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The past, the future and the biology of memory storage

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We here briefly review a century of accomplishments in studying memory storage and delineate the two major questions that have dominated thinking in this area: the systems question of memory, which concerns where in the brain storage occurs; and the molecular question of memory, which concerns the mechanisms whereby memories are stored and maintained. We go on to consider the themes that memory research may be able to address in the 21st century. Finally, we reflect on the clinical and societal import of our increasing understanding of the mechanisms of memory, discussing possible therapeutic approaches to diseases that manifest with disruptions of learning and possible ethical implications of the ability, which is on the horizon, to ameliorate or even enhance human memory.

Keywords: explicit; implicit; memory

1. INTRODUCTION

In the past several decades of this century we have witnessed a remarkable increase in the explanatory power of biology, including the biology of the brain. The ability of biology to address central issues of brain function will only increase in the 21st century and is likely to have a broad impact on many aspects of our lives. As a result, when the intellectual historians of The Royal Society look back on these decades, they are likely to acknowledge that the deepest insights into the nature of mental processes will not have come from the disciplines traditionally concerned with mind. They will have come not from philosophy, from the arts, or even from psychology or psychoanalysis, but from biology. This is because in the past two decades biology has participated not simply in one but in two major unifications of thought which bear on our understanding of mind.

First, there has been a remarkable scientific synthesis, achieved through molecular biology, that has brought together the disciplines of cell biology, biochemistry, developmental biology and oncogenesis. This unification derives from major advances in our understanding of the gene, which have revealed how its structure determines heredity and how its regulation determines development and function. These remarkable insights have given us a marvellous sense of the conservation between different cells in any one organism and different organisms across evolution.

Second, there has been a parallel unification between neurobiology, the science of the brain, and cognitive psychology, the science of the mind. This second unification is far less mature than that brought about by molecular biology, but it is potentially equally profound, for it promises to provide us with a new framework for the analysis of a variety of mental functions, such as perception, action, language, learning and memory.

These two independent unifications stand at the extremes of the biological sciences: the one at the interface between biology and chemistry; the other at the interface between biology and psychology. This raises a question: To what degree can these two disparate strands be brought together? Can molecular biology enlighten the study of mental processes as it has enlightened the study of development and oncogenesis? Can we anticipate an even broader synthesis in the 21st century, a synthesis ranging from molecules to mind? In this article we outline the possibility of a molecular biology of cognition, and suggest that it will occupy centre stage in the early part of the 21st century. We outline this development by using as an example the study of memory.

We begin with a historical perspective because many of the themes that dominate current research on memory—including the distinction between systems and molecular approaches to memory—emerge best in a historical overview. We will then describe more recent molecular biological investigations of memory. Here we will focus in particular on one component of the memory process: the switch from short- to long-term memory. This component can in fact now be analysed by combining cognitive psychology with modern molecular biology. Finally, we will suggest some possible future directions in memory research, focusing on the drawing together of strands of research that have historically been separated.

2. THE PROBLEM OF MEMORY HAS A SYSTEMS COMPONENT AND A MOLECULAR COMPONENT

The work of Ramon y Cajal at the beginning of the century (Cajal 1893) and of Donald Hebb in 1949 (Hebb 1949) established a useful conceptual framework for the study of memory, based on the idea that memory is stored as changes in the strength of specific synaptic connections. This framework divides the study of memory into

two components: the systems problem and the molecular problem. The systems problem of memory is concerned with where in the brain memory is stored and how neural circuits work together to create, process and recall memories. The molecular problem of memory is concerned with the mechanisms whereby synapses change and information is stored. Most early work on memory focused on the systems problem, focusing on the question 'Where is memory stored?' We therefore begin our historical perspective with this question.

3. THE SYSTEMS COMPONENT OF MEMORY

(a) *Early attempts to localize mental function in the brain*

The attempt to understand where memory is stored goes back at least 200 years and is part of a larger question: can any mental process be localized to a specific region of the brain? The modern debate about the localization of brain function began in the early part of the 19th century with Franz Joseph Gall, a German physician and neuroanatomist who taught for a long while at the University of Vienna. Gall started the school of 'organology' (later 'phrenology'). He made two remarkable and enduring contributions to our understanding of brain (Gall & Spurzheim 1810). First, he appreciated that all mental processes are biological and arise from the brain. He therefore opposed the Cartesian mind–brain dualism of his day and argued for a materialist view of the mind (a view that later led to his expulsion from the University of Vienna). Second, he posited that mental functions are localized to different regions within the brain. Gall argued that the brain (and specifically the cerebral cortex) is not homogeneous but is subdivided into functionally distinct regions, each of which serves as the organ for one or another mental function. Based on contemporary academic psychology, Gall ascribed each of 35 mental faculties to a specific cortical region. Thus, Gall argued that even the most abstract and complex of human behaviours, such as cautiousness, secretiveness, hope, sublimity and parental love, are mediated by different, individual cortical regions. Gall therefore was the first strong proponent of the localization of function within the cortex, and he thereby initiated a debate that persisted for the next century (figure 1) (Cooter 1984; Young 1970).

While Gall's theory of localization was prescient and is echoed in the functionally identified areas that tile much of the cortex in today's neuroanatomy texts, his theory was flawed in two ways. First, as we now appreciate, the 35 abstract properties for which Gall was seeking to identify cortical organs, such as parental love and conscientiousness, are too complex to be represented by individual discrete areas. Second, his method for identifying the function of specific areas proved simplistic. Gall distrusted lesion experiments and therefore ignored clinical findings. He rather was guided by the theory that the size of a given area of the brain is related to usage of that area by the mental faculty which it represents. Exercise of a given mental faculty causes the corresponding critical brain region to grow. This growth in a brain region would in turn cause the overlying skull to protrude. By examining the bumps and ridges on the skulls of people

(*a*)

(*b*)

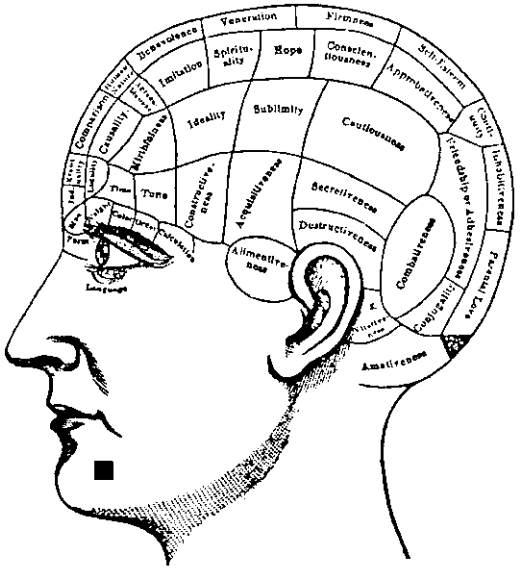

Figure 1. (*a*) Franz Joseph Gall theorized that cortical regions have specialized function. (*b*) Gall divided the cortex into 35 regions, each dedicated to a specific psychological characteristic. Taken from Kandel *et al.* (1995).

well endowed with specific faculties, Gall sought to identify the centres for those faculties. This led to 'phrenology,' with its preoccupation with the shape of the skull as indicating the structure of personality and character.

In the late 1820s, Gall's ideas were subjected to experimental analysis by the French experimental neurologist Pierre Flourens, who readily showed that the bumps on a person's skull bear little relation to the shape of the brain underneath (figure 2). Flourens removed cortical areas corresponding to certain of the functional areas defined by Gall from experimental animals and failed to find any of the deficits that Gall's theories predicted. In fact, Flourens was unable to identify any specific deficits in behaviour that were associated with specific lesions of the

Figure 2. Pierre Flourens tested Gall's theories and concluded that the cortex is equipotential.

Figure 3. Pierre-Paul Broca concluded from patients with localized brain lesions that language is localized within the cortex.

neocortex. He thus concluded that the nervous system in general, and the cerebral hemispheres in particular, are equipotential: any part of the cerebral cortex participates in and is able to perform all the functions of the whole (Flourens 1893). Injury to any part would, according to this theory, not affect any one capacity more than others.

The debate between the advocates of functional localization and the advocates of an equipotential view of cortex coloured thinking about the brain in the first half of the 19th century. This debate was largely resolved in the second half of the century by two great neurologists, Paul Broca and Carl Wernicke. In studies of patients with specific language deficits (aphasias), Broca and Wernicke identified cortical areas whose lesion produced remarkably specific disorders of language, thereby demonstrated convincingly the localization of at least some higher functions within the cerebral cortex.

(b) *Broca, Wernicke and the localization of language*

In 1861, Pierre-Paul Broca, a French neurologist much influenced by Gall (figure 3), described the first of nine patients who suffered from a language impairment (now known as Broca aphasia) in which they could understand language but could not speak fluently. These defects were specific to the expression of language rather than to motor control of the vocal tract, as the patients could hum or whistle a tune but could not write fluently. Postmortem examination of the patients' brains revealed in each case a lesion in the posterior region of the frontal lobe (a region of cortex now called Broca's area). Thus, for the first time, Broca was able to assign a well-defined higher function to a specific region of cortex (Broca 1865). Since all of these lesions were in the left hemisphere, Broca was also able to establish that the two hemispheres, although apparently symmetrical, have slightly different functions.

A decade later, in 1876, Carl Wernicke, a German neurologist (figure 4), described a second type of aphasia (Wernicke's aphasia) that is in a way the opposite of Broca's: an impairment not of the production of speech but of comprehension. Wernicke found that this syndrome was caused by a lesion in the posterior superior portion of the temporal lobe of the left hemisphere, a lesion distinct from that described by Broca, in an area we now call Wernicke's area.

Taking his findings together with those of Broca, Wernicke put forward a theory of how the cortex is organized for language that, although simpler than our current understanding, is still central to how we view the brain. Wernicke proposed that any complex behaviour requires the activity not of one but of a number of different brain areas, and that these areas are interconnected in various ways. Mental activity is not unitary or seamless as might intuitively appear to be the case, but can be broken down into multiple components, and each component can be assigned to a more or less specific brain region, much as the organologists had insisted. However, these different specialized areas do not function by themselves but as part of large, interconnected networks. By application of this model Wernicke predicted, correctly, the possibility of a third kind of aphasia, conduction aphasia, resulting from lesion not of Broca's area or Wernicke's area but of the fibres (the arcuate fasciculus) passing between the two. Thus, while specific functions are localized as Gall had insisted, the

Figure 4. Carl Wernicke developed the idea of interconnected specialized areas of cortex that is still the dominant framework today.

function of the brain as a whole requires distributed processing somewhat reminiscent of that propounded by Flourens (Wernicke 1908). Wernicke's model of a distributed network of specialized areas has emerged as a dominant theme in the study of the brain.

(c) *Can memory storage be localized to specific regions of the brain?*

The finding that the language can be localized within the brain led to the hunt for other areas concerned with specific higher functions. Areas concerned with motor control and with each of the senses were soon identified. It was only a matter of time before efforts to localize cognitive function would turn to memory (Ferrier 1890; Jackson 1884). However, attempts in the first half of the 20th century to localize memory failed. The dominant figure in this period was Karl Lashley, Professor of Psychology at Harvard (figure 5). Lashley began the experimental search for the locus of memory storage by training rats on specific memory tasks, systematically removing portions of cortex, and then testing the rats' recall. He repeatedly failed to find any particular brain region that was special to or necessary for the storage of memory. On the basis of these findings, Lashley formulated the law of mass action, according to which the extent of a memory deficit is correlated with the size of a cortical lesion but not with the specific site of that lesion (Lashley 1929). This law was reminiscent of the views of Flourens a century earlier.

The first clear evidence for the localization of memory came not from experimental animals but from clinical

Figure 5. Karl Lashley investigated the effects of cortical lesions on memory in rats and determined that no portion of cortex is specialized for memory.

studies. In 1938, the neurosurgeon Wilder Penfield (figure 6), working at the Montreal Neurological Institute, developed methods for the surgical treatment of focal epilepsy, a form of epilepsy in which seizure is restricted to a relatively small region of the cortex. To functionally map the areas surrounding the epileptic centre so as to avoid later damage to critical areas, such as Broca's and Wernicke's, Penfield electrically stimulated the cortical surface. Because the brain contains no pain receptors, the patients were unanaesthetized and could report what they experienced during stimulation. In this way Penfield studied over 1000 patients and mapped out in each of them most of the exposed cortical surface. On rare occasions during such mappings, Penfield found a region of temporal cortex where stimulation gave rise to specific experiential responses, memory-like perceptions that the patients could describe. Penfield concluded that portions of the temporal lobe were specifically involved in memory (Penfield & Perot 1963).

More conclusive evidence for the involvement of temporal lobe structures in memory came in 1957, when William Scoville, a neurosurgeon influenced by Penfield, and Brenda Milner, a psychologist and long-term collaborator of Penfield's (figure 7), reported the now famous case of H.M. (Scoville & Milner 1957). At age 9, H.M. sustained a head injury after being hit by a bicycle; over the next 18 years he suffered progressive seizures until he was completely incapacitated. As a last resort, H.M. underwent complete bilateral removal of the medial temporal lobe (where his seizures initiated). The surgery relieved his epilepsy, but he was left with a profound memory deficit: from the time of his surgery until this day he has been unable to form any new memories of people, facts or events.

Brenda Milner studied H.M. and demonstrated that structures in the medial temporal lobe that Scoville had

Figure 6. Wilder Penfield, a neurosurgeon working at McGill University, used stimulation of the cortical surface of epilepsy patients to map functional areas.

Figure 7. Brenda Milner's seminal studies of the patient H.M. conclusively identified the medial temporal lobe as a region specialized for memory.

removed are specialized for memory. Her further studies with H.M. not only controverted Lashley's influential views but also cast new light on the systems problem of memory: there are, within the brain, multiple, functionally specialized memory systems.

(d) *Memory is not a unitary faculty of mind: there are multiple memory systems*

The idea that there may be multiple memory systems is old, but it did not enter mainstream psychological thinking until Milner's work in the 1960s. In the early parts of the 19th century, the French philosopher Maine de Biran argued that memory can be subdivided into different systems for ideas, feelings and habits (Copplestone 1977). William James emphasized the idea that memory has distinct temporal phases (James 1890). In the 20th century, Henri Bergson developed the distinction between conscious memory and habit (Bergson 1913). In 1949, the British philosopher Gilbert Ryle proposed a similar distinction between 'knowing that' (conscious recall of knowledge for facts and events) and 'knowing how' (knowledge of performance or skills without recourse to conscious awareness) (Ryle 1949). A similar distinction was made by the psychologist Jerome Bruner, who termed 'knowing that' a memory with record and 'knowing how' a memory without record (Milner *et al*. 1998). The defining characteristic of memories with record is the ability to summon up a more or less detailed conscious recollection of facts and events about persons, places and objects. The defining characteristic of a memory without record, by contrast, is a change in the way an organism responds to a situation or a stimulus, without access to the specific circumstance under which the memory was formed. The idea of distinct memory systems is, in a sense, implicit in Freud's psychoanalytic writing. Central to Freud's view of the brain is the distinction between conscious and unconscious memories.

Thus, even prior to 1960 fractionation of memory had already been proposed on the basis of content, function and temporal profile. Nevertheless, the concept of multiple memory systems only drew the attention of the scientific community with the studies of H.M. After the profound nature of his memory deficit was recognized, Milner made the further discovery that despite his impairment he could learn a surprising amount of new information. First, H.M. was found to have perfectly good short-term memory: he could accurately repeat back a telephone number and he can carry on a normal conversation. It is only when he is distracted from the topic or task at hand that his memory deficit reveals itself. Thus, the temporal lobe structure, which H.M. lacks, is not required for short-term (or 'working') memory. This finding validated the early distinction between short- and long-term memory.

Second, Milner found that H.M. has reasonably good long-term memory for events prior to his operation. He maintains his overall intelligence and has good command of English. He remembers events from his childhood and adult life before his surgery. There is a period of retrograde amnesia for events shortly before the surgery, but for the most part H.M.'s symptoms revealed that the medial temporal lobe is not the ultimate storage site for previously acquired knowledge. This finding supports the idea implied in Wernicke's model that knowledge is ultimately stored in whatever area of the cortex processes the relevant sort of information (see, for example, Zeki 1993).

Third, and most surprising, H.M. is able to form certain types of long-term memory. In 1962, Milner and the psychologist Suzanne Corkin found that H.M. was able to acquire new motor skills (specifically, the ability to trace a complex figure in a mirror) (Corkin 1965). When asked, he would deny that he had encountered or practised the task before; but his performance showed unequivocal improvement over time. This finding showed that learning of this skill is preserved after severe temporal lobe damage and in the presence of profound amnesia for facts and events, and thus it demonstrated for the first time a fractionation of memory on the basis of content rather than just duration. Milner and Corkin thus validated the distinction between conscious memory and habit propounded by Bergson 50 years earlier and by Ryle in 1949.

The learning tasks that amnesic patients like H.M. are capable of mastering have several things in common. They have an automatic quality, and the formation or expression of the memories is not dependent on awareness or cognitive processes such as comparison and evaluation. This type of memory typically builds up slowly over many trials and is expressed primarily by improved performance on certain tasks. The psychologist Lawrence Weiskrantz has noted that the spared learning skills are reflexive rather than reflective—typically the patient need only produce a physical response to a stimulus or cue.

This distinction was soon validated on normal subjects. Larry Squire has framed the distinction particularly well by emphasizing the ability of humans to report verbally the contents of explicit memory but not of implicit memory: explicit memory is thus declarative whereas implicit memory is non-declarative (Squire & Zola-Morgan 1991). Daniel Schachter framed Bruner's distinction using the terms implicit for 'knowing how' and explicit for 'knowing that' (Schacter 1996). These are the most widely used terms, and while specific definitions of these various terms can differ in different contexts, all these authors are describing the basis distinction that was revealed in the studies of H.M.

(e) *Implicit and explicit memory systems can be further subdivided*

The distinction between implicit (non-declarative) and explicit (declarative) memory, as foreseen by James, Bergson, Ryle and Bruner early in the century and then revealed by studies of H.M. and other patients with amnesia, is now generally accepted as being well-founded biologically. Indeed, once the non-unitary nature of memory had been established, the search was on to discover further subdivisions. In 1972, the cognitive psychologist Endel Tulving proposed that explicit memory can be further subdivided into episodic and semantic memory (Tulving 1972). Episodic memory is autobiographical memory for specific events; semantic memory is more abstract memory for facts, be they autobiographical or more general. Different humans are predisposed to remember their own pasts in one or the other way; and over time autobiographical memory often shades into semantic memory, as we can no longer remember the experience of a specific event but can unmistakably remember that it did happen to us. Tulving has described a patient, K.C., who after a motorcycle accident possesses intact semantic memory but no episodic memory (Tulving *et al*. 1988).

Implicit memory similarly is not a single memory system but a large collection of systems, as is made more clear by Squire's designation non-declarative memory (Squire & Kandel 1999). Fractionation of implicit memory is made on the basis of specific learning tasks, most of which can be assigned to specific systems. Habituation, sensitization and classical conditioning involve sensory and motor systems recruited for the specific task tested. These are the most elementary forms of learning and can be studied effectively even in invertebrates. Motor learning involves the cerebellum (Thompson *et al*. 1998). Emotionally based memory involves the amygdala (LeDoux 1996). Operant conditioning, the association of a stimulus or action with a reward, involves the ventral striatum, or nucleus accumbens, which is also the structure most often implicated in drug addiction (Robbins & Everitt 1999). Acquisition of certain forms of habit, by contrast, requires the dorsal striatum (Graybiel 1998). Finally, the various sensory cortices are involved in the phenomenon of priming (Tulving *et al*. 1982). Implicit memory is therefore a heterogeneous collection of memory functions, each performed by a different brain structure, adapted to a different role, and dissociable from the others by lesions or clever task design.

4. THE MOLECULAR PROBLEM OF MEMORY STORAGE

As we have just summarized, an examination of memory storage at the systems level revealed that memory is not a unitary faculty but has at least two forms: implicit (non-declarative) and explicit (declarative). This distinction raises a question: what are the cell and molecular mechanisms by which implicit and explicit memories are stored in the brain? Are the molecular storage mechanisms as different as is the logic of the explicit and implicit memory systems?

Our most detailed knowledge about the mechanisms of implicit memory storage has come from studies of simple forms of implicit memory storage in the invertebrates *Aplysia* and *Drosophila* and from parallel studies of simple memory storage in mammals. We shall first consider the studies in invertebrates.

(a) *Implicit memory storage is best studied in simple associative and non-associative forms of learning*

Implicit memory refers to memory for perceptual and motor skills. The simplest instances of such storage are elementary forms of non-associative and associative memory. These were first clearly identified in studies of the family of learning processes related to classical conditioning.

Learning is the acquisition of new information about the world, and memory is the retention of that information over time. Classical conditioning is a form of learning in which an animal learns to associate two sensory stimuli, a neutral initiating sensory stimulus (called the to-be-conditioned stimulus or CS by behaviourist psychologists), which produces little or no response in a naive animal, and a highly effective sensory stimulus (the unconditioned stimulus or US), which

produces an unlearned reflex response (the unconditioned response or UR). Upon pairing the two sensory stimuli, the animal learns to strengthen its pre-existing response to the neutral sensory stimulus (the CS) or to develop a completely new response to the CS.

In the course of studying classical conditioning, Ivan Pavlov and others discovered that when each of these two stimuli were repeatedly presented alone, they each gave rise to distinctive forms of learning and memory storage. Repeated presentations of the neutral stimulus (CS) by itself gives rise to a form of learning called habituation, wherein the animal learns to recognize a stimulus as innocuous and comes to ignore it. By contrast, presentation of the aversive stimulus (US) alone gives rise to sensitization, a form of learning wherein the animal enhances its defensive and escape responses to otherwise innocuous stimuli (CS). Thus, simple forms of learning take two forms: (i) non-associative learning, such as habituation and sensitization, in which the animal learns about the properties of a single stimulus; and (ii) associative learning, in which the animal learns about the relationship between two stimuli (Squire & Kandel 1999).

What are the cellular changes that result in the brain when animals learn these simple tasks? Initial insights into the cell biological mechanisms of each of these three forms of memory storage first came from studies of the marine snail *Aplysia*. *Aplysia* lends itself to the study of implicit memory storage. First, the animal can learn in a number of different tasks and has both short- and long-term memory (as we will discuss further below). Second, the animal has a relatively simple central nervous system, consisting of only about 20 000 neurons. Third, the neurons of *Aplysia* are particularly large, which allows them to be uniquely identified so that one can return to the same cell in every animal of the species. Fourth, it is possible to map in detail the synaptic connections between individual cells and between a given cell and the sensory and motor periphery. As a result of these advantages, it is possible to work out significant parts of the neural circuitry of a given behaviour—such as the gill and siphon withdrawal reflexes—in terms of uniquely identifiable cells and their pattern of interconnections (Kandel 1976). One can culture *Aplysia* neurons and construct with them *in vitro* in ways that are not yet possible in other systems.

Studies of memory in *Aplysia* also first illustrated the advantages that accrue from using elementary forms of non-associative and associative learning for studying memory storage. These simple forms of memory consist of a modification of the response to a specific test stimulus (the CS) and can be examined at any time after learning by simply examining the time-locked reflex response to that stimulus (Kandel *et al*. 1995). The key requirement for a neurobiological analysis of memory is to work out, in cellular detail, the neural circuitry mediating the behaviour and how it is modified by learning. One needs in particular to work out the reflex pathway whereby the sensory stimulus leads to a behavioural response. In learning tasks in which there is a clearly defined alteration in the behavioural response to the CS, all the important learning-related changes are contained within this circuit.

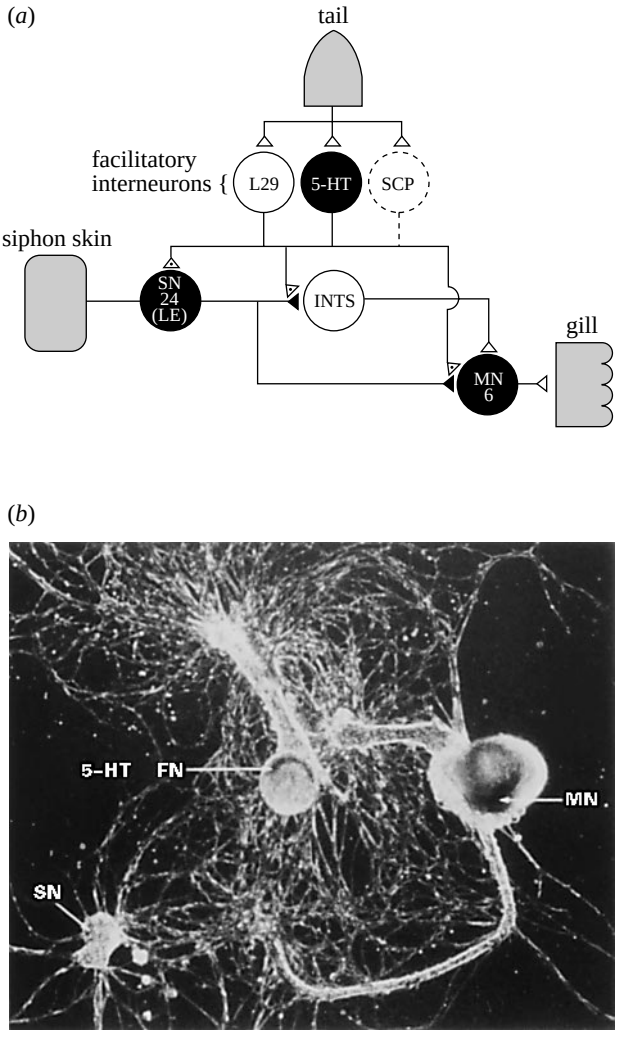

Figure 8. (*a*) The gill withdrawal reflex in *Aplysia* is mediated by a simple circuit. Approximately 50% of the learning observed in sensitization of this reflex by tail shock results from potentiation by serotonin of the direct synapse between the sensory neuron innervating the siphon and the motor neuron innervating the gill. Individual identified neurons are indicated. (*b*) The circuit controlling this reflex can now be studied in reconstituted cell culture. (SN, sensory neuron; 5-HT FN, facilitatory interneuron; MN, motor neuron.)

5. SHORT-TERM STORAGE INVOLVES FUNCTIONAL CHANGES IN THE STRENGTH OF PRE-EXISTING SYNAPTIC CONNECTIONS

In *Aplysia*, most work has been carried out on the withdrawal reflex of the gill and the siphon to a weak tactile stimulus applied to the siphon. This withdrawal reflex is mediated by both monosynaptic and polysynaptic connections. The sensory neuron that innervates the siphon makes direct monosynaptic connections on the motor neuron that withdraws the gill. In addition, the sensory neurons make polysynaptic connections to motor neurons via interneurons (figure 8). While the polysynaptic connections contribute importantly to both the basal reflex and to learning, most studies have concentrated on the monosynaptic connections. These connections form a significant component of the behaviour, their electrophysiological properties recapitulate the basic properties

of both short- and long-term memory for various forms of learning, and they can be reconstituted in culture and therefore studied in great detail morphologically and biochemically.

Although the gill and siphon withdrawal reflex is quite simple, it exhibits all three simple forms of learning. In each, the animal learns to alter its behavioural response to the tactile stimulus to the siphon (which is the CS for these forms of learning), and in each case repetition converts a short-term form to a long-term form of implicit memory.

With habituation of the gill and siphon withdrawal reflex, the animal learns about the properties of a single, novel stimulus, a weak tactile stimulus to the siphon. When this stimulus is first presented, the animal perceives it as novel and responds to it with a brisk reflex response. But when the same weak stimulus is repeated, the animal comes to recognize the stimulus as trivial and learns to stop responding to it. As a result, the weak siphon stimulus that once produced a brisk response now produces little or no response at all. This progressive decrease in the response to the weak siphon stimulus is reflected in reflex circuit as a weakening of the synaptic connections between the siphon sensory neurons and their central target cells—the interneurons and motor neurons (figure 8). This weakening results from a decrease in the amount of the transmitter glutamate released from the presynaptic terminals of the sensory neurons.

With sensitization, *Aplysia* learns about the properties of an important aversive stimulus, a noxious shock to the tail (an unconditioned stimulus). The animal recognizes the stimulus as aversive and learns to enhance its gill and siphon withdrawal responses to the CS, the weak touch to the siphon. Sensitization is reflected in the neural circuit as an increase in the synaptic strength in the input connections of the reflex, between the siphon sensory neurons and their target cells (the CS pathway). This strengthening is due to an increase in the release of glutamate from the terminals of the sensory neurons (Kandel 1976; Byrne & Kandel 1996; Carew & Sahley 1986; Hawkins *et al*. 1993).

Aplysia also can learn to associate these two stimuli; it can learn classical conditioning. When a weak (CS) stimulus to siphon is repeatedly paired with a shock to the tail (the US), the reflex response to the siphon stimulus is enhanced. The enhancement of the response to the CS in classical conditioning is substantially greater than with sensitization, when the weak siphon stimulus (CS) and the tail shock (US) are not paired. This classical conditioning is reflected in the neural circuitry as a greatly enhanced strengthening of the input connections of the sensory neurons to their target cells, an enhancement that is greater than that of sensitization. Whereas in the increase in synaptic strength with sensitization the principle change is presynaptic (an increased release of glutamate from the sensory neuron), in classical conditioning both presynaptic and postsynaptic mechanisms participate (Squire & Kandel 1999).

There is now a reasonably good understanding of how the association in between CS and US is achieved on the cellular level (Glanzman 1995). As we have seen, the sensory neurons release glutamate from their presynaptic terminals. Glutamate acts on two types of postsynaptic receptors: an AMPA receptor and an NMDA receptor. Under normal circumstances and with non-associative learning such as habituation and sensitization, only the AMPA receptors are activated, because the mouth of the NMDA receptor channel is blocked by the ion Mg^{2+}. To remove the Mg^{2+} block and activate this channel, two events need to happen simultaneously: glutamate needs to bind to the receptor; and the postsynaptic membrane needs to be depolarized substantially so as to expel Mg^{2+} from the NMDA receptor channel mouth. Coincident binding of glutamate to the NMDA receptor and postsynaptic depolarization only occur when the weak siphon stimulus (CS) and the strong tail shock (US) are paired. Only then is each of the postsynaptic motor cells sufficiently depolarized to allow activation of the NMDA receptors (Lin & Glanzman 1994). As was shown earlier for sensitization and habituation (Kandel 1976), changes at this synapse have now been observed after learning in the intact animal (Murphy & Glanzman 1997). This compellingly supports the notion that changes in this synapse are importantly, causally involved in the learning of new information.

The NMDA receptor is particularly permeable to the ion Ca^{2+}, so when it is activated, Ca^{2+} enters the cell. The Ca^{2+} influx in turn activates a signalling cascade in the postsynaptic cell. One component of long-term potentiation in *Aplysia* is a retrograde signal that is generated in the postsynaptic cell and sent back from it to the presynaptic neuron. This signal enhances transmitter release to an even greater extent than occurs in sensitization. Thus, in *Aplysia*, facilitation of the connections between the sensory and motor neurons that occurs with classical conditioning has an additional, associative component superimposed on the facilitation produced by sensitization (Squire & Kandel 1999).

These several studies in *Aplysia* generate three insights into short-term memory storage that have proven quite general and apply to explicit as well as to implicit memory storage. First, these studies show that learning can lead to alterations in synaptic strength and that the persistence of these synaptic changes in both the mono- and polysynaptic component of the neural circuit of the reflex circuit represents the cellular storage mechanisms for memory. Second, a single synaptic connection can participate in, and be modified by, different forms of learning and participate in different types of short-term memory storage. Finally, each of these three simple forms of learning—habituation, sensitization and classical conditioning—can give rise to either a short- or a long-term memory, depending on number of training repetitions, as we will discuss further below. Each of the long-term forms of memory is associated with a long-term change in synaptic strength in the monosynaptic connection between the sensory and motor neuron of the reflex. Thus, not only can a single synaptic connection participate in different types of short-term memory storage, but the same connection can also be the site of both short- and long-term memory storage.

6. LONG-TERM STORAGE INVOLVES THE SYNTHESIS OF NEW PROTEIN AND THE GROWTH OF NEW CONNECTIONS

Given that a single connection can participate in both short- and long-term memory, what are the molecular

mechanisms for these different phases of memory storage? In particular, how is short-term memory converted to long-term memory? Can molecular biology reveal similarities in the mechanisms of storage of explicit and implicit memory?

The first insight into the molecular mechanisms of memory storage came from the discovery that there are phases of memory storage. The study of memory phases dates to 1885 and the work of Herman Ebbinghaus. By forcing himself to memorize lists of nonsense syllabus and then testing his own recall, Ebbinghaus determined that there are at least two phases to memory: a short-term phase which contains much information but is transient, lasting minutes; and a long-term phase which is far more stable (Ebbinghaus 1913). This is consistent with our everyday experience: we have access to far more information from the recent past (say, the last few minutes) than we will be able to remember a few hours hence. Ebbinghaus's distinction is also consistent with observations of victims of injury: a person who is struck on the head or shocked will typically lose memory for events that occurred shortly before the insult but not for more remote events (e.g. Zubin & Barrera 1941).

In the 1960s, Louis Flexner and his colleagues first found that short- and long-term memory are not only distinguished by their time-course but also by their biochemical mechanism. Long-term memory differs from short-term memory in requiring the synthesis of new protein (Flexner *et al*. 1965; Agranoff 1976). This requirement for protein synthesis for long-term memory has a specific time-window, during and shortly after training, which is called the consolidation phase. Blockade of protein synthesis during the consolidation phase will disrupt long-term memory, but blockade before or after will have no effect (Bourtchouladze *et al*. 1998; Freeman *et al*. 1995).

The requirement for protein synthesis for long-term but not short-term memory, and the existence of a consolidation window during which memories are sensitive to disruption, has proven to be very general. It has been demonstrated in explicit as well as implicit storage and in different vertebrates as well as in invertebrates. This conservation in turn suggests that the proteins involved in the switch to long-term memory may also be conserved. If that were true, then a detailed study of the molecules involved in the switch in any given memory storage process in any animal is likely to yield proteins that are of general importance. Moreover, molecular study of several different instances of memory storage is likely to reveal the general nature of a cognitive process: the switch whereby a transient short-term memory is converted to a persistent, self-maintained long-term memory (Pittenger & Kandel 1998). During the last decade, studies in *Aplysia*, *Drosophila* and mice have begun to reveal some of the proteins essential for this switch.

(a) **Aplysia** *and* **Drosophila** *use some of the same genes and proteins for converting short- to long-term memory*

The initial molecular insights into long-term storage of implicit memory came from studies of sensitization in *Aplysia*. As with other forms of learning, repetition of the sensitizing protocol in *Aplysia* increases the duration of the memory for sensitization. Thus, one tail shock produces an enhancement of the withdrawal response that lasts a few minutes. Five shocks produce an enhancement that lasts several days, and further training gives rise to memory that lasts weeks. This long-lasting sensitization requires protein synthesis during a critical time-window, whereas short-term sensitization does not (Montarolo *et al*. 1986). Here, then, is a very simple model system of long-term memory that shares some of the key mechanistic properties of more complex vertebrate systems.

Sensitizing tail stimuli activate three different classes of modulatory interneurons that synapse on the axon terminals of the siphon sensory neurons; all three have similar actions. Of the three, the interneurons that release serotonin (or 5-hydroxytryptamine, 5-HT) are thought to be particularly important (Kandel 1976). In the intact animal and in reduced preparations, serotonin acts on the sensory neuron by increasing the intracellular concentration of the second messenger cAMP and by activating the cAMP-dependent protein kinase (PKA) and protein kinase C (Braha *et al*. 1990; Brunelli *et al*. 1976). Transient activation of these intracellular signalling cascades with one tail shock or one pulse of 5-HT leads to a transient facilitation of the synapse between the sensory and motor neurons (Castellucci & Kandel 1976). Repeated training or five pulses of 5-HT produce long-lasting facilitation that can persist for 72 h or more and is accompanied by the growth of new synaptic connections (Castellucci *et al*. 1986; Montarolo *et al*. 1986; Bailey & Kandel 1993).

Whereas a single pulse of 5-HT activates the kinases PKA and PKC transiently, five pulses lead to a persistent activation of PKA and to the recruitment of the MAP kinase signal transduction pathway (Michael *et al*. 1998). Both PKA (Bacskai *et al*. 1993) and MAP kinase (Martin *et al*. 1997*b*) then translocate to the nucleus, where they activate the transcriptional activator CREBla (Bartsch *et al*. 1998) and inactivate the transcriptional repressor CREB2 (Bartsch *et al*. 1995). The CREB family of transcriptional activators has been implicated in plasticity in many systems, as we will discuss below, and may be one of the most conserved molecular components of the mechanisms for switching on long-term plasticity. Once CREBla is activated and the repressive action of CREB2 is removed, a set of immediate-early genes is activated, including the transcriptional activator ApC/EBP (Alberini *et al*. 1994). ApC/EBP forms both homodimers and heterodimers with another factor (activating factor 1). The homodimers and heterodimers act differently on downstream genes that initiate the growth of new synaptic connections. This structural change, which represents the long-term, stable form of memory, is associated with a rearrangement of structural proteins such as the cell adhesion molecule ApCAM (Bailey & Kandel 1993). This internalization of ApCAM is thought to be a necessary prerequisite for the growth of neuronal processes (figure 9).

The requirement for transcription in long-term facilitation in *Aplysia* explains why long-term memory requires the synthesis of new protein. However, this requirement poses a cell biological puzzle: since long-term plasticity relies on the activation of genes in the nucleus, one might expect that long-lasting changes in the connectivity of the

neuron would be cell-wide. Recent experiments have revealed that each synapse or group of synapses can be modified independently. This spatially restricted plasticity requires the activity of CREBl in the nucleus as well as local protein synthesis in the processes that were modulated by 5-HT. The mechanism of this specificity is revealed by a second phenomenon: synaptic capture. Certain *Aplysia* sensory neurons have bifurcated axons and can be cultured such that they synapse on two widely separated motor neurons. When synapse-specific, long-term facilitation is initiated at one of the two branches, a single pulse of 5-HT—which normally is able to induce only transient facilitation—can induce long-term plastic changes when applied to a second branch (figure 10) (Martin *et al*. 1997*a*). This phenomenon suggests that the new genes that are being activated in the nucleus have their products distributed widely, but that the products only persistently strengthen those synapses that have been somehow marked by short-term facilitation. A similar phenomenon has been observed in the vertebrate brain (Frey & Morris 1997).

A similar set of genes important in the switch from short- to long-term memory has emerged from studies of *Drosophila*. As an experimental system, *Drosophila* is in many ways the complement of *Aplysia*. The great advantages of *Aplysia* are that it is tractable for cell biological studies and that synaptic circuits can be reconstituted in cell culture. Genetics, however, is currently impossible in *Aplysia*. In *Drosophila*, on the other hand, cell biological and electrophysiological studies are difficult due to the tiny size of the neurons, but the genetics are extremely tractable and mature. The great strength of the *Drosophila* work in learning is therefore in its genetic analysis. Both genetic screens and reverse genetic analysis, in which specific genes have been disrupted to investigate their function, have been fruitful.

The pioneering work of Seymour Benzer, and the subsequent studies of his students Quinn, Tully and Davis, have led to the identification of a number of genes required for memory storage (Weiner 1999). Many of the genes identified in this way are the same as those implicated in plasticity in *Aplysia*. For example, the *Drosophila* genes *dunce*, *rutabaga* and *amnesiac* all encode components of the cAMP-PKA cascade (figure 11) (Davis 1996). Other genes identified encode participants in other signal transduction cascades (for example, the gene *leonardo*; Skoulakis & Davis 1998) or cell–cell adhesion molecules similar to ApCAM (Grotewiel *et al*. 1998). Moreover, a protein synthesis-dependent phase of learning has been described by Tully and his colleagues, and Yin and Tully have shown that, as in *Aplysia*, CREB has a critical role in the induction of long-term memory. They found that a dominant-negative CREB allele blocked the formation of long-term memories (Yin *et al*. 1994), while overexpression of the wild-type allele enhances long-term memory stabilization (Yin *et al*. 1995). This work shows that CREB has a role in learning in *Drosophila* that is similar or identical to its role in *Aplysia*, demonstrating striking evolutionary conservation.

Finally, Corey Goodman and his colleagues have used the neuromuscular junction of *Drosophila* to examine the developmental plasticity of nerve–muscle synapse. Goodman and colleagues have found that at this synapse CREB is also required for the induction of transcription-dependent plasticity (Davis *et al*. 1996). However, whereas CREB is required for functional plasticity, it is not sufficient for morphological plasticity. For morphological plasticity, the loss of the cell adhesion molecule Fasl, a homologue of ApCAM, is required, much as the internalization of ApCAM is required for learning-related growth in *Aplysia* (Schuster *et al*. 1996).

7. IMPLICIT MEMORY STORAGE AND SYNAPTIC PLASTICITY IN THE MAMMALIAN BRAIN

Implicit forms of memory in mammals, such as habituation, sensitization and classical conditioning, resemble learning in *Aplysia*. As in *Aplysia*, the most successful analyses of implicit memory storage in mammals have been based on a delineation of the reflex circuit of the learned response, so the synapses at which plasticity may be important can in principle be identified. This approach has been particularly fruitful in classical conditioning of the eyeblink reflex to a puff of air, which depends on the cerebellum, and in conditioned fear, which depends on the amygdala.

(a) *The cerebellum and motor memory*

The cerebellum is necessary for a form of implicit memory: it is required for the learning of coordinated motor skills, and in particular for the acquisition of classical conditioning of motor reflexes such as eyeblink in the rabbit (Thompson *et al*. 1998). The cerebellum has an elegantly simple circuitry. Inputs to the cerebellar cortex are of two sorts: the mossy fibres, which carry the information relating to the conditioned stimulus, and the climbing fibres, which represent the unconditioned stimulus. The mossy fibres converge on the large cortical Purkinje cells. (They do this indirectly by synapsing on small granule cells, so it is the granule cell axons, the parallel fibres, which actually synapse on the Purkinje cell.) Whereas each climbing fibre synapses (approximately) one to a Purkinje cell, granule cells are massively convergent, with as many as 100 000 parallel fibres synapsing on a single Purkinje cell (figure 12) (Llinás & Walton 1998). The Purkinje cell is ideally situated to form the connections required for classical conditioning, between arbitrary sensory conditioned stimuli represented by the mossy fibres and fixed responses represented by the climbing fibres (Marr 1969). Indeed, when a single climbing fibre is stimulated it will sometimes produce a muscle contraction, and when this stimulation is repeatedly paired with an arbitrary neutral stimulus, that neutral stimulus will come to elicit the contraction. (That is, direct stimulation of the climbing fibre can substitute for the US in classical conditioning to a CS.) (Brogden & Gantt 1937; Swain *et al*. 1992.)

In this stereotyped circuit, one site of plasticity critical for learning appears to be at the parallel fibre–Purkinje cell synapse (Thompson *et al*. 1998). This synapse exhibits long-term depression, or LTD. At the parallel fibre synapse, induction or LTD appears to require simultaneous depolarization of the Purkinje cell and activation of the metabotropic glutamate receptor mGluR1 (a glutamate receptor which, rather than passing an ion, initiates

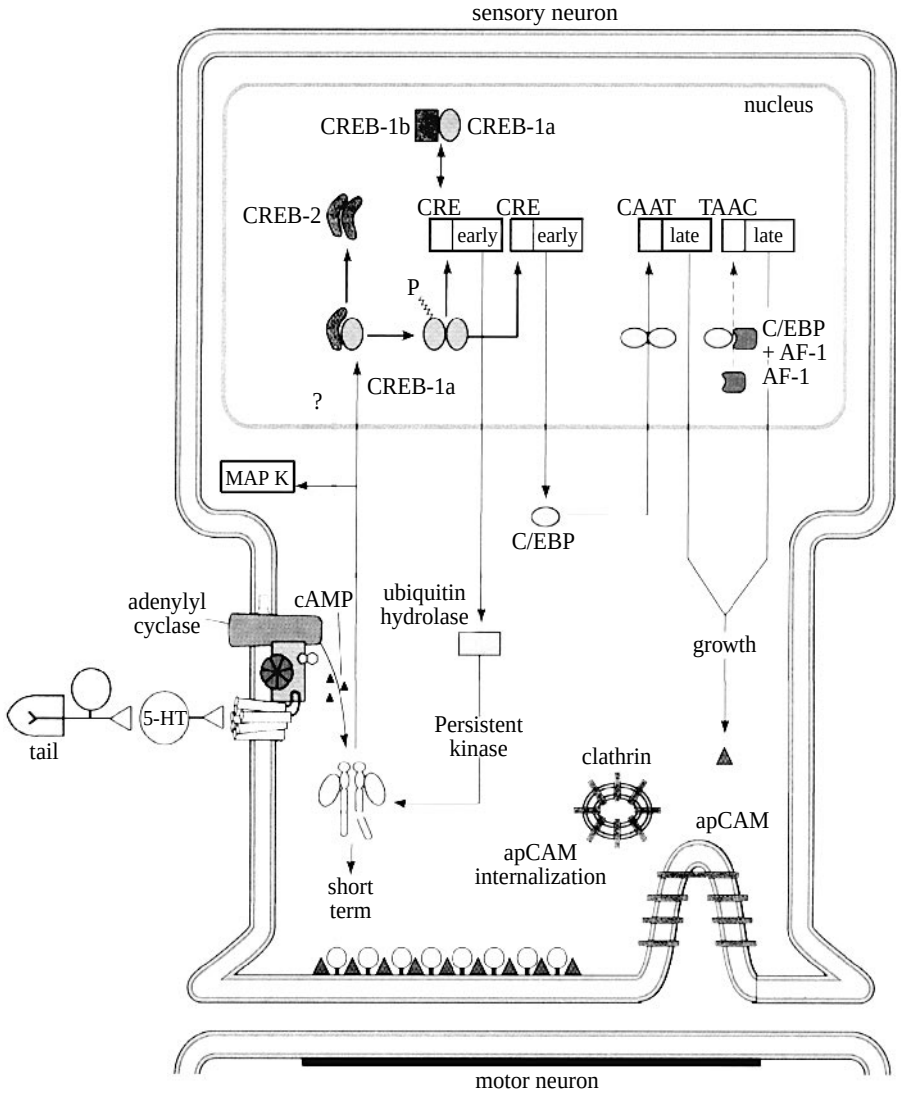

Figure 9. Multiple molecular pathways are involved both in short-term facilitation and in long-term facilitation of the sensory neuron-motor neuron synapse in *Aplysia*.

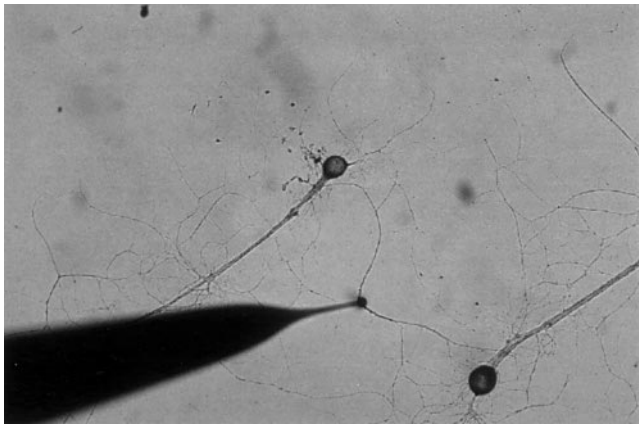

Figure 10. The ability to study two synapses from the same sensory neuron on to widely separated motor neurons in *Aplysia* cell culture allows synaptic tagging and synaptic capture to be explored for the first time at the cellular level.

a cascade of intracellular events) (Linden & Connor 1995). This requirement for two simultaneous activating phenomena is analogous to the associative properties of the NMDA receptor in *Aplysia* classical conditioning, as we have discussed above. Downstream of mGluR1, and critical for LTD, is an isoform of the kinase PKC; inactivation of PKC specifically in the cerebellum in a transgenic mouse interferes with both parallel fibre LTD and cerebellum-dependent learning (De Zeeuw *et al*. 1998). Recently, in another exciting convergence of molecular mechanisms, a transcription-dependent late phase of cerebellar LTD has been described, which requires the kinase CaMKIV and, once again, the transcription factor CREB (Ahn *et al*. 1999).

(b) *The amygdala and emotional memory*

Various aspects of emotional and motivational behaviour require the amygdala, a complex, multifunctional structure (Alheid *et al*. 1995). In the late 1950s, Lawrence Weiskrantz first demonstrated that lesions of the amygdala impair learned fear (Weiskrantz 1956). Subsequent work by M. S. Fanselow, J. E. LeDoux and their collaborators indicated that one subnucleus of the amygdala, the lateral nucleus, is required for implicit memory for fear conditioning to a neutral tone (LeDoux 1998). Information about the CS, the tone, is carried to the lateral nucleus via two pathways: the thalamo-amygdala pathway from the auditory thalamus and the cortico-amygdala pathway from the auditory cortex. Long-term potentiation has been observed *in vivo* in the projection

from the thalamus to the amygdala after tetanization of the thalamic input (Clugnet & LeDoux 1990). Importantly, synaptic change resembling LTP has been observed in this pathway after naturally occurring fear conditioning (Rogan *et al*. 1997).

Because of the anatomical complexity of the amygdala (especially when compared with more regular structures like the hippocampus and the cerebellum), precise molecular characterization of the plasticity at defined synapses has been difficult. However, blockade of noradrenergic β receptors (which, like 5-HT receptor in *Aplysia*, are coupled intracellularly to the cAMP pathway) interferes with the formation of emotional memory in humans, suggesting that the cAMP pathway in the amygdala may be required (Cahill *et al*. 1994). More recently, LTP has been described at the synapse from cortex to lateral amygdala, and, like facilitation in *Aplysia*, it has an early phase and a protein synthesis-dependent late phase. The induction of the early phase requires the activation of postsynaptic NMDA receptors (like *Aplysia* classical conditioning and like the Schaffer collateral LTP in the hippocampus we shall consider below) but also the action of presynaptic PKA, like *Aplysia* conditioning and sensitization and like mossy fibre LTP in the hippocampus (Huang & Kandel 1998).

(c) *The striatum and the memory for habits*

In certain other forms of implicit learning in vertebrates, the CS pathway is less well defined than in the cerebellum and amygdala, and the analysis of the relationship of plasticity to learning is correspondingly less well defined. However, plasticity has been observed in association with such more complex forms of implicit learning, and in many cases it shares molecular components with better-studied systems.

While it has been viewed as primarily a motor structure, recent work has shown that the striatum (caudate and putamen) also has an important role in the learning of habits (Graybiel 1995). Long-term potentiation has been described at projections from the cortex to the dorsal striatum, but it is not as yet known whether this long-term potentiation is correlated to learning. This corticostriatal LTP requires the NMDA receptor and involves intracellular cAMP (Calabresi *et al*. 1997). CREB is once again implicated in the plasticity: stimulation of corticostriatal projections activates CREB (Sgambato *et al*. 1998), and interference with CREB attenuates learning of a striatum-based task in transgenic mice (Pittenger *et al*. 1999). Furthermore, CREB is upregulated in the long-term plasticity induced in the ventral striatum (or nucleus accumbens, an area implicated in depression and in drug addiction) by chronic antidepressant treatment (Nibuya *et al*. 1996). While the mechanisms and role of corticostriatal plasticity remain unclear, it appears possible that some of the same molecular pathways are involved.

(d) *The cerebral cortex, working memory and priming*

The prefrontal cortex is involved in working memory, an important on-line memory that has a restricted capacity for a limited number of items and that allows continuous engagement in complex tasks (Smith & Jonides 1999). Rather than plastic changes, working memory is likely to involve ongoing activity in frontal lobe circuits (e.g. Lisman *et al*. 1998). In addition, humans exhibit a cortex-based implicit memory effect known as priming, in which sensory stimuli bias subsequent interpretation of ambiguous stimuli without conscious awareness (Schacter 1996). Priming and working memory are current topics for systems-level investigations in memory; we will return to them briefly below.

8. EXPLICIT MEMORY STORAGE IN THE MAMMALIAN BRAIN: SPATIAL MEMORY, LONG-TERM POTENTIATION AND THE ROLE OF CREB IN MICE

What about explicit memory storage? As we have seen, explicit memory involves the conscious recall of facts and events. It is therefore more complex than implicit memory storage, for two reasons. First, explicit memory involves conscious participation in memory recall, while implicit recall is unconscious. Second, in the case of explicit memory one cannot readily define a simple CS pathway; rather, explicit memory involves the integration of multiple sensory cues. Nevertheless, the central importance of explicit memory in everyday experience has motivated strong interest in plastic mechanisms related to it.

As technology for manipulating the mouse genome improved, it became clear that mice are likely to be powerful systems for the study of explicit forms of memory. Mice and other rodents, like humans, require the hippocampus for spatial memory and navigation (O'Keefe & Nadel 1978) as well as for object recognition (Gaffan 1998). Spatial memory in mice seems a particularly good model for explicit memory, because it requires for its formation not a simple CS but an arbitrary association between several different sensory cues. Finally, the hippocampus, which is critically involved in spatial and other forms of explicit memory, exhibits several well-studied forms of synaptic plasticity.

The basic hippocampal circuit consists of a three-synapse loop—the perforant pathway, the mossy fibre pathway and the Schaffer collateral pathway—that runs from the entorhinal cortex to the CAl region of the hippocampus (figure 13). In groundbreaking work, Bliss & Lømo (1973) found that when the input from the entorhinal cortex to the dentate gyrus (the first cell field of the hippocampal formation) is stimulated repetitively at high frequency, the synapse is persistently strengthened. They termed this phenomenon long-term potentiation, or LTP. Subsequent work has shown that each of the three synapses in the hippocampal loop exhibits long-term potentiation, and that at each synapse LTP has an early and a late phase, which can be induced by different stimulus protocols (figure 13). In each case, the late phase differs from the early phase in that it is blocked by drugs that inhibit protein or mRNA synthesis. Moreover, the induction of this late phase requires cAMP and PKA at all three synapses. The similarities to the molecular mechanisms involved in *Aplysia* provided the first suggestion of conserved mechanisms for converting short-term memory to long-term across phylogeny (Abel & Kandel 1998; Huang *et al*. 1994).

Most work has focused on the third synapse in the loop from the entorhinal cortex through the hippocampal

formation, the Schaffer collateral synapse, for several reasons. First, it is technically a particularly easy synapse to study. Second, studies in humans by Squire and colleagues have found that a patient with a hypoxic lesion limited to the CA1 field (the location of the Schaffer collateral synapse) had a significant amnesia, suggesting that damage restricted to this area is sufficient to disrupt memory formation (Zola-Morgan *et al*. 1986). Third, whereas dissociations between synaptic plasticity and behaviour in mice have been described at other synapses in the hippocampal formation (see, for example, Huang *et al*. 1995), the efforts of many laboratories have built up a long list of correlations between disruptions of Schaffer collateral LTP and disruptions of hippocampus-dependent memory. Although there are occasional dissociations even at this synapse (see, for example, Zamanillo *et al*. 1999), this synapse seems a good place to start disentangling the role of plasticity in the complex hippocampal circuit.

There is consensus that induction of LTP in the Schaffer collateral pathway involves activation of NMDA receptors (Nicoll & Malenka 1999). As we have seen, unlike other glutamate receptors, which are activated whenever the presynaptic neuron releases glutamate on to the postsynaptic membrane, the NMDA receptor is only activated when the release of glutamate by the presynaptic cell is accompanied by substantial depolarization of the postsynaptic cells. The NMDA receptor is therefore ideally suited to a role in initiating plasticity, where synaptic strengthening can result from coincident firing of the pre- and postsynaptic neurons (Hebb 1949). Activation of the NMDA receptor leads to influx of Ca^{2+} into the postsynaptic cell and activation of CaMKII. Despite much work, there continues to be significant controversy as to subsequent events. Whether the expression of early LTP is post- or presynaptic or both remains unclear. We will bypass this controversy and again focus on the late, transcription-dependent phase.

Whereas a single tetanus activates CaMKII, which induces early-phase LTP, multiple tetani increase intracellular cAMP and thus also recruit PKA and MAP kinase, which induce nuclear events (Impey *et al*. 1998*a*). Parallelling the finding that MAPK is required for long-term facilitation in *Aplysia*, David Sweatt and his colleagues have found that MAPK is activated both *in vitro* on induction of LTP (English & Sweatt 1996) and *in vivo* after certain forms of learning (Atkins *et al*. 1998). Although the mechanism remains obscure, there is currently a consensus that PKA activates the MAPK cascade in neurons and that MAPK is particularly important in the activation of nuclear factors required for the late phase.

The *sine qua non* of L-LTP in mice is, of course, the requirement of transcription and induction of genes in the potentiated cell. In another example of evolutionary conservation, CREB is once again implicated. This was first suggested in a study by A. J. Silva and colleagues, who found L-LTP and hippocampus-dependent learning to be disrupted in a mouse with a knockout of the two most prevalent forms of CREB (Bourtchuladze *et al*. 1994). However, this study is complicated by the nature of the knockout, which only eliminated some CREB alleles (Blendy *et al*. 1996), and by the complex nature of CREB-regulated transcription in mammals. Indeed, a complete knockout of all mouse CREB isoforms is embryonically lethal (Rudolph *et al*. 1998). Furthermore, genes related to CREB are altered in the partial CREB knockout mice in compensation for the missing CREB (Hummler *et al*. 1994).

Despite these complications, recent work from Daniel Storm and his colleagues has provided strong, albeit still circumstantial, evidence that CREB is indeed involved in mouse plasticity. They produced a transgenic mouse in which a *lacZ* reporter gene is activated by a CREB-responsive promoter, and they found that this reporter is activated both by L-LTP *in vitro* (Impey *et al*. 1996) and by certain forms of hippocampus-dependent learning *in vivo* (Impey *et al*. 1998*b*). This demonstrates that CREB or CREB-like transcription factors are in fact activated under circumstances that lead to plasticity and suggests a causal role similar to that seen in *Aplysia*.

As we have noted above, the transcriptional regulation of genes required for LTF in *Aplysia* is more complicated than the regulation of a single transcription factor, involving several other elements. Substantially more complex interactions are likely to be encountered in mice. To work out in detail the molecular switch for converting short-term to long-term facilitation in the Schaffer collateral pathway, much more work will be required.

(a) *Hippocampal plasticity beyond the Schaffer collateral pathway*

While a great deal of work has focused on the Schaffer collateral synapse, neuronal plasticity also has been described elsewhere in the hippocampus. As we note above, LTP is readily demonstrated in the two other major hippocampal fibre tracks, the perforant path from the entorhinal cortex to the dentate gyrus and the mossy fibre pathway from the dentate gyrus to the CA3 cell field (see figure 13). In both of these pathways LTP has an early, protein synthesis-independent phase and a late, protein synthesis-dependent phase, just as in the Schaffer collateral synapse (Huang *et al*. 1994; Nguyen & Kandel 1996). In addition, dense collateral projects from CA3 cells to other CA3 cells may exhibit plasticity; these projections, while doubtless important for the network properties of the hippocampus, are problematical to study (Rolls & Treves 1998). Despite an overall similarity in LTP in these pathways, the molecular specifics differ. For example, at the mossy fibre synapse PKA also is required for the early phase, which is similar to *Aplysia* but contrasts with CA1 plasticity.

9. STRUCTURAL CHANGES AND THE BIOLOGICAL BASIS OF INDIVIDUALITY

As we have seen, structural changes activated by CREB-1 are the defining features of long-term memory storage in invertebrates. There is beginning to be evidence as well for structural changes with LTP in the hippocampus. Thus, several studies suggest that a stably potentiated synapse can release multiple packets of neurotransmitter (or quanta), whereas an unpotentiated synapse releases either zero or one (Bolshakov *et al*. 1997; Bolshakov & Siegelbaum 1995). Consistent with this, some ultrastructural studies have found that in tissue in

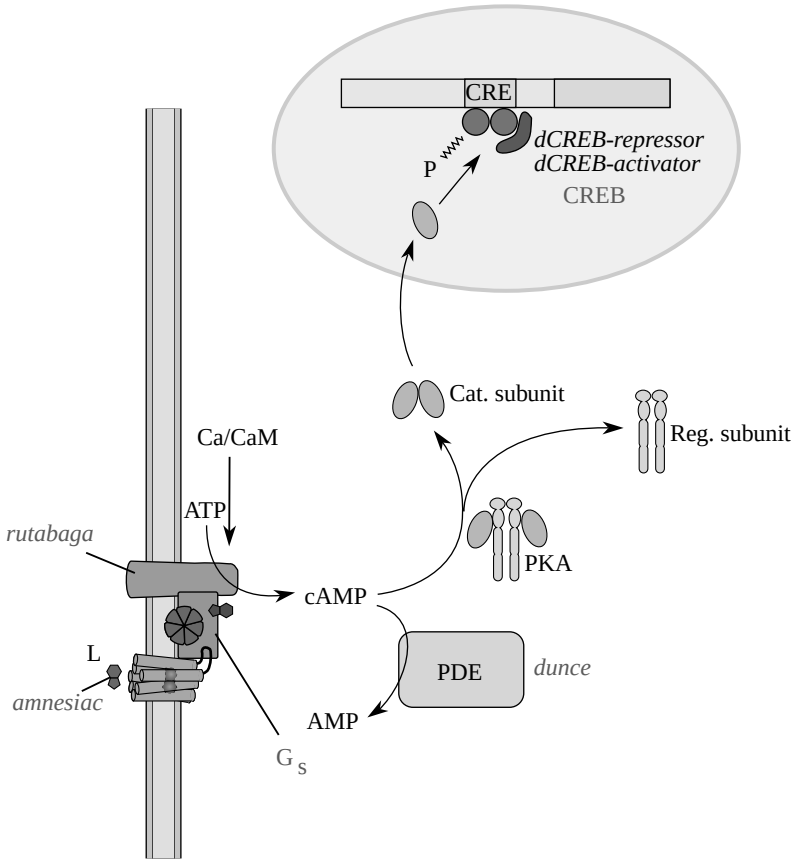

Figure 11. Many of the learning-related genes identified by screens in *Drosophila*, including *dunce*, *rutabaga* and *amnesiac*, participate in the cyclic AMP–PKA pathway that is also implicated in *Aplysia*. From Milner *et al*. (1998).

which potentiation has been induced, synapses can have multiple active release zones, or even be split into two (Muller 1997). Given the widely held belief that each active zone can release at most one quantum of neurotransmitter, this sort of splitting is precisely what may be needed to explain the observed release of multiple quanta (however, see Sorra & Harris 1998). Recent studies indicate that synaptic activation can lead to the outgrowth of dendritic processes that appear to be percursors of dendritic spines, providing a possible mechanism for such morphological change (Maletic-Savatic *et al*. 1999).

Anatomical plasticity has also been studied in cerebral cortex during development and in sensory rewiring in the adult. While ultrastructural change of the type described above may well occur after plasticity in the cortex, the changes that have been well characterized are rearrangements of connections over larger populations of neurons. Developmental plasticity has been studied in the visual system of frogs, cats, ferrets and monkeys, and in the whisker-dedicated portion of the somatosensory cortex, or barrel cortex, in rodents. Most often studies involve systemically perturbing the relevant sensory input, such as by blinding one eye or trimming a subset of whiskers, and observing the results. Such studies in kittens have revealed that plasticity in the developing visual system, like adult plasticity at the Schaffer collateral synapse, requires activation of the NMDA receptor (Gu *et al*. 1989). Furthermore, in the mouse, monocular removal of visual input stimulates expression of a CREB-driven reporter gene in the portion of visual cortex where synaptic plasticity is occurring (Pham *et al*. 1999).

Cortical plasticity in adults has been demonstrated in both sensory and motor cortices in several systems. The clearest demonstrations come from work by M. M. Merzenich and J. H. Kaas in the somatosensory cortex of the monkey. When somatosensory input is altered by nerve section (Kaas *et al*. 1983) or by amputation (Merzenich *et al*. 1984), a portion of the corresponding primary somatosensory cortex is deprived of its input. Over time, the surrounding regions, which still receive normal input, expand into the deprived area, so that the somatotopic map over the surface of the cortex is altered. In the converse experiment, Merzenich and colleagues showed that when a normal monkey is trained to preferentially use only some fingers, the cortical representation of those fingers expands (Jenkins *et al*. 1990).

Dramatic evidence for cortical reorganization in normal humans has been provided by studies from E. Taub and colleagues. They scanned the brains of string instrument players. During performance, string players are continuously engaged in skilful hand movement. The second to fifth fingers of the left hand, which contact the strings, are manipulated individually, while the fingers of the right hand, which move the bow, do not express as much patterned, differentiated movement. Brain images of these musicians revealed that their brains were different from the brains of non-musicians: the cortical representation of the fingers of the left hand, but not of the right, was larger in the musicians (Elbert *et al*. 1995). Such structural changes are more readily achieved in the early years of life. Indeed, Taub and his colleagues found that musicians who learned to play their instruments by the age of 12

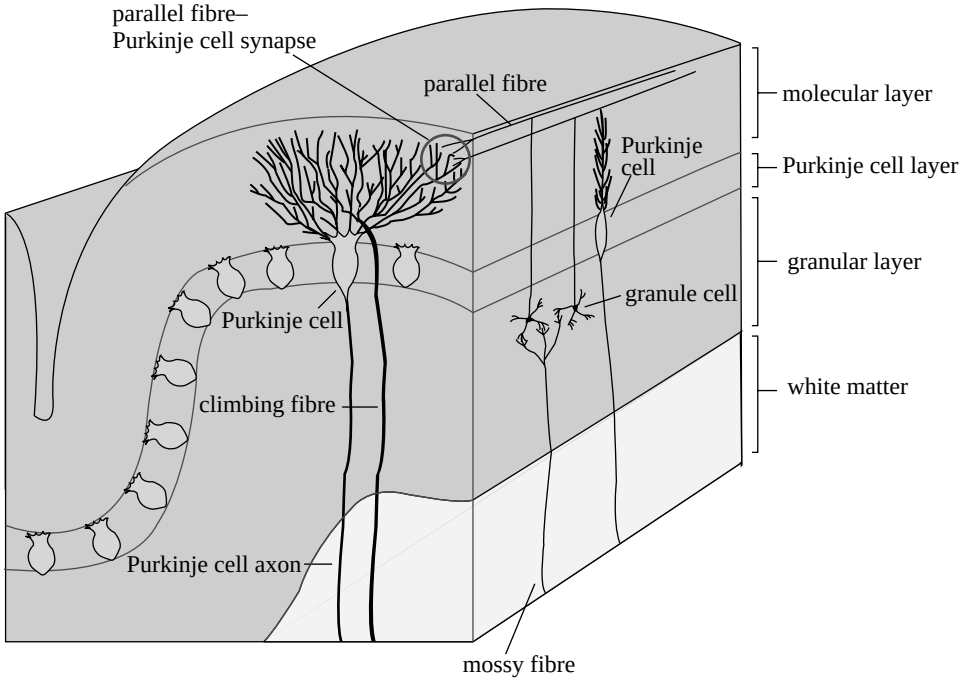

Figure 12. The elegant circuitry of the cerebellum is involved in motor coordination, the learning of motor skills, certain classical conditioning tasks and oculomotor learning. Long-term depression has been intensively studied at the synapse between parallel fibres and Purkinje cells and appears to be involved in the learning of cerebellum-based implicit tasks. Adapted from Kandel *et al*. (1991).

years had a larger representation of the fingers of the left hand than did those who started later in life.

These several studies suggest that long-term memory storage leads to anatomical changes in the mammalian and even the human brain much as it does in *Aplysia*. These anatomical changes are potentially significant for understanding the biological basis of individuality, for they suggest that even identical twins, who share a genome, are likely to have somewhat different brains because they are certain to have somewhat different life experiences.

10. THE QUESTIONS CONFRONTING THE SYSTEMS COMPONENT OF MEMORY STORAGE ARE LIKELY TO OCCUPY OUR ATTENTION EVEN FOR THE DISTANT FUTURE

At the end of the decade of the brain and the beginning of the new millennium, it is appropriate to contemplate possible future directions of memory research. The most difficult questions of memory storage are the systems questions. These are likely to occupy us into the distant future. Here we discuss a few of the important systems-level questions and discuss how they may be addressed in the coming century.

(a) *What is the relationship of medial temporal lobe to permanent memory storage?*

One of the most fascinating systems issues in the study of memory is the question of the how the hippocampus interacts with other structures that are the final repository of stored memories of semantic and episodic knowledge. As we note above, Milner's studies of H.M. revealed that damage to the hippocampus disrupt the formation of new memories but leave more remote established memories largely intact. This observation, which has more recently been substantiated by L. R. Squire and others in other patients and in non-human primates (Squire & Kandel 1999; Rempel-Clower *et al*. 1996; Teng & Squire 1999) and by Fanselow and colleagues in rats (Anagnostaras *et al*. 1999), suggests that whereas the hippocampus is essential for the formation of new explicit memories and possibly for their early storage and recall, it is not the final storage location. An elegant recent study in mice confirms this interpretation. R. Jaffard and colleagues have used a radioactive metabolic labelling technique to show that recall of a spatial memory a short time (five days) after learning recruits the hippocampus and the associated posterior cingulate gyrus, while recall of the same memory a long time (25 days) after learning activates the frontal cortex and associated anterior cingulate gyrus but not the hippocampus or posterior cingulate (Bontempi *et al*. 1999). This study dramatically demonstrates the change over time of the structures required for recall of an explicit memory; it is also consistent with functional imaging findings in humans, to which we will return below, that indicate a role for frontal cortex in the recall of explicit memories.

The final locus of storage of memory is widely assumed to be the cerebral cortex, though this is a difficult assertion to prove. How, then, does the role of the hippocampus relate to that of the cortex in memory formation, storage and retrieval? If explicit memories are initially stored in the hippocampus, how and when are they transferred to cortex or another repository?

There are two major hypotheses on this issue. The first is that memories are never in fact stored in the hippocampus but that its role is to form associations between representations of different aspects of a memory in different regions of cortex—a form of binding. According to this theory, connections between the different components of a memory in different regions of cortex are initially weak, so the hippocampus continues to be necessary to bind together the components of recent memories. As time passes, however, the connections between representations of the components strengthen, so more remote explicit memories can be recalled independently of the

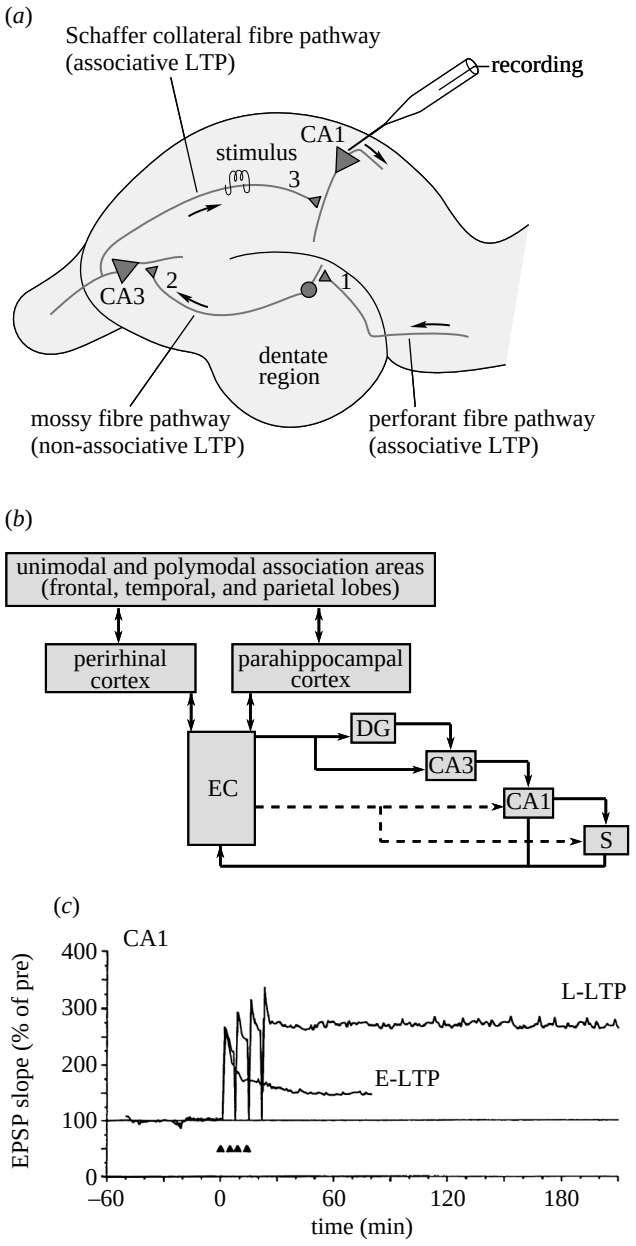

Figure 13. (*a*) In the mammalian brain, plasticity has been most intensively studied in the hippocampus, and particularly at the Schaffer collateral synapse between CA3 and CA1 pyramidal cell fields. (*b*) Systems properties of memory storage. The hippocampus consists of a series of interconnected cell fields. Plasticity has been described at all synapses in this circuit. (*c*) LTP at the Schaffer collateral synapse has an early and late phase. The early phase, induced by a single high-frequency train of stimuli, lasts 60–90 min and is independent of macromolecular synthesis. The late phase, induced by repeated high-frequency trains, lasts many hours and depends on gene induction and the synthesis of new proteins.

hippocampus (Marr 1971). The second hypothesis is that explicit memories are originally stored in their entirety in the hippocampus but that they are somehow transferred at a later time to cortical storage. The leading idea as to the mechanism of this transfer is that it occurs during sleep (Crick & Mitchison 1983); the finding of recapitulation during rapid eye movement (REM) sleep of hippocampal firing patterns observed during waking behaviour has been taken as support for this notion (Skaggs & McNaughton 1996). (It should be noted that recapitulation of hippocampal firing patterns during sleep might also be consistent with the first model for the relationship of hippocampus to cortex.) Distinguishing between these two possible models for the relationship of the hippocampus to the cortex will be one of the major long-term tasks of the future. All studies of plasticity in the hippocampus implicitly suppose that some aspect of memory is at least transiently stored there; otherwise there is no reason to suppose that plasticity is related to memory in any way. The resolution of this systems problem will therefore have profound implications for molecular investigations of memory.

(b) *Do the subregions of the medial temporal lobe serve distinctive functions?*

Studies of humans with lesions in the medial temporal lobe structure suggest an equipotential view of hippocampal function. Patients with lesions in different areas of the hippocampal formation have similar deficits, and deficits in patients with large lesions are more severe but qualitatively similar to those in patients with small lesions (but see Vargha-Khadem *et al*. (1997) for a recent departure from such a view). Such an equipotential view is reminiscent of that of Flourens in the 19th century and of Lashley in the first half of this century and, like theirs, may arise from inadequate experimental tools.

The appearance of equipotentiality in patients with lesions of the subregions of the medial temporal lobe system may result from the generally disruptive effects of brain lesions. It therefore will be of interest to use genetic manipulation in experimental animals and to focus on specific subregions of the medial temporal lobe. Recent advances in regional control of gene expression (Mayford *et al*. 1997) should permit one to selectively disrupt individual components of synaptic plasticity in a subregion of the hippocampus without interfering with other components or with basal synaptic transmission through the circuit.

It will be important to use regional restriction to explore which aspects of memory map onto which portions of the medial temporal lobe system, if indeed any straightforward mapping is possible. A mapping of memory onto structure could take one of at least two forms: a mapping of different types of memory tasks or of different components of a given memory task. Since the hippocampus is concerned with storing information about places, objects, people and other living things, different types of information could be distributed such that that some parts of the system are more concerned with place and spatial recognition while others are more concerned with object recognition. Alternatively, different regions of the systems could be concerned with one or another component or operation of a given memory: (i) encoding of information that can be maintained; (ii) consolidation and storage of that information over time; and (iii) retrieval of stored information at a later point.

(c) *How is human memory best studied?*

Most of the detailed work in memory has of necessity been performed in experimental animal systems. While human memory, both explicit and implicit, is likely to

employ similar basic mechanisms as that of simpler animal systems, it is also doubtlessly unique in other respects. As we note above, important insights from H.M. and other patients have shed a great deal of light on the mechanisms of memory, but until recently the resolution of human studies was limited. With advancing technology it will become possible to examine human memory in a more and more rigorous way. For example, imaging studies have shown that the frontal lobe is critically involved in human explicit memory formation in a modality-dependent way (Buckner *et al*. 1999), and imaging of the hippocampus has confirmed that in humans, as in rodents, this structure is involved in spatial navigation (Maguire *et al*. 1997). Imaging technology is improving rapidly. In particular, spatial and temporal resolution are increasing, although still far away from the ideal of single-neuron visualization (Posner & Raichle 1998).

In the long run, some insight into these issues will come from studying similar tasks in monkeys and humans and then examining them by a combination of interventional studies in monkeys and non-invasive studies in humans. However, because of the brain's extraordinary complexity and the sheer number of nerve cells it contains, we will never be able to achieve a complete understanding of its information-processing functions without a computational theory. Only convergence of these different modes of analysis will lead to a satisfactory understanding of human memory.

(d) *Consciousness and the recall of explicit memory*

An overarching question in human memory research will be to what extent mechanisms of human memory are unique. Important changes in the mechanisms of memory storage and recall may be among the evolutionary adaptations that set humans apart. Addressing this question will require significant advances both in memory research and in other aspects of cognitive neuroscience, such as the study of a language, attention, imagery and self-awareness. The defining feature of explicit memory storage is that recall requires a conscious attention. Here memory research touches on one of the deepest problems in biological science, and one before which we stand in relatively complete ignorance. However, this problem highlights the key issue to emerge from a consideration of the systems problem of memory: it is impossible to dissociate the system problems of memory research from the general agenda of neuroscience to understand perception, action and conscious awareness.

11. SOME OF THE QUESTIONS CONFRONTING THE MOLECULAR COMPONENT OF MEMORY STORAGE SHOULD BE SOLVABLE IN THE NEAR FUTURE

What is in store for the molecular biology of memory in the new century? As the discussion above makes clear, although the molecular biology of memory is in its infancy, intellectually satisfying insights into the family of mechanisms that contribute to different forms of memory-related plasticity are in sight. Here we outline some of the most pressing issues confronting the immediate future.

(a) *The search for a mechanistic framework: do implicit and explicit storage share a common set of molecular mechanisms?*

It is already clear that the mechanisms of synaptic plasticity involved in different forms of explicit and implicit memory storage differ in detail. This is perhaps not surprising given the fact that implicit and explicit memory differ in at least three ways: (i) they use very different logical strategies for recall—conscious awareness for explicit knowledge and unconscious recall for implicit knowledge; (ii) they store different types of information; and (iii) they use very different neuronal systems. Despite these differences, different systems for memory storage seem to use an overlapping set of cellular and molecular components.

Studies thus far available indicate that both implicit and explicit memory storage have phases, a short-term phase lasting minutes and a long-term phase lasting days or longer. In both implicit and explicit storage, repetition converts the short-term to the long-term form. For both types of storage, the short-term form involves covalent modifications of pre-existing proteins mediated by one or another second messenger kinase (usually CaMKII, PKC, PKA and MAP kinase) leading to an alteration of pre-existing connections. By contrast, long-term memory requires PKA, MAPK and CREB-mediated transcription, and anatomical changes in the number and size of the synaptic connections involving cell adhesion molecules such as ApCAM and Fasl. It seems likely that different synapses involved in both implicit and explicit memory storage achieve their plastic capabilities by using subsets of a fairly small family of molecular transducers and effectors, though the precise members of this group employed will differ from synapse to synapse. This overlap is reminiscent of a central finding in developmental biology: related molecules or even functional cassettes of interacting molecules are used in rather different contexts to do different jobs.

(b) *What is the nature of the synaptic changes responsible for the initial expression of plasticity?*

Although the forms of synaptic plasticity that contribute to short-term memory all involve covalent modifications of pre-existing proteins, different forms of plasticity recruit different signalling systems. LTP is not a singular process but a family of processes, and slight variations in the inducing stimulus or learning protocol may alter the signalling pathways recruited. For example, at the Schaffer collateral pathway, high-frequency stimulation (100 Hz) triggers Ca^{2+} influx through the NMDA receptor; Ca^{2+} activates CaMKII, and this activation is critical for the initiation of LTP (Giese *et al*. 1998; Lisman & Goldring 1988). PKA has no role in this form of LTP. By contrast, when this same synapse is stimulated at low frequency (5 Hz), it induces a form of LTP that also is NMDA receptor dependent but now requires PKA (Winder *et al*. 1999). Thus, even the NMDA-dependent form of LTP at the Schaffer collateral synapse has several forms. It therefore seems possible that different learning processes recruit different combinations of programs for LTP.

How is LTP expressed? In the Schaeffer collateral pathway, there is evidence for both presynaptic and postsynaptic mechanisms in the expression of the early phase

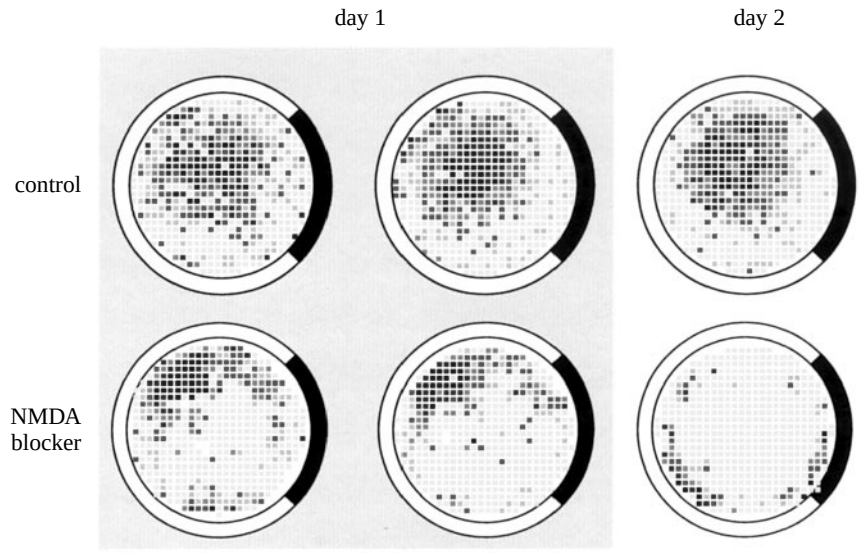

Figure 14. Effect of NMDA antagonist on place cells. Place cells in the hippocampus represent the most advanced current example of a fusion of systems and molecular investigations of memory. Firing rates of two place cells are shown here. Each circle corresponds to an environment the animal explored; coloured pixels correspond to firing rate at different points in the environment. Firing rate is highest in a particular region of the environment, the place field. In the upper cell, from a control rat, the place field is stable across three recording sessions on two days. In the lower cell, from a rat treated with the NMDA blocker CPP, the place field is stable over two training sessions on the first day (separated by 1 h) but is dramatically altered 24 h later. Adapted from Kentros *et al.* (1998).

of LTP, but the issue remains highly controversial, as we note above. Good electrophysiological evidence suggests a presynaptic mechanism (Bolshakov *et al.* 1997; Bolshakov & Siegelbaum 1995). The molecule nitric oxide seems to be capable of serving under some circumstances as a retrograde messenger from the postsynaptic site of induction of LTP to initiate presynaptic change, much as occurs in NMDA-dependent facilitation in *Aplysia* (Arancio *et al.* 1996). However, there also is very good evidence that with 100 Hz stimulation there is a critical postsynaptic contribution through activation of previously silent AMPA receptors (Liao *et al.* 1995). With recent advances in imaging (Denk *et al.* 1996), we can expect new light to be shed on this issue in the near future. Given the high quality of the data on both sides, it seems likely that the final picture will contain elements of both pre- and postsynaptic expression, perhaps with different elements being invoked under different circumstances.

(c) *How is the late phase of LTP induced and maintained?*

While PKA seems to be required for L-LTP in all regions of the hippocampus as well as in the lateral nucleus of the amygdala, PKA does not act alone. For example, PKA activates the MAP kinase cascade in neurons, and both PKA and MAPK are required for CREB activation. There is increasing reason to believe that the neurotrophin BDNF is involved in various aspects of LTP (Kang & Schuman 1995; Patterson *et al.* 1996), both as a possible retrograde signal and as a contributor to growth, but its role has yet be integrated with other mechanistic details (Patterson *et al.* 2000). Much evidence points to CREB-1 as a key, conserved regulatory element in the activation of the genes required for implicit memory in *Aplysia* and *Drosophila*, but its role in L-LTP in the mouse is on much less firm ground.

Presumably, genes activated by CREB or related factors are involved in the effector mechanisms of late phase plasticity. Some downstream genes have been identified in *Aplysia*: C/EBP (Alberini *et al.* 1994), ubiquitin hydrolase (Hegde *et al.* 1997), and elongation factor 1 alpha (Hegde *et al.* 1999). Work in hippocampal LTP has identified the neurotrophins BDNF and NT3 (Patterson *et al.* 1992) and the secreted protease tPA (Qian *et al.* 1993); pharmacologically induced seizure, which is a still less refined way of turning on depolarization-induced genes, induces a larger number (Hevroni *et al.* 1998). These examples suggest what some of the categories of induced genes will be. Some, such as ubiquitin hydrolase and C/EBP, will function to extend or modulate the intracellular signalling events required for L-LTP. The protease tPA also serves as a step in a signalling cascade (Mars *et al.* 1993). Proteases, cell adhesion molecules and neurotrophins may prove important for the morphological plasticity that may occur in L-LTP. Indeed, the *Aplysia* cell adhesion molecule gene ApCAM is transcriptionally regulated in the induction of LTF, though it is downregulated rather than increased (Mayford *et al.* 1992). A related category might be the cytoskeletal molecules and that also participate in morphological plasticity at the cellular level.

We anticipate that a major thrust of work in the near future will be the identification of more downstream genes important for the stabilization of memory. Searches for these genes could take several different approaches. First, candidate genes can be screened for induction in *in vitro* potentiated hippocampal slices or in other models of plasticity (such as kindling or seizure) (Hevroni *et al.* 1998). Second, genetically altered animals with specific defects in memory storage and specific alterations in the transcriptional apparatus known to be induced in LTP and restricted in expression to some part of the medial temporal lobe region can be screened against controls to identify novel genes that may be induced. Finally, cells from models of plasticity such as LTP can be specifically screened against controls to identify in a wholly unbiased manner the genes that are induced. The last two of these possible approaches will be greatly facilitated by recent advances in technology. In particular, gene chip technology that has recently become available allows for the first time the genes expressed by two cell populations to be rapidly and quantitatively compared (Editorial 1999). Comparison of cells in which LTP has been induced against controls using this sort of technology should allow the exhaustive identification of induced genes; these genes

can serve as candidates for disruption, and their identities may provide immediate clues for the ultimate mechanisms of late phase LTP.

(d) *What are the molecular mechanisms for stabilizing memory storage, for synaptic targeting, synaptic tagging and synaptic growth?*

In *Aplysia*, and in the hippocampus, the stable self-maintained forms of long-term plasticity seem to require the induction of a cascade of genes and the growth of new synaptic connections (Bailey & Kandel 1993). The finding of a transcriptional switch in converting short- to long-term memory explained why long-term memory requires new protein synthesis. As we have described above, the products of transcription are targeted to the appropriate synapses by some sort of synaptic tag. Early evidence in *Aplysia* suggests the marking process for long-term growth has two components: a covalent, PKA-mediated marking signal for the growth of new connections, and a local protein synthesis-dependent stabilization signal (Martin *et al*. 1997*a*). Determining the nature of this covalent mark will necessarily overlap with studies of short-term synaptic plasticity such as E-LTP on the one hand (because establishment of the tag is presumably one aspect of early LTP), and with the mechanisms of late LTP on the other (because proteins bound for the synapse must interact with the tag to establish stable potentiation at the appropriate sites).

(e) *Are there non-synaptic mechanisms of neuronal plasticity?*

Ever since the time of Ramon y Cajal (Cajal 1893), investigators have focused on the synapse as the site of memory-related change. Sufficient evidence has accumulated, both experimental and theoretical, that it is nearly impossible to doubt that synaptic plasticity is important in memory storage; however, there is no strong reason to believe that the synapse is the only important locus of change. In particular, modulation of the electrical properties of the dendrite proper could lead to plasticity over large portions of the dendritic arbour. Likewise, modulation of the biochemical cascades involved in synaptic plasticity could change the subsequent behaviour of the neuron on a large scale. Finally, any alterations in integrative sites within the neuron, such as the sites of active conductances in the dendrites or the axon hillock, could obviously alter the input–output relationships of the neuron. The contributions, if any, of these potential alternative sites of plasticity in neurons have scarcely been investigated to date and may prove to be an important area of future research.

The research that we have emphasized is limited in another way: it focuses on plasticity at single synapses. Despite its advantages, such a focus neglects not only the function of multiple neurons in a circuit but also the function of single neurons as an integrated whole. Since the discovery of active dendritic conductances (Llinás & Walton 1998; Spencer & Kandel 1961), it has been clear that the larger geometry and electrophysiological properties of neurons significantly influence the processing of individual synaptic inputs. Therefore, identical plastic processes operating on synapses at different points in a neuron's dendritic tree may have rather different outcomes. Recent work has begun to emphasize the contributions of dendritic geometry, internal calcium stores and active conductances to the induction of synaptic plasticity (Linden 1999), but such work still tends to focus on potentiation at single synapses. A broader context of the relative contributions and behaviour of multiple synapses at different sites on a neuron will greatly aid our understanding of physiological plasticity in the future.

(f) *The important distinction between models and mechanisms of memory*

A pressing question that needs to be addressed for all forms of plasticity is this: to what extent do the conclusions we draw about experimentally induced forms of synaptic plasticity studied *in vivo* or *in vitro* apply to the forms of plasticity that underlie learning in an intact brain? This question has complicated the analysis of the role LTP in hippocampus-based explicit memory in particular because the cell physiological study of this type of storage has taken a bottom-up rather than a top-down approach.

In the cases of implicit memory for habituation, sensitization and classical conditioning in *Aplysia*, a top-down approach has been used. The analysis began with a characterization of learning at the behavioural level, then proceeded to the neural circuitry that mediates the behaviour, and only then focused on the cell and molecular biological changes in the neural circuitry produced by learning that serve as sites of memory storage. As we have emphasized above, many implicit forms of memory storage result from modifications in the response to the input (or conditioned stimulus) pathway. Implicit forms of memory, such as eyeblink conditioning and fear conditioning, therefore lend themselves to a top-down approach: once the wiring diagram of the behaviour that is modified has been delineated, the sites altered by learning, the pathway of the conditioned stimulus and response can be examined quite readily. In such cases, the task of matching synaptic change to behaviour has been met, at least partially, by the analysis that has preceded it.

Studies of the hippocampus-based spatial memory have taken a reverse, bottom-up approach. There has been little progress, for example, in defining the neural circuitry for spatial navigation in the mouse. In addition, we do not know whether the animal uses LTP in order to learn a new spatial task, or even at which synapses the important changes occur. LTP was not found in any behavioural context but by a fairly artificial examination of synaptic physiology (Bliss & Lømo 1973). This bottom-up approach greatly complicates attempts to relate synaptic physiology to behaviour.

LTP suffers from other weaknesses as an experimental model. It is generally studied in a slice of tissue, not in intact brain (though much *in vivo* LTP work has also been carried out). It typically involves an inducing stimulus so strong as to verge on the pathological. It is most frequently measured in populations of neurons, from which the behaviour of single synapses can be inferred only with difficulty. It evokes an extraordinary number of molecular signalling responses, making it difficult to discriminate mediators from modulators (Sanes &

Lichtman 1999). A recent description of a knockout mouse with little or no LTP (in at least one inducing paradigm) and normal hippocampus-dependent learning (in at least one behavioural task) forces reconsideration of the relevance of experimentally induced LTP to learning (Zamanillo *et al*. 1999). It seems very likely that prototypical LTP, produced by a 100 Hz train, is not an important physiological phenomenon underlying learning-related plasticity, any more than the pathfinding of developing axons in culture is identical to the pathfinding of axons in a developing nervous system. Rather, LTP is an experimental model that may share characteristics and mechanisms with the actual mechanisms used for learning.

Despite these and other weaknesses, recent work suggests that some aspects of LTP are indeed likely to represent a valid model for some aspects of memory storage. We have already noted that both certain forms of learning and LTP have a protein synthesis-independent phase and a protein synthesis-dependent phase and that both can be blocked by antagonists of the NMDA receptor. In addition, many different genetically altered mice have been generated in the past decade, and molecular deficits in Schaffer collateral LTP correlate surprisingly well with deficits in hippocampus-dependent learning. Finally, recent studies have shown that learning of hippocampus-based task correlates with the activation of CamKII, PKA, and MAP kinase (Atkins *et al*. 1998), with the turning off of phosphatases, and with the induction of CRE-mediated gene transcription (Impey *et al*. 1998*b*), all of which are predicted from using LTP as a model system.

However, all of this evidence is correlative. A conclusive answer to the question of what aspects of memory storage in fact is being measured by LTP will require a better appreciation of the family of processes that LTP represents, but also a better understanding of the functional circuitry of spatial memory where these processes are expressed. We need a more detailed understanding of how sensory information about the spatial environment enters the medial temporal lobe system and how it modulates the navigational system activated during spatial search.

Making direct mappings from behaviour to plasticity, and vice versa, is particularly challenging in the hippocampus because of the complex nature of the sensory input of explicit memory storage. In contrast to the siphon withdrawal reflex in *Aplysia* and other simple reflex systems, the neural input to the hippocampus is multimodal, highly processed and often not time-locked. Spatial learning involves not a change in the response to a defined conditioned stimulus but rather a change in strategy—in how the animal processes a complex constellation of sensory events. Until we have a better understanding of the nature of these inputs and how they participate in spatial navigation, the relationship between hippocampal plasticity and spatial memory-based tasks is likely to remain correlational.

12. MOLECULAR COGNITION: COMBINING THE SYSTEMS AND MOLECULAR PERSPECTIVES

As we have emphasized, studies in the biological basis of memory can be divided into systems and molecular perspectives, both historically and in current work. These perspectives on the study of memory differ in the questions they ask, their methodology and their conceptual framework. However, our understanding of the mechanisms of memory will not be complete until we can unite both perspectives into a single, unified framework. Early steps towards such a synthesis are already apparent in some current work. Completing this synthesis is one of the final goals of memory research in the future. We illustrate an early attempt at this unification by considering two examples: (i) LTP, place cells and spatial memory, and (ii) molecular therapeutics. In considering the relationship of LTP, place cells and spatial memory, we will also re-examine a question we considered earlier: How can we relate models of memory to mechanisms of memory?

(a) *Place cells as a synthesis of molecular and spatial models of memory in the hippocampus*

How can we bridge the gap between the molecular mechanisms of synaptic plasticity and the systems problems of spatial memory? In spatial learning, the animal is thought to use the sensory input—visual, proprioceptive, vestibular, olfactory—to develop an internal representation of its spatial environment and then to use that representation for spatial navigation. Thus, one step towards understanding will be working out representation of space in the hippocampus. This representation is thought to exist in the pyramidal cells of the hippocampus, which can form a spatial map of the environment.

Hippocampal pyramidal cells function as place cells: they fire when an animal occupies a specific area in its environment (called the place field for a specific cell). Any pyramidal cell can function as a place cell and, in any given environment, about half of the cells in the hippocampus function as place cells. These place cells form a 'cognitive map' of the environment, and learning a new environment and storing that new representation in the hippocampus involves the creation and stabilization of a new cognitive map (O'Keefe & Nadel 1978). In a new environment, the firing field of a place cell forms within a period of minutes (comparable to the acquisition of a learning task), and once formed can be stable for months (Muller *et al*. 1987), comparable to long-term memory. The map is environment specific, in that a cell's place field in one environment does not in any way predict the place field in another environment (Muller & Kubie 1987). A variety of manipulations of the animal can lead to a remapping, in which the place fields of all cells change. Such a remapping may correspond to disorientation, in which the animal no longer recognizes that it is in a familiar environment.

Research on how the network properties of place cells in the hippocampus are remapped in a new environment by learning represents perhaps the best example of research on the plasticity in an important cellular network involved in explicit memory storage. An analysis of plasticity in a system of place cells during spatial learning represent an important advance in the molecular biology of cognition that we advocate for the next century. With such research the top-down and bottom-up modes of analysis begin to converge (Rolls & Treves 1998).

To bring this fine-grained systems level analysis together with a molecular one requires investigating the

mechanisms of plasticity in a population of place cells that may be involved in the learning of a new environment. There is now evidence based on such an approach that hippocampal LTP may be involved in the stabilization of place fields as an environment becomes familiar. When an NMDA blocker (the drug CPP) was given to rats during exposure to a novel environment, the animals formed normal place fields, and (surprisingly) those fields were stable for an hour. However, when the animals were returned to the environment after 24 h, a complete remapping occurred: the place cell map of the environment was not stable. Importantly, the place cells in a control environment with which the animal had been familiarized before drug treatment remained stable (figure 14) (Kentros *et al*. 1998). This study demonstrates that pharmacological treatment that blocks hippocampal LTP can disrupt the stability of hippocampal place cells and supports the idea that an LTP-like phenomenon underlies spatial learning in rodents.

Studies with genetically modified animals also support this general conclusion. Genetic manipulations of the NMDA receptor (McHugh *et al*. 1996), CaMKII (Rotenberg *et al*. 1996) and PKA (Rotenberg *et al*. 1997) all show that place fields form fairly normally without NMDA-dependent plasticity, but are not stable in the long term. These observations amount to significant evidence (albeit still correlational) for a connection between the molecular mechanisms of synaptic plasticity, place cell stability and spatial learning as assessed by behavioural measures. If this correlation is substantiated it will represent a major step towards a unification of molecular and systems approaches to the study of spatial memory formation in rodents, and a major step towards the development of a meaningful molecular biology of cognition.

We have here only considered one point in the medial temporal lobe circuitry. To expand understanding of place cells will require moving backwards from the CA1 region to work out the nature of the sensory input into the hippocampus that gives rise to a spatial map. How is the relevant sensory information processed and encoded in the cortex? How is it transformed at various relays in the medial temporal lobe, and how is that transformed information used by the motor system for spatial navigation? This analysis is extremely ambitious and will occupy us into the distant future.

(b) *Disorders of memory and steps toward a molecular therapeutics*

A second effort in molecular cognition is evident in attempts to analyse and treat disorders of memory. Throughout its century-long history, the investigation of the biological basis of memory has been informed not only by studies of normal memory storage processes in humans and experimental animals, but also by studies of disorders of memory. In the 21st century we anticipate that the study of memory will follow other biological sciences in expanding into an applied as well as a basic science and that one day our expanding understanding of the mechanisms of memory formation will lead to treatments for disorders of cognition.

One example of a memory disorders for which treatments may be available in the future is the gradual weakening of memory with age, a difficulty often referred to as benign senescent forgetfulness. This memory loss is probably the most bothersome and frequently mentioned complaint of the elderly (Adams *et al*. 1997). Age-related memory loss is typically more pronounced in explicit than in implicit tasks, reminiscent of the deficits seen after lesions to the hippocampal system. Indeed, we now know that important aspects of age-related memory loss does involve a reduction in functioning of the hippocampus (Uttl & Graf 1993).

Age-related memory deficits are not limited to humans. Useful rat and mouse models have recently been developed to study the effects of normal ageing on explicit and implicit forms of memory (Barnes 1979). As is the case with humans, when tested in explicit spatial memory tasks, some aged animals perform as well as youthful controls, but others are strikingly impaired (Gallagher & Pelleymounter 1988). These aged mice also show a decline with age in Schaffer collateral LTP. This decline selectively affects the late, protein synthesis-dependent phase of LTP. This defect may reflect a loss of dopaminergic modulation of the hippocampus with age, since dopamine agonists, which increase intracellular cAMP, ameliorate both the defect in LTP and the spatial learning deficit in aged mice. Such pharmacological amelioration of an age-related defect may be the first step towards pharmacological treatment of this widespread complaint in humans (Bach *et al*. 1999).

Perhaps the most tragic disease of our time is Alzheimer's disease. This dramatic weakening of memory with time is not simply an acceleration of benign senescent forgetfulness. Alzheimer's is associated with distinct pathological changes. While there are no effective treatments for Alzheimer's disease at this time, a close examination of the molecular biology of the disease over the last decade has led to an accelerating understanding of its mechanisms, and effective therapies or prevention will surely come from this understanding. Genetic screens of families in which the disease occurs at an elevated frequency have revealed alleles of five different genes—the amyloid precursor protein (APP); presenilins 1 and 2; the Apo-E4 allele of Apo-E; and alpha 2 macroglobulin—that are associated with an increased risk of Alzheimer's. Examination of the products of these genes is leading to an understanding of the etiology of the underlying pathological lesion (Selkoe 1999). For example, there is preliminary evidence to suggest that all five genes may participate in a biochemical cascade concerned with the degradation of the toxic beta amyloid peptide. Alzheimer's disease therefore represents a prime example of how a molecular, mechanistic approach can lead to an understanding of pathologies whose principal symptom is a behavioural degeneration. In the future, we can hope that this mechanistic understanding of Alzheimer's will become more complete and lead to therapies, and that other, less common, dementias will quickly follow.

We have speculated above that an increased understanding of the molecular mechanisms of senescent decline and Alzheimer's may lead to pharmacological treatment. One would hope that these therapeutic insights would extend to various forms of mental retardation. For example, Down's syndrome, autism and Fragile X syndrome are often presumed to result from developmental errors but may in fact also result, at least in part,

from acute abnormalities in the mechanisms of synaptic plasticity (Bartsch *et al.* 1999). An increased understanding of the molecular mechanisms of such diseases may allow for their pharmacological treatment as well. Alternatively, gene-based therapies, either through viral transfer or through recombination, may provide novel modes of treatment for hitherto intractable diseases of memory, either *in utero* or in children or even adults.

13. ETHICAL ISSUES IN THE STUDY OF MEMORY

As our knowledge and interventional ability increase, ethical issues will arise from advances in memory research, as they have in so many other areas of the biological sciences. The most obvious of these is also the simplest to evaluate: the misuse of any advances in memory research to the detriment of patients or others, by damaging or manipulating their memories, is clearly wrong. However, more subtle issues exist.

Several products are currently on the market, which claim to increase memory performance. While currently available products have little if any proven efficacy, it is only a matter of time before bona fide memory-enhancing substances become available. The simplest issues raised by such products are those of side effects: If such drugs act by increasing synaptic potentiation, will they increase the propensity for epilepsy, stressor-related depression or post-traumatic stress disorder? Will these drugs make it difficult to forget even unpleasant events? A more subtle societal issue will be more difficult to evaluate. If such drugs are developed, they are most likely to be developed by an established drug company, and once available they are likely to be costly. Since insurers seem unlikely initially to deem memory enhancement a medical necessity, this implies that memory-enhancing drugs will be available primarily to the affluent. Since memory enhancement would have obvious advantages in education and in almost any profession, such a development might concretely enhance the gap in opportunity for education that already exists between rich and poor. The societal implications are troubling.

Another ethical issue may arise in the context of drug addiction and treatment. As we briefly note above, drug addiction may involve plastic change in the reward circuits of the ventral striatum. An enhanced understanding of the mechanisms of such plastic change might lead eventually to therapeutic techniques to reverse such change or to avoid it entirely. Such a development would be of obvious societal benefit; the effective treatment of addiction could lead to a great reduction in violent crime. If addiction becomes easily treatable or completely avoidable, much of the motivation for the stigmatization and criminalization of drugs in our society will be removed. If the addictive aspects of drugs of abuse were dissociated from their pleasurable effects, a serious reconsideration of their place in our society might ensue. It is not clear what the ethical or societal consequences of such a development would be.

14. CONCLUSION

The 1990s were proclaimed as the 'decade of the brain' in acknowledgement of both the unique challenges posed by the human nervous system and the rapid pace of discovery in neuroscience. In the past ten years we have indeed experienced an accelerating rate of discovery in many fields of neuroscience, including memory research. The gradual unification of neurobiology with cognitive psychology and the subsequent emergence of a molecular biology of cognition have been very fruitful and increased substantially our understanding of the mechanisms of memory formation. We now have reached the end of both the 20th century and of the decade of the brain. We have at this point a clearer understanding of biologically meaningful subdivisions of memory storage and clearer understanding in outline of some molecular mechanisms of storage relevant to each of these subdivisions. Most impressive is the finding that explicit and implicit storage seems to use a common and limited set of mechanisms to convert short- to long-term memory.

Whereas satisfactory insight into even the details of the storage mechanisms are in sight, the systems problems are much more difficult and will continue to occupy us for many decades. This is because the anatomical system that stores explicit memory is complex, as is the nature of the memory that is stored. Moreover, explicit memory is intimately joined with conscious recollection, an area of neuroscience into which we have little insight. Because the complexity of explicit memory will take time to dissect (probably another century) it will be advisable to continue to analyse instances of implicit memory storage, including the simple implicit memory systems of vertebrates and invertebrates, and use them as prototypes for understanding more complex explicit systems. Because explicit memory storage is so deeply embedded in perception, action and consciousness, its future is the future of neuroscience. As a result, insofar as understanding explicit memory will require understanding much of the brain, the mass action arguments of Flourens and Lashley and the localization arguments of Gall, Penfield and Milner will, in the long run, be joined. We may realize that, even though memory is localized, in its explicit form it can have an extremely distributed representation that, in the limit, may involve much of the brain.

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