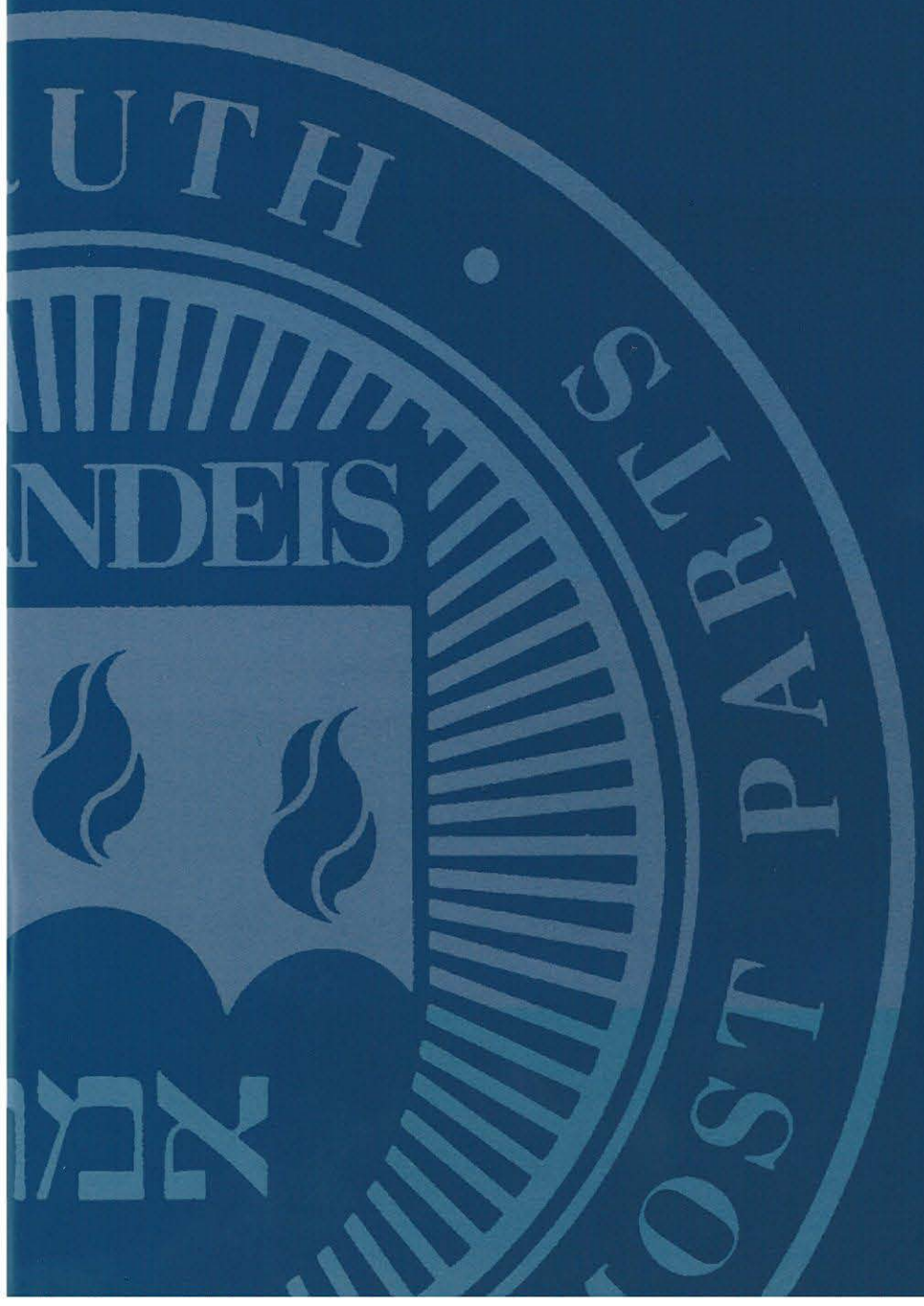
Brandeis University

The Volen National Center for Complex Systems

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

August 2002



The M.R. Bauer Foundation Colloquium Series, Distinguished Lecturer Series, and Scientific Retreat 2001-02 Summary

Brandeis University

Benjamin and Mae Volen National Center for Complex Systems

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Introduction

The 2002 M.R. Bauer Foundation Colloquium Series, Scientific Retreat, and Distinguished Guest Lecturer Series

I am very pleased to present this year's proceedings of the M.R. Bauer Foundation Colloquium Series, Scientific Retreat, and Distinguished Guest Lecturer Series at Brandeis University's Volen National Center for Complex Systems. Now in its eighth year, the generous support of the M.R. Bauer Foundation has enabled the Volen Center to mount an impressive series of lectures and informal interactions that reflect some of the most exciting new developments in neuroscience. An important part of the Volen Center's mission is to make known the results of its quickly advancing work and to offer a forum to discuss them. My colleagues and I at the Center would like to express our thanks to the M.R. Bauer Foundation for its ongoing support that has facilitated learning and communication among the faculty and students with many of the leading practitioners of neuroscience.

The M.R. Bauer Colloquium Series hosted six speakers in 2001-02. Focusing on learning and memory, a group of speakers highlighted advances in understanding the plasticity, or adaptability, of the brain during these dynamic processes. **Howard Schulman**, Ph.D., from the Department of Neurobiology in Stanford University's School of Medicine, spoke about the role of a critical regulatory molecule, CaM kinase II, that may help to form the biochemical "switch" for memory. Synaptic connections between neurons form during the brain's development according to their ongoing use. During learning and memory, the effectiveness of the information transmitted through the synapses is modified and strengthened. Calcium serves as an intracellular signal that triggers a switch as the neuron is stimulated. **György Buzsáki**, M.D., a member of Rutgers University's Department of Neuroscience, discussed the maintenance of firing rates and patterns in hippocampus cells. Although the brain is constantly changing, humans maintain a subjective sense of continuity in who they are and what they can do. Buzsáki suggested that bursts of firing neurons act as a mechanism that creates the equilibrium necessary for this continuity. **David Linden**, M.D., from the Department of Neuroscience at The Johns Hopkins University School of Medicine, considered the molecular basis of motor learning in the brain's cerebellum. The goal of his work is to create a comprehensive model of learning that links molecules, synapses, cells, networks, and behaviors.

Vision is one of the most challenging areas of neuroscience. A number of Bauer Colloquium speakers described recent research developments in the neuroscience of vision. **Richard Thompson**, M.D., from the University of Southern California's Department of Brain and Cognitive Sciences, has studied the eyeblink as a model of Pavlovian conditioning. In a series of experiments using electrophysiological recordings, lesions, stimulation, and anatomical pathway tracing, he identified a brain circuit necessary for this behavior in the anterior interpositus of the cerebellum. When protein synthesis in this region of the brain is blocked, learning of this response is prevented. **Dan Pollen**, M.D., a member of the Department of Neurology at the University of Massachusetts Medical School, addressed the elusive and controversial neural correlates of visual perception. Although some scientists have claimed that a necessary condition of visual experience is the interaction between neural representations of an image with representations of a sense of self, scientists have not yet specified the sufficient conditions of vision. **Alvaro Pascual-Leone Garcia**, M.D., affiliated with Beth Israel Deaconess Medical Center and Harvard Medical School, talked about a closely related subject, visual awareness.

Now in its fourth year, the M.R. Bauer Distinguished Guest Lecturer Series brought two well-known scientists to campus in the spring for extended visits. **Julian Jack**, M.D., a Fellow of the Royal Society of London, is affiliated with the University Laboratory of Physiology at Oxford in England. One of the senior figures in neuroscience, he is known for his outstanding theoretical and experimental contributions to our understanding of synaptic transmission, the way in which impulses are sent between nerve cells, in the central nervous system. He has made the important analysis of the spread of electric signals along the dendritic cable system of neurons in the spinal cord. Jack's development

of quantal analysis, or the measurement of the electrical output of neurons, has been important for understanding how nerve cells receive information and integrate it. His public lecture, "Fifty Years of Quantal Analysis: What Have We Learnt?" addressed the ongoing questions about the reliability of this research. Because the readings sometimes produce spurious peaks in output, the approach may be unable to support any conclusions. However, Jack asserts that quantal analysis is ideal for separating pre- and post-synaptic effects, the transmissions recorded on the input or the output sides of the synapse. More recent quantal recordings may be helpful in sorting out pre- and post-synaptic events.

The year's second M.R. Bauer Distinguished Guest Lecturer was **Mary B. Kennedy**, M.D., professor of biology at the California Institute of Technology. Kennedy has done some of the most important biochemical work in neurobiology on CaM kinase II, the calcium molecule that may be the key to how the brain stores new information. Her research has focused on the molecular structure and function of synapses in the central nervous system. While her work has been devoted to taking apart the synapse and describing each of the molecules that plays a role there, her public lecture took a different tack—"Putting the Synapse Back Together." Different regions of the brain have been identified as the locations of different kinds of memory—the dorsolateral prefrontal cortex, for example, with working memory of the most recent 10 to 20 seconds, and the hippocampus with memory in the range of days to weeks. Is there synaptic specialization that underlies specialization in different parts of the brain? No one knows yet whether different signaling systems are the basis of different functions, but answering this question will help us understand how the brain functions as a whole. Kennedy advised that simulations of synapses must include the spatial arrangements of proteins in the membrane because they determine the specificity of the reactions that create the post-synaptic density relevant to memory storage.

The 2002 Volen Center Retreat sponsored by the M.R. Bauer Foundation addressed "Cellular and Molecular Approaches to Neuroscience." As in previous years, the retreat was held at the Marine Biological Laboratory at Woods Hole, Massachusetts, on March 15 and 16. Among this year's speakers, **Michael Welte**, Ph.D., W.M. Keck Assistant Professor of Biology and Rosenstiel Basic Medical Sciences Research Center at Brandeis, described his work on the tiny motors that move organelles within cells. While scientists understand the motors, they do not yet understand the mechanisms that allow cells to deploy them in a regulated way. Using genetic, biochemical, and biophysical methods to study the regulation of lipid droplet transport in cells, Welte has isolated mutations in the regulator gene called *klar* that may be responsible. Because this gene is also important for nuclear migration during eye development, it is likely essential for intracellular transport in general. **Donald Katz**, Ph.D., who was recently appointed an assistant professor of psychology at Brandeis, spoke about taste perception. While a single neuron may be maximally responsive to different tastes at different times, there may in fact be three times as many neurons involved than previously reported. Taste perception is a dynamic process, Katz asserted, involving interactions at multiple spatial and temporal scales. **John S. Satterlee**, Ph.D., a postdoctoral researcher in the laboratory of Associate Professor of Neurobiology **Piali Sengupta**, spoke about his work on the function and development of neurons that control our sense of temperature. Thermosensation is one of the most poorly understood senses, even though it plays a critical role in regulating behavior and metabolism. Satterlee has identified four genes that regulate the thermosensory neuron, and has linked one of the genes to a role in adaptation to some odorants as well. **Sacha Nelson**, Ph.D., an associate professor of biology and Volen National Center for Complex Systems at Brandeis, presented his work elucidating how neurons in the visual cortex respond to special features or patterns in the world. He is examining single cells in order to determine how their structures affect their ability to integrate visual information, as well as networks of cells, in order to understand how the brain's selective response to visual stimuli may arise from cooperative interactions. Scientists are debating whether cortical neurons transmit information primarily in their firing rates or in the precise timing of their spikes. Nelson addressed the related issue of which features of spike trains control

plasticity at cortical synapses. His results, showing joint dependence on the rate and the relative timing of firing, hold important implications for which parts of the neural code are most readily stored for retrieval. **Matthias Soller**, Ph.D., a postdoctoral researcher in the laboratory of Professor of Biology **Kalpana White**, spoke about his work on the posttranscriptional mechanisms of gene regulation involved in the development and function of the nervous system. This process can substantially change the outcome of the information encoded on the DNA. His work focuses on the erect wing protein in the fruit fly, which he shows is necessary for proper neuronal function underlying coordinated movement. Finally, **Michael Rosbash**, Ph.D., professor of biology and a Howard Hughes Medical Institute Investigator at Brandeis, described some of the most important discoveries he has made about the molecular and genetic components of biological clocks, crucial for sleep disorders and depression. His work is responsible for bringing the study of circadian rhythms into the modern molecular era. He is now exploiting new microarray technologies to show how certain genes control biological rhythms, enhancing our understanding of fruit flies as well as of mammals.

Over the past eight years, the M.R. Bauer Foundation Colloquium and Scientific Retreat have helped to promote the exchange of ideas and methods and to advance the study of neuroscience. In the past four years, the M.R. Bauer Distinguished Guest Lecturer Series has brought some of the most accomplished neuroscientists to the University. Both programs have helped to create a sense of community among the neuroscientists at Brandeis and those who come to Brandeis from elsewhere. These proceedings comprise an essential part of the Volen Center's effort to encourage scientific collaboration and discussion. I am especially pleased to recognize the support of the M.R. Bauer Foundation for continuing to make these activities possible through its generous funding.

Laurence F. Abbott, Ph.D.
Nancy Lurie Marks Professor of Neuroscience and Director, Volen National Center for Complex Systems

Howard Schulman, Ph.D.
Department of Neurobiology
Stanford University School of Medicine
Stanford, California
October 8, 2001

Spatial and Temporal Regulation of Calcium Signals by CaM kinase II

The nervous system consists of billions of neurons, each making thousands of contacts, termed synapses, with other neurons. Unlike an electrical circuit in a computer chip, however, the nervous system is not hard-wired based on a predesigned template. Rather, synaptic contacts between neurons are formed and modified during development of the brain based on their ongoing utilization. Even after contacts are made, the effectiveness of the information transmitted through such synapses is modified during learning and memory. Neuronal networks with modifiable synaptic contacts enable us to be conscious thinkers, to learn from experience, have complex emotions, and be creative in the arts and sciences. One of the central tenets of molecular and cellular neurobiology is that even complex functions such as learning and memory are composed of elementary units subject to experimental discovery.

The collective efforts of many neuroscientists in the past three decades have successfully reduced certain forms of learning and memory to a fundamental biochemical switch that regulates the efficacy of synaptic transmission at individual synapses. Work in my laboratory has focused on the identification of a critical regulatory molecule, termed CaM kinase II, which may be intimately involved in this memory switch.

Nerve cells respond to sensory inputs originating in the environment by "firing" an electrical pulse that travels to the tips of the nerve cell to elicit release of chemical transmitters onto receptive neurons at synapses. It is intriguing that receptive neurons respond to a relatively brief increase in input firing frequency with a long-lasting change in efficiency of synaptic transmission, an elementary form of memory. A transmitter molecule called glutamate is released onto the neuron. A recognition protein on the receiving neuron (termed NMDA receptor) detects glutamate and responds to high frequency glutamate release by allowing calcium ions to flow into the neuron. Calcium serves as an intracellular signal for the presence of glutamate outside and triggers a switch in the response of the neuron to subsequent stimulation.

Many years ago, I found the link between calcium entry and the ultimate change in synaptic strength, a decoder of calcium called CaM kinase II. It is a protein catalyst that is activated by elevated calcium, leading to several consequences. First, CaM kinase II is very cleverly designed to allow high frequency stimulation to produce a prolonged "on" state. Calcium transiently activates the kinase by displacing an inhibitory gate that normally suppresses its catalytic activity. A segment of the NMDA receptor recognizes the activated enzyme and recruits it to the synapse by wedging itself in the "hinge" of this gate. This keeps the gate open and the kinase active even after calcium levels return to baseline, a short-term potentiation. Furthermore, the active kinase chemically modifies its inhibitory gate with a phosphate group that disables the gate and keeps it open until the phosphate is removed by another catalyst. In fact, Lisman and Zhabotinsky at Brandeis have suggested conditions under which the autocatalytic addition of phosphate exceeds its removal, producing a memory switch. Other investigators have shown that the "on" state of the kinase is necessary and sufficient to produce some of the alterations at synapses that are responsible for the increase in synaptic efficacy that is seen in this form of learning and memory. These studies demonstrate features of CaM kinase II that enable it to maintain
an "on" state that supports a synaptic switch. It explains why mice with mutations that block the autocatalytic function of CaM kinase II are deficient in learning and memory.

David Linden, M.D
Department of Neuroscience
Johns Hopkins University School of Medicine
Baltimore, Maryland
February 4, 2002

Cellular Substrates of Cerebellar Motor Learning

This presentation will focus upon the molecular basis of motor learning in the cerebellum. The cerebellum is an unusually good model system where it is possible to build a comprehensive model of learning that flows from molecules and synapses continually through cells and circuits, culminating in behavior. Recent work from my lab using electrophysiological and imaging techniques will be combined with that of others to create a complete and testable hypothesis for a form of learning in the mammalian brain.

Over the last 20 years, a series of experiments that have used behavioral tasks together with extracellular recording, reversible inactivation, and transgenic manipulations have produced a strong case that the cerebellum is critical for these forms of motor learning. In particular, LTD and LTP of the parallel fiber-Purkinje cell synapse have been implicated in the acquisition and extinction of eyeblink conditioning, respectively.

The goals and objectives of the research performed in this lab are: to provide an overview of the cerebellar circuit and its proposed role in motor learning; to summarize recent molecular insights into synaptic phenomena such as long term potentiation and depression which are suggested to underlie memory; and to look towards the future in considering how molecular genetics can be used to ultimately provide a test of the present comprehensive hypothesis.

This laboratory has used both electrode and optical recording in cerebellar slice and culture model systems to explore the molecular requirements for induction and expression of these phenomena. In particular, we (and others) have found that induction of LTP in the parallel fiber synapse requires a presynaptic cascade of Ca influx/adenylyl cyclase I/cAMP/PKA and that its expression is also presynaptic.

In contrast, induction of LTD at this synapse is triggered by postsynaptic activation of mGluR1 and AMPA receptors together with Ca influx, resulting in activation of PKC and consequent clathrin-mediated internalization of AMPA receptors. In addition, inhibition of postsynaptic protein phosphatase activity through a cascade involving NO, cGMP, and cGMP-dependent protein kinase may be important.

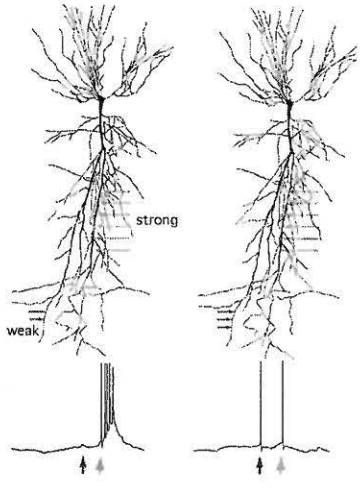
Along the way, we discovered a new form of plasticity, LTD at the climbing fiber-Purkinje cell synapse, which was not anticipated in models of cerebellar learning and which appears to share some induction requirements with parallel fiber LTD. In addition, we have expanded our analysis to include use-dependent synaptic as well as non-synaptic plasticity in the cerebellar output structure, the deep nuclei. At the level of basic science, these investigations are central to understanding the cellular substrates of information storage in a brain area where the behavioral relevance of the inputs and outputs is unusually well defined. In addition, these investigations have potential clinical relevance not only for cerebellar motor disorders, but also for disorders of learning and memory generally.

György Buzsáki, M.D
Department of Neuroscience
Rutgers University
Newark, New Jersey
February 11, 2002

Maintenance of Firing Rates and Patterns in Hippocampal Pyramidal Cells in Vivo

Anatomical and molecular biological observations suggest that the brain is in a perpetual state of change. In contrast to this change of the substrate is our subjective experience of certain constancy of our biographical data and skills we acquired. I suggest that constancy can be maintained in the ever-changing anatomical substrate by rehearsal or repetition. Reuse of the network storing information can occur consciously or while we are asleep. To address this issue we examined how the long-term firing rates and patterns of pyramidal cells are maintained and regulated in different behavioral states. In a familiar environment, the discharge frequency of simultaneously recorded individual CA1 pyramidal neurons and the coactivation of cell pairs remained highly correlated across sleep-wake-sleep sequences in rats. However, both measures were affected when new sets of neurons were activated in a novel environment. Nevertheless, the grand mean firing rate of the whole pyramidal cell population remained constant across behavioral states and testing conditions. The findings suggest that long-term firing patterns of single cells can be modified by experience. We hypothesize that increased firing rates of recently used neurons are associated with a concomitant decrease in the discharge activity of the remaining population, leaving the mean excitability of the hippocampal network unaltered. Next we discuss the physiological basis of this homeostatic process.

Pyramidal neurons fire not only single spikes but also bursts of spikes. Other investigators suggested that bursts are particularly important in the induction of synaptic plasticity. However, the conditions necessary for burst induction are not known. CA1 pyramidal cell burst activity was examined in behaving rats. The fraction of bursts was not reliably higher in place field centers, but rather in places where discharge frequency was 6-7 Hz (theta oscillation frequency). Burst probability was lower, and bursts were shorter, after recent spiking activity than after prolonged periods of silence (100ms-1s). Burst initiation probability and burst length were correlated with extracellular spike amplitude and with intracellular action potential rising slope, indicating that intrinsic properties of the neurons are responsible for the "competition" between single spikes and complex spike bursts. Thus, single spikes triggered by a weak afferent input may suppress the later induction of a burst by a strong input. Thus, the subthreshold or suprathreshold nature of one input determines whether another (strong) input produces a burst or not. Given the suggested importance of burst discharges in synaptic plasticity, our observations provide some interesting possibilities for the regulation of discharge rate in pyramidal neurons.



(Figure) Several findings support the importance of the temporal order of presynaptic and postsynaptic activity in neuronal plasticity: a weak input, eliciting an EPSP, followed by a strong, burst-inducing input is a necessary and sufficient condition for strengthening the weak input (Hebbian rule). The shorter the time interval between the weak and strong input, the larger the magnitude of synaptic potentiation. Conversely, reversing the temporal order of the weak and strong can lead to depression of the weak input or depotentiating its previously gained weight increase.

Given the Hebbian rule, we propose that bursts may be conceived as a homeostatic mechanism to maintain synaptic strength. Once the weak input becomes suprathreshold, the consequent reduction of Na^+ channel availability as a result of the action potential will reduce the ability of strong inputs to induce a burst. The shorter the time between the weak (but now suprathreshold) and strong inputs, the stronger the "veto effect" of the single spike. Should the strength of the synapse decay with time or get depotentiated actively, the weak synaptic input may become subthreshold again. At this point the strong input becomes instantly effective in inducing a burst, which can then potentiate the weakened synapse. In essence, I propose that the veto effect of single spikes on burst probability is a potential mechanism for regulating synaptic strength. The proposed homeostatic mechanism is operative in a single cell and depends primarily on the immediate spiking history of the neuron.

Richard Thompson, M.D.
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University of Southern California
Los Angeles, California
March 11, 2002

A Memory Trace Found

Some years ago, we adopted classical conditioning of the eyeblink response as a prototypic model of Pavlovian conditioning and the rabbit as our initial preparation, largely because so much work had been done with this animal and paradigm. All mammals studied, including human, exhibit the same basic properties of associative learning in eyeblink conditioning.

Several brain systems become massively engaged in the paradigm, particularly the hippocampus and cerebellum; if the US (unconditioned stimulus) is sufficiently aversive, the amygdala is also engaged. However, using the basic delay procedure (conditioned stimulus and unconditioned stimulus overlap and coterminate) only lesions of the cerebellum abolish the CR (conditioned response). The critical lesion is to the anterior cerebellar interpositus nucleus ipsilateral to the trained eye. The lesion has no effect on the reflex eyeblink. Neuronal unit activity increases massively in this critical region of the nucleus with training and electrical stimulation of the region elicits eyeblink in untrained animals; the circuit is hard-wired from interpositus to behavior.

In a long series of studies using eletrophysiological recordings, lesions, stimulation, and anatomical pathway tracing, we identified the essential (necessary and sufficient) circuit for this form of learning and memory. Reversible inactivation (drugs or cooling) of the following structures does not prevent learning at all: motor nuclei, red nucleus, superior cerebellar peduncle (output from cerebellum). Reversible inactivation of the anterior interpositus nucleus completely prevents learning and inactivation of the cerebellar cortex impairs but does not prevent learning. So the anterior interpositus appears to be the critical structure and the probable locus of the basic or primary memory trace. Blocking protein synthesis in the interpositus nucleus completely prevents learning.

Use of mutant and KO (knock out) mice has demonstrated that eyeblink conditioning can develop, albeit with some impairment, in the complete absence of functional cerebellar cortex (ped mouse) so long as the interpositus nucleus is not lesioned (if it is, there is no learning at all). In the cerebellar cortex, several mutant and KO mice studies support the following observation: if cerebellar cortical LTD is impaired, so is eyeblink learning.

Recordings from identified cerebellar Purkinje neurons in the behaving animals are convenient because the CS evoked mossy-parallel fiber activation is recorded as simple spikes and the US evoked climbing fiber activation is recorded as complex spikes and the two types are easily separable. In trained animals, although several patterns of learning-induced simple spike responses are observed (and many Purkinje neurons are of course not interested in the form of learning), the most common pattern is a decrease in simple spike frequency in the CS period. This result is completely consistent with Ito's phenomenon of LTD (Long Term Depression) in cerebellar cortex (decrease in parallel fiber synaptic efficacy on Purkinje neuron dendrites as a result of repeated pairing of the CS [parallel fiber activation] and US [climbing fiber activation]).

For complex spikes, those Purkinje neurons that are influenced by the US consistently show evoked complex spikes to the onset of the US on US alone trials and to the US impaired trials early in training. As learning develops, these US evoked complex spikes are suppressed, just as is US-evoked activity in the inferior olive. Since we argue that this circuit (trigeminal to inferior olive to cerebellum as climbing fibers) is the essential reinforcing or teaching pathway—these results are completely consistent with the elegant formulation by Rescorla and Wagner for acquisition of classically conditioned responses.

Cerebellar cortical lesion studies and studies on mutant and KO mice with cerebellar cortical abnormalities all produce the same two effects: (1) the CR peak latency is no longer adaptive, i.e., no longer occurs at the onset of the US; instead it has a significantly shorter latency, as we showed many years ago (McCormick and Thompson, 1984, *Science*, 223, 296-299); and (2) acquisition of the behavioral CR is slower and develops to a lesser extent. We have argued as noted above, that the basic or primary memory trace is established in the interpositus nucleus; we suggest that secondary traces develop in cerebellar cortex that serve to modulate the interpositus (via Purkinje neuron inhibition of interpositus neurons) to achieve adaptive timing and normal learning.

In a series of experiments we were able to identify the brain circuit necessary for the behavioral phenomenon of blocking, discovered by Kamin. If animals are first trained, e.g., to a tone CS (corneal airpuff US) until they are well trained and then given additional training to a compound tone-light CS (corneal airpuff US), and then tested to the light CS, they show no learning to the light. In contrast, if animals are only given training to compound tone-light CS, they learn to respond to the tone and the light. Prior training to the tone blocks subsequent learning to the light in the compound stimulus training. In cognitive terms, the light adds no new information and so is ignored.

We are not satisfied with such "mentalistic" explanations and set out to identify the essential circuit for blocking in eyeblink conditioning. As it happens, the interpositus has a strong direct GABAergice inhibitory projection to the inferior olive, as well as a strong excitatory projection to the red nucleus (and from there to motor nuclei). Since neuronal activity in the interpositus grows markedly over training in the CS period preceding the onset of the behavioral CR, we reasoned that the growing inhibition of the inferior olive would shut down its climbing fiber projection to the cerebellum normally evoked by the US onset; so after the animal is well trained to tone, additional training to tone-light will not result in additional learning because the reinforcing or teaching input to the cerebellum, the climbing fiber system, is shut down at the inferior olive. This argument is consistent with the more general formulation of the Rescoria-Wagner algorithm. Recordings from the inferior olive supported this possibility, as did recordings of complex spikes from Purkinje neurons in cerebellar cortex (evoked by climbing fibers).

In the critical test, we completed the blocking paradigm with an additional group given tone training first, then compound tone-light training for 5 days with infusions of picrotoxin in the inferior olive to block GABA inhibition from the interpositus (a control group received the same training but with only vehicle infused in the inferior olive). The control for blocking received no prior tone training. The result was as expected. The group not given prior tone training showed clear learning to the light CS; the blocking group with vehicle infusion in the inferior olive showed blocking, i.e., no learning to light. The critical group, receiving picrotoxin in the inferior olive during compound tone-light training after tone training showed responding to light just like the control group that had no prior tone training. Blocking GABA inhibition in the inferior olive completely blocked the behavioral phenomenon of blocking. This is a most satisfying result in that we were able to show that the cerebellar circuit itself instantiated the phenomenon of blocking—blocking is an emergent property of the network itself and not a result of some specialized molecular processes.

Dan Pollen, M.D.
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Amherst, Massachusetts
March 25, 2002

Neural Correlates of Visual Perception

Identification of the neural correlates that comprise the *necessary* substrata for visual experience remains elusive and controversial. Neurological findings suggests that the human striate cortex (V1) is an indispensable component of a neural substratum subserving static achromatic form perception in its own right and not simply as a central distributor of retinally derived information to extrastriate visual areas. This view is further supported by physiological evidence in primates that the finest-grained *conjoined* representation of spatial detail and retinotopic localization that underlies phenomenal visual experience for local brightness discriminations is selectively represented at cortical levels by the activity of certain neurons in VI.

At first glance, however, support for these ideas would appear to be undermined by incontrovertible neurological evidence (visual hemineglect and the simultanagnosias) and recent psychophysical results on "crowding" that confirm that activation of neurons in VI may, at times, be insufficient to generate a percept. Moreover, a recent proposal suggests that neural correlates of visual awareness must project *directly* to those in *executive space,* thus automatically excluding VI from a related perceptual *space* because V1 lacks such direct projections. Both sets of concerns are, however, resolved within the context of *adaptive resonance theories.*

Recursive loops, linking the dorsal lateral geniculate nucleus (LGN) through successive cortical visual areas to the temporal lobe by means of a series of ascending and descending pathways, provide a neuronal substratum at each level within a modular framework for mutually consistent descriptions of sensory data. At steady state, such networks obviate the necessity that neural correlates of visual experience project *directly* to those in *executive space* because a neural *phenomenal perceptual space* subserving form vision is continuously updated by information from an object *recognition space* equivalent to that destined to reach executive space.

Within this framework, activity in VI may engender percepts that accompany figure-ground segregations only when dynamic incongruities are resolved both within and between ascending and descending streams. Synchronous neuronal activity on a short timescale within and across cortical areas, proposed and sometimes observed as perceptual correlates, may also serve as a marker that a steady state has been achieved which, in turn, may be a requirement for the longer time constants that accompany the emergence and stability of perceptual states compared to the faster dynamics of adapting networks and the still faster dynamics of individual action potentials. Finally, the same consensus of neuronal activity across ascending and descending pathways linking multiple cortical areas that in anatomic sequence subserve phenomenal visual experiences and object recognition may underlie the normal unity of conscious experience.

However, as Damasio (1999) has emphasized, there is a necessity for a neural representation of an image to interact with representations of a sense of self not only to secure a sense of image ownership but perhaps also to engender an image interpretation. Pertinent questions arise as to whether the representations of sense of self are coextant with and possibly equivalent to parietal lobe representations that command selective spatial attention to visual details with respect to maps of eye, head, or body position. Finally, although such sense of self-image interactions may provide yet another *necessary* condition for the emergence of phenomenal vision, the *sufficient* conditions to specify such experience remain unknown.

Alvaro Pascual-Leone Garcia, M.D.
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Boston, Massachusetts
March 25, 2002

Rolect Feedback Projectrons in Visual Awareness

The problem of conscious experience has been one that has been difficult for neuroscience to study, but recently new approaches have been devised that make the study of consciousness open to experimental investigation. One such approach has to do with where in the brain the signals responsible for perception arise. Dr. Dan Pollen, in a recent review, summarized the evidence that conscious experience appears to occur in primary visual cortex, V1; to the extent that higher cortical areas can influence elementary perception, they may do so by the backprojections from these areas to V1.

To explore this issue, the methodology of transcranial magnetic stimulation is used. This is a noninvasive procedure in which a magnetic coil is placed on the scalp. Activation of this coil produces electrical activation of the underlying brain region. Using two such coils, it is possible to separately stimulate V1 and the region, V5, that has been implicated in movement detection. When V1 alone is stimulated the subject generally reports a stationary phosphene. When V5 alone is stimulated the subject generally reports a moving phosphene.

To address the issue of whether V5 stimulation produces perception by sending a signal to V1, the following experiment was done. A few milliseconds after V5 was stimulated, V1 was stimulated weakly. The weak V1 stimulation did not by itself cause a phosphene. However, it did interfere with the normal perception produced by V5 stimulation: specifically, the response to V5 stimulation often appeared to be non-moving. This surprising result suggests that some signal must go from V5 to V1 where its processing can be interfered with by weak V1 stimulation; the alteration in this processing affects perception.

The M.R. Bauer Distinguished Lecturer Series Summaries 2002

Introduction

One of the highlights again this year has been the M.R. Bauer Distinguished Guest Lecturer Series. This program, now in its fourth year, brought to campus two outstanding people, both well known neuroscientists—Julian Jack, affiliated with University Laboratory of Physiology at Oxford in England, and Mary Kennedy, a professor in the Department of Biology at the California Institute of Technology. Both guests spent a week at Brandeis. Their schedules were full with a public lecture, class sessions, presentations at journal clubs, meetings with graduate students and postdoctoral fellows, and spending time in many neuroscience laboratories. Feedback from our students clearly indicate what a privilege it is to have these world class scientists spending this amount of time on campus, getting to know the students, and providing invaluable advice to these younger scientists. Both weeks were very busy, informative, and enjoyable for all.

Julian Jack, M.D.
University Laboratory of Physiology
Oxford, United Kingdom
January 22-28, 2002

Fifty Years of Quantal Analysis: What Have We Learnt?

The goal of understanding the workings of the brain is still very far from being reached. One area where significant progress has been made is the study of the detailed mechanism by which the connections between two individual brain cells operate. For the great majority of these connections—called synapses—the signaling is achieved by the incoming message causing the release of a chemical, the—"transmitter." Once released, the transmitter diffuses across a very narrow cleft and binds on to specific molecules, the receptors, on the surface of the target cell. Although there are a variety of mechanisms by which the transmitter receptor complex can influence the target cell, the best studied mechanism is one in which membrane channel opens, allowing the flow of ions into or out of the cell. The net ion flow leads to a direct change in the membrane potential of the cell, affecting its excitability, and hence the likelihood of the incoming message being relayed.

Historically, the understanding of the working of the synapse has been influenced by the technical ease with which these connections can be studied. Much of the early work was performed on the junction, in vertebrates, between the motor nerve and muscle. Subsequently, the connections to the large nerve cells in the spinal cord, giving rise to the motor nerve to muscle, became the "model" synapse. It is only in the last two decades that the techniques have developed so that the most numerous type of brain synapse, that between two nerve cells in regions of

the brain such as the cerebral cortex, can be studied in adequate detail. It still remains a problem that much of the work on this last type of synapse has, for reasons of technical tractability, been performed on immature, developing brains rather than those of the adult. The importance of drawing attention to these issues is that the functional task performed at these three types of synapse can be quite different, and hence some features of the operation of the synapse may reflect its specific job (or state of development).

In a series of papers starting 50 years ago, Bernard Katz and his colleagues revealed a then startling feature of the way in which the transmitter was released at the nerve-muscle junction. Instead of an expected mechanism in which variations in the amount of transmitter released was continuous, it was found the amount was in units of approximately 5,000 molecules. Taking a term from physics for an "irreducible minimum," Katz called these units quanta. The underlying structural mechanism turned out to be that the transmitter was concentrated inside small intracellular organelles, called vesicles, and release was achieved by enabling these vesicles to empty their content into the intracellular space, adjacent to the receiving cell's specialized receptors. Quantal analysis refers to the methods used to deduce, for a particular synapse, how many quanta of transmitter are released and what the average effect of each quantum is on the target cell, measured in terms of amount of ions flowing (charge) or change in the membrane potential. The importance of such an analysis is partly related to the insight it gives into the detailed mechanism of the signaling process and partly because it provides an effective method to quantify the extent to which the change in the strength of a synapse (for example, as a memory mechanism) reflects primarily a change in the number of quanta released (presynaptic) or a change in the response of the target cell to each quantum (postsynaptic change).

What have we learnt from the last 50 years? At the synapse between nerve and muscle, under normal conditions, a large number of quanta of transmitter are released producing a very large effect on the membrane potential, which is more than sufficient to activate the muscle. At a more detailed level, the released transmitter in a single quantum has ample opportunity to bind to the adjacent postsynaptic receptors, so that the factor which is dominant in setting the size of the postsynaptic effect is the exact number of molecules shared in the particular vesicle which releases its content. By contrast, when brain cells are the postsynaptic target, there is a restricted number of such receptors so that the limitation on postsynaptic effect is not necessarily the number of molecules, but can be the number of receptors. Why this difference? There is good evidence for the motor nerve cell (motoneurone) and for other brain cells that this feature has been used functionally in two ways. In the motoneurone, where the synapses show no long-term plasticity (i.e., no "memory" mechanism), it is used exclusively to ensure a synaptic "democracy." In nerve cells, unlike muscle, the synapses can end at various distances from the zone where the cell makes the decision to fire, sending the message onwards. Everything else being equal, those synapses further away have less of a voice. However, the postsynaptic cell has found a mechanism, still mysterious, by which it adjusts the number of postsynaptic receptors, with larger numbers of receptors the further the synapse is from the decision point. This adjustment is made in such a way that, quantitatively, the efficacy of the synapse in causing firing of the cell are equal, whatever their location. This result was first reported for the motoneurone 20 years ago and it is an indication of how difficult and slow it has been to achieve progress that the same result has only now been found in a looser form for brain cells in the cortex. Apart from technical difficulties, there is an additional factor in these latter cells; that the synapses also show long-term changes in their efficacy. One of the mechanisms by which this occurs is postsynaptic, as an increase in the number of response receptors. Thus, depending on the "memory-state" of the synapse, there are more or less postsynaptic receptors than would be expected if the only factor operating was the mechanism of synaptic "democracy."

One issue, about which there has been considerable controversy, is whether the only memory mechanism at the synapse is a change in the postsynaptic receptors. There is also evidence that there is an additional, presynaptic mechanism viz. a greater likelihood of releasing more quanta. This evidence was reviewed and it was concluded that it is compelling, not just from quantal analysis but from other methods as well. Thus, in

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April 29-May 3, 2002

Putting the Synapse Back Together

principle, the synapse is modifiable, both presynaptically and postsynaptically. Although there is some preliminary evidence about the rules governing which process predominates, further research is required. What the result does emphasize is that the synapse is a unified device, with memory mechanisms being available for changes in both the amount of transmitter released and the postsynaptic response to a fixed amount of transmitter.

How does your brain store new information; the face of an acquaintance, the license plate of your new car, or the movements required to throw a baseball? Neurons communicate primarily through chemical synapses that transmit signals by releasing transmitters that cause electrical changes in target neurons. Many of these same transmitters also initiate biochemical changes in the signaling machinery of the synapse itself. Such biochemical "plasticity" is fundamental for information processing and storage in the brain. For example, it is now thought that memories are encoded when the signalling strength of appropriate synapses is permanently increased through biochemical mechanisms triggered by the repeated use of the synapse.

Neurotransmitters can trigger the activation of several signal transduction pathways. We are studying the molecular organization of signal transduction systems in central nervous system synapses. We have found that the postsynaptic density, a specialization of the submembranous cytoskeleton seen at postsynaptic sites in the central nervous system, contains signal transduction molecules that may control the sensitivity of transmitter receptors, the size of receptor clusters, or perhaps the integrity of the adhesion junction that holds presynaptic terminals in place. Employing a combination of microchemical and recombinant DNA techniques, we have determined the structure of several proteins associated with postsynaptic densities. We are presently studying

the associations of these proteins with each other and their specific roles in control of synaptic transmission with the ultimate goal of illuminating the function and the biochemical diversity of this specialized organelle.

In a related project, we found that a neuronal calcium/calmodulin-dependent protein kinase (CaM kinase II), which transfers phosphate from ATP to specific proteins, is concentrated in the postsynaptic density and may play an important role in controlling changes in synaptic strength that underlie memory formation in the mammalian hippocampus. This enzyme is activated by autophosphorylation of a threonine located near the calmodulin-binding site. We have developed a new technique to correlate changes in phosphorylation of CaM kinase II and other proteins at synapses in situ with changes in neuronal physiology. This technique involves the use of antibodies that bind only to a particular phosphorylated site on a protein to visualize changes in phosphorylation of the protein in cultured neurons and in brain slices. It provides unprecedented spatial resolution of protein phosphorylation in tissues.

The 2002 Volen National Center for Complex Systems and Biology Scientific Retreat

Cellular and Molecular Approaches to Neuroscience

Marine Biological Laboratory
Woods Hole, Massachusetts
March 15-16, 2002

On March 15-16, 2002, the Volen National Center for Complex Systems held its 14th annual scientific retreat. This year's retreat was titled "Cellular and Molecular Approaches to Neuroscience." The topics covered a wide range of approaches to studying neuroscience, and our speakers themselves ranged from established faculty such as Professors Michael Rosbash and Sacha Nelson, to new faculty hires such as Michael Welte and Don Katz (Katz is currently at Duke University but will join Brandeis's neuroscience group this August), to two postdoctoral fellows currently doing research in Brandeis neuroscience laboratories.

Approximately 75 people attended this year's retreat, which was held once again at the Marine Biological Laboratory (MBL) in Woods Hole, Massachusetts. The MBL facility includes lecture halls, function rooms, cafeteria-style dining, and overnight dorm room accomodations. Bringing the researchers (faculty, postdocs, and students) together off-campus for a 24-hour retreat was again tremendously successful. The MBL provides a wonderful environment for stimulating scientific discussions as well as a scenic site for walking and relaxing.

Friday, March 15, 2002

2:00 pm
Arrival and check-in

4:30 pm
Poster session and refreshments

6:00 pm
Dinner

7:00-7:45 pm
Michael Welte, Ph.D.
W.M. Keck Assistant Professor of Biology and Rosenstiel Basic Medical Sciences Research Center
Brandeis University
"Development Control of Organelle Transport: How Fabulous Fly Fat Manages Multiple Microtubule Motors"

7:45-8:30 pm
Don Katz, Ph.D., soon to be Assistant Professor of Psychology and the Volen National Center for Complex Systems
Brandeis University
"Time and Taste Perception: Cortical Gustory Responses"

8:30-11:30 pm
Music and dancing

Saturday, March 16, 2002

7:00- 8:45 am
Breakfast

8:45-9:20 am
John Satterlee, Ph.D.
Postdoctoral Fellow
Sengupta Laboratory
Brandeis University
"CaMKI and Thermosensation"

9:20-10:05 am
Sacha Nelson, M.D., Ph.D.
Associate Professor of Biology and the Volen National Center for Complex Systems
Brandeis University
"A Presynaptic Coincidence Detection Mechanism for Neocortical Longterm Depression"

10:05-10:40 am
Break

10:40-11:15 am
Matthias Soller, Ph.D.
Postdoctoral Fellow
White Laboratory
Brandeis University
"Post-Transcription Mechanism of Gene Regulation in Neuronal Development and Function"

11:15-12:00 pm
Michael Rosbash, Ph.D.
Professor of Biology and the Volen National Center for Complex Systems
Howard Hughes Medical Institute Investigator
Brandeis University
"A Molecular Geneticist's Feeble (and Febrile) Attempts at Neuroscience: *Drosophila* and Circadian Rhythms"

12:00 pm
Lunch

1:00 pm
Departure

Michael Welte, Ph.D.
W.M. Keck Assistant Professor of
Biology and Rosenstiel Basic Medical
Sciences Research Center
Brandeis University
Waltham, Massachusetts
March 15-16, 2002

Developmental Control of Organelle Transport: How Fabulous Fly Fat Manages Multiple Microtubule Motors

The motors that drive intracellular transport are increasingly well understood, but the mechanisms that allow cells to deploy them in a regulated manner remain a mystery. Lipid-droplet transport in *Drosophila* embryos provides a unique model system to unravel these control mechanisms because it is amenable to genetic, biochemical, and biophysical analysis.

Droplets move bi-directionally along microtubules, employing opposite-polarity motors in quick succession. To investigate how multiple motors work together, we impaired transport in one direction genetically and determined the effect on motion in the opposite direction. Our data suggest that in the wild type the motors driving transport are not simply engaged in a tug-of-war, but that their activities are coordinated.

Despite the constant back-and-forth motion of individual droplets, the population as a whole displays uni-directional transport. In a genetic screen for factors that determine net transport directionality, we isolated mutations in the regulator *klar* and in several new loci. The fact that Klarsicht is also important for nuclear migration during eye development suggests that principles of motor regulation discovered in the lipid droplet system are likely important for intracellular transport in general.

Donald Katz, Ph.D.
Assistant Professor
Department of Psychology and
the Volen National Center for
Complex Systems
Brandeis University
Waltham, Massachusetts
March 15-16, 2002

Time and Taste Perception: Cortical Gustatory Responses

In gustation, a single number—the average firing rate across the three to five seconds following tastant delivery—has often been viewed as the appropriate measure of a gustatory neuron's response to tastant stimuli. There are reasons both empirical and theoretical, however, to develop more dynamic descriptions of the neural bases of gustatory perception and learning.

In this talk, I will describe our initial examinations of gustatory cortical (GC) single-unit and ensemble responses to controlled delivery of tastant samples to awake rats. The data demonstrate that GC single-neuron tastant responses evolve across the 2.5 seconds following tastant application, such that a single neuron may be maximally responsive to different tastants at different times. When such responses are accounted for, the percentage of GC neurons that are modulated by tastants is seen to be three times that previously reported. The observed single-unit dynamics reflect, in part, multimodal inputs to GC.

Our analyses of GC firing rate modulations reveal three separate "epochs" of gustatory responses, the first of which is purely somatosensory, the second chemosensory, and the third related to stimulus palatability (a process that is itself partly somatosensory). Cross-correlations among taste-specific assemblies of GC neurons make it plain that firing patterns of GC neurons are also in part governed by between-neuron interactions, however; in response to tastants,

assemblies of GC neurons go through coupled progressions of firing rate changes. Gustatory perception is thus a dynamic process, involving interactions at multiple spatial and temporal scales.

The ensemble processing of tastants seems to develop through post-stimulus time, as tastant responses are first made more distinctive and then arranged into internally generated categories. This process is plastic on a trial-by-trial time scale, depending on an animal's experience with the stimuli.

John S. Satterlee, Ph.D.
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March 15-16, 2002

CaMKI and Thermosensation

Thermosensation is perhaps the most poorly understood of the sensory modalities, despite its critical role in modulating the behavior and metabolism of organisms. To investigate thermosensory neuron (AFD) function and development, we isolated mutants with defects in AFD-specific gene expression, and have identified four genes required for this process. ttx-1 encodes an OTX/OTD-like transcription factor which is both necessary and sufficient to specify AFD fate. tax-2 and tax-4 have been previously shown to encode subunits of a cGMP-gated channel that functions in thermosensory signal transduction. cmk-1 encodes the *C. elegans* ortholog of CaMKI, a calcium/calmodulin dependent protein kinase I. Although CaMKI has been well characterized biochemically, the in vivo role of CaMKI is not well characterized.

cmk-1 animals have reduced AFD-specific expression of the receptor guanylate cyclase gene gcy-8, and the nuclear hormone receptor homolog nhr-38, but not ceh-14, a LIM-homeodomain protein. The cmk-1 promoter drives GFP expression in many neurons, including AFD, and CMK-1-GFP protein is cytosolic.

cmk-1 animals have altered thermotaxis behavior, and are thermophilic, preferring temperatures warmer than the cultivation temperature. The gene expression and thermotaxis defects of cmk-1 can be rescued by a cmk-1 cDNA specifically expressed in AFD, indicating that cmk-1 function is cell

autonomous for these phenotypes. Does loss of cmk-1 function have other behavioral consequences? We have preliminary evidence suggesting a role for cmk-1 in adaptation to some odorants.

cmk-1 functions in a novel pathway regulating AFD gene expression and function. Our working hypothesis is that cmk-1 does not function in thermosensory signal transduction directly, but rather is involved in adaptation to thermal cues. This adaptation may occur in the short term via CMK-1 phosphorylation of AFD signal transduction components, and in the long term by modulation of the type and quantity of signaling molecules expressed in the thermosensory neurons. For the future, we are very interested in identifying additional genes which function upstream and downstream of cmk-1.

Sacha Nelson, Ph.D., M.D.
Associate Professor of Biology and the Volen Center for Complex Systems
Brandeis University
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March 15-16, 2002

A Presynaptic Coincidence Detection Mechanism for Neocortical Longterm Depression

Debate has raged over the last few years as to whether cortical neurons transmit information primarily in their average firing rates or in the precise timing of their spikes. I will address the related question of which features of spike trains control plasticity at cortical synapses.

Using paired recording in slices we have developed a quantitative and predictive description of the joint dependence of cortical plasticity on the rate and relative timing of pre- and postsynaptic firing. The results hold important implications for which parts of the neural code are most readily stored for later retrieval. In addition, we have examined the complimentary question of how plasticity changes the coding properties of cortical synapses. Prior work suggested that LTP in neocortex acts mainly by changing short-term plasticity, which changes the way cortical spike trains are read out by their postsynaptic targets. In contrast, work in the hippocampus suggests that LTP affects mainly the gain of transmission, without altering synaptic dynamics. We find that neocortical LTP has mixed effects, altering both short-term plasticity and the overall gain of transmission. In contrast, LTD has essentially pure effects on response dynamics. Finally, we have identified the signaling pathways required for induction of spike-timing-dependent LTD. Surprisingly, this form of plasticity appears to require retrograde signaling by endogenous cannabinoids. Not only do blockers of cannabinoid receptors block LTD, but agonists at these receptors induce LTD when paired with presynaptic

activity. The requirement for presynaptic activity is due to activation of presynaptic NMDA receptors since activity dependent LTD induced by cannabinoid agonists is still NMDA-dependent. Our experiments suggest a model in which the coincident activation of presynaptic CB1 and NMDA receptors leads to a reduction in subsequent transmitter release.

Matthias Soller, Ph.D.
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March 15-16, 2002

Post-Transcription Mechanism of Gene Regulation in Neuronal Development and Function

Post-transcriptional regulation of gene expression can substantially alter the outcome of the primary information encoded on the DNA. For example, information can be added or deleted through alternative RNA processing (splicing, polyadenylation, and editing). The flow of information encoded by a particular mRNA can be regulated at the level of transport to the cytoplasm, at the level of mRNA stability or by directing the mRNA to a subcellular location. Finally, translation of the mRNA into protein can be regulated at temporal, spatial, and efficiency levels. Thus, post-transcriptional mechanisms contribute to an increase of the proteome and account for its complex spatial and temporal control of expression. For example, 40-60% of human genes are alternatively spliced in at least one exon.

Post-transcriptional regulation of gene expression is mediated to a large portion by transacting factors, proteins that bind RNA. ELAV (Embryonic Lethal Abnormal Visual System) of *Drosophila melanogaster* is the founding member of a large family of RNA-binding proteins containing three RRMs (RNA Recognition Motif). ELAV is pan-neurally expressed and is one of the first signs of neuronal differentiation. Human homologues of ELAV were shown to affect RNA-stability and RNA-translatability by binding to AU-rich sequences found in the three-foot UTR of some growth factor mRNAs. ELAV, however, has been shown to be a gene specific regulator of alternative splicing and targets identified so far are *armadillo*, *neuroglian*, and *erect wing*.

ELAV is necessary in photoreceptor neurons and sufficient in non-neuronal wing disc cells for the expression of Erect Wing (EWG) protein, a transcriptional regulator. Restriction of EWG protein expression to the nervous system is achieved through splicing of broadly expressed primary transcripts of *ewg*. The role of ELAV in mediating nervous system specific expression of EWG is most pronounced in regulating splicing of the last intron. To show that ELAV regulates alternative splicing of *ewg* directly we have developed an in vivo system. Using a neuronally restricted *ewg* rescue construct, we show that splicing of the last intron of *ewg* in photoreceptor neurons is dependent on ELAV. In the absence of ELAV, *ewg* transcripts are prematurely cleaved and polyadenylated in the last intron. Deletion analysis of the last *ewg* intron narrowed the cis-sequences necessary for ELAV-dependent processing to about 25% of the total intron length. To complement these in vivo studies we analyzed binding of ELAV in nuclear head extracts by UV-crosslinking to the remaining intron, as well as to *ewg* three-foot UTR sequences. ELAV binds to AU-rich elements close to the intronic polyadenylation site, as crosslinking can be reduced by mutations. ELAV, however, does not bind to three-foot UTR sequences. Next, we tested intronic *ewg* RNAs in a cleavage/polyadenylation assay for in vitro processing. Consistent with ELAV being necessary to prevent premature polyadenylation in vivo, an ewg intronic RNA with impaired ELAV binding sites is efficiently processed in nuclear head extract while wild type RNA is not. To test if ELAV inhibits three-foot end formation in intron 6 in vivo, mutations impairing ELAV binding were introduced in the ewg rescue reporter construct. Analysis of transgenic flies carrying these reporter constructs shows that ELAV binding is necessary to inhibit polyadenylation in ewg intron 6 and to promote splicing in vivo. The *ewg* gene encodes an essential transcription factor with homologues in sea urchins and vertebrates, but not in yeast or *C. elegans*. Although *ewg* RNA is broadly expressed and alternatively spliced, only one major EWG protein (SC3 ORF) is made. This EWG isoform is restricted to the nervous system and transiently occurring in indirect flight muscles. Expressing the SC3 ORF in the nervous system is sufficient to rescue viability. Null mutant embryos for *ewg* develop with no gross morphological defects, however, fail to hatch. Electroretinograms from whole eye clones of an *ewg* null allel reveal a functional impairment in photoreceptor neurons, rescueable with the SC3 ORF.

To define the role of EWG in neuronal function, we aimed in identifying direct target genes of EWG, since knowing these genes will help understanding the importance of its unusual post-transcriptional regulation. Therefore, RNA profiling was done using cDNA microarrays, revealing two sets of putative target genes: metabolic genes and genes implicated in forms of synaptic plasticity.

To analyze the role of EWG in synaptic growth and function at the third instar neuromuscular junction, transgenes were developed allowing the analysis of the lethal null allel in mosaic animals.These transgenes contain a rescue construct flanked by FRT sites. Flipase mediated recombination causes the loss of the coding sequence and the promotor drives a membrane-targeted GFP-marker. Although *ewg* is vital, recombinationally induced loss of the rescuing transgene in most neurons still allows for larval and pupal development. Emerging adult flies, however, are severely impaired in any coordinated movement underlining the importance of EWG for proper neuronal function.

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March 15-16, 2002

A Molecular Geneticist's Feeble (and Febrile) Attempts at Neuroscience: *Drosophila* and Circadian Rhythms

One of our goals is to define the biochemical machinery that underlies the mysterious yet ubiquitous process of circadian rhythmicity. Our entrèe into the process was the period gene (per) of *Drosophila melanogaster.* The per protein (PER) mutants have profound effects on circadian rhythms of locomotor activity and on circadian rhythms of eclosion (emergence of adults from the pupal case). More than a decade ago, we discovered that per mRNA as well as PER undergoes circadian fluctuations in level during the circadian cycle. These observations and others indicate that there is a negative-feedback loop, in which PER and its partner protein TIM inhibit the transcription of their own mRNAs. Negative feedback at the transcriptional level is now accepted as a central feature of circadian timekeeping in plants, cyanobacteria, *Neurospora,* and mammals. This indicates that the principles—if not the components—of the *Drosophila* clock are widely conserved.

Since 1998, the number of known *Drosophila* central clock genes has increased to approximately eight; the precise number depends a bit on the definition of "clock gene," which is interpreted differently in different quarters. Two of these genes are kinases, and there is increasing evidence for the importance of post-transcriptional regulation in circadian timekeeping. My laboratory is studying the role of the circadian transcriptional factor genes, Clock (Clk) and cycle (cyc). The two protein products, CLK and CYC, comprise the key heterodimeric transcription factor that drives per and tim transcription. It also drives the transcription of a number of downstream clock functions. We have been actively pursuing the identification as well as function of circadian genes, using microarray and other methods. Our intention is to subdivide new circadian genes into those that are direct targets of the CLK-CYC complex and those that are indirect targets. Some may even turn out to be central clock genes, and this approach should complement our continuing efforts to identify central clock genes with more traditional genetic strategies. The mammalian CLK ortholog was first identified as a mouse circadian clock mutant in Joseph Takahashi's laboratory (HHMI, Northwestern University). The mouse and fly mutants have strikingly similar phenotypes and etiologies, making it likely that target genes as well as mechanisms will be shared between flies and mammals.