Therapie, 2000, 55 : 461-466.

Cellular and Molecular Approaches to Memory Storage

Serge Laroche

Laboratoire de Neurobiologie de l'Apprentissage, de la Mémoire et de la Communication,
CNRS UMR 8620, Université Paris-Sud, 91405 Orsay, France

Summary : There has been nearly a century of interest in the idea that information is stored in the brain as changes in the efficacy of synaptic connections on neurons that are activated during learning. The discovery and detailed report of the phenomenon generally known as long-term potentiation opened a new chapter in the study of synaptic plasticity in the vertebrate brain, and this form of synaptic plasticity has now become the dominant model in the search for the cellular bases of learning and memory. To date, considerable progress has been made in understanding the cellular and molecular mechanisms underlying synaptic plasticity and in identifying the neural systems which express it. In parallel, the hypothesis that the mechanisms underlying synaptic plasticity are activated during learning and serve learning and memory has gained much empirical support. Accumulating evidence suggests that the rapid activation of the genetic machinery is a key mechanism underlying the enduring modification of neural networks required for the laying down of memory. Below I review these advances.

Résumé : On admet généralement que l'information en mémoire est encodée sous forme de configurations spatio-temporelles d'activité dans des réseaux de neurones distribués et que le stockage de ces représentations repose sur des modifications acquises de la force synaptique au sein des réseaux activés par l'apprentissage. Depuis une quinzaine d'années, de nombreuses études se sont attachées à éprouver l'hypothèse selon laquelle un des mécanismes de l'apprentissage et de la mémoire au niveau cellulaire repose sur une forme particulièrement durable de plasticité, connue sous le nom de potentialisation à long terme. Aujourd'hui, des progrès considérables ont été accomplis dans la connaissance des mécanismes cellulaires et moléculaires de cette plasticité et dans l'identification des circuits neuronaux qui l'expriment. Parallèlement, l'idée selon laquelle ces mécanismes sont activés pendant l'apprentissage et servent effectivement les processus d'apprentissage et de mémoire à acquis un support empirique incontestable. On découvre aussi que l'activation rapide de l'expression de gènes agissant comme des commutateurs moléculaires permet le remodelage durable des réseaux neuronaux à la base de la formation de traces mnésiques stables. Les récents développements dans ces domaines sont résumés.

Introduction

Several hundreds of billions of interconnected neurons communicating through a code formed by electrical impulses, and an amazing property - that of being able to remodel, reconfigure its own circuits using a mechanism of synaptic plasticity - make a formidable neuronal machine that has the ability to remember, one of the most significant feature in our mental lives. From the simplest animal to the most complex of humans, the capacity of the brain to process, store and use information subserves such a considerable number of functions that it is generally believed that there is no cognition without memory. The issue of how information is represented and organized permanently in the brain and what are the processes that control the encoding, storage, retrieval and utilisation of these representations - how in sum we learn and remember - remains a major challenge to contemporary neuroscience. From a neurobiological point of view, it has long been postulated that memories are represented in the brain as spatio-temporal patterns of cellular activity within distributed networks of cells, or cell assemblies, and that changes take place at the cellular level to store these representations. The basic assumption is that specific patterns of activity flowing through neural networks strengthen their component synapses, and activity-dependent changes in the weight or strength of these synapses constitutes a basic mechanism underlying the laying down of memories. It gained coinage with the discovery by Bliss and Lømo in 1973 of an enduring form of synaptic plasticity in the hippocampus, known as long-term potentiation, or LTP (1).

Properties and mechanisms of long-term potentiation

In their original discovery, Bliss and Lømo showed that brief bursts of high-frequency stimulation to the perforant path, one of the major input pathways to the hippocampus conveying pretreated information from cortical areas, induces a robust and long lasting increase in the efficacy of synaptic transmission, measured by an increase in the extracellular field response of the target neurons, the granule cells of the dentate gyrus (1). The most remarkable about this form of plasticity is its extremely persistent nature. While it can be induced within fractions of seconds using short bursts of tetanic stimulation, the modification can last for periods of weeks or even months, thus leaving a quasi-permanent trace of the past activation of the synapse. In addition, LTP displays properties of synapse-specificity, cooperativity and associativity between co-activated synapses that correspond to what is expected from a neural mechanism for the encoding memories at the cellular level.

To date, it is known that most glutamatergic synapses in many cortical and subcortical brain structures support LTP and are thus modifiable synapses. The primary mechanism for the induction of LTP in most of these pathways is the activation of a membrane protein assembly, the NMDA subtype of glutamate receptor. This is a slow-acting, voltage-gated receptor which is largely inactive during normal synaptic transmission but is selectively involved in the induction of LTP. This has

been demonstrated with the selective NMDA receptor antagonist, AP5, which blocks the induction of LTP without otherwise affecting normal synaptic transmission mediated by monovalent ion fluxes through the AMPA receptor channel complex (2). The NMDA receptor acts as a coincidence detector requiring two simultaneous events, the binding of glutamate released from the presynapse, and a sufficient level of postsynaptic depolarization to relieve the magnesium block of the channel. When these conditions are met, calcium invades the postsynaptic neuron, the triggering event in LTP. Certain subtypes of metabotropic glutamate receptors coupled to G-proteins contribute to this process. They interact with the IP3 system to mobilize calcium from the intracellular stores and amplify the calcium wave locally at the activated spines. It is known now that calcium induces a rapid and transient activation of several second messenger cascades and intracellular kinase signalling pathways that lead to the enduring modification of the synapse (see Ref 3 for review). Some of the kinase pathways that have been identified are PKC and CaMKII, the cAMP-PKA pathway, the tyrosine kinase pathway, and the MAPK pathway. The phosphorylation of these kinases is believed to be important for the conversion of short-term to long-term potentiation. Each of these kinase cascades leads to the activation of selective downstream protein subtrates and there is abundant cross-talk between them which certainly plays a crucial role in the coordinated changes occurring at the synapse.

The mechanisms of the expression of LTP have not been fully clarified, but both pre- and postsynaptic modifications are required and the coordinated changes seem to involve retrograde messengers such as nitric oxyde or arachidonic acid which target presynaptic terminals after being released from the postsynapse (3). Presynaptically, this will lead to an increase in the capacity of the potentiated terminals to release transmitter, so that each subsequent activation of the input pathway will release more glutamate. This is mediated by an increase in the turnover of several second messengers, kinase activation and the modification of proteins of the exocytotic machinery. In the postsynaptic neuron, the activation of certain kinases lead to the phosphorylation and increased sensitivity of several receptors and channels, which will contribute to the larger response of the postsynaptic neurons after the initial induction of LTP. More recently, as will be discussed below, it has been shown that the phosphorylation of kinases plays a role not only in maintaining the potentiation of the synapse for several hours, but they also act as transient intermediaries between the signal at the cell surface and the downstream activation of the genetic machinery leading to an end point : the enduring reorganisation of the synapse and the growth of new synaptic connections. Although no single type of morphological or biochemical change has been attributed to the reorganisation of the synapse, many possibilities have either been demonstrated or suggested (3), such as changes in the shape and size of dendritic spines and synapses, the realignment of receptors to the presynaptic release sites of transmitter, the unmasking of silent synapses and the growth of new synapses.

On the role of synaptic plasticity in memory formation

In the early 1970s, several groups, including our own laboratory, were studying simple forms of associative learning in the rat. The basic task consist in presenting pairs of stimuli such as a tone, acting as a conditioned stimulus, coupled to a mild footshock as an unconditioned stimulus. After a few pairing trials, the tone, as a predictor of the shock, triggers the unconditioned response thus reflecting acquisition of an associative representation of the tone-shock relationship. The research has shown that many neurons in various structures of the brain, including in the hippocampus, display robust and selective increases in activity in response to the significant event of the task (4). In the hippocampus, this selective neuronal response develops in parallel with behavioural learning and can be reactivated several weeks after training, thus showing a long-term memory of the significant stimulus. Using several behavioural paradigms, it became apparent that the learning-specific response of hippocampal neurons reflects the activation of an explicit representation of the learned association which may or may not be implicated in the control of the behavioural response depending on the demands of the task at hand. We also demonstrated that the synapses of hippocampal circuits are strengthened during this form of associative learning (see Ref 4 for review). For example, by monitoring variations in synaptic efficacy in the dentate gyrus during learning, it was shown that potentiation develops rapidly after a few pairings in a substantial set of perforant path synapses. This behaviourally-induced change in synaptic strength parallels behavioural learning and the occurrence of learning-specific changes in neuronal activity. Pharmacological studies then demonstrated the crucial role of synaptic plasticity. For example, in the presence of AP5, a selective NMDA receptor antagonist that does not alter normal synaptic transmission but blocks the capacity for synaptic changes, the neurons in the hippocampus become unable to acquire the capacity to respond to the significant events of the learning task (4). Thus, synaptic plasticity is necessary for the formation of a neuronal representation of the learned information in these circuits.

In other learning tasks such as spatial learning in the water maze, widely used as a prototype task to study components of episodic memory, and which is particularly sensitive to hippocampal lesions, blocking NMDA receptors was shown to produce learning deficits at doses which blocks hippocampal LTP (5). Similar memory deficits are also observed using electrophysiological procedures to saturate LTP before learning in a manner that prevents the capacity for further synaptic changes during learning. In the late 1970s, O'Keefe and colleagues discovered that some hippocampal pyramidal neurons, called place cells, fire selectively when an animal is in a particular location in the environment (6). Each cell has its own region of firing, the place field, relative to remote environmental landmarks, such that ensembles of connected hippocampal place cells filling each environment with overlapping place fields would represent the whole environment. This has formed the basis of a theory postulating that hippocampal neurons are an important component of brain circuits encoding spatial cognitive maps used for navigational learning (6). Two groups of

studies examined the effect of blocking the NMDA receptor on the firing properties of place cells. Using pharmacological blockade of the receptor or mice carrying a targeted deletion of the NMDAR1 gene in CA1 pyramidal cells of the hippocampus, it was shown that the spatial selectivity of place fields and the stability of place cell firing was radically altered in correlation with spatial memory deficits (7). These results imply that NMDA receptor-dependent synaptic plasticity is necessary for the normal construction and stability of neuronal representations in the hippocampus.

Molecular mechanisms of memory formation

Capitalizing on identified biochemical and molecular mechanisms underlying the induction and expression of synaptic plasticity, it rapidly became a subject of intense interest to investigate the molecular mechanisms of memory and at the same time ask whether the mechanisms of LTP are activated during learning and serve learning and memory processes. In the earlier studies, Laroche and colleagues found a long-lasting increase in the potential to release glutamate from the presynaptic terminals of several subregions of the hippocampus after associative learning (8). In a striking parallel with the mechanisms underlying the increase in glutamate release in LTP, this was shown to be associated with activation of the second messengers diacylglycerol and IP3, and to be blocked by NMDA receptor antagonist. Analogous results have been obtained after spatial learning in the water maze and further studies have provided compelling evidence that many of the changes associated with plasticity, such as cAMP activation, phosphorylation of protein kinases, increases in the sensitivity of glutamate receptors, to name but a few, are also observed after learning and interfering with these mechanisms perturbs memory formation (see Ref 9 for review).

More recently, the development of the gene deletion or mutation technologies in mice has allowed these ideas to be tested and has provided an impressive corpus of knowledge about the molecular mechanisms of memory and the role of synaptic plasticity. For example, electrophysiological and behavioural studies in mice carrying deletions of genes encoding receptor subunits, kinases such as CaMKII, PKC, PKA, several tyrosine kinases, phosphatases, presynaptic proteins, and many other proteins, have shown correlated deficits in hippocampal LTP and in memory tasks that require the hippocampus (9-11). Using a complimentary strategy, a transgenic mouse overexpressing the NR2B subunit of the NMDA receptor was shown to have enhanced LTP in the hippocampus and better learning performances in several tasks (12), in a similar manner that neuromodulatory treatments to facilitate LTP were shown to improve learning (4). To date, the genetic approach is in its infancy and is not exempt of caveats. There is no doubt, however, that refinements in these technologies, in particular with the development of structure and neuron-specific and temporally-regulated transgene expression, coupled with a precise investigation of the phenotype at the behavioural, electrophysiological and neuroanatomical levels and the use of rescueing strategies, will provide powerful approaches for elucidating the cellular and molecular mechanisms

of memory. A related area of interest is in the development of transgenic mice carrying specific gene mutations or chromosomic alterations identified in human neuropathological diseases. This is examplified by the recent research effort placed on the development of animal models of, for example, Alzheimer's disease, fragile X, or autism; a major step in the investigation of the cellular and molecular mechanisms of memory dysfunction. In a recent work, it was shown that mice overexpressing the amyloid precursor protein APP develop aggregated amyloid material in the hippocampus during the course of ageing, in correlation with altered LTP in the hippocampus and the development of memory deficits (13).

Molecular mechanisms of long-term memory

One important distinction, well known from experience in the every day life, is that between short-lasting and long-lasting memories. The idea that different sets of mechanisms underlie these two forms of memory comes from early studies showing that protein synthesis inhibitors impair long-term memory, leaving short-term memory intact (14). Thus, the synthesis of new proteins is necessary for mechanisms of consolidation to occur and, as shown more recently, protein synthesis is also necessary to maintain the longer lasting phases of synaptic plasticity. In the presence of inhibitors of transcription or of protein synthesis, LTP in the hippocampus decays rapidly, within 3-6 hours (15). This suggests that new genes might be expressed that are necessary for the long-term strengthening of synaptic connections required for long-term memory.

It is in fact known now that transcription of genes and translation of both newly-transcribed and pre-existing mRNAs occur within hours after the induction of LTP and play an important role in maintaining LTP. In the 1990s, several groups have shown that certain immediate early genes (IEG) such as *zif268*, *c-fos*, members of the *jun* family, and more recently dendritic mRNAs such as *arc* or *Homer*, are turned on very rapidly after the induction of LTP (see Refs 9,10 for reviews). The gene products of some of these IEGs can act as nuclear transcription factors to regulate the transcription of target genes. What are these genes is still to date largely unknown. In series of experiments, however, we discovered that following the transient activation of IEGs, there is a succession of overlapping waves of expression of other genes encoding proteins that are known to play an important role in LTP (10). Some of the genes identified are those encoding important kinases such as γPKC and αCaMKII, proteins of the exocytotic machinery such as syntaxin and synapsin, or growth factors, which are overexpressed between a few hours and 24 hours after the induction of LTP. This is followed by upregulation of components of the MAP kinase pathway, peaking at 24 hours, and by specific subunits of glutamate receptors such as the NR1 and NR2B subunits which show a wave of expression two days after the initial induction of LTP. The importance of these genes has been demonstrated in mice carrying specific gene deletions. Their exact functional role, however, is not known, but they are likely to be implicated in the final reorganisation of the synapse

and the growth of new synaptic connections. Empirical evidence suggests that structural remodeling of brain circuits such as the realignment of synapses on dendritic shafts and the increase in the number of synapses occur in the hippocampus after spatial learning.

An important question that has been the subject of recent interest is that of the molecular switch which triggers gene activation to produce a lasting change in synaptic strength. We know that the initial influx of calcium after the induction of LTP activates a whole host of kinases cascades that maintain LTP for some time. Different kinases also converge in promoting the activation of the MAP kinase (MAPK). This kinase is rapidly phosphorylated in LTP and blocking the upstream kinase MEK has been shown to result in a rapidly decaying LTP (16). We have recently shown that the phosphorylation and nuclear translocation of MAPK is required for initiating the transcriptional response associated with LTP. The mechanisms involve a dual pathway by which MAPK activates two transcription factors, Elk-1 and CREB, which in turn binds to the SRE and CRE sites on the promoters of several IEGs, thereby triggering their transcription. Other studies have shown that MAPK is also phosphorylated after fear conditioning or spatial learning and that blocking MAPK phosphorylation results in retention deficits (17). In related studies in drosophila, Aplysia or mice, CREB transcription was shown to be required for the establisment of long-term memories (18). For example, α/ΔCREB knockout mice show a rapid decay of LTP in the hippocampus and selective deficits in long-term memory in several tasks, while the initial learning and short-term memory are intact (19). They also have a reduced place cell selectivity (20). Taken together, these results suggest that activation of the MAPK pathway and of the SRE and CRE DNA binding sites is the critical switch that engages the turning on of genes and initiates the long-lasting changes at the synapse necessary to encode a lasting memory trace.

Molecular imaging of brain networks activation during memory formation

One of the most exciting aspect of the regulated expression of genes and proteins in synaptic plasticity is that they can be used as markers of specific aspects of plasticity in an attempt to visualize the circuits and structures that are activated and express these mechanisms during the laying down of memories or during recall. To date, many of the studies have used the IEGs *c-fos*, *c-jun, fos-B,* or *zif268* as markers of cell activity to examine the activation of specific regions of the brain during different forms of learning (21). Examples of these are *c-fos* expression which is increased in the amygdala during fear conditioning and in the hippocampus during the acquisition of a brightness discrimination task, recall of an appetitive conditioning task, spatial alternation and spatial learning, or odour discrimination. However, the exact role of these genes in synaptic plasticity is not known and their expression is not restricted to learning *per se* as many IEGs, in particular *c-fos*, are extremely sensitive to stress, behavioural state and sensory stimulation. In a recent experiment, Impey and colleagues used a transgenic mouse in which several copies of the CRE element were

linked to a *LacZ* reporter gene, thereby allowing them to visualize CRE-induced gene trancription by measuring *LacZ* expression in neurons. They found that both the induction of LTP in the hippocampus and training in a contextual fear conditioning task stimulated *LacZ* expression in the hippocampus (22).

Late effector genes implicated in specifically defined mechanisms of plasticity have been up to now rarely used to address these questions. In our own studies, we have selected two genes of particular interest, those encoding αCaMKII and the presynaptic protein syntaxin 1B involved in regulated exocytosis. The mRNA encoding αCaMKII is located both in the nucleus and dendrites and appears to be directly associated with plasticity of the activated synapses. As mentioned above, this gene is upregulated after the induction of LTP (23), and we also found that the gene encoding syntaxin 1B is upregulated in granule cells bodies in the dentate gyrus after LTP induction (24). Further studies revealed that the regulation of this gene is not involved in LTP at the site of induction, but in a molecular mechanism mediating transsynaptic plasticity, i.e. the propagation of synaptic plasticity beyond a single synapse (10). In theory, transsynaptic plasticity is a candidate mechanism for configuring specific spatially distributed circuits during the laying down of memory. It is in fact implicit in most models of memory that synaptic changes should occur at successive, spatially separated regions within a distributed network of connected neurons. Thus, these genes can serve as markers of two specific aspects of plasticity that are predicted to be important for the construction of a memory-encoding neural network : αCaMKII for the local conversion of short-term to long-term plasticity at the activated synapses and syntaxin 1B for the propagation of plasticity in a distributed network of connected cells. When rats were submitted to different forms of spatial learning in a radial-arm maze, we showed that these two genes were turned on in specific regions of the brain during temporally restricted phases of learning (25). With syntaxin for example, the gene is upregulated in granule cells of the dentate gyrus and pyramidal cells of the hippocampus during a spatial working memory task. Activation of this gene however, takes place in more widespread circuits comprising hippocampal circuits, the nucleus accumbens and the prefrontal cortex during a spatial reference memory task. More importantly, activation of the gene in these circuits is observed when rats are reaching the brink of learning, but not early in training or in overtraining, and the level of gene activation correlates with the actual performance in the learning task. We know also that there is a direct pathway connecting the hippocampus to the prefrontal cortex and previous research has shown that this pathway supports LTP (26). Given the role of syntaxin in transsynaptic plasticity, this mechanism may thus play an active role in configuring distributed circuits in the hippocampus and in hippocampo-cortical networks in spatial learning with an anatomical specificity that is a function of the type of memory involved.

A second group of studies examined the regulation of these genes during ageing as malfunctionning of the genetic machinery may account, at least in part, for some of the memory

deficits observed in ageing. Normal ageing is usually associated with a decline in cognitive ability, in particular a decline in the ability to acquire and store new information. The memory deficit observed in aged rodents is similar to that observed with hippocampal lesions, suggesting that cellular and subcellular dysfunction in the hippocampus may contribute to the overall deficits in cognition that occur during ageing. Many structural and functional alterations occur during ageing that include loss of neurons, decrease in receptor function and neural transmission, alterations in calcium homeostasis, decrease in second messenger activity and protein synthesis (27). Importantly, Barnes and colleagues have shown that hippocampal synaptic plasticity is compromised during ageing and that the rapid decay function of LTP correlates with that of forgetting (28). In our own studies, we found that although LTP in the dentate gyrus can be induced in the aged rat, it decays to basal levels within 3 hours after induction, suggesting a disruption of the late phases which require gene expression and protein synthesis. In support of this idea, we found that the regulation αCaMKII and of syntaxin after the induction of LTP was defective in the aged rat. Moreover, while the expression of both genes was increased after spatial learning in the young rat, there was no such regulation in the aged rat, in correlation with deficits in learning. Thus, a dysfunction of activity-dependent gene transcription necessary both for efficient long-term plasticity in hippocampal neurons and for the propagation of plasticity in neuronal circuits, appears to be a candidate mechanism for ageing-dependent cognitive deficits.

Thus, the recent advances in molecular biology, together with the refinements in the concepts and methods in various fields of neuroscience, have increased our understanding of the cellular and molecular mechanisms of memory at an astonishing rate. Although still clearly in its infancy, with more and more open questions, it is easy to predict an even more promising future, not only for understanding the mechanisms of memory, but also to open new avenues in the design of novel therapeutic strategies to alleviate memory dysfunction.

References

1. Bliss TVP, Lømo T. Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path. *J. Physiol. (Lond.)* 1973; 232: 331-356
2. Collingridge GL, Kehl SJ, Mclennan H. Excitatory amino acids in synaptic transmission in the schaffer collateral-commissural pathway of the rat hippocampus. *J. Physiol. (Lond.)* 1983; 334: 33-46.
3. Bliss TVP, Collingridge GL. A synaptic model of memory: long-term potentiation in the hippocampus. *Nature* 1993; 361: 31-39.
4. Laroche S, Doyère V, Rédini-Del Negro C, Burette F. Neural mechnanisms of associative memory: role of long-term potentiation. In: McGaugh JL, Weinberger NM, Lynch G, eds. *Brain and Memory: Modulation and Mediation of Neuroplasticity* New York: Oxford University Press, 1995: 277-302.

5. Davis S, Butcher SP, Morris RGM. The NMDA receptor antagonist, D-2-amino-5-phosphonopentanoate (D-AP5) impairs spatial learning and LTP in vivo at intracerebral concentrations comparable to those that block LTP in vitro. *J. Neurosci.* 1992; 12: 21-34.
6. O'keefe J, Nadel L. *The hippocampus as a cognitive map*. Oxford: Oxford University Press, 1978.
7. McHugh TJ, Blum KI, Tsien JZ *et al.* Impaired hippocampal representation of space in CA1-specific NMDAR1 knockout mice. *Cell* 1996; 87: 1339-1349.
8. Laroche S, Rédini-Del Negro C, Clements MP, Lynch MA. Long-term activation of phosphoinositide turnover associated with increased release of amino acids in the dentate gyrus and hippocampus following classical conditioning in the rat. *Eur. J. Neurosci.* 1990; 2: 534-543.
9. Davis S, Laroche S. What can gene activation tell us about synaptic plasticity and the mechanisms underlying the encoding of the memory trace. In: Holscher C, Richter-Levin G., Bliss TVP, eds. *Neuronal Mechanisms of Memory Formation*. Cambridge University Press, 1999, in press.
10. Davis S, Laroche S. A molecular biological approach to synaptic plasticity and learning. *C.R. Acad. Sci. (Paris)* 1998; 321: 97-107.
11. Sanes JR, Lichtman JW. Can molecules explain long-term potentiation. *Nat. Neurosci.* 1998; 2: 597-604.
12. Tang YO, Shimizu E, Dube GR *et al.* Genetic enhancement of learning and memory in mice. *Nature* 1999; 401: 63-69.
13. Chapman PF, White GL, Jones MW *et al.* Impaired synaptic plasticity and learning in aged APP transgenic mice. *Nat. Neurosci*. 1999; 2: 271-276
14. Davis HP, Squire LR. Protein synthesis and memory: A review. *Psychol. Bull.* 1984; 96: 518-559.
15. Frey U, Morris RGM. Synaptic tagging and long-term potentiation. *Nature* 1997; 385: 533-536.
16. Impey S, Obrietan K, Storm DR. Making new connections: role of ERK/MAP kinase signaling in neuronal plasticity. *Neuron* 1999; 23: 11-14.
17. Blum S, Moore AN, Adams F, Dash PK. A mitogen-activated protein kinase cascade in the CA1/CA2 subfield of the dorsal hippocampus is essential for long-term spatial learning. *J. Neurosci.* 1999; 19: 3535-3544.
18. Mayford M, Kandel ER. Genetic approaches to memory storage. *Trends in Genetics* 1999; 15: 463-470.
19. Bourtchuladze R, Frenguelli B, Blendy J *et al*. Deficient long-term memory in mice with a targeted mutation of the cAMP-reponsive element-binding protein. *Cell* 1994; 79: 59-68.
20. Cho YH, Giese KP, Tanila H et al. Abnormal hippocampal spatial representations in αCaMKII^{T286A} and CREB$^{\alpha\Delta-}$ mice. *Science* 1998; 279: 867-869.
21. Tischmeyer W, Grimm R. Activation of immediate early genes and memory formation. *Cell. Mol. Life Sci*. 1999; 55 : 564-574.
22. Impey S, Smoth DM, Obrietan K. *et al.* Stimulation of cAMP response element (CRE)-mediated transcription during contextual learning. *Nat. Neurosci*. 1998; 1: 595-601.
23. Thomas KL, Laroche S, Errington ML *et al.* Spatial and temporal changes in signal transduction pathways during LTP. *Neuron* 1994; 13: 737-745.
24. Hicks A, Davis S, Rodger J *et al.* Synapsin I and syntaxin 1B: Key elements in the control of neurotransmitter release are regulated by neuronal activation and long-term potentiation in vivo. *Neuroscience* 1997; 79: 329-340.
25. Davis S, Rodger J, Stéphan A *et al.* Increase in syntaxin 1B mRNA in hippocampal and cortical circuits during spatial learning reflects a mechanism of trans-synaptic plasticity involved in establishing a memory trace. *Learn. Mem.* 1998; 5: 375-390.
26. Laroche S, Jay TM, Thierry AM. Long-term potentiation in the prefrontal cortex following stimulation of the hippocampal CA1/subicular region. *Neurosci. Lett.* 1990; 114: 184-190.

27. Geinisman Y, DeToledo-Morrell L, Morrell F, Heller RE. Hippocampal markers of age-related memory dysfunction: Behavioral, electrophysiological and morphological perspectives. *Prog. Neurobiol.* 1995; 45: 223-252.
28. Barnes CA. Memory deficits associated with senescence: A neurophysiological and behavioural study in the rat. *J. Comp. Physiol. Psychol.* 1979; 93: 74-104.