throughput methodology for identifying chemical compounds that regulate neuronal morphology. Such compounds are able to change the patterns of neurite outgrowth and the size distributions of cell clusters. Studies examining the effects of treating neurons with these chemicals are expected to be an important approach in examining the nervous system, as well as aiding drug discovery. A typical high-content neuronal screening project generates hundreds of thousands of high-resolution images containing many interconnected neurons whose morphologies are complex. Therefore, it is a great challenge to be able to accurately and automatically analyze these data. In his presentation, Dr. Hong explained how his group is tackling this problem using their own automated method of analysis.

This method has been one of the key innovations to enable high-throughput genetic and drug discovery screening using neuronal cells. It was applied to the data provided by his collaborators around the world. Their computational results have led to successful follow-up studies. For example, Dr. Hong and his collaborators have identified a set of neural outgrowth genes in a genome-wide gene knock-down screen. Several of these genes were then validated using mice or fruit flies as model systems. In a follow-up study, Dr. Hong's collaborators characterized one of these genes, the *Drosophila* homolog of *Phocein*, as a regulator of axonal transport, membrane excitability and organization of microtubule networks. This successful finding served as a proof-of-principle for Dr. Hong's approach.

Dr. Hong went on to describe two other successful applications of this method. First, Dr. Hong and his collaborators were able to identify several promising drug candidates in a chemical-compound screen using a *Drosophila* model of Huntington's disease. Second, the group gained new insights into the impact of three compounds: phenylalanine, phenylpyruvate and phenylacetate on the activity of the myelin basic protein promoter and the production of myelin sheaths. These successes demonstrate that the high-throughput analysis methods employed by Dr. Hong and his colleagues have promising implications for our understanding of the nervous system, as well as for developing therapeutics related to neurological disorders.

David DeRosier, Ph.D.
Professor Emeritus
Abraham S. and Gertrude Burg
Chair of Life Sciences
Brandeis University

Cryo-PALMing the Synapse

Science is firmly rooted in observation, and it is observation that ultimately serves as the foundation of the scientific method. For centuries light microscopy has allowed biologists to observe the structure or form present at very fine scales enabling them to hypothesize how these observed structures might be linked to important life functions. So it is appropriate that the last speaker at this year's retreat was Dr. David DeRosier, who has been developing ways to enhance light microscopy that may allow us to observe biological processes on an even smaller scale than previously thought possible.

Dr. DeRosier's work centers on determining the organization for the hundreds of different proteins at the synapse, a question that is of great interest in the field of neuroscience. A fundamental problem with examining the location of these proteins lies within the physical limitations of various imaging techniques. For example, the electron microscope is limited in its ability to produce images of "large" structures like the synapse. Furthermore, electron microscopy severely restricts a researcher's ability to examine proteins of interest. However, electron microscopy can provide images of the morphological features at molecular resolution. Conversely, super-resolution, fluorescence light microscopy, not being limited by Rayleigh resolution, can visualize at least two fluorescently labeled proteins at once, as well as survey many synapses in a single field of view and can localize the labeled proteins within a few nanometers. However, it cannot visualize the protein components but, rather, only determine their locations. Thus a combination of the two methods provides what each method alone cannot. Dr. DeRosier is developing a cold stage that will enable researchers to carry out super-resolution fluorescence microscopy while preserving the specimen for subsequent electron cryo-microscopy.

Super-resolution fluorescence light microscopy has one main underlying idea: the centroid of a photon can be mapped based on the distribution of light coming from a single fluorophore. This method works best at extremely cold temperatures. Lowering the temperature increases the number of photons each fluorophore emits prior to bleaching. The large increase in the number of photons means an improvement in the precision of fluorophore localization. To accomplish this goal, Dr. DeRosier and colleagues have designed a cold stage, which cools the specimen during imaging but allows the user to operate the microscope at room temperature. The cryo-stage, when complete, will be compatible with a conventional upright fluorescence microscope. The plan is to set up a steady-state situation in which cold gas flows under the specimen to keep it cold, while room-temperature immersion water flows between the objective and the cover slip so as to prevent the water from freezing. The stage consists of a copper finger cooled by cold-flowing nitrogen gas. The frozen-hydrated sample sits atop the copper finger. A collar is fitted over the objective lens, and the immersion water flows between the objective lens and the cover slip, which forms the bottom of the collar. The objective and cover slip move as a unit relative to the specimen. A cryogenic liquid optically couples the bottom of the cover slip to the frozen-hydrated sample so that the full numerical aperture of the objective lens is available. Dr. DeRosier has built a prototype version of this stage and is currently in the process of testing it.

Acknowledgments

We thank the speakers who came to the Brandeis campus this past year to share with us their research and to engage in many hours of stimulating discussion and exchanges of ideas with Volen Center faculty, graduate students and postdoctoral fellows. We are also grateful to our visitors for forwarding to us their lecture summaries, which form the basis of this report.

We especially acknowledge Justin Slawson, Ph.D., a recently graduated doctoral student in biology, and Sean O'Toole, a current graduate student in neuroscience, for their valuable contributions and editorial assistance in the preparation of this report.

The text of this summary of the Bauer Foundation series, along with summaries from previous years, can be found at: www.bio.brandeis.edu/bauer/previous.html.

Office of Publications

C079/TPI