The Changing Brain: Synapse Formation and Plasticity

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The brain is often described as a massive computer, with its network of neurons like the wiring of a vast circuitry. Dr Morgan Sheng has suggested that a more apt metaphor for the brain's behavior is a highly efficient multinational corporation. Its success is built upon its ability to change—to reorganize when needed, to redirect resources among its departments, to take risks and respond to new environments. Like a corporation, the brain's structure and function change dynamically over time.

The communication between neurons is also far more subtle and nuanced than electrical circuits, allowing the brain to achieve its extraordinary complexity. One of the most exciting areas of today's neuroscience has focused on how the special plasticity of synapses—the junctions between neurons—holds a key to the brain's surprising adaptability and underlies the functions of learning and memory.

In this public symposium entitled **The Changing Brain: Synapse Formation and Plasticity**, The Giovanni Armenise Harvard Foundation brought together some of the world's leading researchers on the biology of synapses. Though each has looked at different aspects of synaptic function and plasticity, all are concerned with the fundamental question of how the brain is able to change, to learn, to remember, and how our understanding of these processes can help inform our approach to neurodegenerative and psychiatric disorders that afflict the brain.

The Synapse: Structure and Function. In presynaptic neurons, vesicles containing neurotransmitters are packaged, unloaded into the synapse, and subsequently recycled. Dr Pietro De Camilli, Dr Flavia Valtorta, and Dr Morgan Sheng discussed the system of proteins involved in this tightly controlled process.

Constructing the Synapse. The events that guide synapse formation during development can shed light on subsequent plasticity. Dr Joshua Sanes showed how communication between presynaptic and postsynaptic cells guide the formation of synapses. Dr Carla Shatz has focused on the events that occur during development that lay out the patterns of the adult brain.

Synaptic Plasticity. The last half of the day built upon what is known about synaptic structure and function to determine how synapses change in response to activity. The speakers presented evidence that several players are involved in synaptic plasticity: Dr Richard Huganir discussed how neurotransmitter receptors are regulated during synaptic plasticity; Dr Erin Schuman presented evidence that new proteins are synthesized locally at synapses; Dr Tobias Bonhoeffer let the audience see movies demonstrating that visible changes occur in the shape and size of synapses; Dr Antonio Malgaroli suggested that the recruitment of nonfunctional synapses can modulate synaptic strength. The presentations suggest that many aspects of synapse structure and function can dynamically adapt to experience.

The Synapse: Structure and Function

The Biology of Endocytic Zones of Synapses
Pietro DeCamilli, MD

> The astounding speed and flexibility of communication in the nervous system depends on the rapid transmission and reprocessing of chemical messages at synapses. Dr De Camilli's lab studies how this feat is accomplished, focusing on the packaging of these messages in secretory vesicles. Defects in how these packages are delivered and subsequently recycled can lead to disability and disease. His research has included work on Stiff Man syndrome, a rare but severe autoimmune disease in which the body attacks its own synaptic proteins. Dr De Camilli is Chairman of the Department of Cell Biology at Yale University School of Medicine, and has been an Investigator at the Howard Hughes Medical Institute since 1992. Before his tenure at Yale, Dr De Camilli held faculty positions at the University of Milan and the Rockefeller University.

In studying how the synapse functions, much attention has been paid to how neurotransmitters are released from synaptic vesicles at axon terminals. But an equally critical part of synaptic transmission is the subsequent recycling of vesicle proteins through endocytosis back into the cell. While synaptic vesicles ready to be secreted congregate at the cell membrane in clusters called active zones, endocytosis occurs largely in peripheral rings around these sites. Endocytic zones contain a dynamic pool of actin, a protein critical to cell motility and cell structure that helps drive synapse formation by guiding the ends of axons toward a postsynaptic cell.

To understand how actin is regulated in endocytosis, Dr De Camilli's lab has looked at a class of proteins called phosphoinositides, which are also concentrated in endocytic

zones. Phosphoinositides have the ability to be phosphorylated and dephosphorylated at several different positions, a flexibility that allows them to play important regulatory roles in signaling pathways and membrane traffic. Dr De Camilli's work has shown that the phosphoinoside Pip2 helps to recruit and anchor clathrin coats—the envelopes for many secretory vesicle at the plasma membrane—and promote actin nucleation. His team has also identified a negative regulator of phosphoinositides, synaptojanin 1. Biochemical and genetic disruption of this enzyme has shown that it is critical to vesicle recycling by removing the clathrin coat and blocking the nucleation of actin that Pip2 oversees.

Why is actin always associated so closely with clathrin coats? A promising connection between the two is dynamin, which helps to pinch off clathrin coats from the membrane. Recent evidence has shown that dynamin interacts closely with actin, and dynamin may have a role in forming a matrix of actin and facilitating actin movement.

Single-Synapse Analysis of Protein-Protein Interactions in Live Neurons
Flavia Valtorta, MD

> The release of neurotransmitters into the synapse is a critical event in all the processes in the brain, and imbalances in these signals are linked to psychological disorders and neurological disease. The process of neurotransmitter release is tightly regulated, requiring sequential interactions between a host of proteins. More than simply identifying these proteins, researchers need to know the sequence of their interactions. Dr Valtorta, Professor of Pharmacology at San Raffaele Vita-Salute University in Milan, and head of the Unit of Experimental Neuropharmacology at DIBIT-San Raffaele Scientific Institute, has been working to characterize the process of neurotransmitter release on the level of individual protein interactions. Dr Valtorta, who received the Novartis Award for Basic Research in 1999, has previously held faculty positions in Pharmacology at the University of Bari, the University of Milan Medical School, and the Rockefeller University.

Synaptic messages are delivered from one cell to another in the process of exocytosis, the complementary action to endocytosis that Dr Pietro DeCamilli discussed. Synaptic vesicles containing neurotransmitters inside the presynaptic cell fuse with the synaptic membrane and form a pore, through which the contents of the vesicle are unloaded into the synapse. Although most vesicle proteins and cytosolic factors involved in synaptic vesicle function are known, the small size of synaptic compartments has made it difficult to study protein-protein interactions in vivo.

Dr Valtorta's lab has been using a technique called fluorescence resonance energy transfer (FRET) digital video imaging to observe molecular interactions in neurons at the level of a single synaptic terminal. Synaptic proteins of interest are fused to a form of green fluorescent protein and introduced into cultured hippocampal neurons. Fluorescence is emitted when energy is transferred, allowing her team to view where interactions between proteins are occurring.

Dr Valtorta's research has used this method to focus on two proteins, synaptophysin 1 (Syp 1) and Vamp2, both membrane proteins in synaptic vesicles. Using FRET, her team wanted to test the interaction of these proteins under dynamic conditions in the

synapse—first under resting conditions and then after stimulation of exocytosis. Vamp2 is involved in fusing a vesicle to a cell membrane by forming a complex with membrane-associated proteins. Syp 1 also seems to be involved in forming the fusion pore through which neurotransmitters are released, and in vitro can form channels in artificial membranes. Dr Valtorta's lab has shown that Syp 1 interacts with Vamp2 in vivo; the two proteins then disassociate shortly before fusion with the membrane. By following the timing of these interactions, this method helps to determine the sequence of actions that proteins undergo.

Molecular Dynamics of the Postsynaptic Specialization
Morgan Sheng, MD, PhD

> The brain's ability to learn and form memories implies that changes must occur on the molecular level that allow neurons to adapt to experience. Dr Sheng's lab has been studying the elements in synaptic junctions that might provide a mechanism for these underlying changes. They have focused on the protein components of synapses and how they interact, the trafficking of glutamate receptors, and the formation of dendritic spines, small protrusions from the branches of neurons onto which synapses form. Dr Sheng is the Menicon Professor of Neuroscience in the Center for Learning and Memory at Massachusetts Institute of Technology, and an Associate Investigator at the Howard Hughes Medical Institute. Dr Sheng has also had a long affiliation with Harvard Medical School, at the Department of Neurobiology where he had a lab at Massachusetts General Hospital.

The anatomy of a synapse is typically depicted in a very asymmetric way: one neuron sends the signal, the other passively receives it. But the two sides of this interaction are able to mirror each other exquisitely, and research over the past few years has shown that the cell biology of these junctions is surprisingly symmetric. In looking at some of the components of postsynaptic cell, Dr Sheng's lab has recently focused on liprin, a protein that seems to be involved in delivering other proteins to both sides of excitatory synapses.

At both ends of the synapse, there is a high turnover of proteins that undergo constant recycling—synaptic vesicles at the pre-synaptic membrane, receptors at the post-synaptic membrane. AMPA receptors are recycled particularly rapidly and the formation of receptor pools can contribute to changes in synaptic strength, as Dr Richard Huganir later discussed. Dr Sheng's team found that AMPA receptor subunits interact with specific intracellular scaffolding proteins, such as GRIP, that might help regulate this process.

The team looked for proteins that associate with GRIP that might help control AMPA receptor delivery and recycling. They identified the protein liprin as interacting with GRIP and AMPA receptors in the postsynaptic cell. Liprin is known to have a role in synapse formation in the active zones of presynaptic neurons. Liprin and GRIP seem to be involved in delivering receptors to the surface of the postsynaptic cell, and when their interaction is disrupted, there is a reduction in AMPA receptors clustering at the membrane. Dr Sheng's team believes that the complex of proteins including liprin and GRIP is involved in transport of proteins to both sides of the synapse, perhaps one of

several mechanisms that could account for the uncanny symmetry between the two cells.

Constructing the Synapse

Imaging Forming Synapses in Fluorescent Mice
Joshua R Sanes, PhD

> One of the struggles that researchers face in studying synaptic formation is how to access and observe changes in individual neurons in the brain. Dr Sanes has bypassed this problem and enhanced current understanding of synapse formation by focusing instead on a more accessible synapse: the contact point between the axons of motor neurons and skeletal muscle fibers. Because they form in less complicated patterns, are larger, and can be studied more easily, neuromuscular junctions can serve as an excellent working model for the behavior of neurons in the brain. Dr Sanes is Endowed Professor of Neurobiology and Director, Neuroscience Graduate Program, at Washington University.

Synapse formation at the neuromuscular junction is guided both by internal genetic properties of the presynaptic neuron as well as factors from the postsynaptic muscle cell and the underlying tissue environment. Earlier studies by Dr Sanes' lab and others established that the nerve-derived signal agrin is necessary for formation of the postsynaptic membrane. Agrin works through an interaction with the muscle-specific receptor tyrosine kinase, MuSK, and an effecter molecule, rapsin, that is able to induce the clustering of acetylcholine receptors, a component of synapse formation. More recently, Dr Sanes' research has focused on the events prior to this pathway that regulate differentiation as well as the later events that allow the postsynaptic membrane to mature and become stable.

Studies using a yellow fluorescent protein mouse model make it possible to observe synapses as they form in live animals. This method allowed the team to identify a brief intervening period when acetylcholine receptors cluster at the postsynaptic membrane in the absence of agrin signals or even motor neurons. This suggested that the agrin hypothesis needed to be reworked, that there was an initial nerve-independent initiation of synapse formation in the postsynaptic cell, still involving MuSK and rapsin. It also appears that the nerve presents a second signal to the muscle besides agrin, a dispersal signal that can get rid of postsynaptic specializations that have not been induced or stabilized by agrin. Dr Sanes' team is now trying to determine the exact role of agrin in these processes.

At the later stages of synapse formation, over long periods of time after birth, the synapses undergo several dramatic changes in shape, all while maintaining the perfect apposition of elements at both sides of the synapse. Dr Sanes' group has focused on a complex of proteins called distroglycans that, when mutated, lead to muscular dystrophy. These proteins also seem to play a role in the structural maintenance and stabilization of synapses.

Brain Wiring in Development: Regulation of Gene Expression and Connectivity by Neural Activity
Carla J Shatz, PhD

The brain is an intricately delineated structure, with neurons laid out in precise patterns that correspond to function. How neurons know to structure themselves in specific patterns is still mysterious—some are outlined by genetic patterns in development, some are molded later by learning and outside inputs, others seem to arise spontaneously by neuronal activity. Dr Shatz's research has yielded important clues about the ordering of neuronal circuitry, and has overturned some notions about the underlying processes of neural development and the role of the immune system in the brain. Dr Shatz is Chair of the Department of Neurobiology and the Nathan Marsh Pusey Professor of Neurobiology at Harvard Medical School. Her research has been widely recognized for its relevance to child development and learning.

Although much of the wiring of neural connections in the adult brain is laid out during development or reconfigured later by outside stimuli, Dr Shatz's research has shown that spontaneous neural activity also can re-wire the brain. In the adult visual system, retinal ganglion neurons connect with neurons in the brain in a specific layered pattern, a pattern that is absent during development but is not dependent on visual input from the eyes. Dr Shatz's lab discovered that this patterning depends on spontaneous firing of ganglion cells, which generates waves of activity that patterns the brain before the eye ever sees.

Another surprising discovery has pointed to an unexpected player in brain remodeling: the immune system. By blocking the self-generated activity in ganglion cells, Dr Shatz's team found that one of the gene families that were altered in the target area of the brain were Class I major histocompatibility complex (MHC I), proteins that work in the immune system to help the body recognize foreign invaders. In mice engineered to be deficient in MHC I or CD3 zeta, a related signaling molecule, the structure of this region of the brain is altered. Some layers are larger than normal, suggesting that there may be an inability to weed out improper or unneeded connections. In adult mice lacking these proteins, long-term potentiation was above normal in the hippocampus, while long-term depression was absent.

The findings raise a host of questions about the role of immune proteins in the brain. The brain has often been thought to be outside the realm of immune activity because the blood-brain barrier separates it from many actions of the immune system. This discovery suggests that the immune system may have a broader role in brain function and development—and immune defects could be implicated in dyslexia and other learning disorders.

Synaptic Plasticity

Regulation of Neurotransmitter Receptor Function During Synaptic Plasticity
Richard L Huganir, PhD

In all forms of communication, the outcome of the message depends on the receiver as well as the sender. When neurotransmitters are released into the synapse, they must meet specific receptors on the postsynaptic cell in order for the message to be received. Dr Huganir, a Professor of Neuroscience at the Johns Hopkins School of Medicine, has approached the question of synaptic plasticity by looking at the mechanisms that regulate neurotransmitter receptors. The ability of these receptors to be modulated by other proteins seems to play a critical role in synaptic communication and brain function. Dr Huganir is also an investigator for Howard Hughes Medical Institute and a member of the American Academy of Sciences.

A powerful controller of synaptic plasticity are the neurotransmitter receptors at postsynaptic neurons, which receive and relay the chemical signals sent across the synapse. Focusing on the two major excitatory neurotransmitter receptors, AMPA and NMDA, Dr Huganir's team has looked at how phosphorylation of different subunits of the receptors changes their activity and their ability to interact with other proteins. They found that phosphorylation of AMPA receptor subunits correlates with changes in activity during long-term potentiation and depression, two events that are implicated in learning and memory. Furthermore, mice that had AMPA receptors with mutated phosphorylation sites showed a significant decrease in these functions.

The AMPA receptor complex contains a variety of interacting proteins, including protein kinases that help regulate how receptors function. For instance, phosphorylation of the GLUR1 subunit of the AMPA receptor by protein kinases like PKA and CaM kinase II may contribute to long-term potentiation and depression. The different phosphorylation states of the receptors can be toggled through, offering a potential mechanism for regulating synaptic communication and boosting synaptic plasticity.

Dr Huganir's research has also explored how receptors are then targeted to the synapses. There are several processes that effect how receptors populate the synapse—how they are synthesized, trafficked, or stabilized at the membrane for instance. Dr Huganir's team has identified several proteins that interact with AMPA receptors, such as the proteins PICK1 and GRIP, the importance of which Dr Morgan Sheng has shown. These proteins bind directly to the GLUR2/3 subunits of AMPA receptors and influence how receptors cluster at the synapses. Such a modulation may serve as a major mechanism for synaptic plasticity, and the finding pinpoints a specific molecular change that may account for observed differences in brain function and behavior.

mRNA Trafficking and Protein Synthesis at the Synapse
Erin M Schuman, PhD

Unlike the simple currents that pass through electrical wiring, neuronal signals are products of a complex array of biological mechanisms. Dr Schuman, Associate Professor of Cellular, Molecular, and Developmental Neurobiology at California Institute of Technology, studies the array of molecular signals that contribute to synaptic transmission in the hippocampus. Her lab tries to elucidate how neuronal activity such as protein synthesis and signaling contribute to synaptic plasticity. Dr Schuman is also Assistant Investigator at the Howard Hughes Medical Institute.

The changes that occur in synaptic plasticity are highly localized: what happens in one synapse can be very different from those of neighboring synapses. These changes also involve the synthesis of new proteins. Given these two requirements, the traditional model of proteins synthesized in the cell body and sent out to the synapses seems inadequate to account for the responsiveness and specificity of change. Many researchers have speculated that some proteins needed at synapses may be synthesized locally.

Dr Schuman's lab has been using time-lapse confocal microscopy to observe protein synthesis in the dendrites of living hippocampal neurons. By adding a growth factor to neurons, her team could induce protein synthesis of a fluorescent reporter mRNA out in the branches of the dendrites—even when they were cut off from the cell body. The next question is whether this local protein synthesis can be a response to local stimulation of the synapses, as it would in synaptic plasticity. When Dr Schuman's group infused a tiny area with growth factor, the new protein synthesis was limited to this region of stimulation, confirming the link between the two events.

Neurobiologists have focused on the role of protein synthesis in controlling changes at the synapse, but it is also possible that protein degradation may have a regulatory role. After all, the degradation of specific proteins is a key factor in other cellular systems. One area of Dr Schuman's research has been to investigate the role of ubiquitination, an important degradation pathway in which chains of ubiquitin are added to proteins as a cellular signal to send them to the proteosome to be degraded. They found that proteosome activity is necessary for glutamate receptors to be endocytosed into the cell following stimulation. It may be that the processes of protein synthesis and degradation work in concert to regulate the levels of proteins at synapses.

Spines to Remember: Neurotrophins and Anatomical Changes Associated with Synaptic Plasticity
Tobias Bonhoeffer, PhD

> Postsynaptic neurons of the hippocampus are able to become more responsive to signals from presynaptic neurons after a burst of stimulation. This adaptive ability, long-term potentiation, may underlie our ability to form and retain memories. While one approach has been to characterize the molecular signals involved in this process, Dr Bonhoeffer has focused on how the actual structure of the synapse can be altered during long-term potentiation, encoding the information of memory in a physical form. Dr Bonhoeffer is Director and Scientific Member of the Max Planck Institute for Neurobiology. He has also held positions at the Max Planck Institute for Psychiatry and Brain Research and the Rockefeller University.

Changes in synaptic strength may not account for the entire story of synaptic plasticity—changes in the morphology at the synapses may also have a role in retaining information. It is known that long-term potentiation (LTP) occurs when activity in both neurons raises calcium levels in the postsynaptic cell of hippocampal neurons, but how this leads to stable changes in the shape of the synapse is unclear. Evidence from

animal models shows that brain-derived neurotrophic factor (BDNF), a neuronal survival signal, is a key component in this process. BDNF-knockout animals have severe defects in LTP that can be rescued by locally introducing the BDNF gene with an adenovirus vector. To if BDNF has a direct effect on LTP, Dr Bonhoeffer's team used photosensitive caged antibodies to disrupt the function of BDNF at the time of LTP induction, and were able to pinpoint an immediate effect.

Because BDNF is also known to influence the morphology of neurons, it may provide a link between form and function in synaptic plasticity. Research has shown that LTP induction leads to the formation of new dendritic spines, the main points of synaptic interaction. Understanding the relationship between synaptic function and shape will help determine the structural differences that underlie memory formation and loss.

The idea that structural changes follow activity in synapses has not been easy to prove because of the difficulties of isolating the exact synapses expected to change in response to stimulus. Two-photon microscopy has now made it possible to view new dendritic spines emerging from a living cell in response to LTP induction. Stimulating only a tiny region of the dendrite, Dr Bonhoeffer's lab has found that new spines only occur where the cell is stimulated. Though the importance of the spines in synaptic plasticity has not been proven, there is a strong correlation between the two events.

Analysis of Silent Synapses in CA3-CA1 Hippocampal Culture
Antonio Malgaroli, MD

Dr Antonio Malgaroli's research focuses on the cellular and molecular basis of memory formation. In addition to the instantaneous changes that take place in a neuron when it is stimulated, synapses in hippocampal neurons have the ability to make long-term changes in their strength and responsiveness. This phenomenon offers a likely explanation for our ability to form lasting memories. Dr Malgaroli, who is Associate Professor at San Raffaele Vita-Salute University, has been exploring the mechanisms of synaptic signaling and how synapses respond to change. He was previously an investigator at the Marine Biological Laboratories at Woods Hole and Professor of Physiology at the University of Milan.

One approach to make communication stronger is to recruit new mouthpieces to transmit a message. Synapses are the mouthpieces that transmit messages between neurons. It is still unclear whether the changes that take place in long-term potentiation can be accounted for by modulations in the strength of this communication or whether new connections are made. Dr Malgaroli's group has found evidence that some of these modulations can be explained by the recruitment of non-functional or mute synapses. It has been established by several groups that there are synapses that cannot detect or respond to the released neurotransmitter—these might be described as "deaf" synapses unable to hear messages. However, there seem to also be "mute" synapses, in which the presynaptic cell is unable to release neurotransmitters.

Using a fluorescent reporter for synaptic vesicle exocytosis, his team found that in cultured hippocampal neurons, around thirty percent of the synaptic terminals seem to be nonfunctional after stimulation. However, these mute synapses are not being

removed from the system. Furthermore, the number of silent terminals decreases when the frequency of the stimulation is increased. Perhaps these silent synapses are integral to the process of synaptic plasticity, and are recruited or silenced according to levels of activity.

Another project in Dr Malgaroli's lab has been to determine the molecular mechanisms underlying synaptic plasticity. Dr Joshua Sanes published an eloquent review of the long list of proteins have been implicated in plasticity. But which of these proteins are involved in the initial, primary events of plasticity, and which ones are later products of the influx of calcium into the cell after stimulation? Dr Malgaroli's team has taken synaptic proteins from a cell in which this second wave of activity has been blocked. Using the technique of mass spectronomy, nineteen proteins have been separated from this mix and a current project of the lab is to identify and analyze these initial players in synaptic change.