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## Conference Report

# The Biology of Memory and Learning

Highlights From the Annual Meeting of the American Society for Cell Biology; December 13-17, 2003; San Francisco, California

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## Introduction

Contradicting the old axiom that the brain doesn't "grow" and that all changes occurring in adults are only for the worse (with loss of neurons over time), new data hint at the possibility that, in suitable circumstances and in receptive subjects, "renewal" of certain brain regions may indeed be possible, with acquisition of new memories and new functional potential.<sup>[1]</sup>

Interest in de novo synapse formation and synapse plasticity, as suggested by Dr. Eric Kandel, is more than a transitory scientific fad, as they represent the structural and functional correlates of our memory, learning ability, and, ultimately, our intelligence. Beyond cells, signaling pathways, and higher-order systems, plasticity may be the operative word when we look at the future of neurobiology.

Leading experts in neurobiology discussed these issues at the symposium "Cell Biology of Learning" recently held in San Francisco, California, at the annual meeting of the American Society for Cell Biology. Dr. Eric Kandel<sup>[2]</sup> presented the latest insights on how memory is stored and accessed, Dr. Karel Svoboda<sup>[3]</sup> proposed a new view of synapse plasticity, and Dr. Yukiko Goda<sup>[4]</sup> dissected the cellular structures underlying the generation and performance of such complex neural wiring.

## Memory Storage and Persistence

Although some believe that only those who have a faulty memory can achieve true happiness, memory is a higher brain function almost essential in everyday life. How do experience and inputs influence synaptic activity in the brain? What are the molecules that are critically involved in storing and processing memory within neurons?

These are the questions asked by Dr. Eric Kandel,<sup>[2]</sup> of Columbia University, New York, at the beginning of his presentation. Memory can be explicit and declarative (eg, conscious recollection of people and places), and it maps to the mediotemporal lobes of the brain and the hippocampus. Implicit memory may be procedural memory (skilled, mapping to the skeletal muscles and the cerebellum), associative (emotional memory, involving the amygdala), or nonassociative (habituation and sensitization, linked to the reflex pathway).

Short-term memories may become long-term ones, and repetition has been found to be one of the critical factors in this process. In other words, "practice makes perfect." From a biological standpoint, long-term memory stems from a series of covalent modifications and stabilization of synaptic connectivity.<sup>[5-7]</sup> But what are, precisely, the molecular mechanisms underlying persistence of memory storage?

Aplysia has represented a very accessible model for neurobiologists: for instance, tactile stimuli

lead to organ retraction (the gill withdrawal reflex) and synaptic activation. Following a train of noxious stimuli, animals have been shown to learn how to better react with a more powerful retraction. With a fairly long training, memory and the associated retraction reflex can last for weeks. Of note, sensitization during prolonged training strengthens the monosynaptic connection leading to gill withdrawal, with growth of new synapses.

Thus, in the presence of 1 shock, only transient facilitation occurs with induction of serotonergic activity and increased cAMP. Following multiple shocks, a far more substantial increase in cAMP leads to increased transcription, augmented enzymatic activity (MAP kinase activation, CRE protein activation, increased activity of ubiquitin hydrolase and proteasome), as well as enhanced phosphorylation of presynaptic targets. Ultimately, repeated noxious stimulations lead to growth of new synaptic connections.

Transient facilitation, induced by 1 shock, was found to be restricted to 1 target neuron, with increase in synapse varicosity (synapse-specific facilitation), in a transcription-dependent fashion. On the other hand, protein synthesis and covalent modifications were necessary for continuation and perpetuation of memory in this model. In fact, rapamycin, a selective protein inhibitor, blocked maintenance of growth at 72 hours, with inhibition of the increase usually observed in synaptic strength and in synaptic varicosities. With protein synthesis inhibition, "facilitation and growth disappeared in front of our eyes," recalled Dr. Kandel.<sup>[2]</sup>

If maintenance of long-term memory requires protein synthesis, what proteins, which mRNAs are then necessary in this process? Proteins of the cytoskeleton are critical for neurite outgrowth (eg, tubulin, actin, spectrin, and others). Similarly, proteins responsible for translations are involved in the maintenance of long-term memory (eg, CPEB, EF1A, S6 kinase, ubiquitin, S6, S15, S16, L8, S27, L40.1, etc).<sup>[8,9]</sup> How are they regulated? The cytoplasmic polyadenylation element-binding protein may have a critical function, as it can positively regulate dormant transcripts in the local environment.

The neuronal isoform of CPEB in aplysia lacks a canonical phosphorylation site, while it contains a very unusual sequence in the aminoterminal region. CPEB activity is usually regulated by the amount of protein present, and, in aplysia, by serotonin. Of note, local inhibition of CPEB blocked long-term facilitation and maintenance of long-term memory.<sup>[10]</sup> A train of 5 stimuli would lead to the release of serotonin, an increase in CPEB polyadenylation activity, and then, through PI3kinase activation, to the increased protein synthesis required for the generation of long-term memory.

One problem with this sequence of events is that CPEB has a quick turnover (only a few hours). But there is a prion-like domain at the amino-terminal of the aplysia CPEB, which consists of a polyglutamine stretch with interspersed aminoacids, rich in polar residues (up to 50%), but poor in charged residues. Might it have prion-like activity?<sup>[11]</sup> The unique structure of CPEB seems to endow the protein with flexibility. Like prion proteins, CPEB seems, in fact, to switch between 2 conformations with one being predominant.

CPEB in conformation A cannot bind the CPE element, as detected through a Gal (blue marker) tracking system. Interconversion between these 2 protein conformations is, however, possible, with cells turning from blue to white. In the presence of serotonin, CPEB undergoes translocation and part of its pool acquires an active conformation (B) leading to formation of aggregates (dimers) and mRNA polyadenylation. CPEB was found to form aggregates both in aplysia and in sensory neurons.<sup>[11]</sup>

Thus, in the model proposed by Dr. Kandel,<sup>[2]</sup> following 1 shock and serotonin release, activation of PI3 kinase would lead to a switch in CPEB conformation from A to B, and formation of aggregates that would, in turn, promote polyadenylation and protein translation. It cannot be excluded that conversion of CPEB into the active form might be facilitated by a so far unknown chaperone. Hence, Dr. Kandel is proposing CPEB as an example of a novel class of proteins with regulatory

properties.<sup>[2]</sup>

## Experience and Synaptic Plasticity in the Adult Brain

The neocortex, where memories reside, is of extreme complexity. In fact, cognitive, declarative memory is spread throughout the brain. Studies in animals have shown that sensory and visual memories can be changed by training. For example, whiskers play a significant role in a rodent's life, and dissection of the pathway from whiskers to the cortex revealed that whiskers' representation is fairly large in the somatosensory cortex of mice. Of note, following sensory deprivation, the spared whisker takes over the deprived territory and such plasticity is associated with increased performance. What underlies this adaptive remodeling of neuronal circuits in an adult brain?<sup>[12]</sup>

As illustrated by Dr. Karel Svoboda,<sup>[3]</sup> of Cold Spring Harbor Laboratory, New York, new technologies are now allowing a better understanding of the dynamic changes that may occur in vivo during memory formation and learning. One such technique, 2-photon excitation laser scanning microscopy, for example, allowed in vivo tracking of green fluorescent protein expressed by a recombinant Sindbis virus in different areas of the brain.<sup>[13-15]</sup> For many years, we have been used to a mostly structural view of the different regions of the cortex. But what is indeed stable and what is plastic in the adult brain?

A number of events occurring during the development of the central nervous system may underlie plasticity of the adult brain:

- Modification of existing synapses;
- Formation of new synapses with growth of dendritic branches;
- Formation of new synapses with growth of axonal branches; and
- Formation of new synapses at the intersection of dendrites and axons.

Dendritic branching in L5 neurons is known to be stable, as shown by comparisons of 16- vs 32-day-old mice. Similarly, axonal branching is stable in the adult cortex, as shown by comparison of the cortex in 7-, vs 67- and 95-day-old mice. Hence, plasticity in cortical circuits appears to be predominantly local.

Conversely, the researchers found an incredible turnover of spines (tiny dendritic protrusions) while examining cortical excitatory synapses in individual pyramidal neurons of the mouse barrel cortex, with an approximately 20% gain/loss of spines per day.<sup>[16,17]</sup> Only about 50% to 60% of the spines survived for more than 1 month. Spines with a "mushroom-like" morphology appeared to be the ones with a tendency toward persistence, while smaller spines tended to disappear rather quickly.<sup>[18]</sup>

Spine growth occurred in the absence of synapse formation in the context of an existing synapse or concomitantly with synapse formation. Time-lapse imaging showed that most new spines were associated with synapses, and only a minority contacted axons without presynaptic specialization.

Does this spine growth and retraction (spine remodeling) underlie experience-dependent plasticity (EDP)? If this were the case, spine growth should be more pronounced in brain areas involved in EDP. And in developmental terms, spine plasticity and EDP should occur in parallel.<sup>[18]</sup> Analysis of the novel sensory experience induced in mice with whiskers deprived of sensory input revealed that such experience was indeed associated with an increase in spine remodeling. The sensory manipulation had induced a shift in the receptive field of the experimental animals' cortices.

The canonical view sees synapses in a deterministic pattern, as a fixed configuration of partners,

stabilized by synaptic growth.<sup>[19]</sup> Instead, according to the hypothesis put forward by Dr. Svoboda,<sup>[3,12]</sup> synapses could also occur in a far more transient fashion. Might they represent a way for neurons to "sample" potential partners before building more stable synaptic connections? They appear, in fact, to be activity-dependent. Stabilization may then follow in such transient synapses in the presence of long-term sensory deprivation.

As proposed by Dr. Svoboda, spine growth and retraction could then represent one of the mechanisms mediating memory, owing to their own plasticity and dynamics. Spines would not be trapped by the constraints affecting dendrites, where dendrites and axons are "stuck" with each other and can connect only when they are in same neighborhood. Hence, he concluded, "Spine growth and retraction with synapse formation and elimination occurs in adult brain, and such remodeling may be one of the substrates underlying EDP and long-term memory."<sup>[3]</sup>

## **Regulation of Synapse Plasticity**

Dr. Yukiko Goda,<sup>[4]</sup> of University College, London, United Kingdom, illustrated how cellular structures and molecular complexes functionally interact in supporting synaptic connections and ensuring plasticity over time. Pre- and postsynaptic terminals are highly coordinated during activity-dependent synaptic plasticity and homeostatic compensation.<sup>[20]</sup> From a mechanistic point of view, different systems mediate these processes:

- Synaptic skeleton;
- Synapse adhesion proteins (eg, n-cadherin/catenin complex); and
- Diffusible messengers.

A number of functions can be ascribed to the synaptic actin cytoskeleton. In fact, at postsynaptic levels, the cytoskeleton contributes:

- A scaffold for neurotransmitters;
- Formation and maintenance of dendritic spine shape;
- Spine motility; and
- Retrograde communication in the presynaptic terminal.

Similarly, at presynaptic levels, the actin cytoskeleton ensures:

- A molecular scaffold;
- Modulation of neurotransmitters' release; and
- Formation and maintenance of presynaptic terminal.

Use of fluorescent protein markers now allows visualization of targets' activity on both sides of a synapse (eg, docking, priming, fusion, and endocytosis). Owing to its central role in forming and maintaining synapses, depolymerization of actin within the synaptic cytoskeleton leads to increased release of neurotransmitters. Of note, both pre- and postsynaptic changes in the actin cytoskeleton are associated with activity and neurotransmitter release as well as structural remodeling.

A new and highly selective way to stimulate neurons is the use of silicon chips that undergo an increase in conductance when exposed to light. Such noninvasive, photoconductive stimulation

elicits synaptic activation of the neurons laid on the silicon chip.

When the light is on, synaptic activation proceeds through a series of coordinated, reversible changes that occur on both sides of the synapse. Condensation occurs at a presynaptic level, while postsynaptically, synapse activation is associated with lateral expansion and spreading of actin toward the base of the spines. Multiple stimulations promote formation of new presynaptic actin puncta and extension of processes from the postsynaptic spines. Formation of the new presynaptic actin puncta requires activation of glutamate receptors and calcium/cAMP signaling. Durable remodeling often engages filopodial outgrowth.

Cadherins modulate cell-cell adhesion and provide a link to the actin cytoskeleton. In so doing, they regulate dendritic spine morphology and synaptic efficiency.<sup>[21]</sup> Studies with overexpression of dominant negative forms of N-cadherin or beta-cadherin, as well as analysis of alpha-catenin deficient mice, have been pivotal in proving how significantly these systems are involved in synapse formation and functioning. Of note, neuronal activity can reciprocally regulate activity of the cadherin/catenin system.

How does the cadherin/catenin complex coordinate synaptic strength with pre- and postsynaptic structural changes? Cultured neurons from mice with knocked-down beta catenin have offered a few clues. Presynaptic inputs were maintained following loss of beta-catenin in postsynaptic cells. But loss of beta-catenin led to reduced mEPSC, although with unchanged frequency of these spontaneous events. In other words, cells were still able to receive inputs coupled to activity and modulation of glutamate receptors.<sup>[4]</sup> More studies on the role of beta-catenin in modulating pre- and postsynaptic function are in progress, both in terms of trans- or cis-modulatory activity.<sup>[4,22,23]</sup>

## Conclusion

Far more insight into how synapses actually work and how they are constantly modified by interaction with the surrounding environment, during development and in the adult age, is expected to come in the next few years. Sophisticated and highly sensitive approaches will be needed to catch the "elusive" activity of all these dynamic processes, but the preliminary results reported here hint that this may indeed become possible in the not-so-far-away future.

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