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Visions & Reflections

The genetic enhancement of memory

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Introduction

The ability to memorise varies from person to person but some individuals are particularly gifted. A. R. Luria, in his famous book *The Mind of a Mnemonist* [1] described the case of a Russian memory master called Shereshevsky whose capacity for learning and remembering word lists was apparently unlimited. In part, his performance reflected an aptitude for the mnemonic technique of creating imaginary environments. This aptitude in turn stemmed from a peculiar capacity known as synaesthesia that imbues words with a taste, feel, sound or smell, enabling him to experience each word in the list as a precisely defined entity within the imaginary environment. Other mnemonists, such as Charles F. Stromeyer's 'Elizabeth' [2], lack synaesthesia but instead possess eidetic memory, allowing images to be vividly recalled using the 'mind's eye', as if they were being seen for the first time. These individuals may be genetically different from most of the human population in a way that endows them with superior memory skills. Many children have a similar capacity for eidetic recall but it fades with the advent of adulthood [3]. This transition suggests that changes in the expression of particular genes during normal development affect learning and memory. Various manipulations of genes in mice and other animal models result in memory impairments. Can genetic manipulation of this sort lead to an enhancement of memory and, if so, what are the prospects for enhancing human memory?

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Memory molecules

Research over the past two decades has shown that there are a wide variety of different molecules in the human and animal nervous system that may participate in the processes of information storage that we call memory. These molecules range from those that mediate neurotransmission at synapses to those that signal cellular events to the cell nucleus and thereby influence the expression levels of different memory-related genes. Such molecules may malfunction, resulting in a variety of human memory disorders, but they are also potential targets for the enhancement of memory through genetic engineering. This review will describe evidence implicating three very different but important sets of molecules in memory processes and discuss them in the chronological order in which they have come to the attention of the scientific community. In all cases, animals models have been used, although the first set of studies discussed here have used invertebrates while the others have used mammals. These studies have culminated in the genetic engineering of organisms with enhanced learning and memory.

Enhanced switching from short- to long-term memory

Experiments using protein synthesis inhibitors have demonstrated that the transition from short- to long-term memory storage requires the synthesis of new proteins [4–7]. Many intricate transduction mechanisms ensure that cells recognise direct and modulatory influences as

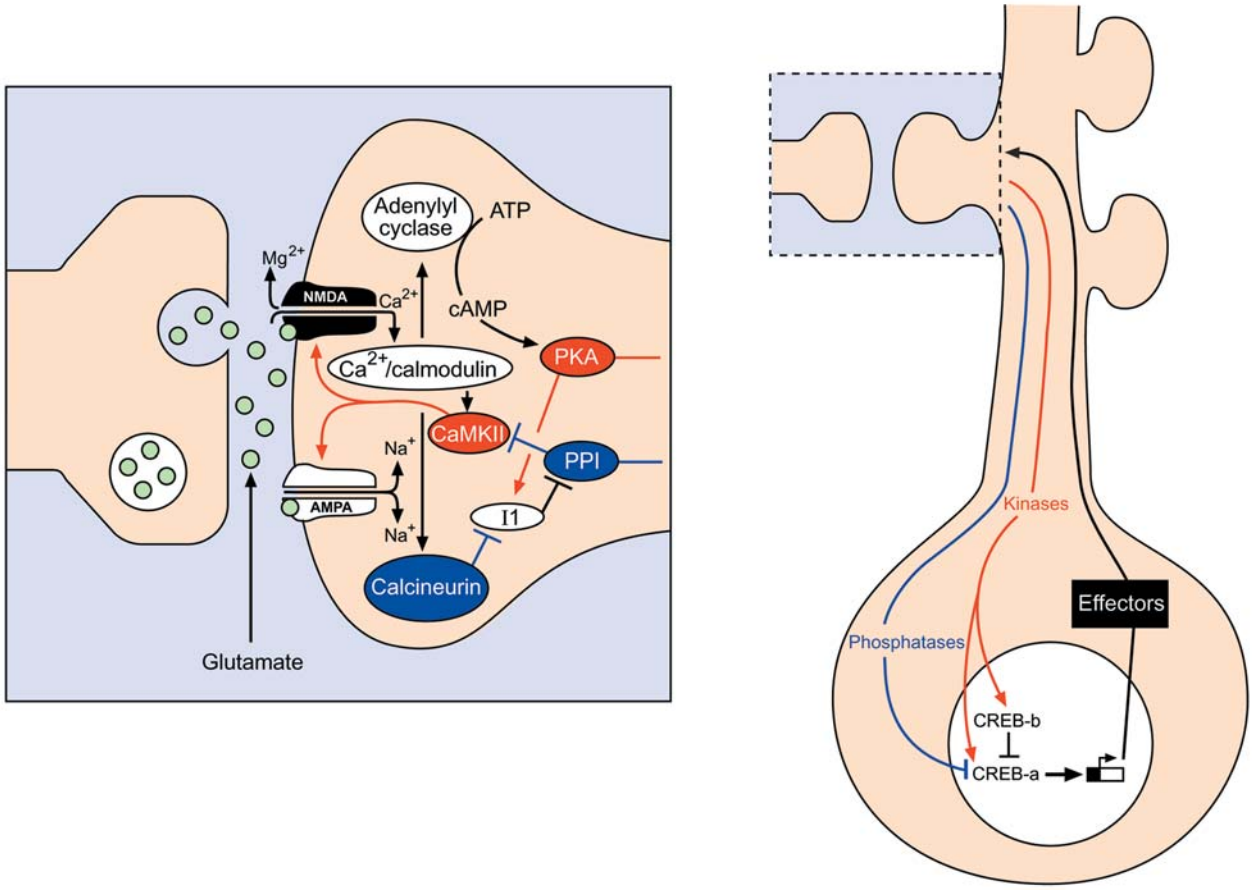

Figure 1. The balance of kinase and phosphatase cascades that governs the expression of long-term potentiation. An important subset of these molecules are discussed in this review and are depicted here. Kinases (coloured in red) phosphorylate both receptors and other signalling proteins and, in so doing, alter their conformation and activity. Phosphatases (coloured in blue) dephosphorylate proteins and return them to their former conformation and activation state. Longer-lasting changes in synaptic function require changes in the level of largely unidentified effector proteins and their selective transportation by unknown mechanisms to the potentiated synapse. Transcription factors in the nucleus, such as CREB, respond to incoming signals from altered synapses and mediate changes in gene expression. These signals are initiated by some of the same kinases and phosphatases as those acting directly within the synapse. Again, a balance of inhibitors (flat-ended lines) and activators (arrows) controls the expression process.

they store information through signalling cascades that affect the expression levels of particular proteins (see fig. 1). Changes in cell structure, receptor number or enzyme activity are orchestrated by certain key proteins. One such molecule is the transcription factor cAMP-responsive element-binding protein (CREB). There is strong evidence that activation of CREB plays a critical role in the molecular switch between short- and long-term memory [8–9].

CREB has various splice variants (alternative mRNA sequences derived from the same DNA template). These sequences in turn yield different isoforms of CREB that have opposing functions. CREB-a is an activator isoform and CREB-b a repressor. The fruit fly *Drosophila melongaster* has some well-publicised advantages over the mouse as a model organism and the introduction of temperature-inducible transgenes, specifically encoding one or other of these two isoforms, into the *Drosophila* genome revealed the function of CREB in memory. Transgenic flies over-expressing the repressor CREB-b isoform were incapable of forming long-term memory of an association between an odour and an aversive shock stimulus [10]. In contrast, flies over-expressing the activator CREB-a isoform were able to commit this odour discrimination memory to long-term storage after just a single training session – much faster than non-mutant flies [11]. These apparently enhanced memorisers show that the activator form of CREB contributes to the transition from short- to long-term memory, although the circumstances governing expression of the CREB splice variants and why activator and repressor forms exist are as yet unclear [12].

‘Smart’ mice

The publication in 1999 of a paper describing a genetically altered mouse, called *doogie,* with an apparently im-

proved capacity for learning and memory [13], created a major public impact. The world's media responded with largely uncritical enthusiasm to the paper's claim that 'genetic enhancement of mental and cognitive attributes such as intelligence and memory in mammals is feasible'. These 'smart' mice performed better than their wild-type littermates on a variety of standard learning and memory tests including the Morris watermaze, novel-object recognition and extinction of contextual fear. *Doogie* contains a transgene encoding the NR2B subunit of the NMDA subtype of glutamate receptor. NMDA receptors are ligand-gated ion channels that allow the permeation of cations, including Ca^{2+} ions, only at depolarised synapses. These properties may allow the receptor to function as a detector of the coincident activation of two connected neurons. In 1949, Donald Hebb proposed an influential model for the neural basis of memory, in which the strength of the connection between a neuron A and a target neuron B becomes increased if neuron A persistently contributes to the firing of neuron B [14]. The discovery of long-term potentiation (LTP), in which transmission between cells that are simultaneously active becomes selectively enhanced, showed that the mammalian brain can undergo such changes [15]. LTP was discovered in a forebrain structure known as the hippocampus, which was already thought to be critical for episodic memory processing [see ref. 16 for review]. This form of hippocampal LTP relies upon the NMDA receptor [see ref. 17 for review] and is increased in 'smart' mice expressing the NR2B transgene.

NMDA receptors are heteromeric proteins that comprise five subunits. In the hippocampus, these are predominantly a mixture of NR1 subunits, which contain the ligand-binding site and are obligatory, and NR2 subunits [18]. The eight splice variants of the NR1 subunit and four NR2 subunits can combine in various ways to form several functionally variant NMDA receptors [19–21]. Receptors that contain the NR2B subunit have more prolonged kinetics and contribute more persistent excitatory post-synaptic potentials (EPSPs) than those containing NR2A subunits. The creators of *doogie* speculated that this property would allow a wider time window for coincidence detection by receptors containing the NR2B subunit. The proportion of NMDA receptors containing NR2B subunits declines with age in the normal animal. In contrast, the expression of the NR2B transgene in *doogi*e is driven by a continuously active, forebrain-specific promoter and is therefore maintained throughout life. The implication of this study is that the enhancement of learning and memory seen in *doogie* mice is related to the continuing expression into maturity of NMDA receptors containing the NR2B subunit.

Reduced forgetting

An understanding of the process of forgetting may provide some insight into reducing memory loss. Recent work has revealed that dephosphorylation of proteins by phosphatases may govern the duration of a memory trace. Calcineurin is one such phosphatase. This enzyme responds directly to calcium influx through the NMDA receptor and is at the head of a signalling cascade that indirectly, and possibly directly, mediates the dephosphorylation of AMPA receptors that have been phosphorylated during LTP. The balance between the action of phosphatases such as calcineurin and protein phospatase 1 (PP1) and kinases such as protein kinase A (PKA) and Ca^{2+}/calmodulin-dependent kinase II (CaMKII) is thought to determine the degree of LTP in its early phases [22; see ref. 23 for review] (see fig. 1). Over-expression of calcineurin in the mouse hippocampus depresses LTP and impedes the formation of long-term memory in several tasks [24–25]. Given a putative role for calcineurin in forgetting, it seems an ideal target for manipulation in attempts to genetically enhance memory.

A study was recently conducted to address this issue [26]. For these experiments, a mouse was generated with two transgenes inserted into its genome. The combination of these transgenes enabled a conditional and regionally restricted expression of a calcineurin inhibitor peptide. Expression of the inhibitor was limited to the forebrain using the CaMKII promoter, and temporally governed using the reverse tetracycline-controlled transactivator system. These systems enabled the inhibitor of calcineurin to be expressed selectively in the forebrain when doxycycline, an analogue of tetracycline, was added to the animal's food. Not only did this technique circumvent problems of compensation by other genes that may occur in the development of such mutant organisms, but it also introduced a control into the experiment whereby the same animals could be tested while expressing the inhibitor and then later when the expression of the inhibitor was suppressed by withdrawal of doxycycline. The authors found that the early phase of LTP was selectively enhanced when the inhibitor molecule was expressed. Furthermore, short- and long-term memory in the Morris watermaze, and in novel object recognition, were reversibly enhanced by expression of the calcineurin inhibitor. This study confirms the importance of the calcineurin phosphatase cascade both in neural plasticity and learning and memory. It also introduces the possibility of reducing forgetting and thereby enhancing memory.

More recently, our understanding of this cascade has been increased by a study of the role of PP1 in forgetting [27]. This molecule directly dephosphorylates CaMKII and, in all likelihood, AMPA receptors themselves. Mice expressing a constitutively active form of an endogenous

PP1 inhibitor, inhibitor 1 (I1) showed a subtle but intriguing enhancement of memory. Many forms of learning require multiple repetitions during training for a memory to be retained long-term. A notable feature of this multiple-trial learning is that massed repetition is not as effective as spacing each repetition out by an interval of time. A common explanation for the inefficiency of massed training is the interference of information processing during one trial by subsequent processing on the next trial. This retroactive interference was less profound in I1* mutant mice (transgenic animals on doxycycline) than in their wild-type littermates or I1 mutants (not on doxycycline). Performance of I1* mutants was no better than control animals in a novel object recognition task in which mice were simply exposed to objects for an uninterrupted 25-min period. However, I1* mice showed a significant enhancement of memory for objects previously presented, compared with controls, if the training period was prolonged by inserting 5-min rest intervals into the training period. Longer rest intervals entirely removed the retroactive interference effect in both wild types and mutants. Increased PP1 inhibition therefore seems to significantly reduce retroactive interference, perhaps by reducing AMPA receptor dephosphorylation. This interpretation is supported by the additional finding that training in the Morris watermaze produced greater phosphorylation of the AMPA receptor subunit, GluR1, in I1* transgenic mice than in the wild-type mice.

A final point of interest is that these mice do not exhibit the classical decline in memory of wild-type mice as they age. Aged I1* transgenic mice were capable of retaining a memory of the position of a hidden platform in the Morris watermaze for at least a month after intensive training. The platform position had been learned by aged control mice but forgotten by the time they were tested the following day, suggesting that PP1 controls the rate of forgetting. PP1 may therefore be an important target protein for drugs aimed at ameliorating age-related memory decline.

Can we enhance without also impairing?

If genetics can be used to enhance memory then why has natural selection not achieved this apparently desirable goal? One answer may be that, owing to the parsimonious nature of evolution, the molecular components of memory are also crucial constituents of other brain functions. For example, the NMDA receptor is involved in other synaptic mechanisms in addition to hippocampal LTP and cognitive memory. Amongst these are the changes that occur in the forebrain during the induction of pain hypersensitisation. Just as memory capacity decreases during maturation from the juvenile to the adult animal so does sensitivity to pain [28]. *Doogie* mice do not show increased sensitivity to acute pain but they can develop symptoms of chronic pain more easily [29]. Such hypersensitivity may be adaptive in juvenile and not in adult animals. The progressive decline in the expression of NR2B subunits in the forebrain likely reflects a delicate balance between the benefit of an enhanced memory and the cost of enhanced pain to an animal.

The studies described in this article have all considered the enhancement of memory to be a simple facilitation of the process of information storage. Mice have been engineered that can either learn faster or remember longer and flies have been equipped to recognise a smell after a single exposure. These changes, however, may prove to be impediments rather than enhancements of the animal's memory system. Is it really beneficial for humans to possess the memory capacity of mnemonists or eidetikers or is it rather the case that average human memory is perfectly suited to the requirements of normal life? Shereshevsky, as a note of warning, had a famously inflexible and almost savant-like cognitive approach, perhaps as a direct consequence of his remarkable declarative memory. The mutant animals that have been discussed here have not, as yet, been subjected to anything like a comprehensive assessment of memory, cognition or behaviour. As with *doogie*, CREB-a transgenic flies and mice over-expressing phosphatase inhibitors may have deficits that outweigh the immediate benefits of genetic mutation to their declarative memory system. Such animals reveal the key elements of memory systems and in so doing they suggest targets for the genetic treatment of deficient memory but there is, as yet, no obvious method for doing so safely in humans. Other forms of treatment may prove more fruitful, as a further consideration of the *doogie* mouse illustrates. This mutant may have a better declarative memory than its non-transgenic littermates, but enrichment of the environment, by adding toys, tunnels, houses and platforms, can overcome this relative deficit in the control animals [30]. Enriching the environment in this way has no such effect on the mutant mice, suggesting that the mutation has already forced the memory capacity of these animals to its biological limit. With regard to human treatment, this finding reinforces the importance of exposing the infant or child to a stimulating environment – an approach to optimising memory that is surely preferable to genetic manipulation.

The treatment of inherited human memory disorders is an attainable goal and, in this regard, continuing research into the genetic basis of memory is essential. The extent to which normal human memory can be enhanced beyond its already spectacular capacity, without compromising other aspects of our physiology, is a different issue.

1 Luria A. R. (1968) The Mind of a Mnemonist, Harvard University Press, Cambridge, Mass.
2 Stromeyer C. F. and Psotka J. (1970) The detailed texture of eidetic images. Nature **24:** 346–349
3 Kuhl P. K. (1994) Learning and representation in speech and language. Curr. Opin. Neurobiol. **4:** 812–822.
4 Flexner J., Flexner L. and Stellar E. (1963) Memory in mice is affected by intracerebral puromycin. Science **141:** 57–58
5 Agranoff B. W. and Klinger P. D. (1964) Puromycin effect on memory fixation in the goldfish. Science **146:** 952–953.
6 Castellucci V. F., Blumenfeld H., Goelet P. and Kandel E. R. (1989) Inhibitor of protein synthesis blocks long-term behavioural sensitization in the isolated gill-withdrawal reflex of *Aplysia*. J. Neurobiol. **20:** 1–9
7 Freeman F. M., Rose S. P. and Scholey A. B. (1995) Two time windows of anisomycin-induced amnesia for passive avoidance training in the day-old chick. Neurobiol. Learn. Mem. **63:** 291–295
8 Dash P. K., Hochner B. and Kandel E. R. (1990) Injection of the cAMP-responsive element into the nucleus of *Aplysia* sensory neurons blocks long-term facilitation. Nature **345:** 718–721
9 Tully T., Preat T., Boynton S. C. and Del Vecchio M. D. (1994) Genetic dissection of consolidated memory in *Drosophila*. Cell **79:** 35–47
10 Yin J. C., Wallach J. S., Del Vecchio M., Wilder E. L., Zhou H., Quinn W. G. et al. (1994) Induction of a dominant negative CREB transgene specifically blocks long-term memory in *Drosophila*. Cell **79:** 49–58.
11 Yin J. C., Del Vecchio M., Zhou H. and Tully T. (1995) CREB as a memory modulator: induced expression of a dCREB2 activator isoform enhances long-term memory in *Drosophila*. Cell **81:** 107–115
12 Abel T., Martin K. C., Bartsch D. and Kandel E. R. (1998) Memory suppressor genes: inhibitory constraints on the storage of long-term memory. Science **279:** 338–341
13 Tang Y. P., Shimizu E., Dube G. R., Rampon C., Kerchner G. A., Zhuo M. et al. (1999) Genetic enhancement of learning and memory in mice. Nature **401:** 63–69
14 Hebb D. O. (1949) The Organization of Behaviour, Wiley, New York
15 Bliss T. V. and Lømo T. (1973) Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path. J. Physiol. **232:** 331–356
16 Squire L. R. (1992) Memory and the hippocampus: a synthesis from findings with rats, monkeys and humans. Psychol. Rev. **99 (2):** 195–231
17 Nakanishi S. (1992) Molecular diversity of glutamate receptors and implications for brain function. Science 258: 597–603
18 Bliss T. V. and Collingridge G. L. (1993) A synaptic model of memory: long-term potentiation in the hippocampus. Nature **361:** 31–39
19 Monyer H., Sprengel R., Schoepfer R., Herb A., Higuchi M., Lomeli H. et al. (1992) Heteromeric NMDA receptors: molecular and functional distinction of subtypes. Science **256:** 1217–1221
20 Kutsuwada T., Kashiwabuchi N., Mori H., Sakimura K., Kushiya E., Araki K. et al. (1992) Molecular diversity of the NMDA receptor channel. Nature **358:** 36–41
21 Meguro H., Mori H., Araki K., Kushiya E., Kutsuwada T., Yamazaki M. et al. (1992) Functional characterization of a heteromeric NMDA receptor channel expressed from cloned cDNAs. Nature **357:** 70–74
22 Blitzer R. D., Wong T., Nouranifar R., Lyengar R. and Landau E. M. (1995) Post-synaptic cAMP pathway gates early LTP in hippocampal CA1 region. Neuron **15:** 1403–1414
23 Soderling T. R. and Derkach V. A. (2000) Postsynaptic protein phosphorylation and LTP. Trends Neurosci. **23:** 75–80
24 Winder D. G., Mansuy I. M., Osman M., Moallem T. M. and Kandel E. R. (1998) Genetic and pharmacological evidence for a novel, intermediate phase of long-term potentiation suppressed by calcineurin. Cell **92:** 25–37
25 Mansuy I. M., Mayford M., Jacob B., Kandel E. R. and Bach M. E. (1998) Restricted and regulated overexpression reveals calcineurin as a key component in the transition from short-term to long-term memory. Cell **92:** 39–49
26 Malleret G., Haditsch U., Genoux D., Jones M. W., Bliss T. V. P., Vanhoose A. M. et al. (2001) Inducible and reversible enhancement of learning, memory and long-term potentiation by genetic inhibition of calcineurin. Cell **104:** 675–686
27 Genoux D., Haditsch U., Knobloch M., Michalon A., Storm D. and Mansuy I. (2002) The protein phosphatase 1 is a molecular constraint on learning and memory. Nature **418:** 970–975
28 Teng C. J. and Abbott F. V. (1998) The formalin test: a dose-response analysis at three developmental stages. Pain **76:** 337–347
29 Wei F., Wang G. D., Kerchner G. A., Kim S. J., Xu H. M., Chen Z. F. et al. (2001) Genetic enhancement of inflammatory pain by forebrain NR2B overexpression. Nat. Neurosci. **4:** 164–169
30 Tang Y. P., Wang H., Feng R., Kyin M. and Tsien J. Z. (2001) Differential effects of enrichment on learning and memory function in NR2B transgenic mice. Neuropharmacology **41:** 779–790