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DATABASES
Entrez Gene: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene
APOE | BDNF | IGF1 | TRKB| VEGF
OMIM: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM
Alzheimer's disease | Parkinson's disease

FURTHER INFORMATION
Charles H. Hillman's homepage: http://www.kch.uiuc.edu/labs/neurocognitive%2Dkinesiology/default.htm

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OPINION

Synaptic plasticity, memory and the hippocampus: a neural network approach to causality

Guilherme Neves, Sam F. Cooke and Tim V. P. Bliss*

Abstract | Two facts about the hippocampus have been common currency among neuroscientists for several decades. First, lesions of the hippocampus in humans prevent the acquisition of new episodic memories; second, activity-dependent synaptic plasticity is a prominent feature of hippocampal synapses. Given this background, the hypothesis that hippocampus-dependent memory is mediated, at least in part, by hippocampal synaptic plasticity has seemed as cogent in theory as it has been difficult to prove in practice. Here we argue that the recent development of transgenic molecular devices will encourage a shift from mechanistic investigations of synaptic plasticity in single neurons towards an analysis of how networks of neurons encode and represent memory, and we suggest ways in which this might be achieved. In the process, the hypothesis that synaptic plasticity is necessary and sufficient for information storage in the brain may finally be validated.

Multiple strands of evidence suggest an important role for the hippocampus in episodic memory in animals and humans. Most notable among human patients has been H.M., who as a young man suffered from intractable epilepsy and underwent experimental surgery involving bilateral removal of the medial temporal lobe, including large parts of both hippocampi. The procedure left H.M. with an inability to form new episodic memories (anterograde amnesia), coupled with a substantial, but not total, loss of old memories (retrograde amnesia)[1]. Other cases since H.M. have confirmed that the hippocampus is essential for the formation of new episodic memories and might also have a role in their long-term storage. Animal studies reveal that controlled lesions, pharmacological inactivation or molecular knockouts limited to the hippocampus result in either a failure to learn or a loss of spatial memory[2–5]. Electrophysiological recordings[6] and molecular imaging studies in animals[7,8], as well as MRI imaging studies in humans[9–11], provide correlative evidence that episodic or episodic-like learning and memory involves hippocampal activity.

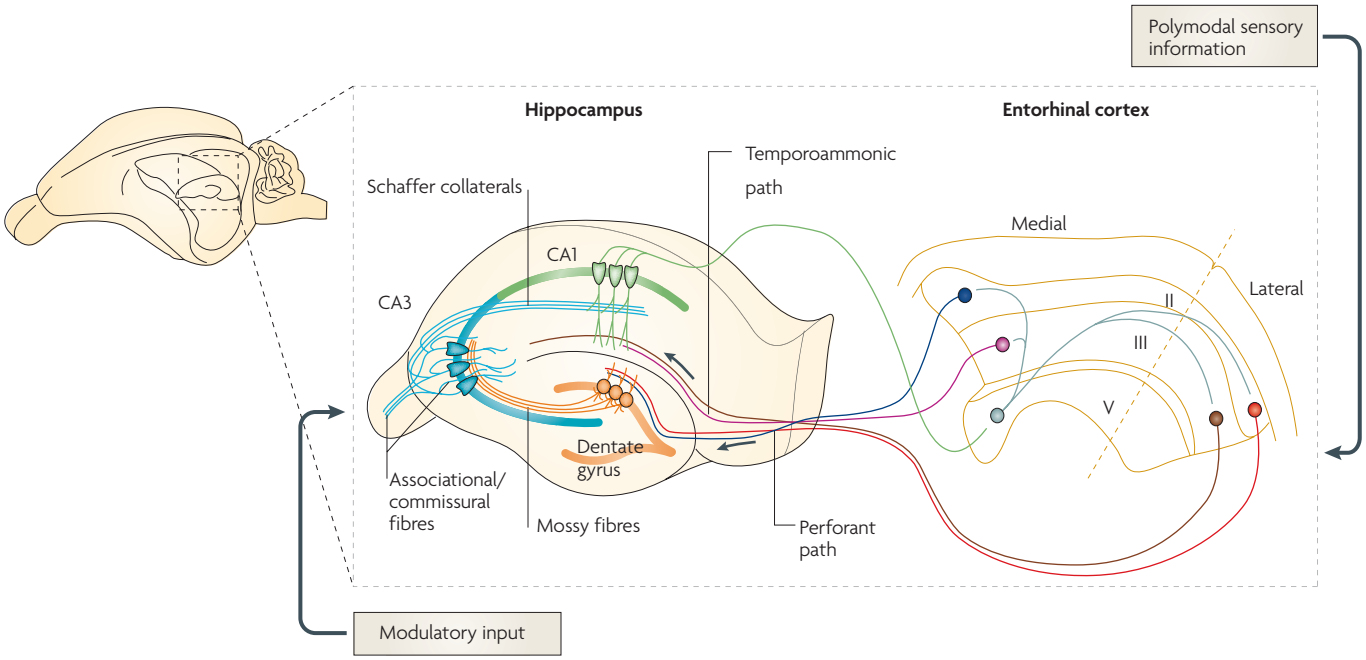


Figure 1 | **Basic anatomy of the hippocampus.** The wiring diagram of the hippocampus is traditionally presented as a trisynaptic loop. The major input is carried by axons of the perforant path, which convey polymodal sensory information from neurons in layer II of the entorhinal cortex to the dentate gyrus. Perforant path axons make excitatory synaptic contact with the dendrites of granule cells: axons from the lateral and medial entorhinal cortices innervate the outer and middle third of the dendritic tree, respectively. Granule cells project, through their axons (the mossy fibres), to the proximal apical dendrites of CA3 pyramidal cells which, in turn, project to ipsilateral CA1 pyramidal cells through Schaffer collaterals and to contralateral CA3 and CA1 pyramidal cells through commissural connections. In addition to the sequential trisynaptic circuit, there is also a dense associative network interconnecting CA3 cells on the same side. CA3 pyramidal cells are also innervated by a direct input from layer II cells of the entorhinal cortex (not shown). The distal apical dendrites of CA1 pyramidal neurons receive a direct input from layer III cells of the entorhinal cortex. There is also substantial modulatory input to hippocampal neurons. The three major subfields have an elegant laminar organization in which the cell bodies are tightly packed in an interlocking C-shaped arrangement, with afferent fibres terminating on selective regions of the dendritic tree. The hippocampus is also home to a rich diversity of inhibitory neurons that are not shown in the figure. For a full description of hippocampal anatomy, see REF. 90.

Synaptic plasticity in the hippocampus

The hippocampus has been a major experimental system for studies of synaptic plasticity in the context of putative information-storage mechanisms in the brain. Its simple laminar pattern of neurons and neural pathways (FIG. 1) enables the use of extracellular recording techniques to record synaptic events for virtually unlimited periods *in vivo*[12]. The much-studied model of synaptic plasticity, long-term potentiation[13,14] (LTP; see FIG. 2a), was first identified in the hippocampus and has been extensively characterized using electrophysiological, biochemical and molecular techniques[15]. Several recent studies have detected LTP-like synaptic changes in the hippocampus[16,17] (FIG. 2b) and the amygdala[18] following learning. Other forms of activity-dependent plasticity have been found, including long-term depression (LTD)[19], EPSP-spike (E-S) potentiation[20,21], spike-timing-dependent plasticity (STDP)[22], depotentiation[23–25] and de-depression[25,26]. The transverse hippocampal slice preparation[27] (FIG. 2a) has been of major importance to this field, enabling pharmacological agents to be rapidly washed on and washed off and allowing intracellular and patch-clamp recordings. In addition, hippocampal neurons can be cultured[28,29], either as transverse 'organotypic' slices or as populations of dissociated neurons, for periods of months, facilitating molecular manipulations such as overexpression or RNAi-based knock-down of specific proteins. These *in vitro* techniques have greatly enhanced our understanding of the molecular mechanisms that underlie synaptic plasticity[15,30]. In the hippocampus it has been possible to track effects such as the phosphorylation of a protein at a specific residue at multiple levels of organization, from isolated synaptic membranes all the way through to the behavioural analysis of intact animals with specific molecular defects[31]. Nevertheless, the larger picture of how synaptic plasticity in extensive networks of cells leads to the storage and recall of information remains dimly illuminated. The Canadian psychologist Donald Hebb posited a role for such assemblies as engrams or memory traces[32]. His famous 'neurophysiological postulate' proposes that connections between co-active neurons are strengthened through mechanisms of synaptic plasticity, so that subsequent activation by incoming stimulation of only a sub-component of the assembly will lead to activation of the whole assembly, thereby recapitulating the activity elicited by the original event. (LTP is a Hebbian process, since its induction requires coincident activity of the pre- and postsynaptic neurons.) The immediate problem is to identify such cell assemblies in the hippocampal encoding of memory.

Place cells

Single-unit recordings from neurons in the hippocampus of freely moving rodents reveal that pyramidal and granule cells show a preference for firing in a particular location of an explored environment, regardless of the direction from which the animal enters the location[33] (BOX 1). Hundreds of such 'place cells' fire in concert as a rat reaches a particular location, and place cells fire in sequence as the animal moves

through a series of locations in a given environment[34,35], suggesting that a network of pyramidal cells can also serve as a cell assembly to encode and store a neural representation of space.

LTP and learning: approaches to causality

The SPM hypothesis. The presumptive causal link between synaptic plasticity and memory has been formalized by Morris and colleagues as the synaptic plasticity and memory (SPM) hypothesis:

> *Activity-dependent synaptic plasticity is induced at appropriate synapses during memory formation, and is both necessary and sufficient for the information storage underlying the type of memory mediated by the brain area in which that plasticity is observed*[36].

It is now over 30 years since the first description of LTP in the hippocampus, 20 years since the first attempt to use pharmacological tools to dissect the relationship between LTP and memory, and over 10 years since the first knockout studies were published. Even though the SPM hypothesis, or a similar model, is enshrined in most neuroscience textbooks, the issue is far from resolved. We next consider the reasons for this impasse, and ask what new approaches are needed if the relationship is ever to be unravelled.

Testing necessity. In order to establish the necessity of synaptic plasticity (taking LTP as our exemplar) for information storage, the ideal experiment would be an intervention that completely blocked the induction or expression of LTP in the hippocampus while doing nothing else. The twin problems in any real-life experiment lie in the precise spatial targeting of the blockade and in the need to affect 'nothing else'. At first glance, the early observation that infusion into the hippocampus of the selective NMDA (*N*-methyl-D-aspartate)-receptor blocker APV (2-amino-5-phosphonovaleric acid) profoundly impairs learning and recall in the Morris water maze[4] is a compelling validation of the hypothesis: the drug is applied directly into the hippocampus and blocks LTP without affecting basal synaptic transmission. In a crucial recent experiment, it was shown that inhibition of the active form of the protein kinase PKMζ by infusion into the hippocampus of its specific inhibitor, ZIP (myristoylated zeta-pseudosubstrate inhibitory peptide), can impair spatial memory and block LTP, even

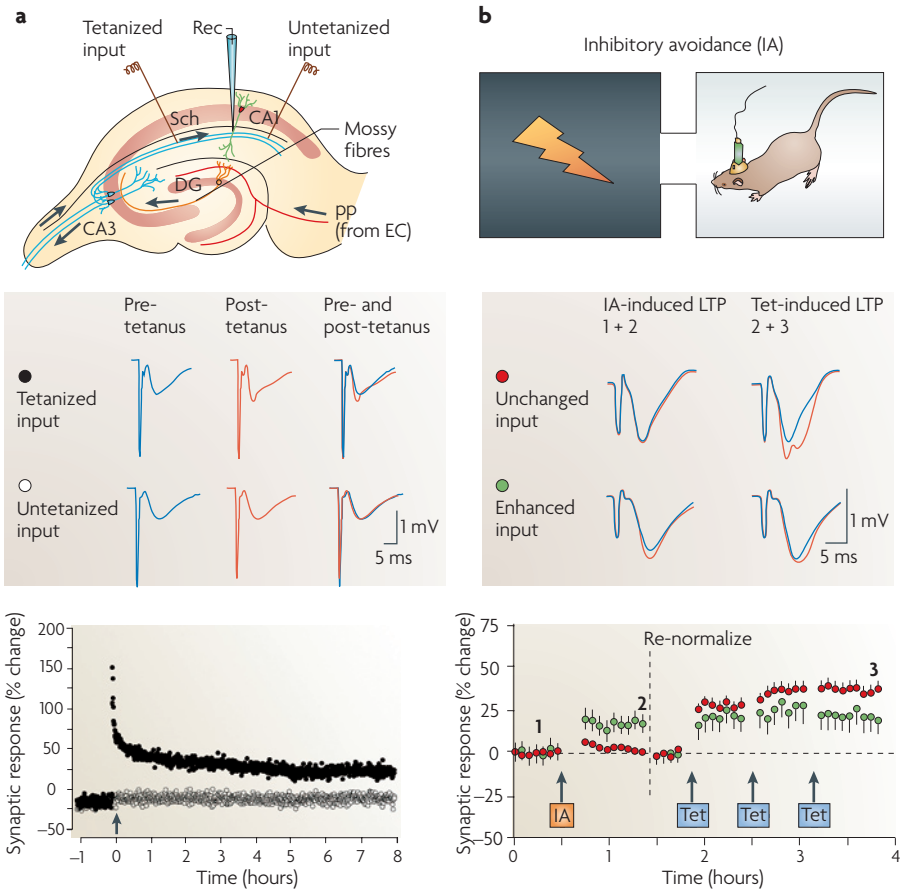
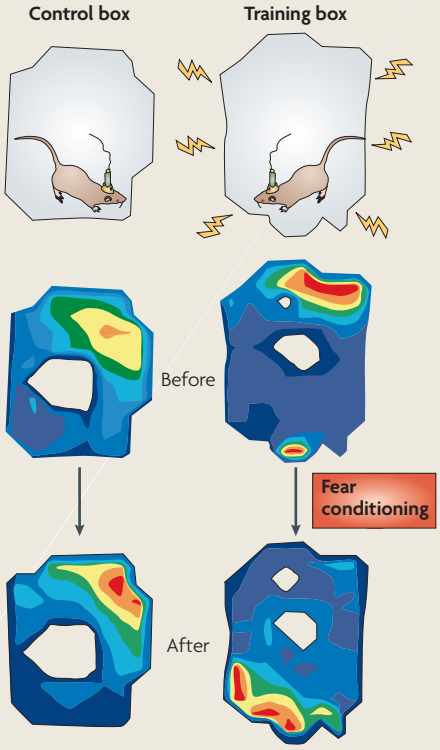


Figure 2 | **Long-term potentiation (LTP) *in vitro* and *in vivo*. a** | Extracellular recordings of LTP induced by tetanic stimulation of the Schaffer-commissural projection (Sch) to CA1 pyramidal cells in a transverse hippocampal slice (shown as a schematic in the top panel). Hippocampal slices can be kept healthy for many hours if a steady flow of oxygen and artificial cerebrospinal fluid is supplied. The laminated organization of the hippocampus lends itself perfectly to extracellular recording techniques, allowing selective pathways to be stimulated and the evoked synaptic responses generated by a population of target neurons to be monitored for prolonged periods of time. The middle panel shows typical synaptic responses recorded from the apical dendritic region of the CA1 subfield following stimulation of the Schaffer-commissural pathway. Two metal stimulating electrodes are placed on either side of the recording electrode to evoke responses in overlapping populations of pyramidal cells through different sets of synapses. A tetanus (a brief, high-frequency train of electrical stimuli) can be used to induce LTP lasting for many hours in the tetanized pathway (bottom panel, closed circles); the second, control pathway (open circles) receives only test stimulation and is not potentiated following the tetanus to the experimental pathway. This demonstrates an important property of LTP, namely input specificity. **b** | *In vivo* LTP induction by learning[17]. Synaptic responses from multiple locations can be recorded in area CA1 of freely moving animals using an array of recording electrodes and a single stimulating electrode (examples in middle panel). Rats were trained in an inhibitory avoidance (IA) task, a hippocampus-dependent form of single-trial learning in which a rodent avoids entering a dark arena where it has received a footshock (top panel). IA training leads to a rapid increase, lasting for hours, in the amplitude of evoked responses in some of the recorded pathways (green circles in lower panel) but not in others (red circles). Training-dependent synaptic enhancement (bottom panel, arrow IA) occludes LTP induced by delivering tetanic stimulation (bottom panel): compare the degree of potentiation induced by tetanic stimulation (arrow Tet) in the pathways that were enhanced by training (green circles) to the pathways that were unchanged (red circles). The numbers 1, 2 and 3 indicate the times at which sample responses were obtained from inputs that were either enhanced (green) or unchanged (red) following learning. Note that post-IA responses are re-normalized before tetanus-induced LTP. Superimposed responses in the middle panel show effects of learning (1+2) and the subsequent effects of delivering three episodes of tetanic stimulation (2+3). These results suggest that experience-dependent synaptic enhancement uses the same molecular mechanisms of expression as tetanus-induced LTP. DG, dentate gyrus; EC, entorhinal cortex; pp, perferant path. Part **a** modified, with permission, from REF. 91 © (2003) Blackwell Science. Part **b** reproduced, with permission, from REF. 17 © (2006) American Association for the Advancement of Science.

Box 1 | **Plasticity in place cells**



Place cells are hippocampal pyramidal or granule cells that fire action potentials in particular locations (place fields) in an environment and which thus collectively carry information about the animal's moment-to-moment position.

Ensembles of place cells probably serve as our best working model of hippocampal function. However, they do not observe any obvious spatial topography and certainly do not conform to a two-dimensional topographical map[76,77]. Adjacent place cells in the hippocampus can encode locations separated by great distances in an environment and, also, an individual place cell's receptive field can be very different from environment to environment. This observation is consistent with the idea that spatial memory is encoded in a distributed fashion in the hippocampus[78]. Plasticity of place cells has been observed as a remapping of either their firing rates or their receptive fields when cues in an environment[79], or the shape of an environment, are changed[80,81]. Remapping can also be triggered by a discrete learning event in the same, unchanged environment[82] — in a form of Pavlovian conditioning called contextual fear conditioning (middle and lower pairs of panels). Here, an electrical footshock is applied as an unconditional stimulus while the environment effectively acts as a conditional stimulus that, after training, can itself elicit a behavioural freezing response. The middle and lower pairs of panels in the figure show the firing rates of one place cell in two different environments. The cell's place field was stable in a control environment (left-hand panels) but remapped from the north east to the south west of the experimental chamber (right-hand panels) after contextual fear conditioning. Firing rates in the environment are colour coded (redder colours indicate higher firing rates).

Assuming that this form of remapping depends on hippocampal plasticity, it could serve as an intermediate electrophysiological assay for effective silencing, erasure or re-installation of memory in the proposed experiments illustrated in FIGS 3,4. Evidence that remapping requires hippocampal plasticity has come from analysis of subfield-specific knockouts. Place cell activity is disrupted in animals with CA1-specific knockout of the NMDA receptor subunit NR1, such that receptive fields do not retain strong location specificity and ensembles of cells with similar receptive fields are not correlated in their firing, consistent with the disruption of a functional representation of space[83]. Similar results were obtained with perfusion of an NMDA-receptor antagonist into the hippocampus[84]. In CA3-specific knockouts of NR1, CA1 place cells have normal place fields in familiar environments but enlarged, unrefined place fields in novel environments[85], suggesting a role for plasticity at CA3 recurrent collateral synapses in remapping of place fields. Place cell remapping in area CA3 is also disrupted when NR1 expression is deleted in the dentate gyrus[54]. Figure reproduced, with permission, from REF. 82 © (2004) Society for Neuroscience.

when the inhibitor is administered days after the acquisition of the memory or the induction of LTP, again without affecting baseline synaptic transmission[5]. However, in both cases it is impossible to be certain that the drug has not spread outside the hippocampus and is not having some effect other than blocking the induction or maintenance of LTP[4] (see below). The use of viral vectors to interfere with the process of glutamate-receptor trafficking suggests that membrane insertion of GluR1-containing AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid) receptors (a candidate mechanism for the expression of LTP[30]) might be necessary for the full expression of amygdala-dependent cued fear conditioning[18]. Again, it is difficult to exclude the possibility that effects on processes unrelated to the maintenance of LTP cause the learning impairment.

The situation is not obviously improved in most experiments using genetically engineered mice, as the effects of knocking out a transcription factor or a protein kinase, for instance, will certainly affect cell processes other than LTP; even inducible systems require several days to take effect, during which time compensatory mechanisms can develop. The most compelling transgenic experiment so far is the reversible inactivation of the NMDA receptor subunit NR1 in the CA1 subfield of the hippocampus[37]. Here the gene product is directly responsible for the induction of LTP, and the molecular deficit is precisely defined, inducible and reversible. It also seems to be mostly confined to pyramidal cells in area CA1. In this mouse both LTP and spatial learning are suppressed, implying that the presence of NR1 receptors in area CA1 is necessary for spatial learning to occur. Is it equally safe to conclude that LTP in area CA1 is necessary for spatial learning? The answer is no, as the blockade of the NMDA receptor is known to affect several other processes, including the induction of E-S potentiation[38] and certain forms of LTD[19], and to reduce postsynaptic responses during short bursts of high-frequency activation[39]; any or all of these processes might contribute to information storage. The closer the experimental intervention gets to LTP itself, however, the more confident we can be that there is a causal link between LTP-like synaptic plasticity and learning and memory. Nevertheless we have to conclude that, despite the wealth of experimental support, definitive evidence that LTP is necessary for hippocampus-dependent learning is still lacking.

Learning without hippocampal LTP?

Given that a single negative result could ostensibly disprove the necessity arm of the SPM hypothesis, a potentially more powerful result would be one in which LTP is suppressed yet learning is unaffected; in such a case, whether or not other processes are affected, the conclusion can be drawn that LTP is not necessary for that particular form of learning. One example is the 'upstairs/downstairs' water maze experiment[40], in which rats were trained in one maze (on a lower floor of the laboratory building) and subsequently were able to learn and retain information about the location of the hidden platform in a second upstairs maze, even when infused with the NMDA-receptor antagonist APV. This experiment is important because it suggests that, at least in some circumstances, conventional NMDA-receptor-dependent LTP is not required for the acquisition and storage of hippocampus-dependent reference memory.

The GluR1 knockout. Another potential counter-example to the predictions of the SPM hypothesis is the GluR1-knockout mouse, which showed a total absence of conventional tetanus-induced LTP in area CA1 without any impairment in acquisition or recall in the standard, reference memory version of the Morris water maze[44]. A problem with reaching a firm conclusion from such an apparently definitive result is that it is very hard to be sure that LTP has in fact been abolished. The experimenter has available a range of protocols to induce LTP, but we do not know what protocols the hippocampus itself is using. It might be that the behaving animal was able to generate 'sharp-wave ripples', a naturally occurring high-frequency waveform generated by synchronous firing of CA3 pyramidal cells that can facilitate the induction of LTP[41]. The initial GluR1-knockout paper reported that LTP induced by a brief burst of 100 Hz stimulation was absent in the Schaffer-commissural pathway, but only reduced to about 50% of controls in the perforant path[42] (FIG. 1). Subsequent analysis revealed that, even in the Schaffer-commissural pathway, LTP could be induced using a theta-burst pairing protocol, in which presynaptic stimulation at 5 Hz was paired with synchronous depolarization of the CA1 pyramidal cell[43]. (Theta-burst stimulation mimics the frequency of theta waves that are generated in the hippocampus of rodents as they explore an environment[44,45].)

In summary, the GluR1 animal has not disproved necessity, and it is hard to see how any other transgenic mouse would get around this problem. Finally, we note that even if LTP is not necessary for learning, it might nevertheless be the brain's default choice when it is available, as in the normal brain.

Testing sufficiency. Turning to sufficiency, can we devise an experiment in which a novel memory is installed by inducing LTP at a selection of hippocampal synapses? The answer is that we know so little about how episodic memories are encoded in the hippocampus or in the neocortex that even if we had the experimental tools to modulate synaptic weights at a spatially distributed set of hippocampal synapses, we would have no idea how to go about selecting which synapses to modify. Conceivably, the situation is more tractable for other forms of memory in the brain. In the cerebellum, for instance, there is a regularly organized circuit that delivers relatively unprocessed somatosensory and motor information to the Purkinje cells of the cerebellar cortex. It is believed that implicit motor learning is mediated by synaptic plasticity in the cerebellar cortex and/or the deep cerebellar nuclei[46–49]. These structures are organized as two-dimensional topographical maps of the body, and it is possible to target specific microzones that mediate particular skeletal muscular responses[50,51]. The best-studied example of this functional organization is probably classical conditioning of the nictitating membrane/eyeblink response in rabbits[49,52]. With such regular, tractably organized and well-characterized circuitry, there might be some hope of developing interventional assays to test the causal role of synaptic plasticity in motor learning, for example, by selectively erasing or installing conditioned eyeblinks. By contrast, the functional organization of the hippocampus is less understood, largely owing to a lack of two-dimensional topography (BOX 1). However, it might be possible to address the SPM hypothesis in the hippocampus by adopting a set of experimental strategies that are largely blind to functional organization. We outline some of our suggested approaches below but, before doing so, we need to summarize what has been learned from mutant mice engineered to express region-specific and/or inducible transgenes.

Subregion-specific deletion of NR1

Over the past decade several studies have used the enzyme Cre recombinase, driven by subregion-specific promoters, to restrict deletion of the gene that encodes the NMDA-receptor-subunit NR1 to particular subfields of the hippocampus, giving rise to different cognitive impairments. Although, as we have seen, NR1 receptors in pyramidal cells of area CA1 seem to be essential for normal performance on the reference memory version of the Morris water-maze[2,37], animals can perform these tasks as well as control littermates if the NR1 deletion is confined to pyramidal cells in area CA3 (REF. 53) or granule cells in the dentate gyrus[54,55]. We can therefore be confident that NMDA-receptor-mediated LTP in the dentate gyrus[54,55] and in area CA3 (REF. 53) is not necessary for the acquisition and storage of reference spatial memory, as this form of LTP is impossible in regions where NR1 has been deleted and yet these animals can learn. However, this is not to say that these subregions have no role in spatial memory. Animals with deletion of NR1 in area CA3 have subtle defects in pattern completion, such that they are unable to use partial presentation of external cues to recall the position of a hidden platform[53]. By contrast, deletion of NR1 in granule cells of the dentate gyrus impairs both working memory in a radial arm maze[55] and discrimination of context in fear conditioning[54], suggesting that a failure of pattern separation occurs.

These results are consistent with the much-cited model of subregional processing in hippocampal function developed by David Marr[56]. The situation is unresolved in area CA1 where, as we have seen, the evidence does not allow us to decide whether or not LTP is necessary for reference learning in the water maze. The role of non-NMDA-receptor-dependent plasticity at mossy fibres also remains to be clarified.

Network approaches

A direct test of the proposition that the neural representation of a memory is encoded in the network of neurons containing synapses that were modified during the acquisition of that memory would be to ask whether the memory is lost when only these neurons are selectively inactivated.

Exploiting immediate-early genes. A feasible approach to silencing potentiated neurons at the network level would be to design a transgenic mouse in which a promoter for a plasticity-marker gene drives the expression of a protein that reduces the excitability of the cell. No such plasticity-marker genes have been identified that are expressed when and only when synaptic plasticity is induced. However, the expression of several immediate-early genes (IEGs), including those that encode c-Fos, Zif268, Arc/Arg3.1 and Homer, are strongly upregulated by LTP-inducing protocols both *in vitro* and *in vivo*, as well as by behavioural training (FIG. 3a) (reviewed in REF. 57). Both Arc/Arg3.1 (REFS 58,59) and the transcription factor Zif268 (REF. 60) are required for the maintenance of LTP that lasts for several days and for the stability of long-term memories. A knock-in mouse line has been generated in which the coding sequence of the Arc/Arg3.1 gene was replaced with that of the gene that encodes green fluorescent protein (GFP). GFP expression in the visual cortex of these mice could be tracked *in vivo*, and was regulated by light exposure in an NMDA-receptor-dependent manner (FIG. 3b), faithfully mimicking the expression of Arc/Arg3.1 (REFS 59,61). In a recent study[62], the promoter of the gene that encodes c-Fos was used to generate a mouse line that permits long-lasting genetic tagging of activated neurons. This study suggested that neurons that are activated in the amygdala during contextual fear conditioning are re-activated during retrieval of the memory.

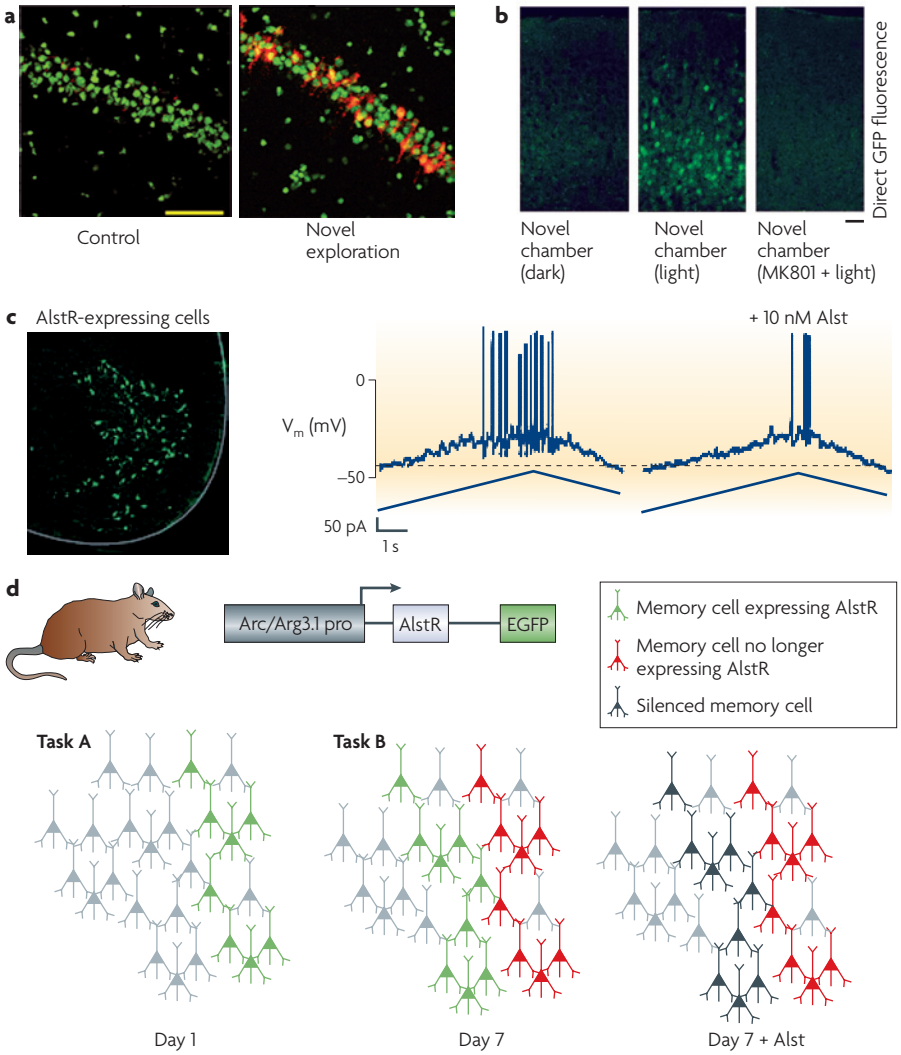


Figure 3 | **A strategy for silencing a hippocampal cell assembly encoding a particular memory.** The approach depends on genetic constructs in which promoters from activity-dependent genes (such as those that encode Arc/Arg3.1 or Zif268) are used to drive the expression of transgenes specifically in recently potentiated cells. These transgenes can be used to silence activated cell assemblies. **a** | Arc/Arg3.1 (immunostained in red) is activated in a subset of hippocampal neurons (in this figure, CA1 pyramidal cells) when an animal explores a novel environment[92] (scale bar 100 µm). **b** | Green fluroescent protein (GFP) mirrors the endogenous expression of Arc/Arg3.1 in a genetically engineered mouse in which the expression of GFP is controlled by the Arc/Arg3.1 gene promoter[59]. In this example, expression in the primary visual cortex is upregulated by light exposure. The NMDA (*N*-methyl-D-aspartate) receptor antagonist MK801 blocks this effect (scale bar 40 µm). **c** | The left-hand panel shows a confocal micrograph of a section obtained from the spinal cord of a transgenic mouse in which the engrailed gene promoter had been used to drive expression of the allatostatin receptor (AlstR) in a specific subtype of interneuron (labelled in green, reflecting expression of GFP). The right-hand panel shows a current-clamp recording from a labelled interneuron: in the presence of 10 nM allatostatin (Alst), neurons have a higher threshold for triggering action potentials and therefore are effectively inactivated[68]. **d** | A way in which to abolish specific memories while sparing others. The activity-dependent Arc/Arg3.1 gene promoter is used to drive the expression of the allatostatin receptor. Cells that, as a result of training in Task A, acquire potentiated synapses (green cells in left panel) will express the receptor and can potentially be silenced by perfusion with allatostatin. Silencing is dependent on allatostatin, but also on the presence of the allatostatin receptor on the cell surface. After a certain period of time the receptors will be internalized and degraded (red cells, day 7). Recent memory, activating a different, possibly overlapping, population of cells (Task B, green cells) should therefore be abolished when these cells become silent (black) in the presence of allatostatin, whereas remote memories (red cells) should be spared. Part **a** reproduced, with permission, from REF. 92 © (2005) Society for Neuroscience. Part **b** reproduced, with permission, from REF. 59 © (2006) Elsevier Science. Part **c** reproduced, with permission, from REF. 68 © (2006) Macmillan Publishers Ltd; courtesy of M. Goulding.

These and other studies[63,64] show that it is possible to activate transgenes specifically in circuits that are activated by particular behaviours. It follows that we should be able to design molecular devices that reversibly activate or inactivate hippocampal neuronal spiking, using the promoters of IEGs to drive the expression of membrane proteins that generate appropriate changes in excitability. Using these devices, it should be possible to test the causal role of plasticity in distributed networks of neurons in the hippocampus during learning and memory. This strategy takes us closer to establishing the importance of Hebbian cell assemblies in information storage. By exploiting the transient and activity-dependent expression of IEGs, it should be possible to discriminate between cell assemblies: the IEG promoter will drive gene expression only in those cell assemblies that represent a newly acquired memory, leaving other hippocampal neurons unaffected (FIG. 3d).

Reversible activation or inactivation

Designs for a new generation of engineered ligand- and light-gated membrane receptors, which can be used to initiate or inhibit neural activity, have come to the fore in the past year or two.

Ligand-gated systems. Many invertebrates express a gated Cl^- channel that is kept open by ivermectin (IVM), a drug that is widely used for the control of worm infections. In the presence of low concentrations of ivermectin, cultured hippocampal neurons expressing this channel are held near the hyperpolarizing Cl^- reversal potential, and thus are effectively silenced[65]. The channel is composed of two subunits, α and β, both of which must be expressed in the same cell to generate an IVM-sensitive current[65]. Inspired by the potential of this approach, we attempted to generate a transgenic mouse that would express the IVM-binding α-subunit under the control of the Arc/Arg3.1 promoter (conferring activity-dependence on the transgene) and the β-subunit under the control of the α-calcium/calmodulin-dependent protein kinase II promoter (to restrict expression to the postnatal forebrain). Further spatial selectivity would be achieved by the direct injection of IVM into the hippocampus. Our attempts have so far been jeopardized by inadequate expression levels. However, this method has recently been successfully used to silence cells in the striatum by viral infection[66], although the silencing that was achieved had a slow activation and inactivation time scale. The approach may

therefore be more suitable for use in the context of neural development or pathological processes.

A more promising approach might be to exploit metabotropic signal transduction pathways to amplify the effect of the transgene on cell excitability. When transfected into mouse neurons, a *Drosophila melanogaster* G-protein-coupled receptor for the peptide hormone allatostatin (AlstR) can couple to mammalian G-protein-activated inwardly rectifying K^+ channels (GIRK or Kir3 channels)[67]. This allows rapid, reversible hyperpolarization and hence silencing of allatostatin-receptor-expressing neurons in response to allatostatin. For example, when transgenic mice expressing the allatostatin receptor in a specific class of spinal cord interneurons were treated with allatostatin, the neurons were quickly and reversibly inactivated[68] (FIG. 3c). Applied to the silencing of cell assemblies in the hippocampus, this approach would require a transgenic animal in which expression of the allatostatin receptor is driven by an activity-dependent promoter (FIG. 3d). Spatial specificity would be gained by infusing allatostatin to the hippocampal region of interest (BOX 2). At least two other methods for ligand-controlled inactivation of neurons have been successfully used in behaving mice[69,70], and all signs are that these methods will continue to develop at a rapid pace.

Light-gated systems. A whirlwind of interest has been generated recently by strategies that rely on light exposure to modulate neural activity. *Chlamydomonas reinhardtii* channelrhodopsin 2 (ChR2) is a cation channel that opens on exposure to blue light (thereby depolarizing neurons that express it), whereas *Natronomonas pharaonis* halorhodopsin (NpHR) is a Cl^- pump that is activated by yellow light (and which thus can hyperpolarize neurons in which it is expressed)[71]. The use of light as a switch allows precise temporal control but presents challenges in getting enough light through the skull and into the brain area expressing the light-sensitive ion channels. However, major strides have been made in solving this problem[72,73]. In one particularly impressive study, the selective optical activation of hypocretin-producing neurons in the hypothalamus, an area that lies deep inside the brain, was shown to awaken sleeping animals[73]. Again, in our context, ChR2 or NpHR would be driven by IEG promoters. The same strategy, in which an IEG promoter would drive the expression of light-dependent ion channels, has been proposed for the study of neural networks that are implicated in disease models[72].

Memory erasure. Another approach to testing the necessity of LTP for learning and memory would be to attempt to erase memory by selectively reversing experience-dependent plasticity. As discussed earlier, perfusion of the specific PKMζ inhibitor, ZIP, erases hippocampus-dependent memory. ZIP only targets activated synapses, but it does not appear to differentiate between recent and old memories. One conceptually simple approach to reversing change at recently potentiated synapses would be to use the phenomenon of depotentiation. In area CA1, synapses that have recently been potentiated can be depotentiated by low frequency stimulation[24]. Depotentiation occurs only at those synapses that were potentiated in the preceding few minutes, after which LTP becomes stabilized and resistant to depotentiation. Thus, depotentiating stimulation could, in principle, be used to erase LTP specifically in the cell assemblies that represent a recently acquired memory. As we discuss below, memory erasure might also prove useful in testing the sufficiency arm of the SPM hypothesis.

Is synaptic plasticity sufficient for memory?

Can synaptic plasticity alone be used to build a memory? To answer this question we need to be able to mimic the natural process of synaptic plasticity by artificial means, at only the subset of synapses that would be involved in storing a particular memory. Our aim is to make memory without the need for learning (memory mimicry).

In the current state of knowledge this is not feasible, and it is unlikely to become so any time soon. However, one can imagine ways in which it might be possible to recreate a lost memory. Two such thought-experiments are described in FIG. 4. In both cases a hippocampus-dependent memory is formed by a standard training procedure. The memory is then erased, but subsequently re-installed by exploiting the knowledge gained about the synaptic changes that occurred during the original learning episode.

The very large multi-electrode array. The first experiment relies on gaining access to a large enough assembly of pre- and postsynaptic neurons to monitor plasticity between pairs of cells participating in encoding a new memory, each with one partner in area CA3 and one partner in area CA1 (FIG. 4a). Initial cross-correlation of action potentials from each CA3–CA1

Box 2 | **The hippocampus and global versus local approaches to neural silencing**

Memory has many forms and is distributed across many brain regions. Although the hippocampus is required for the formation of episodic or episodic-like memory, it remains unclear whether the hippocampus itself acts as a memory store (and, if so, for how long). In some descriptions the hippocampus, although a necessary component of the memory system, does not itself store memories. Rather, it acts as an indexing device in which the hippocampal cell assembly that is activated during learning has access, through reciprocal cortical–hippocampal connections, to the neocortical neural networks where episodic memories are actually stored[86]. How long memories are stored or indexed in the hippocampus is also a topic of debate. In many cases the presence of the hippocampus seems only to be required during an initial period, lasting a few weeks in rodents and perhaps a few years in humans, during which the permanent memory is gradually 'consolidated' in the neocortex. The patient H.M. provides one example of such a case. In other circumstances however, the presence of the hippocampus is required for extended periods, and perhaps indefinitely, as seems to be the case with reference spatial memory in rodents. For a fuller discussion of these issues, see REFS 87–89.

Whatever the exact distribution of the engram or memory store for a given type of memory, our proposed strategy involving the exploitation of immediate-early genes (IEGs; see main text) is sufficiently flexible to encompass these multiple possibilities. The approach would allow us to operate at a systems level, by delivering an exogenous trigger (ideally a systemically deliverable ligand, such as ivermectin, that can cross the blood-brain barrier). In this sense the experiment can be done 'blind' — without the whereabouts of the encoding networks in the brain being known — because the procedure will inactivate all potential components of a memory. However, the strategy is not limited to operating blind at a systems level. If the IEG exploitation experiments we propose were conducted in a targeted fashion using local hippocampal infusions of the silencing ligand ivermectin, rather than systemic infusion, we would hope to parse out the hippocampal contribution to episodic-like memory. A similar approach could be used throughout the nervous system to define causal roles for individual structures in different forms of information storage. In this way, the problem of whether the hippocampus stores memories or merely indexes them also becomes addressable, by monitoring the behavioural effects of successively silencing alternative storage sites.

pair before learning will detect rare pairs of cells that are connected, and the amplitude of the cross-correlogram will be a measure of the strength of the synapse that links the two cells (FIG. 4b); comparison of the peak before and after learning will indicate which of the connected pairs are part of the cell assembly that encodes the memory. Depotentiation by immediate post-training application of low-frequency stimulation would erase the memory. Spike-timing-dependent plasticity, in which LTP or LTD is induced by appropriately ordering the sequence of pre- and postsynaptic spiking, could then be applied to re-tune synapses back to their memory state. In this way, we could selectively reverse synaptic changes that underlie the storage of one memory without affecting either basal transmission or synaptic changes that subserve the storage of other memories, and then, subsequently and on demand, reinstall the lost memory.

The ideal transgenic mouse. The second experiment (FIG. 4c) makes use of an imaginary but not wholly implausible molecular device that serves as a free-standing LTP device. This alien device might be, for example, a Ca^{2+} channel that when operated by an exogenous ligand allows the permeation of sufficient Ca^{2+} to trigger the native LTP induction mechanisms (FIG. 4d). The strategy is to target the free-standing LTP device to recently potentiated synapses

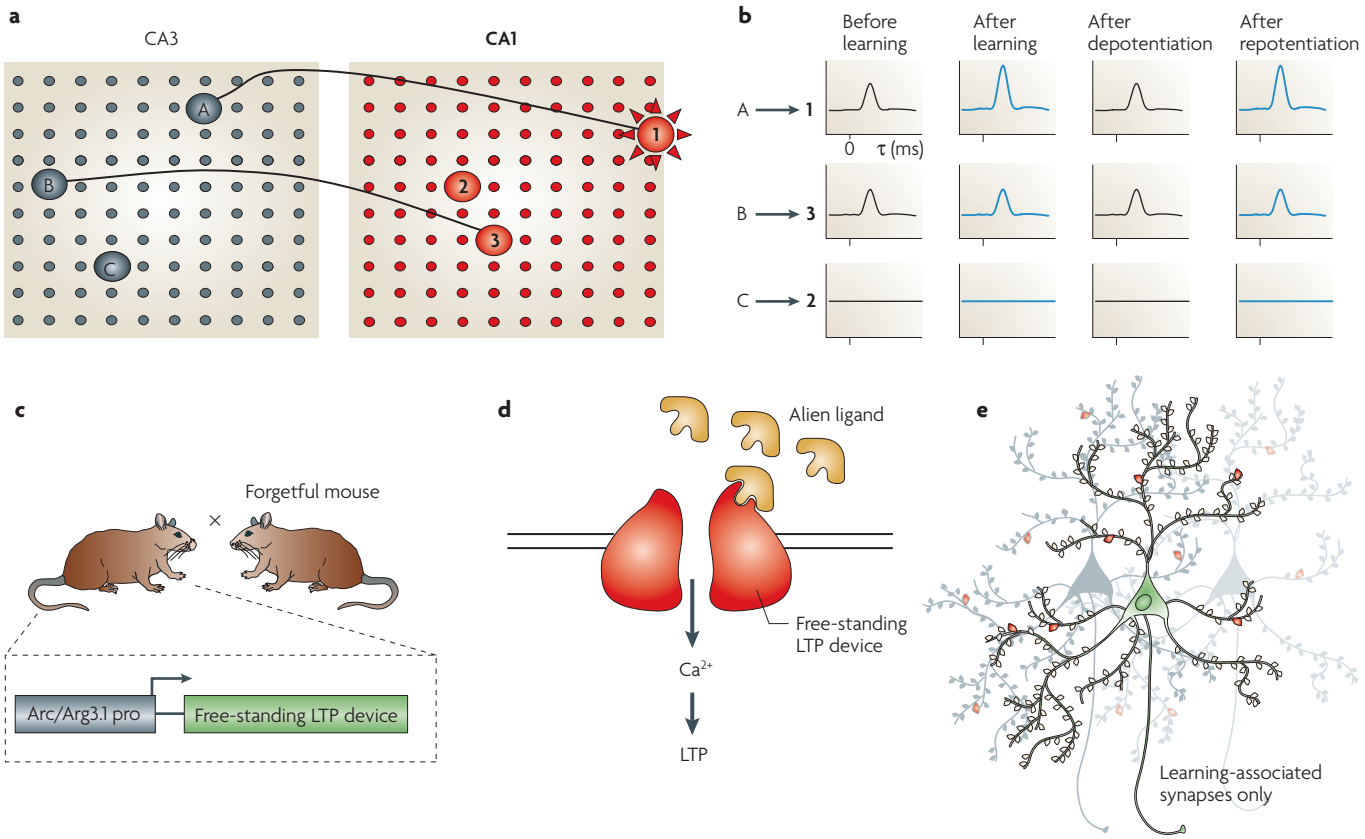


Figure 4 | **Thought experiments: erasing and re-installing a hippocampus-dependent memory. a** | The first thought-experiment requires a very large array of metal electrodes to monitor the spike activity of each cell in areas CA3 and CA1 (connectivity is unidirectional, from CA3 to CA1); the same electrodes can be used to stimulate each cell individually. Connectivity is sparse, and the great majority of CA3–CA1 cell pairs are not connected. **b** | Cross-correlation of spontaneous activity will identify connected pairs of CA3 and CA1 cells (here, A to 1, B to 3 and C to 2). The cross-correlogram plots the number of spikes emitted by a given CA1 cell during a given time interval $(\tau, \tau + \delta\tau)$ after each action potential of a given CA3 cell; a peak at a delay τ of a few milliseconds suggests that the two cells are monosynaptically connected. Following learning, the SPM hypothesis predicts that a subset of synapses will be potentiated (some perhaps will be depressed); these pairs will be identified by an increase (or decrease) in the peak of the cross-correlogram. Each of these affected synapses can be either depotentiated by low-frequency stimulation (which has no effect on unpotentiated synapses) or re-potentiated by appropriately timed spike-timing-dependent potentiation. Returning all synaptic strengths to baseline by depotentiation should abolish the memory. At an arbitrary later time, the memory can be reinstalled by re-potentiating or re-depressing the affected synapses by appropriately timed spike-timing-dependent plasticity. **c** | An attempt to use molecular genetics to achieve the same aim. With currently available technology, the best way to gain access to potentiated synapses is by first training the animal to form a memory that is transient. One way to achieve this is to use mutant animals that fail to form long-term memory, termed here 'forgetful mice', such as mice in which Arc/Arg 3.1 (REF. 58), Zif268 (REF. 60) or α/δCREB[93] have been knocked out. An immediate-early gene promoter can be used to drive transcription of a molecular LTP device in recently activated synapses (shown in red in **e**). The transcript could encode, for instance, an exogenous ligand-gated Ca^{2+} channel (**d**). Infusion of the exogenous ligand would activate the Ca^{2+} channel (free-standing LTP device) in only those synapses that had recently been potentiated, inducing further potentiation in those synapses and thus re-installation of a memory in an animal in which memories were normally only transient. **e** | An important development that existing technologies do not yet allow is the targeting of transgenes to the specific synaptic sites that have undergone plasticity. Arc mRNA[94] and protein[95] are selectively transported to dendritic regions containing recently potentiated synapses, and possibly to the potentiated synapses themselves. The molecular mechanics behind the putative 'tagging' of synapses that allows them to capture recently synthesized proteins remains elusive. When we learn how synapses do this, we may be in a position to target exogenous proteins, including free-standing LTP devices, specifically to synapses that are activated during learning.

expressing synaptic tags, as postulated by Frey and Morris[74]. The LTP device would be driven by a promoter from an IEG, such as from the gene that encodes Arc/Arg3.1, and the transcript would have to contain at least a dendritic targeting sequence and a motif that binds to the putative tag.

We can imagine that learning would leave a trail of silent LTP devices at all potentiated synapses (FIG. 4e), much like Hänsel and Gretel left a trail of pebbles to mark their path through the enchanted forest in the famous story by the brothers Grimm. Subsequent loss of this memory, either through erasure or forgetting, would then leave an animal in a quasi-naive state in which the memory would be re-installed after triggering the LTP device by injection of the ligand, just as Hänsel and Gretel followed the pebbles to find their way home. Transgenic 'forgetful mice', which are unable to form long-term memories (for example, animals in which the IEGs that encode Zif268 (REF. 60) or Arc[58] have been inactivated), would be particularly suited for such experiments.

Limited synaptic states. The strategies we have described will work optimally if the number of synaptic states is limited — ideally, each synapse would adopt one of three discrete states: basal, potentiated or depressed[75]. If this is not the case the problem becomes more challenging, but it does not become a lost cause. In the first case, knowledge about the size of the change would be available, and the STDP stimulus protocol would be adjusted accordingly. In the case of the free-standing LTP device, it is likely that information about the size of the change will be encoded in the synaptic tag, and the design of the device will need to take this into account. Finally, although we have discussed these approaches only in terms of LTP, they can be readily extended to accommodate homosynaptic or heterosynaptic LTD.

Conclusion

The study of the neural basis of memory has been dominated over the past 30 years by investigations into the molecular and cellular basis of synaptic plasticity. We argue here that a full understanding of memory and the neural circuits responsible for its acquisition, encoding and recall will not be achieved until instrumental and conceptual tools have been developed to study neural networks in the large. Further progress in analysing the neural basis of memory will require an approach that emphasizes the importance of the network of neurons that are activated during learning. We predict that new technologies will allow the silencing of the subset of hippocampal neurons that encode a particular memory, allowing questions of causality to be addressed at the level of what Hebb called the cell assembly. Circuit-specific memory erasure would demonstrate that

Glossary

Contextual fear conditioning
A hippocampus-dependent form of Pavlovian conditioning in which a rodent comes to associate a context defined by polymodal sensory cues with an electrical footshock.

Cued fear conditioning
A hippocampus-independent form of Pavlovian conditioning in which a rodent comes to associate a tone cue (conditional stimulus) with an electrical footshock (unconditional stimulus). Learning is assessed by the animal's behavioural freezing.

De-depression
The selective reversal of LTD by high-frequency stimulation.

Depotentiation
The selective and time-dependent reversal of already-potentiated synapses using low-frequency stimulation. Note that depotentiation differs from LTD in that it has no affect on unpotentiated synapses and affects only recently potentiated synapses.

Episodic memory
Event-related memory: the 'what, where and when' memory system. Experiments in rodents are largely restricted to the 'what' and 'where' elements. We define hippocampus-dependent tasks such as contextual fear conditioning and the Morris water maze as requiring episodic-like memory.

EPSP-spike potentiation
(E-S potentiation). A potentiation not of synaptic transmission, as in LTP, but of the likelihood that action potentials will be generated for a given synaptic input. This phenomenon usually occurs in tandem with LTP after high-frequency stimulation.

Forgetful mouse
This is a type of genetically engineered mouse that can learn but not consolidate hippocampus-dependent memory.

Long-term depression
(LTD). The opposing process to LTP, whereby synaptic transmission is weakened by low-frequency stimulation. LTD might serve as a learning mechanism in its own right or might be a means of ensuring homeostatic stability by preventing an increase in overall activity in potentiated networks.

Long-term potentiation
(LTP). An experimental model of synaptic plasticity. In the hippocampus, high-frequency electrical stimulation of afferent-fibre pathways induces an enhancement of synaptic transmission that can last for months.

Memory mimicry
(MM) An experiment designed to test whether LTP-like plasticity alone is sufficient to support memory, by artificially installing a memory of an unexperienced event. Also called the 'Marilyn Monroe' thought experiment, because it could entail creating a false memory of a meeting with her.

Morris water maze
A spatial learning and memory task in which a rodent learns the position of an escape platform placed beneath the surface of a pool of opaque water using a set of distal visual cues.

Nictitating membrane/eyeblink conditioning
A form of classical Pavlovian conditioning in which an animal gradually modifies the timing of an eyeblink to an anticipated unconditional stimulus, using a sound or light conditional stimulus. Rabbits are traditionally used for this task owing to the presence of a third eyelid, or nictitating membrane, which is not under conscious control.

Pattern completion
The phenomenon whereby a memory can be recalled by presentation of only a subset of the cues that were available during the learning episode. There is evidence that the CA3 subregion of the hippocampus is necessary for animals to achieve pattern completion.

Pattern separation
The phenomenon whereby two similar contexts can be discriminated on the basis of subtle differences in the constituent cues. Such pattern separation allows the recall of only those memories that are relevant to one context or the other. There is evidence that the dentate gyrus is necessary for pattern separation.

Radial arm maze
Usually an eight-armed maze that can be used for various memory tasks. Here we refer to it in the context of working memory in which each arm is baited with food. Working memory can be assessed by how often the animal returns to an arm that it has already visited and emptied of food reward.

Reference memory
Long-term spatial memory that involves reference to external cues, as is needed for succesful learning of the standard form of the Morris water maze task, in which the location of the hidden platform is fixed for several days.

Spike-timing-dependent plasticity
(STDP). Plasticity in which pre- and postsynaptic cells are stimulated independently and the timing with which spikes are evoked in the two types of cell determines the direction of plasticity.

Synaptic tagging
Both long-term memory and LTP require mRNA transcription and protein synthesis. However, plasticity changes are specific to activated synapses. A mechanism, termed synaptic tagging, must exist to capture newly expressed plasticity related mRNAs or proteins specifically at activated synapses. One possible solution is the setting of labile 'tags' at activated synapses that would capture recently synthesized proteins.

Working memory
Short-term memory, used here to describe the type of memory that is needed for successful completion of a version of the Morris water maze experiment in which the position of the hidden platform is changed daily (see also radial arm maze).

synaptic plasticity is necessary for storing memories. Moreover, it is becoming possible to envisage techniques that will permit the re-installation, at the network level, of silenced or lost memories; such experiments, if successful, would establish that synaptic plasticity is also a sufficient mechanism for storing memories.

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DATABASES

Entrez Gene: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene

Arc/Arg3.1 | AlstR | c-Fos | ChR2 | GluR1 | Homer | NpHR | NR1 | Zif268

FURTHER INFORMATION

Tim V. P. Bliss's homepage:
www.nimr.mrc.ac.uk/neurophysiol/bliss

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