Before we turn to the genetic studies of memory, it will be useful first to address two preliminary questions. (1) Is memory a distinct property of mind? Can memory be studied independently of other higher-cognitive functions? Or do most manipulations of the brain that interfere with memory also produce a general cognitive decline by producing deficits in motivation, perception, or voluntary movement? (2) If memory can be isolated and studied as an independent process, is there any reason to think that there will be genes that are specifically devoted to memory?

The answer to the first question was first addressed in the 1930s in studies of human amnesic patients, beginning with the now famous patient H.M. Following surgical removal of portions of his medial temporal lobes on both sides, including a structure called the hippocampus, H.M. developed a severe memory deficit. The following excerpt from a clinical examination performed on 26th April 1955 (at a time when H.M. was 29) is illustrative. The patient gave the date as March 1953 and gave his age as 27. Just before coming into the examining room he had been talking to Dr Karl Pribram, yet he had no recollection of this at all. In conversation, he reverted constantly to boyhood events and seemed scarcely to realize that he had had an operation.

What is striking about this case, and typical of memory loss due to large bilateral lesions of the medial temporal lobe, is not only the severity of the amnesia but also the preservation of other cognitive functions. For example, following his operation, H.M.’s language and reasoning abilities were unchanged and his performance on an IQ test actually increased slightly. In addition, remote memories, such as those from childhood, were intact. More-recent anatomical studies of H.M., and other human patients with amnestic syndromes, as well as studies of animal models suggest that the memory deficits in H.M. arise most probably from damage to the hippocampus and the cortical structures immediately surrounding the medial temporal lobe. Thus, it seems that the answer to the first question, at least from an anatomical point of view, is that memory is indeed a distinctive cognitive function that can be studied independently of other higher-cognitive abilities.

The answer to the second question — whether there are specific genes devoted to memory storage — is less clear. As we will see below, most of the genes so far identified as affecting memory are involved in signal-transduction pathways that are recruited for purposes unrelated to memory in other cell types. However, given that memory is stored in certain specific structures in the brain, it is likely that some isoforms exist that affect these structures selectively, either developmentally or for mature cellular function. In addition, conditional genetic approaches allow the selective targeting of genetic modification to structures that are specifically involved in memory, such as the hippocampus, allowing the molecular mechanisms of memory to be examined independently of other brain functions.

Classical genetic approaches

In the early 1970s, Benzer’s group at Cal Tech carried out a genetic screen in Drosophila for mutants that affect learning and memory. They used a simple conditioning task involving two odors, one paired with an electric shock, and the other not associated with a shock. The memory test consisted of presenting the flies with a choice of moving toward the odor that was paired with the shock or toward the other odor. Flies that learned the task tended to avoid the odor that was paired with the shock. Using this memory test, they screened chemically mutagenized flies and found one mutant, which they called dnc2, that seemed to affect learning of this task specifically, without affecting the behavior required for performance of the task, such as locomotion or odor detection. Subsequent work from a number of laboratories identified several other learning and memory mutants. To date, the genetic modifications associated with four mutants obtained from behavioral screens have been characterized (dnc2, rutabaga, anemone, and linotte). It is striking that three of the four mutants affect molecules that are involved in cAMP signaling: in dnc2, the phosphodiesterase
 Genetic approaches to memory storage

In 1970, Kandel and colleagues began to study a simple marine invertebrate called Aplysia, an organism that is amenable to experimental manipulation and is capable of learning and memory storage. The sensory-motor neuron connection is a simple circuit that can produce long-lasting changes in synaptic strength, which is the basis of long-term memory. The memory for this reflex is a function of the number of training trials: a single noxious stimulus to the tail gives rise to a short-term memory that lasts for minutes, but repeated training can produce long-term memory lasting for weeks or more. This long-term memory is resistant to protein-synthesis inhibition and can last for years. What are these genes and how are they turned on? Studies in Drosophila and Aplysia suggest that the cAMP-responsive transcription factor, CREB, is critically involved in the conversion of short-term to long-term memory. In Aplysia, CREB is activated by a heat-shock promoter and is involved in the conversion of short-term to long-term memory. In Drosophila, CREB is activated by a heat-shock promoter and is involved in the conversion of short-term to long-term memory.}

**Long-term memory and CREB**

It is well known from experience of every day life, as well as from psychological studies in the laboratory, that memory can be either short-lasting or long-lasting. A single training trial usually produces only a short-term memory lasting minutes to hours, whereas repeated training trials can produce long-term memory lasting many years. One interesting insight into the possible mechanistic differences between short- and long-term memory is the observation that long-term memory is blocked by inhibiting protein synthesis during the learning trials, whereas short-term memory is resistant to protein-synthesis inhibition. This suggests that new genes might be expressed during learning that are required to establish long-term memories.

**What are these genes and how are they turned on?** Studies in Drosophila and Aplysia suggest that the cAMP-responsive transcription factor, CREB, is critically involved in the conversion of short-term to long-term memory. Injection of a competitive oligonucleotide inhibitor of CREB into Aplysia sensory neurons selectively blocked long-term facilitation without affecting that of short-term facilitation. In Drosophila, heat-shock induction of a dominant-negative CREB transgene impaired the acquisition of long-term memory, even though short-term
memory was unaffected. Taken together, these results suggest that CREB-related transcription is necessary for the formation of long-term memories and that the memories might be encoded in the pattern of strengthening of synaptic connections between neurons.

Is CREB transcriptional activation the rate-limiting step in the conversion of short-term to long-term memory? To address this question, Yin and Tully developed a transgenic fly in which an activated form of CREB (dCREB2) was expressed from a heat-shock promoter. Normally, when
Genetic approaches to memory storage

Previous pharmacological and genetic studies in a variety of species, including flies, rodents, and humans, have implicated CREB in the long-term storage of memories. An obvious next step will be to examine the role of CREB in the mouse, which has proved to be a powerful tool for elucidating the molecular mechanisms underlying memory storage.

The first studies to examine mice with targeted deletions in the CREB gene were performed in Aplysia by Bartsch and colleagues. They used a heat-shock prior to training to induce expression of the CREB activator, which led to long-term facilitation in Aplysia. In rodents, memory for place is commonly tested in a water maze, in which the animal is trained to escape from a large pool of opaque water by finding a hidden platform located just beneath the surface. Lesions of the hippocampus impair this form of memory, and the CaMKIIa and CaMKIIb mice, which lack LTP in the hippocampus, were also severely impaired in this spatial memory task.

A second group of studies examined mice with targeted deletions in the genes that encode three different tyrosine kinases, fyn, src, and yes (Ref. 23). Previous pharmacological studies had shown that tyrosine kinase activity was necessary for the induction of LTP (Ref. 15). In the genetic experiments, only the fyn knockout mouse showed deficits in LTP and also in spatial memory.

More recently, work in the mouse has focused on the cAMP-signaling pathway that is important for learning and memory in Drosophila and Aplysia. Pharmacological experiments indicated that LTP itself has stages. There is an early stage, E-LTP, lasting 1–3 hours, which does not require protein synthesis. It is the stage that requires CaMKII and is the substrate for the second stage, which is a more stable, self-sustained quality that is the growth of new synaptic connections. This synaptic growth is mediated by the transcription factor CREB. Blocking the expression of this single gene blocks the long-term process and the growth of new synaptic connections.

Thus, the work from invertebrates implies that the CREB-mediated induction of transcription is necessary to produce the long-lasting changes in synaptic strength required for the long-term storage of memories. An obvious next step is to ask whether similar mechanisms apply to the more complex learning of the mammalian brain.

Reverse genetics in the mouse

Initial genetic studies of memory in the mouse were influenced by two earlier observations. The first was the observation in humans that damage to the hippocampus seemed to affect declarative memory, while sparing other cognitive functions. The second was the finding that the excitatory neurons in the hippocampus, as well as neurons in many other regions of the brain, display a robust, activity-dependent form of synaptic plasticity, somewhat akin to long-term facilitation in Aplysia, which is known as long-term potentiation (LTP)15.

A great deal is known about the molecular mechanisms of LTP in the hippocampus based on electrophysiological and pharmacological studies in brain slices (Fig. 1). Homologous recombination in embryonic stem cells allowed these ideas to be tested genetically. If LTP was, in fact, a cellular mechanism for memory storage, then mice lacking LTP in the hippocampus should be impaired in memory tasks that require the hippocampus.

The first studies to examine mice with targeted deletions explored two different signaling kinases. Pharmacological studies had implicated Ca2+/calmodulin dependent protein kinase II (CaMKII) as the early stage, E-LTP, of LTP production. Silva and Tonegawa generated a mouse that carried a targeted deletion of the gene that encodes the alpha subunit of CaMKII in the hippocampus. The mice lacking CaMKIIa displayed a nearly complete loss of LTP in the CA1 neurons of the hippocampus. In humans, damage to the hippocampus, and to the CA1 neurons in particular, results in a specific impairment in the ability to form new memories for facts, places and events. In rodents, memory for place is commonly tested in a water maze, in which the animal is trained to escape from a large pool of opaque water by finding a hidden platform located just beneath the surface. Lesions of the hippocampus impair this form of memory16, and the CaMKIIa mice, which lack LTP in the hippocampus, also showed severe impairments in this spatial memory task.

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and the mice survive to adulthood. These alpha/delta CREB-knockout mice show an impairment in LTP and in memory. The memory deficit is similar to that seen in the mice expressing the PKA inhibitor, that is, initial learning and short-term memory are intact while long-term memory is impaired.

These results imply that the encoding of long-term memories in mice, similar to what is seen in Drosophila and Aplysia, involves activation of the cAMP-signaling cascade and an increase in transcription mediated by CREB during or immediately following the behavioral training (Fig. 3). To test this idea, Storm and colleagues developed a transgenic mouse in which several copies of the cAMP response element (CRE) were linked to a lacZ reporter gene. These transgenic mice allow CREB-induced gene expression to be monitored specifically in the whole animal during learning. They found that L-LTP stimulated CRE-linked lacZ expression. Moreover, during behavioral training on the contextual fear-conditioning task, the CRE-linked lacZ reporter was specifically induced in the hippocampus. Thus, experience that leads to the formation of a memory also induces gene expression via the CRE–CREB pathway.

The results from these initial studies suggest that one can use genetic manipulation in the mouse to analyse the molecular mechanisms of synaptic function on the one hand and memory storage on the other. The role of specific isoforms of a gene or class of genes can be examined and changes in synaptic plasticity can be mapped onto changes in short- and long-term memory ability. Subsequent work has produced numerous mouse lines with deficits in several different aspects of synaptic plasticity and in learning and memory. Although these initial studies demonstrate the power of a genetic approach to memory, several problems have become apparent in trying to understand the mechanisms by which these genes influence behavior. These problems generally fall into two categories: complications of genetic background and pleiotropy.

**Conditional knockout**

In one respect, a traditional knockout-mouse or mutant-fly line with an impairment in learning and memory tells us what genes are necessary to develop a normal learning and memory phenotype in the adult, and suggests genes that might vary in a normal population to give rise to normal variation in cognitive ability. On the other hand, these knockout lines do not give us access to all the molecules in the adult brain that participate in memory encoding, because many of these molecules are probably important for normal development and survival as well as adult learning. One example of how to circumvent this problem is provided by work on conditional knockout strategies in the brain.
Glutamate is the major excitatory neurotransmitter in the mammalian brain and the N-methyl D-aspartate (NMDA) receptor is one type of glutamate receptor that is found throughout the nervous system. Activation of the NMDA receptor is the critical first step in the induction of LTP (Ref. 15). However, mice with a targeted deletion in the NR1 subunit of the NMDA receptor die shortly after birth35.

To get around the problem of the lethal phenotype, Tsien and colleagues used the CRE–loxP system to generate mutant mice with a conditional deletion in forebrain neurons (see Fig. 4)28. They created transgenic mice that expressed the CRE recombinase driven by the CaMKII promoter, which normally produces expression in excitatory neurons of the forebrain31. In a second mouse line, the gene encoding NR1 was flanked with loxP sites using gene targeting. Neither expression of the CRE transgene nor the modification of the NR1 locus with loxP sites alone affected memory. However, when both genetic modifications were introduced into the same mouse by crossing the two mouse lines, the CRE recombinase caused the deletion of the NR1 gene in the CA1 neurons of the hippocampus30. This result was especially interesting because LTP is most commonly studied in the CA1 neurons and, in humans, damage restricted to just the neurons in the CA1 region is sufficient to produce a memory impairment56.

As expected, the conditional NR1 knockouts lacked LTP at CA1 synapses in the hippocampus. The hippocampal-specific deletion of the NR1 gene led to a severe impairment in spatial learning ability. Thus, a gene required for the LTP-induced changes in synaptic strength was deleted only in a region of the brain that anatomical studies in rodents and humans show is specifically involved in learning and memory, and this genetic modification impaired memory. Interestingly, recent work from Tsien and colleagues suggests that enhancing NMDA receptor function might actually enhance learning ability56.

The use of this conditional-knockout approach enables not only the study of the role in learning and memory of a molecule that when deleted globally is lethal, but also alleviates many of the concerns about pleiotropic effects on behavior by removing that molecule only from a group of cells that are specifically involved in memory. However, even with a conditional-knockout approach, several questions remain. For example, even though the gene encoding NR1 is deleted postnatally, it is still absent for many weeks while the brain is developing and before memory ability is assessed. Is the phenotype that is observed due to a direct role of NR1 signaling in development? Also, learning and memory are not unitary processes but consist of the initial encoding of information, a consolidation process during which some of the memory can change, and finally the stored memory, which must be accessed during a recall test. Which of these processes is disturbed in a mouse with a targeted deletion in the brain? However, we have had some success in employing the tetracycline system in the brain to regulate expression of a transgene in an anatomically restricted manner and to use this system to explore the various stages of learning and memory formation.

As discussed above, CaMKII is one of the signaling molecules thought to be important for the production of LTP, and mice lacking the alpha isoform lack LTP and show severe memory deficits. CaMKII in the basal state is completely dependent on Ca2+/calmodulin for its activity, but upon activation can rapidly convert to a Ca2+-independent kinase by autophosphorylation at a single threonine residue, Thr-286. It has recently been shown that this autophosphorylation is necessary for the induction of LTP and for normal spatial learning56. We have examined the role of CaMKII signaling in LTP and memory by expressing an enzyme made constitutively active by mutating the normally autophosphorylated threonine to an aspartate (CaMKII-Asp286). The expression of the transgene was controlled using the tetracycline regulatable tTA system, which allows transgene expression to be suppressed by the administration of tetracycline analogues (Fig. 5)57.

Because CaMKII is required for the normal induction of LTP, one might expect that expression of a constitutively active form of the kinase would produce LTP at all synapses or at least reduce the threshold level for the synaptic stimulation required to induce LTP. This proved not to be the case. Rather, expression of the CaMKII-Asp286 transgene in forebrain neurons increased the level of synaptic stimulation needed to induce LTP and caused a deficit in spatial memory in animals that had expressed the transgene throughout development57,58. When the expression of the transgene was suppressed in adult animals by the administration of doxycycline, the LTP deficit and the memory deficit recovered to wild-type levels57. These results demonstrate that the transgene exerts its affect acutely in the adult animal and any affect it might have on development does not contribute to the observed memory impairments.

FIGURE 4. Conditional gene deletion in the brain

Two different mouse lines required for conditional gene targeting. The first line is a transgenic mouse in which expression of the CRE recombinase is driven by a cell-type specific promoter. The second line is a mouse in which an endogenous target gene is flanked by loxP sites (‘floxed’) using homologous recombination in embryonic stem (ES) cells. Introduction of the CRE transgene into a mouse homozygous for the floxed allele leads to deletion of the target gene in those cells that express the CRE recombinase. In CA1 neurons of the forebrain28, lines give deletion only in the CA1 neurons of the hippocampus even though CRE is expressed in other neurons of the forebrain56.
In a second set of experiments, we took advantage of the fact that suppression of the transgene completely reversed the learning deficit to investigate the signaling pathways involved in memory consolidation and recall. In this case, animals were trained with expression of the CaMKII-Asp286 transgene suppressed and then, following learning, the transgene was activated. We found that activation of the transgene in animals that had learned normally impaired the recall of the memory. Thus, an alteration of CaMKII signaling in forebrain neurons after normal learning will disrupt either the initial consolidation of memory or the subsequent ability to access the stored memory during a test of recall.

**rtTA and calcineurin**

Although the TTA system for gene regulation allows transgene expression to be suppressed, it would be useful to have a truly inducible system, in which transgene expression could be suppressed until the administration of an inducer. A mutant form of the tetracycline repressor has been isolated that induces transcription only in the presence of doxycycline. This reverse TTA (or rTTA) can be used to obtain inducible and reversible transgene expression in mice. Mansuy et al. used the rTTA system to control the expression of a CaMKII-activated protein phosphatase calcineurin in forebrain neurons. Calcineurin is interesting because it controls a form of synaptic plasticity called long-term depression (LTD), which is the mirror opposite of LTP. Calcineurin has this role in LTD because it has a high affinity for Ca^2+^, even higher than that of CaMKII. At low-frequency stimulation, the amount of Ca^2+^ coming into the cell through the NMDA receptor is small. This will activate calcineurin but not CaMKII. In turn, calcineurin will dephosphorylate protein phosphatase inhibitor-1, which in turn activates protein phosphatase 1 (PP1) and leads to long-term depression of synaptic transmission (LTD). By contrast, the higher frequencies of stimulation that we considered earlier lead to greater Ca^2+^ influx, which activates kinases, including PKA, that phosphorylate and block inhibitor-1, thereby shutting off the phosphatase cascade. In fact, PKA and calcineurin phosphorylate and dephosphorylate the same residue on inhibitor-1.

Overexpression of calcineurin leads to an impairment in an intermediate form of LTD (I-LTP) in the hippocampus and to a defect in spatial memory on the Morris water maze test. Mutant mice that express the calcineurin transgene transiently, after learning has been acquired and spatial memory has already been stored, have an apparent defect in the retrieval of the spatial information. This retrieval defect is not due to a disruption in memory storage because it could be reversed when the transgene expression was turned off by doxycycline removal. Thus, with the use of regulated genetic modification, one can control for potential developmental abnormalities associated with a genetic change and begin to explore the various phases of memory acquisition, storage and retrieval.

**Future directions**

The use of genetics to investigate complex behavioral traits, such as learning and memory, is at an early stage. The work in invertebrates and mice suggests that memory uses genes and signaling molecules, such as cAMP (PKA, CREB), MAPK, and Ca^2+^ (calcineurin and CaMKII) that are commonly used in many cell types for different purposes. It appears from these results that many of the basic molecular mechanisms for memory might be conserved across species, allowing insights from invertebrates to be applied to the mammalian brain. However, because the developmental integrity of many brain regions is necessary for the proper performance of even the simplest memory task, and because memory storage requires some of the most basic cellular-signaling mechanisms, there will be many developmentally important genes that affect learning and memory. Therefore, the difficult task facing the field will be to distinguish between those mutations that impact on the core cellular mechanisms that are used to encode memories and those mutations that modulate these mechanisms, or affect the development of the basic circuits that are important for performing the learning task or for storing the learned information.

In the fly, the characterization of a greater number of learning and memory mutants will hopefully provide a fuller understanding of the critical genes involved in memory storage.
Genetic approaches to memory storage

For example, one recent study suggests that integral-based signaling is important in the Drosophila olfactory learning paradigm. Together with the data from Aplysia about the importance of NCAM-like cell-adhesion molecules in the growth of new synaptic connections, these studies begin to outline possible molecular candidates for growth. The use of anatomically restricted and temporarily regulated expression of genes in the mouse will, as discussed above, be critical for determining those genes that directly affect memory encoding from those that affect memory indirectly through developmental, motivational, or perceptual mechanisms. The best evidence that memory can be studied independently of other cognitive functions comes from studies of patients with damage to the hippocampus. Thus, the characterization of new promoters that limit genetic modification to the hippocampus or to specific groups of neurons in the hippocampus would be useful in eliminating the pleiotropic effects of many genetic modifications. This can, of course, be extended to other regions of the brain to produce a situation in the mouse that is similar to that in the fly with the GAL4 system, where genetic modification can be targeted to specific structures simply by making effector molecules (i.e., tet-transgenes or floxed chimeromic loci) specific to (TA activator or CRE-deleter lines of mice). This approach should allow one to understand how genetic manipulations affect not only synaptic plasticity at individual synapses in brain slices and behavior in the whole animal, but also to understand how these alterations affect the firing properties of large neuronal circuits in the animal’s hippocampus during behavioral training. Whereas the cellular substrate of memory plasticity may be the object of interest in large groups of neurons within the brain. Several groups have recently applied techniques for recording the firing properties of groups of neurons in the hippocampus in large groups of neurons. Although, as we have seen, there are several strong correlations between LTD defects in the CA1 region and memory deficits, there are also some dissociations. These indicate that, under some circumstances, other behavioral level can compensate for defects in CA1. The use of regulated and anatomically restricted genetic modification, combined with phenotype analysis at the cellular, systemic, and behavioral level should provide a powerful set of tools for elucidating gradually the cellular and molecular mechanisms of memory storage throughout the medial temporal lobe.

References