Howard Hughes Medical Institute

Cell and Molecular Biological Studies of Memory Storage

Eric Kandel's lab is studying selected examples of several major forms of memory storage. The lab is studying explicit memory storage (the conscious recall of information about people, places, and objects) in mice and implicit memory storage (the unconscious recall of perceptual and motor skills) in the snail *Aplysia*. In *Aplysia*, the lab has focused on the implicit memory for sensitization, a simple form of learned fear, and the mechanisms for achieving synapse-specific anatomical changes. In mice, Kandel and his colleagues also examined the synaptic mechanisms contributing to memory storage for learned fear, and, in addition, they have studied memory for space, a complex form of explicit memory storage.

The general finding that long-term plasticity and long-term memory recruit transcription in the nucleus, an organelle shared by all synapses of a neuron, has raised a question that we have begun to explore in *Aplysia* and mice: Are long-term changes cell-wide, or can induced gene products be spatially compartmentalized so that they selectively alter the function of some synapses and not others? In mice, we have also explored the molecular mechanisms whereby attention modifies and stabilizes internal representation of space.

Persistence of Synaptic Facilitation for Learned Fear in *Aplysia*

In both *Aplysia* and mice, we have found that long-term synapse-specific plasticity can occur and that this requires a local marking signal. In *Aplysia* we found that one component of the synapse-specific marking signals requires local protein synthesis at the activated synapse. Local protein synthesis serves two functions in *Aplysia*: (1) it marks the activated synapse that confers synapse specificity, and (2) it stabilizes the synaptic growth associated with long-term facilitation. We have found that a neuron-specific isoform of cytoplasmic polyadenylation element–binding protein (CPEB) regulates this synaptic protein synthesis in an activity-dependent manner. *Aplysia* CPEB protein is up-regulated locally in activated synapses; it is needed not for the initiation but for the stable maintenance of long-term facilitation. Recent work suggests that *Aplysia* CPEB is the stabilizing component of the synaptic mark.

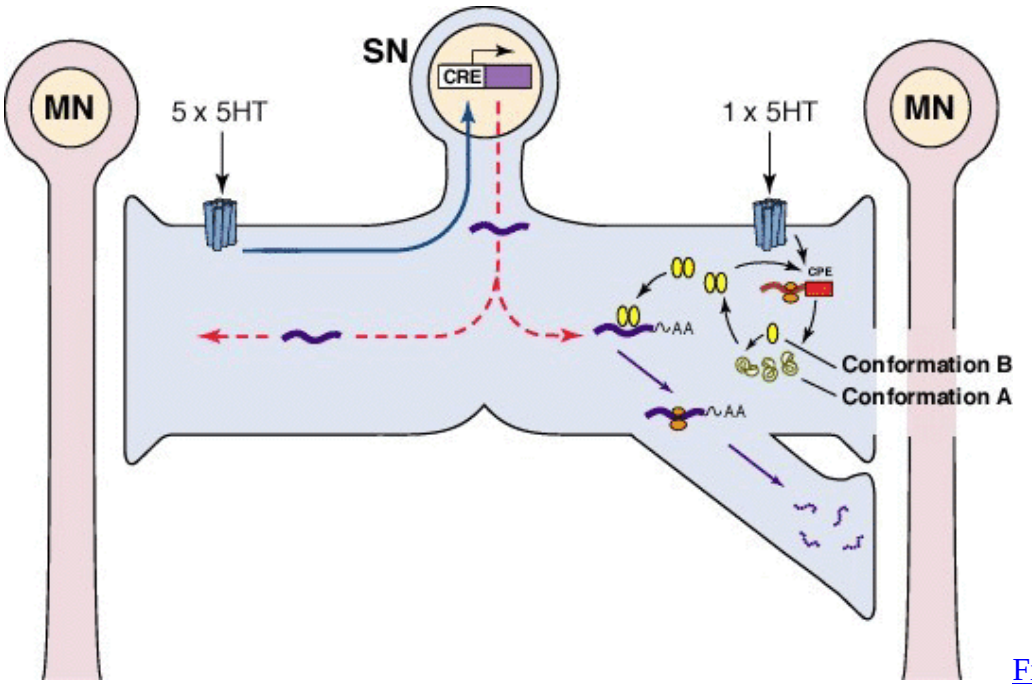

Figure 1: A model for memory and its persistence in Aplysia...

We have found that CPEB may serve as a stabilizer because it has prion-like properties. Prion proteins have the unusual ability to fold into functionally distinct conformations, one of which is self-perpetuating. When prion proteins convert to the self-perpetuating state, they can cause disease (in mammals) or a nonfunctioning protein (in yeast). Compared to other CPEBs, the neuronal form in *Aplysia* has an amino-terminal extension which shares characteristics of yeast prion determinants: a high glutamine content and predicted conformational flexibility. When fused to a reporter protein in yeast, this region is sufficient to confer upon it the prototypical epigenetic changes in state that characterize yeast prions. Full-length CPEB undergoes similar changes in yeast but, surprisingly, the dominant, self-perpetuating prion-like form has the greatest capacity to stimulate translation of CPEB-regulated mRNA. Our preliminary studies suggest that conversion of CPEB to a prion-like state in stimulated synapses helps to maintain long-term synaptic changes associated with memory storage.

Learned Fear and Learned Safety in the Mouse

Fear in mice, monkeys, and people is mediated by the amygdala, a structure that lies deep within the cerebral cortex. To develop a molecular approach to learned fear in the mouse, we identified two genes as being highly expressed both in the lateral nucleus of the amygdala—the nucleus where associations for Pavlovian learned fear are formed—and in the regions that convey fearful auditory information to the lateral nucleus. One of these, the *Grp* gene, encodes gastrin-releasing peptide. We next found that the GRP receptor (GRPR) is expressed in GABAergic interneurons of the lateral nucleus. GRP excites these interneurons and increases their inhibition of the principal neurons of the nucleus. GRPR-deficient mice showed decreased inhibition of principal neurons by the interneurons, enhanced long-term potentiation (LTP), and greater and more persistent long-term fear memory. By contrast, these mice performed normally in the hippocampus-

dependent Morris maze. These experiments provide genetic evidence that GRP and its neural circuitry operate as a negative feedback regulating fear and establish a causal relationship between *Grpr* gene expression, LTP, and amygdala-dependent memory for learned fear.

We also have identified a second gene, *stathmin*, an inhibitor of microtubule formation, as highly expressed in the lateral nucleus of the amygdala as well as in the thalamic and cortical structures that send information to the lateral nucleus about the conditioned (learned fear) and unconditioned (innate) fear. Mice deficient in stathmin show a deficit in LTP. The knockout mice are bold—they exhibit decreased memory in amygdala-dependent fear conditioning and fail to recognize danger in innately aversive environments. These mice also do not show deficits in the water maze, a spatial task dependent on the hippocampus, where stathmin is not normally expressed. We therefore conclude that stathmin is essential in regulating both innate and learned fear.

We have explored the opposite of fear—safety and security. The ability to identify, develop, and exploit conditions of safety and security is central to survival and mental health, but little is known of the neurobiology of these processes or associated positive modulations of affective state. We have studied electrophysiological and affective correlates of learned safety by negatively correlating an auditory conditioned stimulus (CS). This CS came to signify a period of protection, reducing fear responses to predictors of the US and increasing adventurous exploration of a novel environment. In nonaversive conditions, mice turn on the CS when given the opportunity. Thus, conditioned safety involves a reduction of learned and instinctive fear, as well as positive affective responses. In concurrent electrophysiological measurements, we have identified a safety learning-induced long-lasting *depression* of CS-evoked activity in the lateral nucleus of the amygdala, consistent with fear reduction, and an *increase* of CS-evoked activity in a region of the striatum involved in positive affect, euphoric responses, and reward.

A Reductionist Approach to Attention

The hippocampal formation plays a critical role in the acquisition and consolidation of memories. When recorded in freely moving animals, hippocampal pyramidal neurons fire in a location-specific manner; they are "place" cells, and are thought to generate an internal representation of space. To explore the relationship between place cells and spatial memory, we recorded from the hippocampal pyramidal cells of mice under various degrees of task demands. We found that long-term stability of place cells correlates with the degree of task demands and that successful performance of a spatial task is associated with stable place fields. This suggests that the storage and retrieval of place cells is modulated by a top-down cognitive process resembling attention. Consistent with the idea of an attention-like process, conditions that maximize place field stability greatly increase orientation to novel cues. These results suggest that place cells are neural correlates of spatial memory and that the rodent analog of selective attention modulates place field stability. We implicate dopamine in this process and suggest a learning model wherein attention recruits a neuromodulatory input which switches short-term homosynaptic plasticity to long-term heterosynaptic plasticity.

Ion Channels and Learning

In contrast to our increasingly detailed understanding of how synaptic plasticity provides a cellular substrate for learning and memory, how a neuron's complement of voltage-gated ion channels interact with plastic changes in synaptic strength to generate an appropriate output signal to influence behavior is less clear. We have addressed this problem using mice with general and forebrain-restricted deletion of the HCN1 gene, which encodes a voltage-gated nonselective cation channel thought to be important for neural integration. Deletion of HCN1 causes profound learning and memory deficits in visible platform and rotarod tasks which require complex and repeated coordination of motor output, but does not modify acquisition or extinction of eyelid conditioning, a discrete motor behavior that also involves cerebellar synaptic plasticity. Cerebellar Purkinje cells are a key component of the cerebellar circuit required for learning of correctly timed movements. In these cells, HCN1 mediates a large inward current that opposes hyperpolarization below the spike threshold. This ionic mechanism ensures that the integrative properties of Purkinje cells are stable and independent of the neuron's history of activity. Based on these findings, we have proposed and are now testing a model according to which this nonsynaptic integrative function of HCN1 is required for accurate decoding of input patterns and thereby enables synaptic plasticity within the cerebellar cortex to influence, appropriately, the performance of motor activity.

HCN1 channels are also highly expressed on the neocortex and hippocampus, where they are preferentially localized in the distal region of the apical dendrites. We have found that spatial learning and memory are enhanced in mice with forebrain-restricted knockout of the HCN1 gene. This deletion modifies the subthreshold integrative properties of CA1 pyramidal cells by removing a major component of the hyperpolarization-activated current (I_h), preferentially increasing their response to low-frequency inputs and selectively enhancing long-term potentiation of distally located inputs from the entorhinal cortex, but not more proximal Schäffer collateral inputs. Based on these findings, we have suggested that spatial learning and memory mediated by forebrain neurons can be constrained by nonsynaptic influences on neuronal integration mediated by HCN1 channels. These results indicate the behavioral importance of integration at distal dendrite inputs.