Ancient rules of memory

The molecules and mechanisms of memory evolved long before their ‘modern’ use in the brain

The first kiss, the smell of mother’s cooking or the vision of a beautiful landscape; memories such as these are etched firmly into our brains for recall in vivid detail, even decades later. Memories and experience influence our decisions throughout life, both consciously and subconsciously—in a sense, our memories make us what we are. Yet, understanding how information is encoded in our brains and how memories are maintained, both at the conceptual and molecular levels, remains one of the greatest challenges in the life sciences. Through decades of research, biologists have answered many fundamental questions about the brain, and more recent work has revealed the identity of several crucial molecules involved in the storage and retrieval of information. This has at last begun to shed light on the major scientific puzzle: how do we form and maintain long-term memories?

The field of neurobiology had made relatively little progress toward understanding memory since the Canadian psychologist Donald Hebb (1904–1985) introduced the idea of synaptic plasticity nearly 60 years ago (Hebb, 1949). Hebb explained the establishment of memory by changes in the number and strength of synaptic connections between neurons. According to his theory, synapses respond to stimulation by neurotransmitters in a cumulative manner, and repeated stimulation—for example, by learning—leads to ever-stronger connections between specific neurons until they become permanent. Over time, neuroscientists accepted Hebb’s idea of synaptic plasticity, despite a lack of direct molecular evidence. In fact, it was not until recently that scientists—in large part Eric Kandel at Columbia University in New York, NY, USA—discovered several biochemical mechanisms that now support Hebb’s idea.

Kandel himself won the Nobel Prize for medicine in 2000 for his discovery that the syntheses of different proteins relate to short-term and long-term memory in the sea slug *Aplysia*. The foundations for this work

and subsequent discoveries were laid when Kandel made the crucial decision to focus his research on this simple invertebrate, believing—correctly—that this would more readily elucidate the fundamental processes

of memory and learning. At the time—during the 1950s and 1960s—it was widely believed that the mechanisms of memory were qualitatively different in mammals and simple invertebrates, so this was a bold decision to make. It is true that there are quantitative differences: the mammalian brain has a billion nerve cells, whereas *Aplysia* has just 20,000—of which 100 or so are involved in learning processes at any one time. However, it is this simplicity, and the fact that *Aplysia*'s nerve cells are among the largest in the animal kingdom—up to 1 mm in diameter and visible to the naked eye—which make the task of correlating individual neurons with specific behaviour relatively straightforward. It is possible to observe the neuronal changes that occur during the learning process in a way that is still virtually impossible with mammals.

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Kandel's foresight was rewarded; he laid the groundwork for discoveries that have, over time, established a common molecular basis for memory in all animals. "I believe that all of the basic cellular and molecular mechanisms of learning and memory that are present in our brains are also present in the nervous system of *Aplysia*," said David Glanzman of the Departments of Physiological Science and Neurobiology at the University of California, Los Angeles, USA. "In other words, I believe that the mechanisms of learning and memory have been highly conserved over the course of evolution."

Even if the mechanisms of memory are fundamentally the same in lower and higher vertebrates, it is by no means straightforward to extrapolate from a sea slug to mammals, especially humans. The memory of a sea slug is only capable of basic conditioning, whereas the human memory encompasses many different categories, including factual, spatial, visual, linguistic and emotional aspects. All sea slug memories seem to be much the same, which makes it possible to identify the role of individual neurons in particular types of behaviour. Human memories, conversely, have

enormous individual differences. This begs the question: if all memories are created by the same molecular mechanisms, what accounts for the differences?

Identifying the main proteins involved in these mechanisms is the first step to understanding the complex memory of mammals. The next step is to integrate these components into a coherent mechanism of memory storage, access and retrieval. The final challenge—as Glanzman pointed out—is the hardest; memories are encoded not by individual proteins or cells, but by groups of neurons that are configured as circuits. The main problem lies in understanding how these neural circuits change as short-term memories evolve into long-term ones and are subsequently reactivated during recall.

"To my mind, the retrieval of a given memory is not a question of merely activating specific molecules, but, rather, of activating a specific neural circuit, one that was 'constructed' during the consolidation of the memory," said Glanzman. "The basic problem here is that the tools for identifying a specific neural circuit in the brain, and studying the activity of that neural circuit in isolation, are very primitive. Until we develop those tools, our understanding of memory consolidation and memory retrieval will remain seriously incomplete. Knowledge of the molecules is only going to get us part of the way to our ultimate goal."

If there is a common molecular basis for memory, memories in the various categories—for example, spatial or emotional—will differ only at the higher levels of neuronal circuitry. "The molecular mechanisms seem more similar than different across types of learning," agreed Joseph LeDoux, Professor of Neuroscience and Psychology at New York University (NY, USA). LeDoux and colleagues have succeeded in identifying some differences between the circuits involved in emotional memories that are stored in the amygdala area of the brain and other types of long-term memory that reside in the hippocampus. "The amygdala circuits lead to direct expression of behavioural and physiological responses in the body, whereas the hippocampal circuits lead to more processing about the stimulus situation," said LeDoux.

Meanwhile, the task of identifying the molecules is far from complete, which means that it is not yet possible to construct models of memory. "Integrative research

is, of course, important once the different pieces of the puzzle have been worked out," agreed Liliana Minichiello, a group leader at the European Molecular Biology Laboratory in Monterotondo, Italy. "My feeling is that we are getting close, but there are still a few pieces missing."

Even if these pieces were all highly conserved, it would not be possible to find them all by only focusing on primitive invertebrates, such as the sea slug, because this would not reveal how each is involved in correlating the more advanced functions of memory and learning. Conversely, observing molecular changes associated with learning in mammals is significantly more difficult. Minichiello and colleagues recently made progress when they detected a common molecular basis for learning—the ability to execute cumulative increases in synapse strength that lead to long-term memory in mice (Gruart *et al.*, 2007). This phenomenon, known as long-term potentiation (LTP), is associated with a protein called phospholipase C γ (PLC γ), which, in turn, is activated by a receptor molecule, tyrosine kinase receptor B (TrkB), located on the surface of cells in the hippocampus. Mice with a defective version of TrkB were incapable of LTP and of learning even a simple associative task.

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"In this work, we have shown that TrkB through the PLC γ site activated signalling pathway, is central to both LTP and learning," said Minichiello. "This conclusion has been possible to reach because, for the first time, we have combined highly defined genetic mouse models with behaviour and *in vivo* recordings, which have indicated that LTP and learning do in fact have a common molecular basis."

This leads to the next question: how are long-term memories maintained? Often our memories last a lifetime, but the proteins involved in the process typically only last for a few days and have to be continually re-synthesized. A mechanism is therefore needed to recruit newly synthesized proteins

for use in long-term memory storage, and two recent insights are providing answers in this direction. Todd Sacktor's team at SUNY Downstate Medical Centre in New York, NY, USA, made the discovery of the first molecule known to be essential for the maintenance of long-term memory in mammals: protein kinase modulator ζ (PKM ζ).

"The essential difference between the function of PKM ζ and all other molecules important for memory is that PKM ζ is the first identified component of the storage mechanism of memory persistence, whereas other signalling molecules [...] are important in the formation of memory," said Sacktor. His team has shown that if PKM ζ is briefly inhibited, rats lose a previously conditioned aversion to a particular taste, even if the memory had been learned weeks before. Conversely, the inhibition of PKM ζ before the memory is formed had no impact on its subsequent persistence, which confirms a direct link with long-term storage (Shema *et al*, 2007). More recently, Sacktor also identified other molecules that regulate the synthesis of PKM ζ and are subordinate to it. Nonetheless, although his work proves that PKM ζ is maintained in the case of long-term memory, it does not answer the question of how.

This answer might come from Kandel's recent work on a protein called cytoplasmic poly(A) element binding (CPEB). It acts as a type of switch that has to be turned on to activate the synthesis of other local proteins, and it is present in synapses. Kandel noticed that a part of CPEB resembled a component of the PrP prion protein, which is responsible for its infectious properties in degenerative diseases, such as Creutzfeldt-Jakob disease or bovine spongiform encephalopathy. At the time, however, there was no evidence that CPEB actually had the essential prionic property of existing in two alternative conformations, one of which is self-perpetuating.

Kandel thus teamed up with Susan Lindquist, an expert on protein folding in yeast at the Whitehead Institute for Biomedical Research in Cambridge, MA, USA. Together, they showed that CPEB did behave like a prion in yeast cultures and Kandel further demonstrated that CPEB synthesis was triggered simultaneously with the exposure of a synapse to serotonin—the neurotransmitter that initiates synapse strengthening associated with memory formation (Si *et al*, 2003a,b).

These observations led Kandel and Lindquist to propose a simple, elegant, but also controversial theory (Si *et al*, 2004). They hypothesize that the repeated stimulation of synapses during learning increases the production of CPEB, which, in turn, increases the probability that some of the protein will switch to the self-perpetuating prion form. Once this happens, the prion form takes over by recruiting non-prion CPEB into its dominant self-perpetuating configuration, while attracting and activating relevant messenger RNAs for the synthesis of other crucial proteins in long-term memory formation.

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According to Sacktor, this might be one of the initial mechanisms in memory formation, and could well have a role in the persistence of the PKM ζ protein he identified. But not all researchers agree that the prion effect has a crucial role in the persistence of long-term memory. Michael Kiebler, Head of the Division of Neuronal Cell Biology at the Medical University of Vienna, Austria, for example, argues that the CPEB prion hypothesis remains to be proven. "This is semantics, because instead of terming it a prion, we could say that it has a very flexible internal structure. Lots of proteins can be either flexible or stiff, and very likely one form can have an influence on the other. However, whether it can be irreversibly influenced so that one conformation is infectious to the other one is unclear," he said.

Kandel and Lindquist are about to show exactly that. "We have definitive data that *Aplysia* CPEB is a prion, but [it is] not published yet and [we are] about 4 months away," Lindquist confirmed. This will be a major step toward establishing whether a prion is significant for the preservation of long-term memories, although it will require more work to show that CPEB also behaves like a prion in mammals. In any case, it would be an example of a conserved mechanism that perhaps originally evolved in yeast.

Another example of a family of highly conserved proteins that changed their role during the course of evolution are the so-called septins, which are involved in memory structure. In yeast, septins have a crucial role in cell division

by forming rings around the neck of a new bud to prevent the diffusion of components between the cytoplasm of the parent and daughter cells—removal of this ring prevents the daughter bud from forming and breaking off. Kiebler and colleagues have discovered that one protein from this family, septin 7, has a similar role in the formation of dendritic branches in neurons, which is associated with synaptic plasticity and memory formation (Xie *et al*, 2007). Septin 7 forms a ring-like structure around the spines protruding from the nerve cell, similar to those in budding yeast, again to prevent diffusion of cellular components.

Kiebler suggested that this role for septins might have been conserved from yeast to mammals, and was eventually put to a different use in the vertebrate brain. It is also possible that septin is not the only example; more molecules that are crucial to memory might have evolved for simpler reasons in single-celled eukaryotic or prokaryotic ancestors, and were later recruited to the more complex processes of learning, memory and conditioning in vertebrates. It seems, therefore, that although memory has become ever more complex as higher organisms have evolved, its foundations rest, at least in part, on ancient mechanisms that evolved long before brains and memories were even thought of.

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Philip Hunter

doi:10.1038/embor.2008.5