

## 40th Anniversary Retrospective

**Editor's Note:** To commemorate the 40th anniversary of the Society for Neuroscience, the editors of the *Journal of Neuroscience* asked several neuroscientists who have been active in the society to reflect on some of the changes they have seen in their respective fields over the last 40 years.

# The Biology of Memory: A Forty-Year Perspective

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In the forty years since the Society for Neuroscience was founded, our understanding of the biology of memory has progressed dramatically. From a historical perspective, one can discern four distinct periods of growth in neurobiological research during that time. Here I use that chronology to chart a personalized and selective course through forty years of extraordinary advances in our understanding of the biology of memory storage.

## Emergence of a cell biology of memory-related synaptic plasticity

By 1969, we had already learned from the pioneering work of Brenda Milner that certain forms of memory were stored in the hippocampus and the medial temporal lobe. In addition, the work of Larry Squire revealed that there are two major memory systems in the brain: declarative (explicit) and procedural (implicit or nondeclarative). Declarative memory, a memory for facts and events—for people, places, and objects—requires the medial temporal lobe and the hippocampus (Scoville and Milner, 1957; Squire, 1992; Schacter and Tulving, 1994). In contrast, we knew less about procedural memory, a memory for perceptual and motor skills and other forms of nondeclarative memory that proved to involve not one but a number of brain systems: the cerebellum, the striatum, the amygdala, and in the most elementary instances, simple reflex pathways themselves. Moreover, we knew even less about the mechanisms of any form of memory storage; we did not even know whether the storage mechanisms were synaptic or nonsynaptic.

In 1968, Alden Spencer and I were invited to write a perspective for *Physiological Reviews*, which we entitled “Cellular Neurophysiological Approaches in the Study of Learning.” In it we pointed out that there was no frame of reference for studying memory because we could not yet distinguish between the two conflicting approaches to the biology of memory that had been advanced: the *aggregate field approach* advocated by Karl Lashley in the 1950s and Ross Adey in the 1960s, which assumed that

information is stored in the *bioelectric field* generated by the aggregate activity of many neurons; and the *cellular connectionist approach*, which derived from Santiago Ramon y Cajal's idea (1894) that learning results from changes in the strength of the synapse. This idea was later renamed synaptic plasticity by Kornhuber and incorporated into more refined models of learning by Hebb. We concluded our perspective by emphasizing the need to develop behavioral systems in which one could distinguish between these alternatives by relating, in a causal way, specific changes in the neuronal components of a behavior to modification of that behavior during learning and memory storage (Kandel and Spencer, 1968).

## Procedural memory

The first behavioral systems to be analyzed in this manner were simple forms of learning in the context of procedural memory. From 1969 to 1979, several useful model systems emerged: the flexion reflex of cats, the eye-blink response of rabbits, and a variety of invertebrate systems: the gill-withdrawal reflex of *Aplysia*, olfactory learning in the fly, the escape reflex of *Tritonia*, and various behavioral modifications in *Hermissenda*, *Pleurobranchaea*, and *Limax*, crayfish, and honeybees. The studies were aimed at pinpointing the sites within a neural circuit that are modified by learning and memory storage, and specifying the cellular basis for those changes (Spencer et al., 1966; Krasne, 1969; Alkon, 1974; Quinn et al., 1974; Dudai et al., 1976; Menzel and Erber, 1978; Thompson et al., 1983).

A number of insights rapidly emerged from this simple systems approach. The first was purely behavioral and revealed that even animals with limited numbers of nerve cells—~20,000 in the CNS of *Aplysia* to 300,000 in *Drosophila*—have remarkable learning capabilities. In fact, even the gill-withdrawal reflex, perhaps the simplest behavioral reflex of *Aplysia*, can be modified by five different forms of learning: habituation, dishabituation, sensitization, classical conditioning, and operant conditioning.

The availability of these simple systems opened up the first analyses of the mechanisms of memory, which focused initially

Received Aug. 12, 2009; accepted Aug. 17, 2009.

My research is supported by the Howard Hughes Medical Institute. I benefited greatly from comments made by the several colleagues who have read earlier versions of this review: Larry Abbott, Craig Bailey, Tom Carew, Kelsey Martin, Mark Mayford, Russell Nicholls, Priya Rajasethupathy, Steve Siegelbaum, and Larry Squire. I am particularly grateful to Lora Kahn for proofreading the review and helping me with the bibliography.

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DOI:10.1523/JNEUROSCI.3958-09.2009

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on short-term changes lasting from a few minutes to an hour. These studies showed that one mechanism for learning and short-term memory evident in both the gill-withdrawal reflex of *Aplysia* and in the tail flick response of crayfish is a change in synaptic strength brought about by modulating the release of transmitter. A decrease in transmitter release is associated with short-term habituation, whereas an increase in transmitter release occurs during short-term dishabituation and sensitization (Castellucci et al., 1970; Zucker et al., 1971; Castellucci and Kandel, 1974, 1976) (for early reviews, see Kandel, 1976; Carew and Sahley, 1986).

Cell biological studies of the connections between the sensory and motor neurons of the gill-withdrawal reflex in *Aplysia* revealed a biochemical mechanism for the short-term increase in transmitter release produced by sensitization (Brunelli et al., 1976). A single noxious (sensitizing) stimulus to the tail leads to the activation of three known classes of modulatory neurons. The most important releases serotonin. Serotonin acts to increase the level of cAMP in the sensory neurons. This in turn activates the cAMP-dependent protein kinase (PKA), which enhances synaptic transmission. Injecting cAMP or the catalytic subunit of PKA directly into the sensory neurons is sufficient to enhance transmitter release (Brunelli et al., 1976; Castellucci et al., 1980).

Studies of the gill-withdrawal reflex also revealed that even elementary forms of learning have distinct short-term and long-term stages of memory storage. Whereas one training trial gives rise to a short-term memory lasting minutes, repeated spaced training gives rise to long-term memory lasting days to weeks (Carew et al., 1972; Pinsker et al., 1973). These behavioral stages parallel the stages of the underlying synaptic plasticity—a short-term form lasting minutes to hours and a long-term form lasting days to weeks (Carew et al., 1972; Castellucci et al., 1978).

In one of the most surprising and dramatic findings in the early study of long-term memory, Craig Bailey and Mary Chen (1988) found that profound structural changes accompany the storage of long-term memory in both habituation and sensitization of the gill-withdrawal reflex. The sensory neurons from habituated animals retract some of their presynaptic terminals so that they make 35 fewer connections with motor neurons and interneurons than do sensory neurons from control animals. In contrast, following long-term sensitization, the number of presynaptic terminals of the sensory neurons increases over twofold. This learning-induced synaptic growth is not limited to sensory neurons. The dendrites of the postsynaptic motor neurons also grow and remodel to accommodate the additional sensory input. These results demonstrate that clear structural changes in both the presynaptic and postsynaptic cells can accompany even elementary forms of learning and memory in *Aplysia* and serve to increase or decrease the total number of functional synaptic connections critically involved in the behavioral modification (Bailey and Chen, 1988).

Together, these early cellular studies of simple behaviors provided direct evidence supporting Ramon y Cajal's suggestion that synaptic connections between neurons are not immutable but can be modified by learning and that those anatomical modifications serve as elementary components of memory storage. In the gill-withdrawal reflex, changes in synaptic strength occurred not only in the connections between sensory neurons and their motor cells but also in the connections between the sensory neurons and the interneurons. Thus, memory storage, even for elementary procedural memories is distributed among multiple sites. The studies showed further that a single synaptic connection is

capable of being modified in opposite ways by different forms of learning, and for different periods of time ranging from minutes to weeks for different stages of memory.

Studies of memory in invertebrates also delineated a family of psychological concepts paralleling those first described in vertebrates by the classical behaviorists (Pavlov and Thorndike) and their modern counterparts (Kamin, Rescorla, and Wagner). These concepts include the distinction between various forms of associative and nonassociative learning and the insight that contingency—that the conditioned stimulus (CS), in associative learning, is predictive of the unconditional stimulus (US)—is more critical for learning than mere contiguity: the CS preceding the US (see, for example, Rescorla and Wagner, 1972). Moreover, psychological concepts, which had been inferred from purely behavioral studies, could now be explained in terms of their underlying cellular and molecular mechanisms. For example, the finding that the same sensory-to-motor neuron synapses that mediate the gill-withdrawal reflex are the cellular substrates of learning and memory illustrates that procedural memory storage does not depend on specialized, superimposed memory neurons whose only function is to *store* rather than process information. Rather, the capability for simple nondeclarative memory storage is built into the neural architecture of the reflex pathway.

#### *Declarative memory*

The remembrance of things past does require a specialized system involving the medial temporal lobe and the hippocampus. A new era of research was opened in 1971 when John O'Keefe made the amazing discovery that neurons in the hippocampus of the rat register information not about a single sensory modality—sight, sound, touch, or pain—but about the space surrounding the animal, a feat that depends on information from several senses (O'Keefe and Dostrovsky, 1971). These cells, which O'Keefe referred to as “place cells,” fire selectively when an animal enters a particular area of the spatial environment. Based on these findings, O'Keefe and Nadel (1978) suggested that the hippocampus contains a cognitive map of the external environment that the animal uses to navigate.

Independent of O'Keefe, Timothy Bliss and Terje Lomo, working in Per Andersen's laboratory in Oslo, were also investigating the hippocampus and discovered that the synapses of the perforant pathway of the hippocampus have remarkable plastic capabilities that can serve for memory storage (Bliss and Lomo, 1973). It soon became clear that a brief, high-frequency train of action potentials in any one of the three major hippocampal pathways strengthens synaptic transmission. This long-term potentiation (LTP) has several forms. In the perforant and Schaffer collateral pathways, LTP is associative, requiring presynaptic activity closely followed by postsynaptic activity. In the mossy fiber pathway, LTP is nonassociative; it requires no coincident activity (Bliss and Collingridge, 1993).

A key insight into the various forms of LTP derived from Jeffrey Watkins's discovery in the 1960s that glutamate is the major excitatory transmitter in the brain and that it acts on a number of different receptors, which he divided into two major groups: NMDA and non-NMDA (AMPA, kainite, and metabotropic) receptors. In the course of finding specific antagonists for each of these, Watkins discovered that  $Mg^{2+}$  blocks the NMDA receptor (Watkins and Jane, 2006). Philippe Ascher and Gary Westbrook next found that the  $Mg^{2+}$  blockade is voltage-dependent (Nowak et al., 1984; Mayer et al., 1984). This was important because LTP in the Schaffer collateral pathway re-

quires the NMDA receptor, and the receptor is unblocked when the postsynaptic cell is depolarized, which normally occurs only in response to a burst of presynaptic action potentials. Thus, the NMDA receptor has Hebbian associative properties; to release the  $Mg^{2+}$  blockade, the presynaptic neuron must be activated to provide glutamate just before the postsynaptic cell fires an action potential (Bliss and Collingridge, 1993).

Gary Lynch and Roger Nicoll next found that the induction of LTP in the Schaffer collateral pathway requires an influx of  $Ca^{2+}$  into the postsynaptic cell (Lynch et al., 1983; Malenka et al., 1988). The  $Ca^{2+}$  activates directly or indirectly at least three protein kinases: (1) calcium/calmodulin protein kinase II (Malenka et al., 1989; Malinow et al., 1989), (2) protein kinase C (Routtenberg, 1986; Malinow et al., 1988), and (3) the tyrosine kinase fyn (O'Dell et al., 1991; Grant et al., 1992).

A major question remaining is whether LTP is expressed presynaptically or postsynaptically. Nicoll's finding that LTP in the Schaffer collateral pathway is associated with a selective increase in the AMPA-type receptor component of the EPSP with little change in the NMDA-type receptor component provided the first evidence that LTP at this synapse is both initiated and expressed postsynaptically (Kauer et al. 1988). Roberto Malinow next discovered that the increase in response of the AMPA-type receptors is due to a rapid insertion of new clusters of receptors in the postsynaptic membrane from a pool of intracellular AMPA-type receptors stored in recycling endosomes (Shi et al., 1999; see also Carroll et al., 1999 and Nicoll et al., 2006). Other studies, however, have implicated additional presynaptic changes that require one or more retrograde messengers from the postsynaptic cell (Bolshakov and Siegelbaum, 1994; Emptage et al., 2003). These differences may depend on the frequency or pattern of stimulation used, or as suggested by Alan Fine, on the developmental stage of the hippocampus (Reid et al., 2001, 2004).

In 1986 Richard Morris made the first connection of LTP to spatial memory by demonstrating that NMDA receptors must be activated for spatial learning in the rat. When NMDA receptors are blocked pharmacologically, LTP is blocked: the animal can still use visual cues to learn a water maze but cannot form spatial memories (Morris et al., 1982). More direct evidence for this correlation came from genetic experiments a decade later.

### Emergence of a molecular biology of learning-related synaptic plasticity

Beginning in 1980, the insights and methods of molecular biology were brought to bear on the nervous system, making it possible to explore both how short-term memory works and how short-term memory is converted to long-term memory.

#### Procedural memory

Molecular biology also made it possible to see commonalities in the molecular mechanisms of short-term memory among different animals. In 1974, Seymour Benzer and his students discovered that *Drosophila* can learn fear and that mutations in single genes interfere with short-term memory. Flies with such mutations do not respond to classical conditioning of fear or to sensitization, suggesting that the two types of learning have some genes in common (Quinn et al., 1974; Dudai et al., 1976). In 1981, Duncan Byers, Ron Davis, and Benzer found that in most of the mutant flies, the genes identified represented one or another component of the cAMP pathway, which is the same pathway underlying sensitization in *Aplysia* (Byers et al., 1981).

The first clue to how short-term memory is switched to long-term memory came when Louis Flexner, followed by Bernard

Agranoff and his colleagues and by Samuel Barondes and Larry Squire, observed that the formation of long-term memory requires the synthesis of new protein. Subsequent work in *Aplysia*, *Drosophila*, and the honeybee showed that with repeated training, PKA moves from the synapse to the nucleus of the cell where it activates the transcription factor, CREB-1 (the cAMP response element-binding protein). CREB-1 acts on downstream genes to activate the synthesis of protein and the growth of new synaptic connections (Glanzman et al., 1989; Dash et al., 1990; Bailey et al., 1992; Bacskai et al., 1993; Alberini et al., 1994; Martin et al., 1997; Hegde et al., 1997).

Initial studies of the molecular switch from short-term to long-term memory in *Aplysia* and *Drosophila* focused on regulators like CREB-1 that promote memory storage. However, subsequent studies in *Aplysia* and in the fly revealed the surprising finding that the switch to long-term synaptic change and the growth of new synaptic connections is also constrained by *memory suppressor genes* (see Abel et al., 1998). One important constraint on the growth of new synaptic connections is CREB-2 (Bartsch et al., 1995; Yin et al., 1994), which when overexpressed blocks long-term synaptic facilitation in *Aplysia*. When CREB-2 is removed, a *single* exposure to serotonin, which normally produces an increase in synaptic strength lasting only minutes, will increase synaptic strength for days and induce the growth of new synaptic connections.

#### Declarative memory

Long-term potentiation in the hippocampus proved to have both early and late phases, much as long-term synaptic facilitation in *Aplysia* does. One train of stimuli produces the early phase (E-LTP), which lasts 1–3 h and does not require protein synthesis. Four or more trains induce the late phase (L-LTP), which lasts at least 24 h, requires protein synthesis, and is activated by PKA (Frey et al., 1993; Abel et al., 1997).

Unlike the early phase, which can involve separate presynaptic or postsynaptic changes, the late phase depends on a coordinate structural change in both the presynaptic and postsynaptic cell through the action of one or more orthograde and retrograde messengers that assure the orderly and coordinated remodeling of both components of the synapse.

#### Molecular similarities between procedural and declarative memory

Procedural and declarative memory differ dramatically. They use a different logic (unconscious versus conscious recall) and they are stored in different areas of the brain. Nevertheless, once again molecular biology revealed homology relationships between these two disparate memory processes that make us appreciate that both share in common several molecular steps and an overall molecular logic. Both are created in at least two stages: one that does not require the synthesis of new protein and one that does. In both, short-term memory involves covalent modification of preexisting proteins and changes in the strength of preexisting synaptic connections, while long-term memory requires the synthesis of new protein and the growth of new connections. Moreover, at least some examples of both forms of memory use PKA, MAP kinase, CREB-1, and CREB-2 signaling pathways for converting short-term to long-term memory. Finally, both forms appear to use morphological changes at synapses to stabilize long-term memory (Bailey et al., 2008).



## Emergence of a genetics of learning-related synaptic plasticity in mammals

### Declarative memory

In the 1980s and 1990s, genetic analyses of behavior pioneered in *Drosophila* by Seymour Benzer were opened up for the mouse by Ralph Brinster, Richard Palmiter, Mario Capecchi, and John Smythies. It soon became possible to selectively manipulate individual genes in an intact animal to compare the effects of such manipulations on long-term hippocampal-based memory, on the one hand, and on the other, on different forms of LTP in isolated hippocampal slices. These techniques, first used to study memory by Alcino Silva in Susumu Tonegawa's lab (Silva et al., 1992a,b) and by Seth Grant in my lab (Grant et al., 1992), revealed that interfering with LTP by knocking out specific kinases (CaMKII, fyn) also interfered with spatial memory.

However, these initial gene alterations were not restricted spatially. Instead the gene was eliminated in all parts of the brain, and the gene alterations were not restricted temporally. Gene products were eliminated throughout all of development and could have interfered with the formation of the basic wiring diagram of the hippocampus, making it difficult to distinguish between phenotypes stemming from adult expression and those stemming from an interference with normal developmental. To overcome these two limitations, Mark Mayford (Mayford et al., 1996) developed a second generation of genetically modified mice that addresses the problems of spatial restriction and temporal regulation. To achieve spatial restriction, Mayford used the forebrain-specific (CaM kinase II) promoter. He then collaborated with Joe Tsien, who generated a number of different mouse lines expressing CRE recombinase (used to achieve gene deletion) under control of this promoter. Some of these mouse lines were able to restrict gene knock-out within the forebrain. Remarkably, in one line CRE-mediated gene deletion was restricted just to the CA1 pyramidal neurons (Tsien et al., 1996a). Tsien and Susumu Tonegawa used this line to knock out the NMDA receptor only in CA1 pyramidal neurons, which demonstrated the importance for spatial memory of NMDA receptor-mediated LTP localized to the Schaffer collateral pathway (Tsien et al., 1996b). To obtain temporal restriction, Mayford combined the CaM kinase promoter with the tetracycline-off system developed by Hermann Bujard. This further refinement allowed him the temporal control to turn gene expression on and off (Mayford et al., 1996). One could now distinguish the role of genes in the development of the brain versus a specific role in learning-induced changes in the adult.

Genetically modified mice were also used to determine the consequences of selective defects in the late phase of LTP. Ted Abel developed transgenic mice that expressed a mutant gene that blocks the catalytic subunit of PKA, thus eliminating the late phase but not the early phase of LTP (Abel et al., 1997, 1998). Silva and Rusiko Bourtchuladze studied mice with mutations in CREB-1. Both lines of mice had a serious defect in long-term spatial memory and both had roughly similar defects in LTP: the early phase was normal, but the late phase was blocked, providing strong evidence linking the phases of LTP to the phases of memory storage (Silva et al., 1992a,b; Bourtchuladze et al., 1994; Huang et al., 1995; Abel et al., 1997).

### Procedural memory

Important molecular studies of procedural memory for fear in mammals have focused on the amygdala, which is essential for both instinctive and learned fear (Davis et al., 1994; LeDoux, 1995, 1996). We now have a good understanding of the neural

circuit underlying learned fear and of the role of synaptic plasticity in fear memory, thanks to the work of Joseph LeDoux, Michael Davis, Michael Fanselow, and James McGaugh. Both the synaptic changes and the persistence of the memory for learned fear require PKA, MAP kinases, and the activation of CREB (McDonald and White, 1993).

An inverse mechanism to LTP, long-term depression (LTD), was discovered in 1982 by Masao Ito. This proved important in eye-blink conditioning, the study of which was pioneered by Richard Thompson. Eye-blink conditioning involves modification in the inhibition by the cerebellar Purkinje cells of the cells of the interpositus nucleus (one of the deep nuclei of the cerebellum). With conditioning, there is an increase in the frequency of eye blinks to the CS, which results from an inhibition of the Purkinje cells and a resulting disinhibition of the neurons of the interpositus nucleus. Purkinje cell inhibition is mediated by LTD and results in a decrease in the strength of parallel fiber synaptic input on to the Purkinje neurons. This decrease in strength of the parallel fibers occurs when the climbing fiber inputs to the cerebellum are activated in appropriate temporal proximity and at low frequency. Roger Tsien next found that parallel fiber stimulation leads to LTD by generating the gaseous messenger nitric oxide (NO), which elevates cyclic GMP and cAMP dependent protein kinase in the Purkinje cells. As a result, the Purkinje cells become less responsive to input, probably due to reduced sensitivity of their non-NMDA glutamate receptors (Thompson et al., 1983; Malinow and Tsien, 1990).

The parallel enhancement of synaptic plasticity and learning in the hippocampus and the amygdala of the mammalian brain, and in the invertebrate brain of *Drosophila* and *Aplysia* with sensitization and classical conditioning supports the view that an increase in synaptic strength is one general means of memory formation. Studies of eye-blink conditioning, and of modifications of the vestibular-ocular reflex, as well as habituation in *Aplysia* and crayfish, provide support for the role of synaptic depression as a parallel mechanism for memory storage (Lisberger et al., 1987; Boyden et al., 2006).

### Synapse-specific local protein synthesis and learning networks

The finding that long-term memory and synaptic plasticity involve gene expression and therefore the nucleus of the cell—an organelle that is shared by all the synapses of the neuron—initially cast doubt on the assumption that long-term memories, like short-term memories, are synapse-specific and stored in the same synapses where they were formed. Uwe Frey (Frey et al., 1993) and Richard Morris (Morris et al., 1982), who studied long-term potentiation in the mammalian hippocampus, and Kelsey Martin, who studied long-term facilitation in *Aplysia*, erased that doubt (Martin et al., 1997). They found that long-term memory is indeed synapse-specific, can occur only at synapses that are marked (activated), and can capture and use productively gene products shipped to all synapses.

How is a synapse marked? Martin found two distinct components of marking in *Aplysia*, one that requires PKA and initiates long-term synaptic plasticity and growth, and one that stabilizes long-term functional and structural changes at the synapse and requires (in addition to protein synthesis in the cell body) local protein synthesis at the synapse. Since mRNAs are made in the cell body, the need for the local translation of some mRNAs suggests that these mRNAs may be dormant before they reach the activated synapse. If that were true, one way of activating protein synthesis at the synapse would be to recruit a regulator of trans-

lation at the activated synapse that is capable of recruiting dormant mRNA.

Kausik Si began to search for such a regulator of protein synthesis. In *Xenopus* oocytes, Joel Richter had found that maternal RNA is silent until activated by the cytoplasmic polyadenylation element-binding protein (CPEB) (Richter, 1999). Si searched for a homolog in *Aplysia* and found a new isoform of CPEB with novel properties. Blocking this isoform at a marked (active) synapse prevented the maintenance but not the initiation of long-term synaptic facilitation (Si et al., 2003a,b). Indeed, blocking ApCPEB blocks memory days after it is formed. An interesting feature about this isoform of *Aplysia* CPEB is that its N terminus resembles the prion domain of yeast prion proteins and endows similar self-sustaining properties to *Aplysia* CPEB. But unlike other prions, which are pathogenic, ApCPEB appears to be a functional prion. The active self-perpetuating form of the protein does not kill cells but rather has an important physiological function.

The Si lab and the Barry Dickson lab have found, independently, that long-term memory in *Drosophila* also involves CPEB. Dickson found a learned courtship behavior in which males are conditioned to suppress their courtship after previous exposure to unreceptive females. When the prion domain of the *Drosophila* CPEB is deleted, there is loss of long-term courtship memory (Keleman et al., 2007). A homolog of CPEB named CPEB-3 has been found in mice, raising the possibility that CPEB may perform a similar function in vertebrates (Theis et al., 2003). A parallel, self-sustaining mechanism, mediated by PKC- $\zeta$ , has been discovered independently in the mammalian brain by Todd Sacktor. Blocking PKC- $\zeta$  interferes with memory even days or weeks after it is formed (Serrano et al., 2008), indicating that in mammals, as well as in *Aplysia* and flies, memory must be actively sustained for long periods of time.

The finding that memory must be actively maintained raises questions related to the recall and modification of memory through reconsolidation, in which the retrieval of a learned experience transforms memory into a labile state, only to become stabilized again over time. What are the mechanisms for this process in the storage and reconsolidation of long-term memory? Having found that PKC- $\zeta$  and CPEB both exist in vertebrates and invertebrates and are related to the persistence of memory storage raises the question: do CPEB and PKC- $\zeta$  interact with one another, or are they completely independent memory agents? If they were indeed capable of independent actions, they might be able to act in different time domains, which could provide a powerful mechanism for producing a cascade of multiple stable states of activity at the synapse.

### The emergence of a systems approach to memory storage

#### *The hippocampus: grid cells and the spatial map*

In his earlier work on place cells, John O'Keefe had only explored the CA1 region. It was not known what the various subregions of the hippocampus do in representing space, and the accepted view was that sensory information is conveyed from the entorhinal cortex through the trisynaptic pathway to the CA3 and CA1 regions of the hippocampus where it is put together as a spatial map. In 2004, Edvard and May-Britt Moser completely revised this idea when they found a precursor of the spatial map that is formed by a new class of cells known as grid cells. These space-encoding cells have a grid-like, hexagonal receptive field and convey information to the hippocampus about position, direction, and distance (Fyhn et al., 2004; Hafting et al., 2005).

In addition to being activated from the entorhinal cortex by the trisynaptic circuit, the CA1 region of the hippocampus receives direct input from the entorhinal cortex. In this way it can compare information transferred directly from the cortex with information processed through the hippocampus via the trisynaptic pathway. This is important because storage of declarative memory depends on *pattern separation*, the ability to distinguish between two closely related episodes or spatial configurations. Moreover, declarative memory can retrieve stored memories through *pattern completion*, the use of preexisting knowledge to fill in an incomplete pattern. Numerous cell-physiological and computational studies, beginning with the theoretical work of David Marr in 1971, suggest that pattern separation depends on the direct projection from the entorhinal cortex to the dentate gyrus and that pattern completion depends on the recurrent connections between the CA3 pyramidal cells (Marr, 1971). Genetic experiments by Tonegawa and his colleagues now support these ideas (McHugh et al., 2007).

#### *New ways of analyzing neural systems involved in learning*

A major advance in the ability to analyze learning-related neural circuitry in the intact behaving animal has come from the introduction of noninvasive ways of selectively activating or shutting off specific neurons in a learning circuit of the animal with beams of light or by expressing in these cells nonendogenous receptors or channels gated by light such as rhodopsin, halorhodopsin, or opto-XR (Zhang et al., 2007a,b; Zhao et al. 2008; Airan et al., 2009). Also effective for turning neurons on or off are ligand-gated variants with customized binding sites, such as the *Drosophila* allostatin receptor and the G<sub>1</sub> protein coupled designer receptor (Alexander et al., 2009), which are activated by an inert ligand (Arenkiel et al., 2007; Huber et al., 2008).

#### *Consolidation and competition in memory*

Competition between neurons is necessary for refining neural circuitry, but does it play a role in encoding memories in the adult brain? In studies of the amygdala, Sheena Josselyn and Silva found that neurons with large amounts of the CREB switch, required for long-term memory, are selectively recruited in the memory of fear. Indeed, the relative activity of CREB at the time of learning determines whether a neuron is recruited (Han et al., 2007). Conversely, if such neurons are deleted after learning, the memory of fear is blocked (Han et al., 2009).

#### *Animal models of memory disorder*

Our understanding of memory storage has reached the point at which we can begin to explore disorders of memory associated with various neurological and psychiatric conditions. Investigators are now working to understand how Alzheimer's disease is initiated in the entorhinal cortex and whether the accumulation of  $\beta$  amyloid spreads to other areas of the hippocampus and to the neocortex. They are also trying to distinguish Alzheimer's from more benign, age-related memory loss.

Almost all psychiatric disorders are characterized by disorders of memory. Anxiety, schizophrenia, and depression, in particular, are being studied intensively. Studies of the extinction of learned fear have proven to be particularly instructive because the neural circuitry of fear is well established and has proven important for understanding post-traumatic stress disorder. Ressler and his colleagues found that D-cycloserine—a partial agonist of the NMDA glutamate receptor in the amygdala—enhances the extinction of fear in mice and is useful for people with phobias as an effective adjunct to psychotherapy (Ressler et al., 2004).

## Open-ended problems

Memory represents a large family of deep problems. Although there is now a good foundation, we are still at the initial stages of our understanding of the full complexity of storage, perpetuation, and recall. The situation in the neural science of memory in 2009 is somewhat reminiscent of, if not analogous to, that for mathematicians in 1900. In that year, David Hilbert addressed the Second International Congress of Mathematicians in Paris and outlined 23 problems confronting mathematics. “Who of us,” he wrote, “would not be glad to lift the veil behind which the future lies hidden; to cast a glance at the next advance in our science and at the secrets of its development during future centuries?” He indicated that some of these questions were so broad and so deep that they might never be solved. Others were more facile and likely to yield answers in a few years. He goes on to say, in a way that applies to neuroscience, “as long as a branch of knowledge supplies a surplus of problems, it maintains its vitality.” The mathematician Hermann Weyl was so impressed with the nature of Hilbert’s problems that he proposed that anyone who solved one of them should automatically be admitted to the honor-class of mathematicians. I am not David Hilbert. I cannot come up with 23 problems, nor can I guarantee that the problems I list are deep. But I do want to facilitate my colleagues’ entry into the honor-class of neuroscience, so I put forward 11 unresolved problems.

### 1. How does synaptic growth occur, and how is signaling across the synapse coordinated to induce and maintain growth?

An intermediate phase of memory storage that requires the synthesis of new protein (but not new RNA) and coordinated signaling between the presynaptic and postsynaptic cell has recently been identified in both *Aplysia* and the hippocampus. This phase may represent the initial steps leading to the growth of new synaptic connections (Ghirardi et al., 1995; Winder et al., 1998; Sutton and Carew, 2000). What molecular steps make up this intermediate phase? Can they provide insights into the nature of trans-synaptic signaling and its contribution to the maintenance of memory storage?

### 2. What trans-synaptic signals coordinate the conversion of short- to intermediate- to long-term plasticity?

Several molecules—BDNF, nitric oxide, arachidonic acid, and spontaneously occurring miniature synaptic potentials—have been suggested, but definitive evidence is lacking.

### 3. What can computational models contribute to understanding synaptic plasticity?

The influential *cascade model* of synaptically stored memory by Stefano Fusi, Patrick Drew, and Larry Abbott (2005) emphasizes that switch-like mechanisms are good for acquiring and storing memory but bad for retaining it. Retention, they argue, requires a cascade of states, each more stable than its precursor. As their hypothesis predicted, a progressive stabilization of changes in the synapse has been found to take place during the transition from short-term to intermediate-term to long-term memory storage. Moreover, possible interactions between CPEB and PKC- $\zeta$  might provide further semi-stable states within the long-term memory domain.

A major reason why computational neuroscience is rising and becoming more powerful and more interesting, as evident in the cascade model, is that these models lend themselves to experimental testing. In the future, however, computational models will need to broaden their focus to include the role of modulatory transmitters and the molecular components of synapses.

### 4. Will characterization of the molecular components of the presynaptic and postsynaptic cell compartments revolutionize our understanding of synaptic plasticity and growth?

The characterization of all proteins (the “proteome”) in the presynaptic active zone and the postsynaptic density pioneered by Richard Scheller, Thomas Südhof, Reinhard Jahn, Pietro DeCamilli, James Rothman, Mary Kennedy, Seth Grant, Morgan Sheng, and others has opened the door to studying how components of the presynaptic terminal and the postsynaptic density receptor sites are modulated to produce changes in synaptic efficacy. This is an extension of Cajal’s quest to understand synapse specificity and synaptic plasticity, but now on a molecular level.

### 5. What firing patterns do neurons actually use to initiate LTP at various synapses?

It is quite likely that 100 Hz, 200 Hz, and theta burst may not actually be the natural firing patterns for long-term potentiation in the hippocampus or elsewhere. As Bert Sakmann has argued, based on the study of natural firing patterns, physiological patterns for long-term potentiation more likely represent a spike-time-dependent form of synaptic plasticity (STDP) (Markram et al., 1997). This discovery made a number of important advances. First, it showed that one can achieve appreciable LTP with a physiologically reasonable manipulation as opposed to a high-frequency tetanus. Second, in many systems it is the only way to get LTD reliably. Third, it unified LTP and LTD in a single protocol. Fourth, it introduced the idea of causality into Hebb’s rule since STDP potentiation only occurs—as Hebb predicted—when one neuron causes another to fire, not as with conventional LTP, in which the firing of the two cells is simply correlated in time.

### 6. What is the function of neurogenesis in the hippocampus?

Neurogenesis may be important for some aspects of memory storage, such as pattern completion, and it seems to be activated in response to and needed for the effectiveness of antidepressants. How are these two mechanisms related? Are there as yet undetected roles for hippocampal neurogenesis?

### 7. How does memory become stabilized outside the hippocampus?

The hippocampus is not the ultimate storage site of memory. All forms of declarative memory are thought ultimately to be stored in areas of the neocortex and to become independent of the hippocampus. How this occurs is not known. Studies of spatial memory in mice suggest that during non-REM (slow wave) sleep and ripples on EEG, information from recently stored memory is conveyed to the neocortex. Whether this proves to be general and if so, how it occurs, needs to be explained.

### 8. How is memory recalled?

This is a deep problem whose analysis is just beginning. Mayford has made an important start of this problem and found that the same cells activated in the amygdala during the acquisition of learned fear are reactivated during retrieval of those memories. In fact, the number of reactivated neurons correlated positively with the behavioral expression of learned fear, indicating that associative memory has a stable neural correlate (Reijmers et al., 2007). But one of the defining characteristics of declarative memory is the requirement for conscious attention for recall. How does this attention mechanism come into play? Do the modulatory transmitters dopamine and acetylcholine have a role in the recall process?



### 9. What are the role of small RNAs in synaptic plasticity and memory storage?

Micro RNAs are small single stranded RNA's of 21–23 nt in length, which regulate gene expression by inhibiting one or more mRNAs. Since microRNAs are activity-dependent and are also present at the synapse, they are likely to be important in regulating a variety of plastic processes including local protein synthesis.

### 10. What is the molecular nature of the cognitive deficits in depression, schizophrenia, and non-Alzheimer's age-related memory loss?

Animal models of human cognitive disorders will provide new insights into these defects. New approaches to reversing them are desperately needed: no new anti-schizophrenic agent has been developed in the last forty years and no new antidepressant has been developed in the last twenty years. Similarly, one should be able to develop imaging criteria for distinguishing benign age-related memory loss from Alzheimer's disease and develop therapies selective for each.

### 11. Does working memory in the prefrontal cortex involve reverberatory self-reexcitatory circuits or intrinsically sustained firing patterns?

Either mechanism would be novel, and the specific mechanism may vary for different types of working memory. Although learning and memory storage importantly involve changes in synaptic efficacy, this is not the only mechanism. Indeed, changes in excitability often accompany different forms of synaptic plasticity and reverberatory loops may, under some circumstances, also be called into play.

Collectively these questions may seem daunting, but when we consider the remarkable technical and conceptual progress that has been made in the last forty years, we can only imagine what the next forty years will yield.

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