gions may form the larger, irregularly shaped, broken ice-raft terrains called "chaos."

Water may once have been near Europa's surface in chaos and in some rare, circular, pondlike regions. The search for thinner-than-average crustal localities, which may permit future technological penetration to the putative ocean, should concentrate on incipient circular features of internal origin. Whether what underlies the ice is, in fact, a briny ocean or a more viscous slurry remains to be proven.

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PERSPECTIVES: NEUROSCIENCE

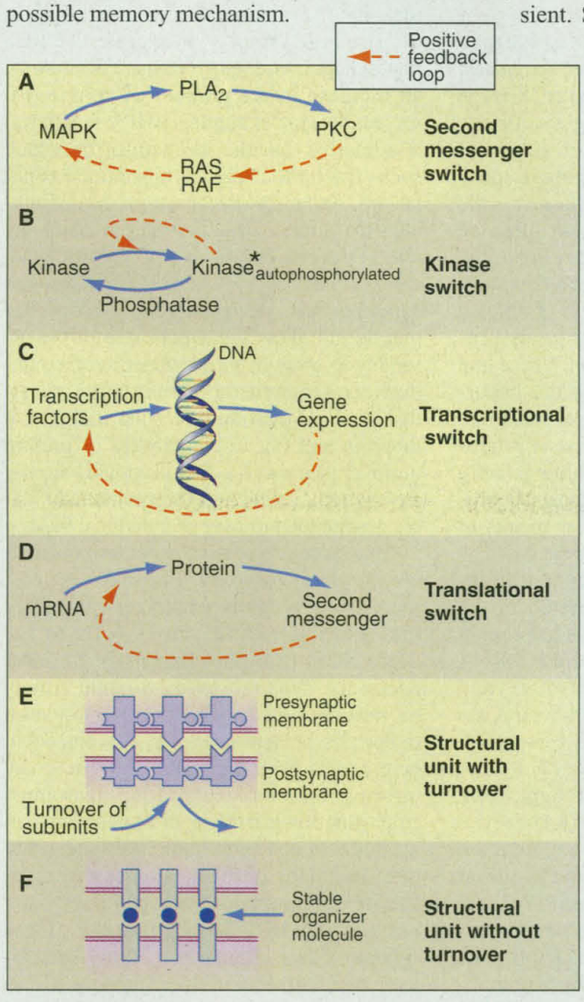
What Maintains Memories?

John E. Lisman and Justin R. Fallon

After we switch on the room lights, we would surely be surprised if the light switch spontaneously turned off. Light switches are designed to remember what we tell them, and they rarely forget. Digital computers elaborate on this principle by using large arrays of binary switches to store information of all kinds. In biological systems there is a similar need to store information. Indeed, a central unsolved problem is to elucidate how memories are stored in the brain.

It is generally thought that electrical activity in neurons leads to long-lasting changes in the strength of synapses and that it is these changes that store memories. But how does the synapse remember whether it is strong or weak? Some type of stable switches must be involved, but the principle by which stability is achieved remains elusive. Several ideas have been proposed, and another intriguing one is put forward on page 381 of this issue by Bhalla and Iyengar (*1*) (see the figure).

Bhalla and Iyengar propose a switch that operates through a positive feedback loop consisting of a cascade of biochemical reactions. Bhalla and Iyengar point to evidence that MAPK can activate protein kinase C (PKC) (through phospholipase A_2). But the reverse is also true: PKC can activate mitogen-activated protein kinase (MAPK) (through Raf and MEK). Clearly there is the potential for positive feedback (see part A of the figure). The authors have used computer simulations to show that this biochemical loop can be bistable. If the enzymes are only weakly activated by an external stimulus, MAPK activity increases, but returns to baseline after the stimulus is removed. With stronger stimulation, the positive feedback becomes strong, and MAPK activity can be sustained in an "on" state long after the stimulus is removed, perhaps indefinitely. This biochemical loop can thus act as a bistable switch and is a possible memory mechanism.



The substance of memories. Molecular mechanisms that can result in stable changes at the synapse, and so are candidates for memory storage devices.

Indeed there is increasing evidence for a role of MAPK in long-term potentiation (LTP) in the CA1 region of the hippocampus, the standard model system for associative synaptic modification. Inhibitors of MAPK block the induction of LTP, and biochemical assays show that the kinase becomes activated. But the available evidence argues that this kinase is not the mechanism by which LTP is maintained. First, the activation of MAPK is only transient. Second, if an inhibitor of this kinase is applied *after* LTP induction, the inhibitor has no effect on the maintenance of LTP (*1*, *2*).

Another form of positive feedback that has been proposed as a potential memory store is a much shorter loop involving a single multisubunit molecule, CaM-kinase II (CAMKII). This molecule is normally inactive, but can be activated by increased Ca^{2+}. Among the substrates of the active enzyme is CAMKII itself. After the molecule becomes "autophosphorylated," it changes its properties and no longer requires Ca^{2+} to be active (*3*). Now comes the role for positive feedback (see part B). Suppose that Ca^{2+} has returned to resting levels and that the job of the kinase is to store the memory of the event that raised Ca^{2+}. Suppose further that a phosphatase dephosphorylates a kinase subunit. Because of positive feedback, other subunits that remain "on" may rephosphorylate this site, thereby retaining the "on-state" of the chemical system. This hypothesis (*4*) has gained substantial support, including the finding that CaMKII is required for

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LTP induction (*5*), that it is persistently activated after LTP induction (*6*), and that a substrate that controls the strength of the synapse, the AMPA channel, is persistently phosphorylated and functionally enhanced by CaMKII (*7*). But on a key test this model has run into trouble: If a kinase inhibitor is applied after LTP induction, the maintenance of LTP is unaffected (*8*).

Although simulations show that covalent modifications that are maintained by positive feedback could potentially store information for years (*4*), it is widely thought that the late phases of memory are stored by other types of processes involving changes in gene expression (*9*). Consistent with this idea is the finding that the late phase of LTP requires the synthesis of new proteins and the action of the transcriptional control element CREB (*10*) (see part C). Recent experiments in hippocampus by Frey and Morris (*11*) nicely demonstrate the importance of the newly synthesized proteins for long-term modification. When one pathway is strongly stimulated, this triggers new protein synthesis and results in a long-lasting synaptic strengthening of the stimulated synapses. If this protein synthesis is blocked, there is only short-term strengthening. Similarly, after weak stimulation, which does not trigger protein synthesis, there is only short-term strengthening. If, however, the weak stimulation is given 1 hour after the strong stimulation, the strengthening of the weakly stimulated pathway becomes long-lasting, presumably because it is able to use the newly synthesized proteins produced by the strong stimulation. These and other experiments demonstrate the importance of transcriptional change, but there remains no evidence for a persistent transcriptional switch. If switching is only transient, the transiently synthesized proteins must work by some other means to provide stable information storage.

One mechanism that is generating increased interest is a local switch in the translation of mRNA. There has been rapid recent progress in the study of the mRNA that is localized near synapses (*12*, *13*) and the control of its translation by neural activity (*14*–*16*). [See the related Perspective on RNA transport in neurons (*17*).] After exposure of dark-reared rats to light, there is a rapid increase in CaMKII in the synaptic compartment. The mechanism of this change has been studied in visual cortex (*18*). Here, translationally dormant CaMKII mRNA is activated by cytoplasmic poly(A) tail elongation. The polyadenylation is regulated by a component of the postsynaptic density, CPEB (cytoplasmic polyadenylation element binding protein). CPEB is likely to be regulated by activity-dependent second messengers, and here again is the potential for a feedback loop (see part D) that could form a stable translational switch localized to individual synapses. No experiments to date have specifically tested whether activity-dependent changes in dendritic translation are persistent.

One of the attractive aspects of feedback loops as an information storage mechanism is that the storage does not rely on any one molecule. Thus if a given molecule becomes degraded, it can be replaced by a newly synthesized molecule, and this molecule will be updated by other molecules that are part of the loop. For example, in the model of Bhalla and Iyengar, a newly synthesized PLA_2 will be phosphorylated by MAPK if the switch is "on" and MAPK is active, but not if the loop is "off." In this way information storage can be stable even though the molecular components are themselves unstable.

This question bears on another class of memory models, ones that are based on the idea that memory is encoded by changes in the structure (size) of synapses. The idea that synaptic strength increases because of an increase in the number of component molecules (for example, AMPA receptors) is attractive, but the mechanism by which such structural change is maintained is not known. Synapses are dense protein assemblies that form a structural unit in which the size of the presynaptic and postsynaptic elements are closely matched (*19*). Perhaps this assembly has special structural properties that do not allow the overall size of the assembly to grow or shrink under basal conditions (as required for stability), but nevertheless allow existing molecular modules to move in and out in the process of protein turnover (see part E). A conceptually similar case is DNA, which is informationally stable despite the turnover of individual bases.

But there are reasons to entertain models in which turnover does not occur. If there were synaptic organizer molecules that were somehow impervious to the degradation processes that make turnover necessary, synaptic strength could simply be determined by the number of such molecules present (part F). The precedent here comes from the study of agrin, a key informational molecule at the neuromuscular junction (*20*). The adult neuromuscular junction is a very stable synaptic structure, and many of the molecules that regulate its differentiation and stabilization are known. Agrin is a component of the synaptic basal lamina, the highly specialized extracellular matrix interposed between the nerve terminal and the postsynaptic membrane. Agrin works through the receptor tyrosine kinase MuSK to organize acetylcholine receptors and other postsynaptic elements on the muscle cell surface (*21*, *22*). Through less well understood mechanisms, agrin can also affect the organization of the presynaptic membrane. A role for agrin in long-term informational storage was revealed by the classic experiments of McMahan in which both the nerve and muscle were destroyed, leaving only the extracellular matrix. Remarkably, when regeneration was allowed to proceed, new synaptic specializations formed at precisely the same sites as the original (*23*). Indeed, the agrin was stably associated with the basal lamina for at least a month (*24*). Because there were no living neurons or muscle cells present at these sites, it can be assumed that agrin must be stable enough to serve as an information store over this period.

An ongoing experimental challenge will be to distinguish the mechanisms that are necessary for the creation of long-term synaptic modifications from those that maintain the synapse in that state. The range of possibilities for memory maintenance is large. None of the proposed models have been firmly excluded, and there seems to be no clear leading candidate. Bookies take note: This would be a good time to take bets on one of the most fundamental problems in neurobiology.

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