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Memory formation and the regulation of gene expression

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Abstract. On a cellular level, formation of memory is based on a selective change in synaptic efficacy that is both fast and, in case of important information, long-lasting. Rapidity of cellular changes is achieved by modifying preexisting synaptic molecules (receptors, ion channels), which instantaneously alters the efficacy of synaptic transmission. Endurance, that is the formation of long-term memory (LTM), is based on transient and perhaps also long-lasting changes in protein synthesis. A number of different methods exist to interfere with the synthesis of specific proteins or proteins in general. Other methods, in turn, help to identify proteins whose synthesis is changed following learning. These mostly molecular methods are briefly described in the present review. Their successful application in a variety of memory paradigms in invertebrates and vertebrates is illustrated. The data support the importance of selective changes in gene expression for LTM. Proteins newly synthesized during memory consolidation are likely to contribute to restructuring processes at the synapse, altering the efficiency of transmission beyond the scope of STM. Increased or, less often, decreased synthesis of proteins appears during specific time windows following learning. Recent evidence supports older data suggesting that two or even more waves of protein synthesis exist during the consolidation period. It is expected that the new molecular methods will help to identify and characterize molecules whose expression changes during LTM formation even in complex vertebrate learning paradigms.

Key words. Neuronal plasticity; consolidation; long-term memory; protein synthesis; gene transcription.

Introduction

For more than a century [1], two forms of memory have been distinguished on the basis of their duration: short-term memory (STM), which is rapidly formed and can outlast the learning situation for minutes to hours, and long-term memory (LTM), lasting from hours to days, weeks or years. With the advent of sophisticated pharmacological, molecular and anatomical tools, it has become possible to establish specific biological characteristics of STM and LTM and to gain insight into the neural mechanisms underlying the storage of information in the nervous system. As a result it is now widely believed that STM in both vertebrates and invertebrates is based on transient modifications of preexisting molecules, most importantly phosphorylation and dephosphorylation of enzymes, receptors and/or ion channels, capable of instantaneously altering the efficiency of synaptic transmission (for review, see Mons et al., Micheau and Riedel, Riedel, all this issue). LTM, in contrast to STM, can be blocked by protein synthesis inhibitors, indicating that it is dependent on de novo synthesis of proteins [2]. It furthermore is—at least in those cases that were accessible to adequate analysis—associated with structural changes of existing synapses or generation of new neuronal circuits through reactive sprouting and synaptogenesis [e.g. 3–6; Moser, this issue]. Proteins newly synthesized during memory consolidation are likely to contribute to restructuring processes and thereby alter the efficiency of synaptic transmission beyond the scope of STM. Structural changes underlying memory formation do not necessarily imply de novo protein synthesis. For example, long-term facilitation at the crayfish neuromuscular junction

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leads to rapid modifications of preexisting active zones as well as insertion of new release sites, in the absence of the neuronal soma and protein synthesis [7, 8]. For more permanent structural changes that outlast the first few hours following stimulation or training, however, protein synthesis seems to be an indispensable prerequisite, as we attempt to illustrate in this review.

Synthesis of most proteins can be regulated through sequence-specific transcription factors (TFs) capable of modulating transcription rates by supporting or inhibiting the formation of a transcriptional preinitiation complex at the promoter region of their target gene. Transcription rates are generally low in the absence of TFs, but greatly increased upon their activation [9]. In neurons, specific spatial and temporal patterns of afferent activity can stimulate intracellular signal transduction pathways that regulate the activity of TFs such as the adenosine 3′,5′-cyclic phosphate (cAMP)-responsive element binding protein (CREB) [10]. Changes in TF activity can thus be indicative of altered transcription, and interference with the function of TFs (e.g. in mouse mutants) may affect the expression of specific sets of proteins during formation of LTM. In addition to regulation at the transcriptional level, modulation of messenger RNA (mRNA) degradation (e.g. through differential polyadenylation or the presence of stabilizing factors such as hormones) may greatly determine the quantity of the final protein. Moreover, the sequence of a gene product may be changed through alternative splicing of primary transcripts or through RNA editing [11], and precise, possibly even synapse-specific, regulation of the translation process may be achieved through the transport of some mRNAs into dendrites [12–14]. Following translation, protein folding and transport modifications such as glycosylation are often necessary to ensure functionality of a newly synthesized protein. During formation of memory, all these cellular mechanisms may be involved in the functional expression of new proteins, some of which (such as inducible TFs and neurotrophins) will play roles in the activation and specification of the cellular response. Others (including receptors, cytoskeleton components and cell adhesion molecules) will modify signal transduction pathways or synaptic structures and thereby contribute to permanent changes of synaptic efficacy.

Methods

A variety of methods have become available to study plasticity-associated changes in gene expression at different molecular levels. Conversely, it is possible to pharmacologically interfere with specific steps of protein synthesis and to selectively modify expression of a protein through transgenic or acute molecular interventions. In the following sections we attempt to briefly describe some of the most common and promising approaches, which were so far mainly used to investigate gene expression in relatively simple models of synaptic plasticity. They are likely to promote our understanding of memory formation in the near future through their increased application to more complex vertebrate learning paradigms.

Detecting changes in gene expression

Transcription factor binding and activity: gel shift assays and reporter genes. The activity of TFs depends on their DNA binding ability, an ability that can be modulated by several factors including the abundance of the TF itself, availability of potential dimerization partners, phosphorylation state or presence of activators such as steroid hormones or Ca^{2+}. Therefore, transcriptional activation may be best monitored by analyzing DNA binding activity of TFs. One method to determine DNA binding activity is the gel-mobility shift (gel-retardation) assay. In this assay, a (usually P^{32}) radiolabeled DNA fragment resembling a certain promoter element is incubated with nuclear extracts from the tissues under investigation. The resulting protein-DNA complexes are separated according to their size by gel electrophoresis and quantified (see e.g. [15]).

Another elegant approach involves the generation of transgenic animals, in which the expression of a reporter molecule, such as β-galactosidase or luciferase, is controlled by the promoter element of interest. Induction of the reporter gene product can then be monitored by incubation of sections or tissue extracts with the appropriate substrate solution [16].

Detection of mRNA by hybridization methods. To detect changes in the expression of a specific gene, its mRNA may be isolated from the tissue and detected using Northern hybridization or an RNase protection assay. In Northern hybridization, the mRNA sample is separated according to size using denaturing gel electrophoresis, blotted to nylon or nitrocellulose filters and then hybridized with a probe of radioactively (P^{32}) or nonradioactively (e.g. digoxigenin) labeled complementary DNA (cDNA) or RNA. In an RNase protection assay, the mRNA sample is first hybridized with a labeled RNA probe. In a second step single-stranded RNA is removed through digestion with ribonuclease A or T1, leaving only double-stranded mRNA/probe hybrids for detection and quantification. Due to its high sensitivity, an RNase protection assay can be used to distinguish even closely related isoforms and to quantify mRNAs present in low abundance.

In situ hybridization (ISH) can moreover provide information about gene expression and mRNA distribution

with a cellular or even subcellular resolution, and may be especially valuable when only a few cells in the brain tissue under investigation express the mRNA of interest. During ISH, RNAs are labeled in tissue sections from either prefixed or freshly frozen specimen through hybridization with a radioactively (S^{35}) labeled antisense RNA, nick-translated cDNA or oligonucleotide probe. Detection of radioactive signals with a cover of photoemulsion allows quantification of the signal by densitometric analysis on a single-cell level as well as detection of mRNA in specific cellular compartments such as dendrites [12]. The development of a similarly sensitive detection method with digoxigenin-labeled probes and enzyme-conjugated antibodies and the availability of custom oligonucleotides have led to a widespread use of ISH in the analysis of behaviorally induced gene expression. Double ISH (radioactive/nonradioactive) and combined ISH/immunohistochemistry protocols have been developed to further identify the labeled cells and to study coexpression of different mRNAs (for a comparison of ISH with blot hybridization approaches, see e.g. [17–20]).

Detection with RT-PCR. Due to its high sensitivity and its handiness, polymerase chain reaction (PCR) has greatly influenced recent developments in almost all biological and medical disciplines. During PCR a piece of DNA can be amplified exponentially in multiple cycles of (i) denaturing the sample DNA (at 94 °C), (ii) annealing of primer oligonucleotides designed to hybridize specifically with the DNA fragment of interest (usually at a temperature between 55 and 72 °C, depending on the sequence and length of the oligonucleotide), and (iii) DNA polymerization through a thermostable DNA polymerase (at 72 °C). For detection of RNA it is necessary to reverse-transcribe the RNA sample into DNA and thereby generate a double-stranded cDNA template for subsequent PCR amplification (RT-PCR). RT-PCR may be used to study expression of mRNAs with extremely small amounts of starting material, such as a single cell, using the patch-clamp method [21]. Products of RT-PCR can be identified by hybridization with suitable probes or by sequence analysis, which allows detection even of subtle changes resulting from alternative splicing or RNA editing.

Reliable quantification of gene products by RT-PCR, however, may often be impeded through fluctuations in amplification efficiency. Multiplex assays, in which the gene of interest is amplified together with an internal (i.e. housekeeping gene) or external control (in vitro transcribed RNA) of different size [22, 23], and competitive PCR, in which the target DNA is competing for amplifcation with a standard of known concentration [24], have successfully been employed to overcome this problem. Recent development of a fluorogenic system now allows continuous quantification of PCR products (including internal controls) as the PCR proceeds, providing an effective tool for routine quantification of mRNAs present in widely differing abundance. On the other side of the spectrum, the development of in situ RT-PCR has opened the exciting prospect of carrying (possibly also in combination with conventional ISH) the sensitivity of PCR detection to the histological level, although at present it may still be hampered by a modest tissue preservation and a vulnerability to non-specific labeling through amplification of genomic DNA.

Differential cDNA cloning: subtractive hybridization, differential display. Many investigators have used differential cDNA cloning strategies to study tissue-specific or treatment-induced gene expression in a variety of organisms. Subtractive hybridization and differential display have become the two most popular and widespread methods to this aim. In subtractive hybridization, mRNA or cDNA from one (the experimental) sample is hybridized with an excess cDNA from a second (control) sample carrying a tag for separation (such as magnetic beads, biotin or digoxigenin). Fragments that are only expressed in the experimental sample will remain single-stranded after the hybridization and will be strongly enriched after removal of tagged double strands. A PCR-based variation of subtractive hybridization involves the ligation of specific adaptors to the experimental cDNA sample and amplification of the enriched differentially expressed messages using primer oligonucleotides directed against these adaptors [25]. Subtracted samples can be used to construct subtractive cDNA libraries, as demonstrated by Nedivi and coworkers [26], who isolated 52 candidate plasticity genes, induced in the hippocampus 6 h after kainic acid treatment.

In differential display, cDNA is synthesized from both experimental and control RNA samples and amplified by PCR with oligo-dT anchor primer oligonucleotides and a mix of up to 256 different 10mer oligonucleotides serving as 5′ primers. Amplification with these primers results in a vast number of different fragments, reflecting the presence of 5′ primer binding sites in the cDNA pool. Banding patterns of PCR products are compared between samples by gel electrophoresis to identify fragments derived from differentially expressed gene products. These fragments can then be rescued from the gel and used for further analysis. Several investigators have applied differential display successfully to identify plasticity-related genes [27, 28]. Such PCR-based cloning methods offer great sensitivity and can be applied to a very small amount of starting material (i.e. 50 ng of total RNA), but commonly require additional screening with conventional methods due to their high probability for false positives (for comparison of these methods, see [29]).

Pulse labeling and detection of proteins. A comparative analysis of proteins selectively expressed after the manifestation of a specific behavior can be performed by pulse labeling of proteins and subsequent identification of labeled products in two-dimensional (2D) gel electrophoresis samples. In this procedure, the organism/slice preparation is supplied at a certain time point (e.g. after a training session) with a radioactively labeled amino acid (such as S^{35}Met). Later, proteins are isolated and separated according to their isoelectric point and size by 2D gel electrophoresis. Comparison of 2D gels from experimental and control samples will lead to identification of proteins generated during the time when the labeled amino acid was available for protein synthesis. This approach was chosen by Barzilai and co-workers [30], who identified 21 proteins whose expression was altered after sensitization training in *Aplysia californica*. Changes in expression of a specific protein may then be further visualized by quantitative Western blotting, which involves separation of proteins on a polyacrylamide gel, transfer to a membrane and detection with a target-specific primary antibody and radiolabeled or enzyme-conjugated secondary antibody. Alternatively, the concentration of a specific protein may be measured by enzyme-linked immunosorbant assay (ELISA) or radioimmunoassay (RIA).

Immunohistochemistry. Immunohistochemistry allows the analysis of protein expression with a cellular resolution. Antigens are detected in situ using a specific antibody and are labeled with a secondary antibody conjugated either with a fluorochrome (FITC, rhodamin) or an enzyme (such as horseradish peroxidase) catalyzing a color reaction. With the use of confocal laser scanning microscopy it is possible to obtain information about the cell type and structure (e.g. dendrites, spines) expressing the epitope, but quantification may be difficult and in many cases involves cell counting (e.g. [31]). In order to obtain higher resolution, immunoelectronmicroscopy with gold- or enzyme-conjugated antibodies can be applied to detect changes in antigen expression in subcellular compartments such as the postsynaptic density [32, 32a]. Functional expression of transmembrane and extracellular proteins usually involves glycosylation, which may be regulated during memory formation. Glycoepitopes can be detected in the same way as the core protein itself. Incorporation of radiolabeled sugars [33] and detection with specific antibodies [34] have already been successfully used to study changes in glycosylation following learning.

When interpreting immunohistochemistry signals it should be considered, however, that structural changes (e.g. at the synapse) might affect access of antibodies to the target protein and that subtle differences (e.g. in sequence) will often remain undetected. Therefore, in many cases it will be advisable to confirm results with RNA hybridization methods, such as ISH or Northern hybridization.

Manipulating gene expression

Inhibitors of transcription and translation. Antibiotics such as 8-azaguanine or actinomycin-D (dactinomycin) can be employed to disrupt RNA synthesis during memory formation. While the former substitutes for the natural nucleoside guanine and becomes incorporated into RNA [35], the latter acts by directly binding to DNA [36]. Actinomycin-D does not cross the blood-brain barrier and, therefore, has to be injected intracranially when blocking transcription in the central nervous system is desired. Unfortunately, doses of these substances sufficient to substantially suppress cerebral RNA synthesis cause rapid and irreversible systemic toxicity as well as necrosis ([37, 38], and references therein). Intracranial injections of low doses (1 μg) of actinomycin-D, on the other hand, had little effect on RNA synthesis but still attenuated retention even when injected as late as 24 h after training [38]. This suggests that retention deficits may be the result of drug-induced hippocampal damage and abnormalities in electrical activity. Thus, experiments with these transcription inhibitors should include anatomical and electrophysiological controls and have to be interpreted with caution.

Three classes of translation inhibitors have been widely used in memory experiments: puromycin (PURO), glutarimides [cycloheximide (CXM), ementine, and acetoxycycloheximide] and anisomycin (ANI). PURO is incorporated into the growing peptide chain at its carboxyl end, resulting in premature release of peptidyl-PURO fragments from the ribosomal complex. Such peptidyl-PURO fragments may by themselves have a long-lasting effect on cell function that causes amnesia (see [2]). In addition to inhibiting protein synthesis, PURO induces hippocampal seizures, swelling of mitochondria and disaggregation of ribosomes (for references see [39]). Due to its numerous side effects, PURO cannot be recommended to investigate the effects of protein synthesis inhibition on memory formation (for discussion see [2]).

ANI interferes with peptide bond formation [40] and is, at doses that successfully block retention, a fairly non-toxic protein synthesis inhibitor. Successive injections of ANI permit an inhibition of variable duration in the range of 2 to 8 h, with the inactive form deacetyl-ANI serving as control compound [39]. CXM, on the other hand, inhibits not only translation (initiation, translocation and steps of elongation processes) but also DNA and RNA synthesis [35]. CXM in effective doses is far more toxic than ANI [41]. Doses inhibiting protein synthesis 80% or more can cause sickness and, under

stressful training conditions, even death. On the behavioral level, changes in locomotor activity occur which are not seen after ANI injections [39]. Both protein synthesis inhibitors can cause diarrhea, but the signs are hardly noticeable after ANI injections [39].

Several hypotheses have been put forward to explain the amnestic effects of protein synthesis inhibitors by mechanisms other than their blockade of protein synthesis (e.g. by producing sickness and conditioned aversion, changes in locomotor activity, inhibition of steroidogenesis and disturbance of catecholamine neurotransmission). A number of careful experiments in different laboratories provided results that make all these alternative explanations unlikely (for review see [2]). However, it still should be considered that inhibitors of protein synthesis may affect behavioral performance not only through their interference with memory formation, but also by acting on learning-related processes involved in, for example, attention or motivation.

Mutant strategies. The development of transgenic and gene-targeting techniques has made it possible to overexpress or delete a specific gene and, thus, analyze its functions in an intact organism. In the transgenic approach a genetic construct—generally consisting of a promoter sequence, a coding sequence for the gene of interest and a polyadenylation signal—is injected into a pronucleus of fertilized oozytes. Injected eggs are implanted into the oviduct of pseudopregnant host females, and as the transgene integrates into their genomes at different copy numbers, transgenic animals showing different levels of transgene expression can be obtained. Generally two or more mutant lines are bred and analyzed to consider phenotypic changes related to different expression levels or to unintended disruption of other genes through the randomly inserted transgene. By choosing a specific promoter, it has now also become possible to restrict the expression of the transgene to a subset of cells or to a developmental stage [42–44].

Gene null mutations (knockouts), on the other hand, are generated through homologous recombination in embryonic stem (ES) cells. To obtain a suitable targeting vector, a genomic clone must be modified such that the critical exons are replaced by a positive selection marker (typically a neomycin resistance cassette). The targeting vector is introduced by electroporation into ES cells, which are grown and screened for homologous integration. ES cell clones carrying the desired mutation are injected into blastocysts, which are then implanted into the uterus of pseudopregnant females. Some of the recombinant ES cells may integrate into the embryo and result in chimeras with germ-cell contribution, which can be further bred to obtain homozygous null mutants.

Both transgenic overexpression and null mutation provide powerful tools to study functions of a specific protein in neural plasticity and LTM, if some considerations are accounted for: for one, mutation of a gene may affect a variety of biological functions, especially if it is active during development, making it difficult to specifically address memory formation. Second, mutant phenotypes may be related to compensatory upregulation or cis activation [45] of other genes, rather than the mutation itself. Third, since null mutant mice are generally generated as hybrids of the ES cell donor strain (e.g. a substrain of 129) and another strain (mostly C57B/6), genetic background effects must be carefully controlled (see [46, 47]). Some of these problems may be overcome with inducible transgenic or knockout mutants responsive to an externally provided substance, such as tetracycline in the drinking water [48, 49]. However, it may still be difficult to clearly distinguish roles of the mutated molecules in STM and LTM, as experimentally induced changes in gene expression require time and can hardly be controlled to occur in an exact time window before, during or after training.

Antisense oligonucleotides. The application of antisense oligonucleotides and the introduction of genes through viral vectors provide ways to not only acutely but also locally alter the expression of specific proteins. After application of antisense oligonucleotides into the superfusion fluid of a slice preparation or directly into the brain and their uptake into cells, they can hybridize with complementary sequences on mRNAs and thereby disturb their translation. This antisense strategy has been successfully applied to a variety of mRNAs, providing additional evidence for, for example, a crucial involvement of c-*jun* and CREB in learning processes [50, 51].

Conversely, genes can be introduced into the mature nervous system through viral vectors [adenovirus, herpes virus, human immunodeficiency virus (HIV-1)]. To that aim, replication-deficient recombinant viruses are generated which carry the gene of interest and can transduce it into cells. This approach has been used to elucidate the roles of Ca^{2+}/calmodulin-dependent protein kinase (CaM kinase II) in LTP [52] and to reintroduce brain-derived neurotrophic factor (BDNF) synthesis into slices of BDNF null mutant mice, thereby rescuing LTP that was disturbed in mutants [53]. However, in many cases it may be difficult to induce or delete a protein both effectively and within a distinct time window. If antisense oligonucleotides or viruses are applied well before training, on the other hand, they are at danger to disturb cellular functions that are not involved in LTM formation.

Models of learning and memory

An involvement of protein synthesis has been demonstrated in several electrophysiological model systems of neural plasticity and in a great variety of vertebrate and invertebrate learning paradigms, all of which share a number of key features. First, a single event or experience is sufficient to modify the way the system or organism reacts in response to an unaltered stimulus (i.e. a stimulation-induced change in response probability appears). Second, changes in synaptic efficacy are accompanied by structural and functional alterations at the cellular and synaptic level. Third, de novo protein synthesis is necessary for long-term modification of synaptic efficacy and behavioral responses to occur as we will show in the following section where we present evidence for this hypothesis collected in both electrophysiological plasticity models and learning paradigms.

Electrophysiological models: kindling and LTP

The term 'kindling' refers to a neural phenomenon of recurrent subconvulsant electrical or chemical stimulation eventually eliciting seizure; this procedure is considered to be an experimental model of epileptogenesis [54]. Kindling-induced seizures are accompanied by a dramatic increase of neuronal activity and changes in structure and function of neurons, mostly in the neocortex and hippocampus. Structural and functional changes have been shown to be accompanied by alterations in gene expression and protein synthesis that commence soon after seizure induction and last from hours to weeks. Kindling-induced structural changes include sprouting and formation of novel synapses (e.g. [55, 56]). The contribution of these morphological changes to the permanent increase in seizure susceptiblity still has to be determined.

Protein synthesis was shown to be important for the development of kindling in vivo (e.g. [57]), as well as maintenance of spontaneous bursting in an in vitro kindling model [58]. In the kindled state, immediate early genes were found to be expressed in hippocampal and neocortical areas within minutes after seizure onset (e.g. c-*fos*, tissue-plasminogen activator [59–61]). Neurotrophic factors and their receptors (NGF, BDNF, trkB, trkC [62, 63]) were also quickly induced and remained altered for hours or even days, as were the expression of peptides [64], neurotransmitter and hormone receptors [mGluR1, subunits of AMPA and NMDA receptors, γ-aminobutyric acid type A receptor ($GABA_A$), mineralcorticoid and glucocorticoid receptors; [65–70], and nitric oxide synthase [71].

Long-term potentiation (LTP) and long-term depression (LTD) are electrophysiological models of learning and memory that can be tested in vivo as well as in vitro. Physiological and molecular events characterizing LTP and LTD resemble those observed in learning paradigms, that is strong parallels exist between hippocampal LTP and spatial learning [72, 73], and amygdala LTP and fear conditioning [74, 75]. Like memory formation, LTP and LTD can be divided into a protein synthesis-independent early phase and a protein synthesis-dependent late phase which is accompanied by synaptic structural changes. Numerous experiments revealed that protein synthesis inhibitors prevent the development of long-lasting LTP (late LTP or L-LTP) as well as long-lasting LTD while leaving short-term potentiation and depression unaffected (e.g. [76–81]). In a study by Otani and co-workers [82], maintenance of LTP for up to 3 h required protein synthesis—but not mRNA synthesis—within 0–15 min following its induction, suggesting that proteins supporting a limited maintenance of LTP were synthesized shortly after induction of LTP from preexisting mRNA, which may at least in part have been located at postsynaptic sites [83, 84].

To maintain LTP for more than 3 h not only translational but also transcriptional activation appears to be necessary. With a transgenic reporter gene containing a CRE promoter it could be shown that stimuli that generate L-LTP also induce CRE-mediated gene expression in the hippocampus [85]. A key role for CREB in establishing L-LTP, but not early LTP, is further suggested by two other experimental approaches with transgenic animals: both mice with a selective inhibition of hippocampal protein kinase A, one of the protein kinases that activates CREB [86], and mice with a null mutation in the CREB gene [87] were deficient in L-LTP.

Activation of CREB and other preexisting TFs was likely responsible for the observed increases in mRNA of immediate early genes (*zif/268*, c-*fos*, c-*jun*, *jun*B, *jun*D) following induction of L-LTP. Among these expression of *zif/268* appears to be most closely correlated with the induction of L-LTP [14, 61, 88, 89]. Other genes that changed their expression rate following induction of LTP include neurotrophins (BDNF, NGF, neurotrophin-3) and their receptors [90–92], proenkephalin [93] as well as glutamate receptors (the presynaptic receptor G33 [94], AMPA receptors [95]). Changes in growth factor expression during unilateral LTP appeared not only in hippocampal cells of the tetanized hemisphere but also in the hippocampus of the nonstimulated hemisphere [91]. Especially the involvement of BDNF in L-LTP is well established. Treatment of slices with antibodies to its receptor, trkB, disrupted L-LTP [96]. Further, almost normal LTP could be recovered in slices from BDNF-deficient mice when BDNF expression was reinstated through virus-mediated gene transfer [53, 97].

Memory formation in invertebrates

A. californica. The natural response of the marine mollusc *A. californica* is to withdraw its siphon and gill when being touched on the mantle shelf, siphon or gill. This reflex habituates with repeated stimulation but is sensitized by a nocious stimulation of the tail. Basic elements of sensitization of the gill-withdrawal reflex can be simulated in an in vitro preparation consisting of sensory and motor neurons. If the sensitizing stimulation is simulated by one brief application of serotonin—which is released in vivo by interneurons—a short-term facilitation of synaptic transmission results that lasts several minutes. Five spaced applications of serotonin can produce a long-term facilitation that lasts for days and involves changes in gene expression and growth of new synaptic connections [98].

Inhibition of protein or mRNA synthesis 1–3 h following serotonin application suppressed long-term facilitation and the accompanying structural changes [30, 99–101]; short-term facilitation was not affected. In a study using cAMP injections as the sensitizing stimulus, injections of ANI around the time of induction (-1 to $+2$ h) and 4–7 h later—but not 12–15 h later—blocked the formation of structural changes associated with long-term sensitization [102]. Further, presynaptic—but not postsynaptic—injection of a protein synthesis inhibitor blocked the branch-specific long-term facilitation and accompanying structural changes. This synapse-specific facilitation was dependent on CREB-mediated transcription [98].

Using 2-D gel electrophoresis, Barzilai and co-workers [30] detected three temporally distinct changes in individual proteins following an application of serotonin that simulated long-term facilitation. There was an increase in 10 and a decrease in 5 proteins at 30 min. A transient increase in 4 proteins was observed at 3 h, and a sustained increase in the expression of two proteins at 24 h. Two other proteins showed a sustained increase that persisted for at least 24 h after long-term facilitation. In another study, altered expression of four proteins was shown during the maintenance phase after long-term sensitization after 4 days of sensitization training [104].

Subsequent identification of the isolated proteins has shown that among the first wave induced in long-term facilitation are the TF C/EBP and C-terminal ubiquitin hydrolase [105, 106]. Proteins decreasing in expression very likely included *Aplysia* cell adhesion molecules (apCAMs), homologs of the neural cell adhesion molecule (NCAM) found in vertebrates [103]. Inhibiting the expression of either protein was sufficient to block induction of long-term but not short-term facilitation. mRNAs for calmodulin and an as yet unidentified protein were also transiently increased immediately after sensitization training [107]. Three hours after the onset of long-term facilitation increases in the mRNA for the *Aplysia* homolog of BiP, an endoplasmatic reticulum resident protein with chaperone function, could be detected [108]. Further, clathrin light-chain, actin and *Aplysia* intermediate filament protein have been identified as potential late effector genes in long-term facilitation [109, 110]. Increases were also observed in the steady-state level of mRNA for calreticulin during the maintenance phase of long-term sensitization [111]. Calreticulin is a major calcium-binding protein within the endoplasmatic reticulum particularly found in presynaptic varicosities. All the above-described changes in gene expression take place presynaptically to the motor neuron. Postsynaptic changes in form of an increase of functional receptors may also occur [112], although they are apparently not required for long-term facilitation measured at the 24-h time point.

Hermissenda crassicornis. An associative memory forms in the marine mollusc *Hermissenda* when light is contiguously paired with rotation, which elicits foot retraction as an unconditioned response. After conditioning, light alone is sufficient to elicit foot contraction as a conditioned response. The conditioned response dissipates within 45 min if two pairings are provided, but can be detected at least 90 min after training with nine pairings. The conditioning is associated with sensitivity changes at the neuronal level (type B photoreceptor neurons), which can be detected electrophysiologically in vitro, and with structural changes of neurons involved in this type of learning [113]. Injections of ANI attenuated the conditioned response 90 min after conditioning in vivo as well as in vitro [114], but injections of ANI 5 min after the $\sim$9.5-min conditioning session failed to attenuate retention of the conditioned response during the 90 min following conditioning.

In a model system of this conditioning paradigm the CS (light) is paired during 5 min with application of serotonin to the exposed nervous system [115]. Short-term (1 h) and long-term enhancement (24 h) of generator potentials recorded from identified lateral and medial B photoreceptors can be detected. When ANI or a transcription inhibitor was present during and shortly after the conditioning trial, but not when given 1 h later, long-term enhancement was blocked while short-term enhancement remained unaffected [114, 116].

Extensive conditioning of the association 'light-rotation' over 3 days suppresses the animals' normal phototactic response measured 24 h after the last training session. Conditioning, but not random presentation of CS and US, was shown to be correlated with RNA changes maximal at 24 h and still detectable after 4 days [117]. Increases appeared in at least 21 high molecular weight mRNAs [118]. An increase in a specific G protein (cp20) in conditioned but not in control animals

could also be detected 24 h after training using high-performance liquid chromatography [119]. Injection of this G protein back into type B photoreceptor neurons resulted in reduced potassium currents (I_K and $I_{K\text{-}Ca}$).

Drosophila melanogaster. Memory formation in *D. melanogaster* can be studied through classical conditioning of an odor-avoidance response. After exposing flies simultaneously to a specific odor and electric shock, the avoidance reaction to the shock-paired odor can be determined in a T maze. In this paradigm, the existence of a protein synthesis-independent form of LTM, in addition to a protein synthesis-dependent form, was observed [120]. The former, CXM-insensitive memory, was anesthesia-resistant but decayed within 4 days after training. The latter, CXM-sensitive memory, required training with spaced trials and usually lasted at least 7 days [120]. The crucial role of CREB-related molecules in the CXM-dependent form of LTM has been shown in transgenic studies. Overexpression of dCREB2-b (a dominant negative form of CREB functionally related to a mammalian CREM isoform) could specifically disrupt the CXM-sensitive, but not the CXM-insensitive, form of LTM [121]. Induced expression of the CREB activator dCREB2-a, on the other hand, was sufficient to enhance LTM following only a single training session [122]. CREB furthermore appears to act in concert with fasciclin II, the *Drosophila* homologue of NCAM, to modify synaptic strength, as shown with different CREB and fasciclin mutants. A mutation, which resulted in constant expression levels of fasciclin II at the synapse, prevented cAMP- and CREB-mediated synaptic plasticity induced by increased neuronal activation in eag Shaker and dunce mutants [123]. Furthermore, expression of dCREB2-a increased synaptic strength in mutants that show a 50% reduction of synaptic fasciclin, but not in wild types [124]. Conversely, these fasciclin II mutants showed an increased number of synaptic boutons, but unaltered synaptic strength in the absence of the CREB activator [125].

Other invertebrates. Passive avoidance learning in the praying mantis could be prevented by protein synthesis inhibition. When tested 24 h after a training session, animals injected with CXM immediately after training—but not those injected with a delay of 2 h—acted like naive animals [126].

LTM of neither olfactory conditioning nor color learning were inhibited by protein synthesis inhibition in the bee [127, 128]. Although CXM suppressed protein synthesis for more than 90% over a period of 3 h and in some cases over a period of up to 7 h, it did not reduce test performance 24 h after learning. The results show that in the bee, as in *D. melanogaster*, LTM of up to 24 h is possible without need of protein synthesis for several hours after training. However, this does not exclude that longer-lasting LTM (for more than 24 h) may be dependent on learning-induced protein synthesis. Protein synthesis-independent LTM in the bee could be related to sustained activation of protein kinase C (PKC). After only three training sessions in an olfactory memory task, PKC activity in the antennal lobe remained activated for up to 3 days [129]. The RNA synthesis inhibitor actinomycin D injected 1 h post-training blocked PKC activation 18 h but not 3 h later. In contrast, actinomycin D-induced performance deficits were not manifest until 3 days later. Thus, at least one protein synthesis- and PKC-independent memory trace lasting for 24 h seems to exist in the bee.

Memory formation in birds

Imprinting. Early during development birds can be imprinted to a variety of arbitrary objects. For example, 24 h after hatching, chicks can be imprinted to a conspicuous moving object (e.g. a red ball with yellow stripes) in a single 10-min exposure. CXM administered 5 min after this imprinting session reduced the time spent near the object, a measure of imprinting strength, 2 days later [130]. Imprinting also induced structural changes in the intermediate medial hyperstriatum ventrale (IMHV), a brain structure known to be essential for different types of visual learning in chicks; however, blocking these changes with protein synthesis blockers has not yet been tried [131].

Changes in mRNA and protein synthesis as a consequence of imprinting have been repeatedly demonstrated (e.g. [132–135]). A selective change was observed in Fos-like immunoreactivity [136] and the mRNA for MARCKS [137], a PKC substrate and cytoskeleton-associated protein, in the IMHV. Some of the cells with increased Fos expression were immunoreactive for GABA [138] or PKCγ [139]. Furthermore, a decrease in the expression of a gene encoding a subunit of the avian $GABA_A$ receptor has been reported [140]. The decrease was apparent at 10 h but not at 5 h after training in the IMHV and selected other areas. A selective increase in clathrin heavy chain (but not light chain) has also been observed in the IMHV of good but not of bad learners [141].

Song learning. Songbirds learn to recognize the song of conspecific individuals with long-lasting retention of songs, for example for up to 8 months in male hooded warblers [142]. In zebra finches, listening to a conspecific song initially elicited increased neuronal activity and expression of the immediate early genes *zenk* (synonym to *zif/268*, *erg*1, NGFI-A and *krox*24) and c-*jun* in the caudomedial striatum [143–146]. With repeated listening to the same song, neuronal activity became smaller, and *zenk* expression disappeared. In contrast, increased neuronal activity and *zenk* expression were

still elicited when other calls or complex sounds were presented. This 'stimulus-specific habituation' can last for up to 2 days [147, 143]. Long-term habituation, that is the reduction in neuronal activity and loss of *zenk* expression, could be blocked by an injection of CXM immediately after presentation of a conspecific song (0.5–3 h) or at multiple subsequent periods (5.5–7 h, 14–15 h and 17–18.5 h [143, 147, 148]).

Passive avoidance learning. Newly hatched chicks quickly acquire a passive avoidance reaction directed towards bitter-tasting fluids. To test this, water droplets forming at differently colored and shaped beads are presented. When one of the beads signals that the water droplet is altered by a bitter-tasting substance, picking at this kind of beads is avoided when tested 24 h later [149]. Critical brain areas for this type of learning have been identified as the IMHV and the lobus parolfactorius [149], both of which show structural changes such as long-term increases (24 h later) in synaptic density (in the lobus parolfactorius [150]) and increases in the density of dendritic spines (in the IMHV [151]). Inhibition of protein synthesis by ANI immediately before or up to 30 min after initial training blocked the formation of LTM tested 24 h later (e.g. [152, 153]) and prevented the structural changes in the lobus parolfactorius when given 30 min before the training [150]. ANI also prevented LTM formation when injected at a second, later time window (4–5 h post-training [154]).

After LTM training, new proteins appear to be synthesized in two distinct waves sensitive to protein synthesis inhibition. The first wave immediately follows training (0–1 h). Interference with protein synthesis during that wave blocks LTM formation as described above. Proteins whose expression have been found increased at this time include the immediate early genes c-*fos* and c-*jun* (30 min post-training [17, 18]), and tubulin (1 h later [155]). A second wave of protein synthesis essential for LTM formation appears 5–8 h post-training. During this time changes were observed in the synthesis of pre- and postsynaptic membrane glycoproteins, some of which are very likely cell adhesion molecules (e.g. [156–159]) and incorporate externally provided [^{3}H]fucose [160]. Inhibitors of protein synthesis as well as inhibitors of fucosylation prevented the formation of LTM when applied during this time window [33, 161]. Whether a strong or weak memory trace forms generally is dependent on both the training procedure and increase in plasma corticosterone levels (e.g. [33, 162]). However, glycoprotein synthesis was only enhanced after a training protocol that leads to the formation of LTM [162].

Memory formation in rodents

Inhibitors of transcription or of protein synthesis have been employed for more than 3 decades to investigate memory formation in rats and mice. These data have been thoroughly reviewed by Davis and Squire [2]. Thus, the following section summarizes a selective number of older studies and emphasizes more recent studies some of which were using molecular methods to investigate the role of de novo protein synthesis in LTM formation.

Avoidance learning. There is ample evidence that protein synthesis inhibitors when injected before or immediately after training can prevent passive avoidance learning in mice (e.g. [39, 41, 163, 164]) and rats (e.g. [165, 166]). The amnestic action of protein synthesis inhibition was shown to decrease with the strength of training (i.e. weak training with low shocks and strong training with high shocks) and to increase with longer duration of protein synthesis inhibition [39, 41, 163]. Performance during acquisition of the task and short-term memory were not affected by the treatment. Similar results were obtained when RNA synthesis was blocked by intracranial injection of actinomycin-D [166]. Immediate posttrial bilateral injections of the drug into the dentate gyrus in rats led dose-dependently to amnesia for the passive avoidance task measured 24 h after training. The dentate gyrus was also the site of an increased polysialylation of NCAM180, 12 and 24 h after passive avoidance training, which occurred without detectable change in the amount of core protein [34]. Intracranial injection experiments suggest that the amygdala may also be a critical structure of protein synthesis in active avoidance learning [167].

Protein synthesis inhibition through systemic injection of ANI also blocked active avoidance learning in mice. However, multiple injections were necessary to achieve amnesic effects [168]. Moreover, retention of the extinction of active avoidance learning was dependent on protein synthesis in a way similar to the initial acquisition of the response [169]. Further, induction of the immediate early gene TFs c-*fos* and *zif/268* was observed in the hippocampus and visual cortex following active avoidance learning [170].

Discrimination learning. Effects of protein synthesis inhibitors on memory formation have been studied in a variety of discrimination tasks. Injections of either CXM or ANI during and following training in an object discrimination task interfered with the establishment of LTM [171]. In contrast, memory formation after olfactory discrimination learning remained unaffected by systemic injections of ANI [165], possibly due to residual protein synthesis or the occurrence of protein synthesis-independent synaptic changes outlasting the training-test interval. On the other hand, the development of a preference for an odor associated with artificially scented rat pups and odor-elicited maternal behavior were prevented by an intraventricular injection of CXM [172].

In a brightness discrimination task the existence of two waves of protein synthesis has been demonstrated by

injecting ANI bilaterally into the hippocampus 10 min before training or at various times after training [173]. Double injections around training (10 min pre- and 80 min post-) or several hours later (4 and 6 h post-training) attenuated retention measured 24 h after the training session. Injections between these two time windows (45 min and 2 h 45 min post-training) had no amnesic effect. Training of rats in a shock-motivated brightness discrimination task was followed by an increased expression of several immediate early genes in the hippocampus (c-*fos*, *jun*B, *zif/268*) and in the cerebellum (c-*fos* [174]). Rats trained in a similar task exhibited increased incorporation of L-[1-^{3}H]fucose into glycoproteins of the hippocampal formation [175]. While labeling of glycoproteins in the dentate gyrus was increased only 0–2 h post-training, labeling of glycoproteins in the CA1 and CA3 regions of the hippocampus was also increased between 3–9 h and 7–9 h post-training, respectively. During the first time window fucose was found to be mainly incorporated into soluble proteins, whereas in the second time window radioactive fucose labeled membrane-bound insoluble proteins.

Spatial learning. Spatial learning can be measured in mazes such as the water maze or radial maze. In the water maze an animal has to learn to locate an escape platform in a fixed position by using only distal spatial cues. The platform is hidden 1 cm below water surface and, therefore, does not provide any local cues. Spatial learning in this test system could be blocked by intraventricular injection of ANI 20 min before, but not at any time point after, training [176]. Protein synthesis during consolidation seems to be at least in part controlled by CREB. In rats, bilateral hippocampal injections of antisense oligonucleotides to CREB before training impaired performance 48 h—but not 4 h—later [177]. Furthermore, mice with targeted mutation of the CREB gene failed to learn the position of the hidden platform [87]. A possible involvement of the TF Fos is suggested by results obtained in mice with ablation of the c-*fos* gene. These mice were deficient in a spatial and a cued version of the water maze task, but not in a simpler learning paradigm [178].

Using RNA fingerprinting, water maze training has been shown to induce the expression of several genes in the hippocampus [179]. Two induced gene products were identified as the rat homologue of the ryanodine receptor type-2 (RYR2), an intracellular Ca^{2+}-release channel that participates in the homeostasis of cytosolic calcium, and glutamate dehydrogenase, an enzyme that catalyzes the conversion of α-ketoglutarate to glutamate. Significant increases were found 6 and 12 h post-training for glutamate dehydrogenase and 6 h post-training for RYR2 in the hippocampal formation. No increases were seen at 2 or 24 h post-training. Less systematically observed outside the hippocampus, glutamate dehydrogenase but not RYR2 was found to show training-induced increases in the cingulate cortex and laterodorsal nucleus of the thalamus. Other groups have observed changes in the expression of polysialylated NCAM in the dentate gyrus and the entorhinal cortex 10–12 h after water maze training [180, 181].

In a radial maze, hungry animals can collect food pellets at the distal end of eight arms radiating from a central platform, and working memory or reference memory can be tested depending on training conditions. When a working memory task was acquired in this system, increased syntaxin 1B mRNA expression was observed in all hippocampal regions. When a reference memory task had to be learned, similar increases were detectable in subregions of the frontal cortex and nucleus accumbens [182].

Classical conditioning. Fear conditioning can be achieved by presenting to rodents pairings of a conditioned stimulus (CS, such as light or tone) and an unconditioned stimulus (US, such as footshock), resulting in a fear response to the presentation of the CS alone (cued version). Alternatively, contextual memory can be analyzed by reexposing animals to the training environment. In rats systemic injection of CXM prevented the formation of such contextual memory [183]. The dependence of fear conditioning on de novo protein synthesis is further supported by evidence for an involvement of CREB in this type of learning. Mice with a targeted disruption of the CREB gene showed deficits in context- and cue-induced freezing behavior when tested 1–24 h (but not 30 min) after training, although genotypes did not differ in their reaction to the footshock itself [87]. This deficit could be overcome through a training protocol with temporally spaced training sessions [184]. Following contextual or cued training, induction of c-*fos* and *zif/268* have been observed by several investigators in the amygdala and a variety of other brain areas (e.g. [19, 20, 31, 185, 186]). Interestingly, ablation of another TF, the CCAAT/enhancer binding protein δ, resulted in specific enhancement of contextual fear conditioning with the performance after auditory cued fear conditioning and spatial learning remaining unaffected [187].

Consolidation of conditioned taste aversion learning, in which a novel taste is followed by the induction of sickness (e.g. through rotation or administration of LiCl) is also dependent on de novo protein synthesis. Intraventricular injection of CXM [188] or bilateral injections of ANI directly into the gustatory cortex [189] of rats prevented the development of aversion against a flavor associated with malaise as measured in a preference test 24 h or 4 days after training, respectively. Injections several hours before training of antisense oligonucleotides to CREB into another structure

critical for this type of learning, the amygdala, blocked formation of LTM but not STM [51].

Discussion

Transcriptional activation and protein synthesis have proven crucial for LTM formation in a variety of behavioral paradigms in both vertebrates and invertebrates. First, several transcription inhibitors (e.g. actinomycin D) and translation inhibitors (e.g. CXM, ANI) were capable of blocking the formation of LTM. Second, learning situations activated preexisting TFs (e.g. CREB) and rapidly induced immediate early gene TFs (e.g. c-*fos* or c-*jun*) that regulate expression of effector genes crucial to the formation of LTM. Further, molecular disruption of TFs (CREB, c-*fos*, c-*jun*) by mutation or by application of antisense oligonucleotides prevented LTM formation [50, 87, 121, 122, 178, 190]. The findings with behavioral learning paradigms are paralleled by similar observations in electrophysiological models of neural plasticity such as kindling and LTP. Furthermore, a strong similarity is apparent between synaptic modulation underlying memory formation and the regulation of growth during development and regeneration in the nervous system. All these processes are activity-dependent, require retrograde messengers and recruit cellular programs of growth including de novo protein synthesis to evoke changes in synaptic structure and function [191–193].

Several studies have suggested the existence of two or more waves of protein synthesis during memory consolidation; only when applied within these time windows are protein synthesis inhibitors able to block the formation of LTM [33, 154, 173, 194]. One of the first steps in the molecular response to a learning situation appears to be the activation of certain preexisting TFs (such as CREB), which trigger the expression of other genetic regulators, such as c-*fos*, *zif/268* etc., ultimatedly leading to the expression of specific sets of effector genes [195]. The responsiveness of both preexisting and stimulus-induced TFs to different signal transduction pathways and their ability to interact with each other provide them with the important ability to integrate temporally and spatially converging patterns of stimulation (coincidence detection, see Mons et al., Lamprecht, this issue).

Neurotrophic factors, including NGF, BDNF, neurotrophin-3, -4 and -6 which regulate survival and differentiation of neurons during development, are among those quickly induced factors in paradigms of synaptic plasticity as diverse as lesion-induced sprouting [196], induction of LTP [91] and spatial learning [197] (for review see [198]). Their mRNA can be localized dendritically (e.g. BDNF and trkB mRNAs in hippocampal neurons [199]), likely supporting a quick as well as locally restricted synthesis upon stimulation. Neurotrophins are able to act on presynaptic neurons and neighboring cells, where they in turn can induce the expression of TFs such as c-*fos*, NGFI-A, and NGFI-C [200] and neuropeptides [201], and are thus perfectly suited to orchestrate the activity-dependent functional and structural reorganization of neuronal networks.

Target genes of transcriptional activators, expressed in later waves of protein synthesis, very likely include effector molecules that participate in the modification of synaptic structure. In addition, changes in signal transduction properties through, for example, differential expression of (mGluR, NMDA, AMPA or $GABA_A$) receptor subunits may occur. Changes in preexisting synaptic structures require at least two steps: (i) an initial breaking (or loosening) of the structures, for example through the activation of proteases or the internalization or glycosylation of cell-adhesion molecules, followed by (ii) a generation (or strengthening) of modified connections through reestablishing cellular contacts. It is clear that these cellular processes require a precisely controlled, locally and temporally restricted expression of proteases and their inhibitors, as well as cytoskeleton components, cell adhesion molecules and extracellular matrix molecules. As one example, the neural cell adhesion molecule (NCAM) and its homologues fasciclin II and apCAM have been implicated in learning-related and other forms of neural plasticity in rodents [202–205], *Drosophila* [123, 125] and *Aplysia* [103]. However, modifications of NCAM expression and function during learning are not restricted to the transcriptional level, but also involve endocytosis [32] and polysialylation [206] of the protein.

Generally, transcriptional activation may be envisaged as a trigger event shortly after stimulation, whereas downstream mechanisms will modify the newly synthesized proteins according to the cellular requirements. For example, several studies have implicated alternative splicing of mRNAs encoding structural proteins and receptor subunits in developmental or learning-related neural plasticity [24, 207–209]. Other mRNAs of molecules involved in memory formation, including 2,6-sialyl transferase [206, 210] and some glutamate receptor subunits [11, 211] can be modified through RNA editing. Direct evidence for an involvement of mRNA editing in neural plasticity, however, is still lacking [68].

In this context it is important to note that transport of mRNA into dendrites appears to be common for plasticity-related molecules, including microtubule-associated protein 2 (MAP2) [12, 13], CaM kinase II [212], arg3.1 [14], brain spectrin [213], trkB and BDNF [199], and probably many others not yet investigated. Such mRNA transport makes it possible that factors of neu-

ral plasticity, such as protein constituents of the postsynaptic junction, are synthesized locally [214] and thus may speed up local reorganization processes. In addition, a compartment-specific stabilization of specific mRNAs through retrograde messengers such as nitric oxide becomes possible [215], providing a mechanism for localized and transcription-independent regulation of protein synthesis. In fact, protein synthesis during the first time window after training appears to be not entirely dependent on transcriptional activation, as a limited LTM can be induced even in the presence of transcription inhibitors (e.g. [82, 98]).

It remains a great challenge to clarify how neurons can obtain the necessary synaptic specificity and guide specific mRNAs and newly synthesized proteins from the cell body to the site of activation. A very appealing hypothesis has recently been summarized by Frey and Morris [81], who suggest that during the induction of synaptic plasticity, molecular changes at the synapses concerned will alter local protein constituents. These modified (e.g. phosphorylated or proteolytically cleaved) molecules may then serve as a tag guiding newly synthesized proteins towards the site of stimulation. One candidate for such a molecular tag is—once again—the adhesion molecule apCAM, which may serve such function during sensitization of the siphon withdrawal reflex in the marine mollusk *A. californica* [216].

Conclusions

In recent years, the use of molecular techniques and mutant strategies have led to the identification of key molecules in memory formation, such as CREB, BDNF and NCAM homologues and have helped to elucidate their molecular and cellular functions [210, 216–219]. Future studies using cell type-specific and inducible mutants and highly sensitive tools for detection of gene expression, such as in situ PCR, are likely to provide more detailed insight into the regulation of gene expression and the roles of specific molecules during memory formation.

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1 James W. (1890) The Principles of Psychology (2 vols), Holt, Rinehart and Winston, New York
2 Davis H. P. and Squire L. R. (1984) Protein synthesis and memory: a review. Psychol. Bull. **96:** 518–559
3 Lee K. S., Schottler F., Oliver M. and Lynch G. (1980) Brief bursts of high-frequency stimulation produce two types of structural change in rat hippocampus. J. Neurophysiol. **44:** 247–258
4 Chang F. F. and Greenough W. T. (1984) Transient and enduring morphological correlates of synaptic activity and efficacy change in the rat hippocampal slice. Brain Res. **309:** 35–46
5 Bailey C. H. and Kandel E. R. (1993) Structural changes accompanying memory storage. Ann. Rev. Physiol. **55:** 397–426
6 Moser M.-B., Trommald M. and Andersen P. (1994) An increase in dendritic spine densitiy on hippocampal CA1 pyramidal cells following spatial learning in adult rats suggests the formation of new synapses. Proc. Natl. Acad. Sci. USA **91:** 12673–12675
7 Atwood H. L., Dixon D. and Wojtowicz J. M. (1989) Rapid introduction of long-lasting synaptic changes at crustacean neuromuscular junctions. J. Neurophysiol. **20:** 373–385
8 Wojtowicz J. M., Marin L. and Atwood H. L. (1989) Synaptic restructuring during long-term facilitation at the crayfish neuromuscular junction. Can. J. Physiol. Pharmacol. **67:** 167–171
9 Tobin A. J. (1994) Gene expression in the mammalian nervous system. In: Basic Neurochemistry, pp. 493–513, Siegel G. J., Agranoff B. W., Albers R. W., Fisher S. K., Uhler M. D. et al. (eds), Raven Press, New York
10 Silva A. J., Kogan J., Frankland P. W. and Kida S. (1998) CREB and memory. Annu. Rev. Neurosci. **21:** 127–148
11 Seeburg P. H., Higuchi M. and Sprengel R. (1998) RNA editing of brain glutamte receptor channels: mechanism and physiology. Brain Res. Rev. **26:** 217–229
12 Garner C. C., Tucker R. and Matus A. (1988) Selective localization of messenger RNA for cytoskeletal protein MAP2 in dendrites. Nature **336:** 674–677
13 Tucker R. P., Garner C. and Matus A. (1989) In situ localization of microtubule-associated protein mRNA in the developing and adult rat brain. Neuron **2:** 1245–1256
14 Link W., Konietzko U., Kauselmann G., Krug M., Schwanke B., Frey U. et al. (1995) Somatodendritic expression of immediate early gene is regulated by synaptic activity. Proc. Natl. Acad. Sci. USA **92:** 5734–5738
15 Nakova B., Kvetnansky R., McMahon A., Viskupic E., Hiremagalur B., Frankle G. et al. (1994) Induction of tyrosine hydroxylase gene expression by a nonneuronal nonpituitary-mediated mechanism in immobilization stress. Proc. Natl. Acad. Sci. USA **91:** 5937–5941
16 Smeyne R. J., Schilling K., Robertson L., Luk D., Oberdick J., Curran T. et al. (1992) fos-lacZ transgenic mice: mapping sites of gene induction in the central nervous system. Neuron **8:** 13–23
17 Anokhin K. V., Mileusnic R., Shamakina I. Y. and Rose S. P. R. (1991) Effects of early experience on c-*fos* gene expression in the chick forebrain. Brain Res. **544:** 101–107
18 Anokhin K. V. and Rose S. P. R. (1991) Learning-induced increase of immediate early gene messenger RNA in the chick forebrain. Eur. J. Neurosci. **3:** 162–167
19 Campeau S., Hayward M. D., Hope B. T., Rosen J. B., Nestler E. J. and Davis M. (1991) Induction of the c-*fos* proto-oncogene in rat amygdala during unconditioned and conditoned fear. Brain Res. **565:** 349–352
20 Campeau S., Falls W., Cullinan W. E., Helmreich D. L., Davis M. and Watson S. J. (1997) Elicitation and reduction of fear: behavioural and neuroendocrine indices and brain induction of the immediate-early gene c-fos. Neurosci. **78:** 1087–1104
21 Berger T. S. C., Kraushaar U. and Monyer H. (1998) Dentate gyrus basket cell GABAA receptors are blocked by Zn2+ via changes of their desensitization kinetics: an in situ patch-clamp and single-cell PCR study. J. Neurosci. **18:** 2437–2448
22 Mack K. J., Yi S.-D., Chang S., Millan N. and Mack P. (1995) NGFI-C expression is affected by physiological stimulation and seizure in the somatosensory cortex. Mol. Brain Res. **29:** 140–146

23 Singh T. D., Mizuno K., Kohno T. and Nakamura S. (1997) BDNF ad trkB mRNA expression in neurons of the neonatal mouse barrel field cortex: normal development and plasticity after cauterizing facial vibrissae. Neurochem. Res. **22:** 791–797

24 Rodger J., Davis S., Laroche S., Mallet J. and Hicks A. (1998) Induction of long-term potentiation in vivo regulates alternate splicing to alter syntaxin 3 isoform expression in rat dentate gyrus. J. Neurochem. **71:** 666–675

25 Diatchenko L., Lau Y. F., Campbell A. P., Chenchik A., Moqadam F., Huang B. et al. (1996) Suppression subtractive hybridization: a method for generating differentially regulated or tissue-specific cDNA probes and libraries. Proc. Natl. Acad. Sci. USA **93:** 6025–6030

26 Nedivi E., Hevroni D., Naot D., Israeli D. and Citri Y. (1993) Numerous canditate plasticity-related genes revealed by differential cDNA cloning. Nature **363:** 718–722

27 Douglass J., McKinzie A. A. and Couceyro P. (1995) PCR differential display identifies a rat brain mRNA that is transcriptionally regulated by cocaine and amphetamine. J. Neurosci. **15:** 2471–2481

28 Huang A. M., Wang H., Tang Y. P. and Lee E. H. (1998) Expression of integrin-associated protein gene associated with memory formation in rats. J. Neurosci. **18:** 4305–4513

29 Wan J., Sharp S. J., Poirier G. M.-C., Wagaman P. C., Chambers J., Pyati J. et al. (1996) Cloning differentially expressed mRNAs. Nature Biotech. **14:** 1685–1691

30 Barzilai A., Kennedy T. E., Sweatt J. D. and Kandel E. R. (1989) 5-HT modulates protein synthesis and the expression of specific proteins during long-term facilitation in *Aplysia* sensory neurons. Neuron **2:** 1577–1586

31 Beck C. H. M. and Fibiger H. C. (1995) Conditioned fear-induced changes in behavior and in the expression of the immediate early gene c-fos: with and without diazepam pretreatment. J. Neurosci. **15:** 709–720

32 Bailey C. H., Chen M., Keller F. and Kandel E. R. (1992) Serotonin-mediated endocytosis of apCAM: an early step of learning-related synaptic growth in *Aplysia*. Science **256:** 645–649

32a Schuster T., Krug M., Hassan H. and Schachner M. (1998) Increase in proportion of hippocampal spine synapses expressing neural cell adhesion molecule NCAM180 following long-term potentiation. J. Neurobiol. **37:** 359–372

33 Rose S. P. R. (1995) Cell-adhesion molecules, glucocorticoids and long-term-memory formation. Trends Neurosci. **18:** 502–506

34 Doyle E., Nolan P. M., Bell R. and Regan C. M. (1992) Hippocampal NCAM 180 transiently increases sialylation during the acquisition and consolidation of a passive avoidance response in the adult rat. J. Neurosci. Res. **31:** 513–523

35 Gale E. F., Cundliffe E., Reynolds P. E., Richmond M. H. and Waring M. J. (1981) The Molecular Basis of Antibiotic Action, 2nd ed., John Wiley, London

36 Reich E. (1963) Biochemistry of actinomycins. Cancer Res. **23:** 1428–1441

37 Chabner B. A., Allegra C. J., Curt G. A. and Calabresi P. (1995) Antineoplastic agents. In: Goodman & Gilman's The Pharmacological Basis of Therapeutics, 9th ed., pp. 1233–1287, Hardman J. G., Limbird L. E., Molinoff P. B., Ruddon R. W. and Goodman Gilman A. (eds), McGraw-Hill, New York

38 Squire L. R. and Barondes S. H. (1970) Actinomycin-D: effects on memory at different times after training. Nature **225:** 649–650

39 Flood J. F., Rosenzweig M. R., Bennett E. L. and Orme A. E. (1973) The influence of duration of protein synthesis inhibition on memory. Physiol. Behav. **10:** 555–562

40 Pestka S. (1971) Inhibitors of ribosome function. Ann. Rev. Microbiol. **25:** 487–562

41 Flood J. F., Rosenzweig M. R., Bennett E. L. and Orme A. E. (1972) Influence of training strength on amnesia induced by pretraining injections of cycloheximide. Physiol. Behav. **9:** 589–600

42 Tsien J. Z., Chen D. F., Gerber D., Tom C., Mercer E. H., Anderson D. J. et al. (1996) Subregion- and cell type-restricted gene knockout in mouse brain. Cell **87:** 1317–1326

43 Kojima N., Wang J., Mansuy I. M., Grant S. G., Mayford M. and Kandel E. R. (1997) Rescuing impairment of long-term potentiation in fyn-deficient mice by introducing Fyn transgene. Proc. Natl. Acad. Sci. USA **94:** 4761–4765

44 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

45 Olson E. N., Arnold H.-H., Rigby P. W. J. and Wold B. J. (1996) Know your neighbors: three phenotypes in null mutnats of the myogenic bHLH gene MRF4. Cell **85:** 1–4

46 Crusio W. E. (1996) Gene-targeting studies: new methods, old problems. Trends Neurosci. **19:** 186–187

47 Gerlai R. (1996) Gene-targeting studies of mammalian behavior: is it the mutation or the background genotype? Trends Neurosci. **19:** 177–181

48 Shockett P., Difillippantonio M., Hellman N. and Schatz D. G. (1995) A modified tetracycline-regulated system provides autoregulatory, inducible gene expression in cultured cells and transgenic mice. Proc. Natl. Acad. Sci. USA **92:** 6522–6526

49 Saez E., No D., West A. and Evans R. M. (1997) Inducible gene expression in mammalian cells and transgenic mice. Curr. Opin. Biotechnol. **8:** 608–616

50 Tischmeyer W., Grimm R., Schicknick H., Brysch W. and Schlingensiepen K. H. (1994) Sequence-specific impairment of learning by c-jun antisense oligonucleotides. Neuroreport **5:** 1501–1504

51 Lamprecht R., Hazvi S. and Dudai Y. (1997) cAMP response element-binding protein in the amygdala is required for long- but not short-term conditioned taste aversion memory. J. Neurosci. **17:** 8443–8450

52 Pettit D. L., Perlman S. and Malinow R. (1994) Potentiated transmission and prevention of further LTP by increased CaMKII activity in postsynaptic hippocampal slice neurons. Science **266:** 1881–1885

53 Korte M., Griesbeck O., Gravel C., Carroll P., Staiger V., Thoenen H. et al. (1996) Virus-mediated gene transfer into hippocampal CA1 region restores long-term potentiation in brain-derived neurotrophic factor mutant mice. Proc. Natl. Acad. Sci. USA **93:** 12547–12552

54 McNamara J. O., Bonhaus D. W. and Shin C. (1993) The kindling model of epilepsy. In: Epilepsy: Models, Mechanisms and Concepts, pp. 27–47, Schwartzkroin P. A. (ed.), Cambridge University Press, Cambridge

55 Sutula T., Xiao-Xian H., Cavazos J. and Scott G. (1988) Synaptic reorganization in the hippocampus induced by abnormal functional activity. Science **239:** 1147–1150

56 Cavazos J. E., Golarai G. and Sutula T. P. (1991) Mossy fibre synaptic reorganization induced by kindling: time course of development, progression and permanence. J. Neurosci. **11:** 2795–2803

57 Cain D. P., Corcoran M. E. and Staines W. A. (1980) Effects of protein synthesis inhibition on kindling in the mouse. Exp. Neurol. **68:** 409–419

58 Jones L. S., Grooms S. Y., Lapadula D. M. and Lewis D. V. (1992) Protein synthesis inhibition blocks maintenance but not induction of epileptogenesis in hippocampal slice. Brain Res. **599:** 338–344

59 Morgan J. I. and Curran T. (1991) Proto-oncogene transcription factors and epilepsy. Trends Pharmacol. Sci. **12:** 343–349

60 Labiner D. M., Butler L. S., Cao Z., Hosford D. A., Shin C. and McNamara J. O. (1993) Induction of c-*fos* mRNA by kindled seizures: complex relationship with neuronal burst firing. J. Neurosci. **13:** 744–751

61 Qian Z., Gilbert M. E., Colicos M. A., Kandel E. R. and Kuhl D. (1993) Tissue-plasminogen activator is induced as an immediate-early gene during seizure, kindling and long-term potentiation. Nature **361:** 453–457

62 Ernfors P., Bengzon J., Kokaia Z., Persson H. and Lindvall O. (1991) Increased levels of messenger RNAs for neurotrophic factors in the brain during kindling epileptogenesis. Neuron **7:** 165–176
63 Bengzon J., Kokaia Z., Ernfors P., Kokaia M., Leanza G., Nilsson O. G. et al. (1993) Regulation of neurotrophin and trkA, trkB and trkC tyrosine kinase receptor messenger RNA expression in kindling. Neurosci. **53:** 433–446
64 Schwarzer C., Sperk G., Samanin R., Rizzi M., Gariboldi M. and Vezzani A. (1996) Neuropeptides-immunoreactivity and their mRNA expression in kindling: functional implications for limbic epileptogenesis. Brain Res. Rev. **22:** 27–50
65 Pratt G. D., Kokaia M., Bengzon J., Kokaia Z., Fritschy J.-M., Möhler H. et al. (1993) Differential regulation of *N*-methyl-D-aspartate receptor subunit messenger RNAs in kindling-induced epileptogenesis. Neurosci. **57:** 307–318
66 Clark M., Smith M. A., Weiss S. R. B. and Post R. M. (1994) Modulation of hippocampal glucocorticoid and mineralocorticoid receptor mRNA expression by amygdaloid kindling. Neuroendocrinol. **59:** 451–456
67 Kamphuis W., De Rijk T. C., Talamini L. M. and Lopes da Silva F. H. (1994) Rat hippocampal kindling induces changes in the glutamate receptor mRNA expression patterns in dentate granule neurons. Eur. J. Neurosci. **6:** 1119–1127
68 Kamphuis W. and da Silva F. (1995) Editing status of the Q/R site of glutamate receptor-A, -B, -5 and -6 subunit mRNA in the hippocampal kindling model of epilepsy. Mol. Brain Res. **29:** 35–42
69 Kokaia M., Pratt G. D., Elmér E., Bengzon J., Fritschy J.-M., Kokaia Z. et al. (1994) Biphasic differential changes of $GABA_A$ receptor subunit mRNA levels in dentate gyrus granule cells following recurrent kindling-induced seizures. Mol. Brain Res. **23:** 323–332
70 Akbar M. T., Rattray M., Powell J. F. and Meldrum B. S. (1996) Altered expression of group I metabotropic glutamate receptors in the hippocampus of amygdala-kindled rats. Mol. Brain Res. **43:** 105–116
71 Elmer E., Alm P., Kokaia Z., Kokaia M., Larsson B., Andersson K. E. et al. (1996) Regulation of neuronal nitric oxide synthase mRNA levels in rat brain by seizure activity. Neuroreport **7:** 1335–1339
72 Bliss T. V. P. and Collingridge G. L. (1993) A synaptic model of memory: long-term potentiation in the hippocampus. Nature **361:** 31–39
73 Richter-Levin G., Collingridge L. and Bliss T. V. (1995) Long-term potentiation and glutamate release in the dentate gyrus: links to spatial learning. Behav. Brain Res. **66:** 37–40
74 Maren S. and Fanselow M. (1995) Synaptic plasticity in the basolateral amygdala induced by hippocampal formation stimulation in vivo. J. Neurosci. **15:** 7548–7564
75 Rogan M. T., Staubli U. and LeDoux J. E. (1997) Fear conditioning induces associative long-term potentiation in the amygdala. Nature **390:** 604–607
76 Frey U., Krug M., Reymann K. G. and Matthies H. (1988) Anisomycin, an inhibitor of protein synthesis, blocks late phases of LTP phenomena in the hippocampal CA1 region in vitro. Brain Res. **452:** 57–65
77 Stanton P. K. and Sarvey J. M. (1984) Blockade of long-term potentiation in rat hippocampal CA1 region by inhibitors of protein synthesis. J. Neurosci. **4:** 3080–3088
78 Huang Y.-Y., Li X.-C. and Kandel E. R. (1994) cAMP contributes to mossy fiber LTP by initiating both a covalently mediated early phase and macromolecular synthesis-dependent late phase. Cell **79:** 69–79
78a Nguyen P. V., Abel T. and Kandel E. R. (1994) Requirement of a critical period of transcription for induction of a late phase of LTP. Science **265:** 1104–1107
79 Namgung U., Valcourt E. and Routtenberg A. (1995) Long-term potentiation in vivo in the intact mouse hippocampus. Brain Res. **689:** 85–92
80 Linden D. J. (1996) A protein synthesis-dependent late phase of cerebellar long-term depression. Neuron **17:** 483–490
81 Frey U. and Morris R. G. M. (1998) Synaptic tagging: implications for late maintainance of hippocampal long-term potentiation. Trends Neurosci. **21:** 181–188
82 Otani S., Marshall C. J., Tate W. P., Goddard G. V. and Abraham W. C. (1989) Maintenance of long-term potentiation in rat dentate gyrus requires protein synthesis but not messenegr RNA synthesis immediately post-tetanization. Neurosci. **28:** 519–526
83 Steward O. and Levy W. B. (1982) Preferential localization of polyribosomes under the base of dendritic spines in granule cells of the dendate gyrus. J. Neurosci. **2:** 284–291
84 Steward O. and Reeves T. M. (1988) Protein-synthetic machinery beneath postsynaptic sites on CNS neurons: association between polyribosomes and other organelles at the synaptic site. J. Neurosci. **8:** 176–184
85 Impey S., Mark M., Villacres E. C., Poser S., Chavkin C. and Storm D. R. (1996) Induction of CRE-mediated gene expression by stimuli that generate long-lasting LTP in area CA1 of the hippocampus. Neuron **16:** 973–982
86 Abel T., Nguyen P. V., Barad M., Deuel T. A. S., Kandel E. R. and Bourtchouladze R. (1997) Genetic demonstration of a role for PKA in the late phase of LTP and in hippocampus-based long-term memory. Cell **88:** 615–626
87 Bourtchuladze R., Frenguelli B., Blendy J., Cioffi D., Schutz G. and Silva A. J. (1994) Deficient long-term memory in mice with a targeted mutation of the cAMP-responsive element-binding protein. Cell **79:** 59–68
88 Abraham W. C., Dragunow M. and Tate W. P. (1991) The role of immediate early genes in the stabilization of long-term potentiation. Mol. Neurobiol. **5:** 297–314
89 Williams J., Dragunow M., Lawlor P., Mason S., Abraham W. C., Leah J. et al. (1995) Krox20 may play a key role in the stabilization of long-term potentiation. Mol. Brain Res. **28:** 87–93
90 Castrén E., Pitkänen M., Sirviö J., Parsadanian A., Lindholm D., Thoenen H. et al. (1993) The induction of LTP increases BDNF and NGF mRNA but decreases NT-3 mRNA in the dentate gyrus. Neuroreport **4:** 895–898
91 Bramham C. R., Sourthard T., Sarvey J. M., Herkenham M. and Brady L. S. (1996) Unilateral LTP triggers bilateral increases in hippocampal neurotrophin and trk receptor mRNA expression in behaving rats: evidence for interhemispheric communication. J. Comp. Neurol. **368:** 371–382
92 Dragunow M., Hughes P., Mason-Parker S. E., Lawlor P. and Abraham W. C. (1997) TrkB expression in dentate granule cells is associated with a late phase of long-term potentiation. Mol. Brain Res. **46:** 274–280
93 Roberts L. A., Large C. H., O'Shaughnessy C. T. and Morris B. J. (1997) Long-term potentiation in perforant path/granule cell synapses is associated with a post-synaptic induction of proenkephalin gene expression. Neurosci. Lett. **227:** 205–208
94 Smirnova T., Laroche S., Errington M. L., Hicks A. A., Bliss T. V. P. and Mallet J. (1993) Transsynaptic expression of a presynaptic glutamate receptor during hippocampal long-term potentiation. Science **262:** 433–436
95 Nayak A., Zastrov D. J., Lickteig R., Zahniser N. R. and Browning M. D. (1998) Maintenance of late-phase LTP is accompanied by PKA-dependent increase in AMPA receptor synthesis. Nature **394:** 680–683
96 Korte M., Kang H., Bonhoeffer T. and Schuman E. (1998) A role for BDNF in the late-phase of hippocampal long-term potentiation. Neuropharmacol. **37:** 553–559
97 Korte M., Caroll P., Wolf E., Brem G., Thoenen H. and Bonhoeffer T. (1995) Hippocampal long-term potentiation is impaired in mice lacking brain-derived neurotrophic factor. Proc. Natl. Acad. Sci. USA **92:** 8856–8860
98 Martin K. C., Casadio A., Zhu H., Yaping E., Rose J. C., Chen M. et al. (1997) Synapse-specific, long-term facilitation of *Aplysia* sensory to motor synapses: a function for local protein synthesis in memory storage. Cell **91:** 927–938

99 Castellucci V. F., Blumenfeld H., Goelet P. and Kandel E. R. (1989) Inhibitor of protein synthesis blocks long-term behavioral sensitization in the isolated gill-withdrawal reflex in *Aplysia*. J. Neurobiol. **20:** 1–9

100 Montarolo P. G., Goelet P., Castellucci V. F., Morgan J., Kandel E. R. and Schacher S. (1986) A critical period of macromolecular synthesis in long-term heterosynaptic facilitation in *Aplysia*. Science **234:** 1249–1254

101 Bailey C. H., Montarolo P., Chen M., Kandel E. R. and Schacher S. (1992) Inhibitors of protein and RNA synthesis block structural changes that accompany long-term heterosynaptic plasticity in *Aplysia*. Neuron **9:** 749–758

102 O'Leary F. A., Byrne J. H. and Cleary L. J. (1995) Long-term structural remodeling in *Aplysia* sensory neurons requires de novo protein synthesis during a critical time period. J. Neurosci. **15:** 3519–3525

103 Mayford M., Barzilai A., Keller F., Schacher S. and Kandel E. R. (1992) Modulation of an NCAM-related adhesion molecule with long-term synaptic plasticity in *Aplysia*. Science **256:** 638–644

104 Castellucci V. F., Kennedy T. E., Kandel E. R. and Goelet P. (1988) A quantitative analysis of 2-D gels identifies proteins in which labeling is increased following long-term sensitization in *Aplysia*. Neuron **1:** 321–328

105 Alberini C., Ghirardi M., Metz R. and Kandel E. R. (1994) C/EBP is an immediate-early gene required for the consolidation of long-term facilitation in *Aplysia*. Cell **76:** 1099–1114

106 Hedge A. N., Inokuchi K., Pei W., Casadio A., Ghirardi M., Chain D. G. et al. (1997) Ubiquitin C-terminal hydrolase is an immediate-early gene essential for long-term facilitation in *Aplysia*. Cell **89:** 115–126

107 Zwartjes R. E., West H., Hattar S., Ren X., Noel F., Nuñez-Regueiro M. et al. (1998) Identification of specific mRNAs affected by treatments producing long-term facilitation in *Aplysia*. Learn. Mem. **4:** 478–495

108 Kuhl D., Kennedy T. E., Barzilai A. and Kandel E. R. (1992) Long-term sensitization training in *Aplysia* leads to an increase in the expression of BiP, the major protein chaperon of the ER. J. Cell Biol. **119:** 1069–1076

109 Hu Y., Barzilai A., Chen M., Bailey C. H. and Kandel E. R. (1993) 5-HT and cAMP induce the formation of coated pits and vesicles and increase the expression of clathrin light chain in sensory neurons of *Aplysia*. Neuron **10:** 921–929

110 Noel F., Nuñez-Regueiro M., Cook R., Byrne J. H. and Eskin A. (1993) Long-term changes in synthesis of intermediate filament protein, actin and other proteins in pleural sensory neurons of *Aplysia* produced by an in vitro analogue of sensitization training. Mol. Brain Res. **19:** 203–210

111 Kennedy T. E., Kuhl D., Barzilai A., Sweatt J. D. and Kandel E. R. (1992) Long-term sensitization training in *Aplysia* leads to an increase in calreticulin, a major presynaptic calcium-binding protein. Neuron **9:** 1013–1024

112 Trudeau L. E. and Castellucci V. F. (1995) Postsynaptic modifications in long-term facilitation in *Aplysia*: upregulation of excitatory amino acid receptors. J. Neurosci. **15:** 1275–1284

113 Alkon D. L., Ikeno H., Dworkin J., McPhie D. L., Olds J. L., Lederhendler I. et al. (1990) Contraction of neuronal branching volume: an anatomical correlate of Pavlovian conditioning. Proc. Natl. Acad. Sci. USA **87:** 1611–1614

114 Ramirez R. R., Gandhi C. C., Muzzio I. A. and Matzel L. D. (1998) Protein synthesis-dependent memory and neuronal enhancement in *Hermissenda* are contingent on parameters of training and retention. Learn. Mem. **4:** 462–477

115 Crow T. and Forrester J. (1990) Inhibition of protein synthesis blocks long-term enhancement of generator potentials produced by one-trial in vivo conditioning in *Hermissenda*. Proc. Natl. Acad. Sci. USA **87:** 4490–4494

116 Crow T., Siddiqi V. and Dash P. K. (1997) Long-term enhancement but not short-term in *Hermissenda* is dependent upon mRNA synthesis. Neurobiol. Learn. Mem. **68:** 343–350

117 Nelson T. J. and Alkon D. L. (1988) Prolonged RNA changes in the *Hermissenda* eye induced by classical conditioning. Proc. Natl. Acad. Sci. USA **85:** 7800–7804

118 Nelson T. J. and Alkon D. L. (1990) Specific high molecular weight mRNAs induced by associative learning in *Hermissenda*. Proc. Natl. Acad. Sci. USA **87:** 269–273

119 Nelson T. J., Collin C. and Alkon D. L. (1990) Isolation of a G protein that is modified by learning and reduces potassium currents in *Hermissenda*. Science **247:** 1479–1483

120 Tully T., Preat T., Boynton S. C. and Del Vecchio M. (1994) Genetic dissection of consolidated memory in *Drosophila melanogaster*. Cell **79:** 35–47

121 Yin J. C., Wallach J., 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

122 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

123 Schuster C. M., Davis G. W., Fetter R. D. and Goodman C. S. (1996) Genetic dissection of structural and functional components of synaptic plasticity. I. Fasciclin II controls synaptic stabilization and growth. Neuron **17:** 641–654

124 Davis G. W., Schuster C. M. and Goodman C. S. (1996) Genetic dissection of structural and functional components of synaptic plasticity. III. CREB is necessary for presynaptic functional plasticity. Neuron **17:** 669–679

125 Schuster C. M., Davis G. W., Fetter R. D. and Goodman C. S. (1996) Genetic dissection of structural and functional components of synaptic plasticity. II. Fasciclin II controls presynaptic structural plasticity. Neuron **17:** 655–667

126 Jaffé K. (1980) Effect of cycloheximide on protein synthesis and memory in praying mantis. Physiol. Behav. **25:** 367–371

127 Wittstock S., Kaatz H.-H. and Menzel R. (1993) Inhibition of brain protein synthesis by cycloheximide does not affect formation of long-term memory in honeybees after olfactory conditioning. J. Neurosci. **13:** 1379–1386

128 Wittstock S. and Menzel R. (1994) Color learning and memory in honey bees are not affected by protein synthesis inhibition. Behav. Neural Biol. **62:** 224–229

129 Grünbaum L. and Müller U. (1998) Induction of a specific olfactory memory leads to a long-lasting activation of protein kinase C in the antennal lobe of the honey bee. J. Neurosci. **18:** 4384–4392

130 Gibbs M. E. and Lecanuet J.-P. (1981) Disruption of imprinting by memory inhibitors. Anim. Behav. **29:** 572–580

131 Horn G., Bradley P. and McCabe B. J. (1985) Changes in the structure of synapses associated with learning. J. Neurosci. **5:** 3161–3168

132 Bateson P. P. G., Horn G. and Rose S. P. R. (1969) Effects of an imprinting procedure on regional incorporation of tritiated leucine into proteins of chick brain. Nature **223:** 534–535

133 Horn G., McCabe B. J. and Bateson P. P. G. (1979) An autoradiographic study of the chick brain after imprinting. Brain Res. **168:** 361–373

134 Rose S. P. R. (1977) Early visual experience, learning and neurochemical plasticity in the rat and chick. Phil. Trans. R. Soc. London B **278:** 307–318

135 Brown M. W. and Horn G. (1990) Are specific proteins implicated in the learning process of imprinting? Dev. Brain Res. **52:** 294–297

136 McCabe B. J. and Horn G. (1994) Learning-related changes in Fos-like immunoreactivity in the chick forebrain after imprinting. Proc. Natl. Acad. Sci. USA **91:** 11417–11421

137 Meberg P. J., McCabe B. J. and Routtenberg A. (1996) MARCKS and protein F1/GAP-43 mRNA in chick brain: effects of imprinting. Mol. Brain Res. **35:** 149–156

138 Ambalavanar R., McCabe B. J. and Horn G. (1993) Fos-like immunoreactivity in γ-aminobutyric acid (GABA)-containing neurones in a forebrain region of the domestic chick. J. Physiol. **467:** 350P

139 Ambalavanar R., Van der Zee E. A., Bolhuis J. J., McCabe B. J. and Horn G. (1993) Co-expression of Fos immunoreactivity in protein kinase (PKCγ)-positive neurones: quantitative analysis of a brain region involved in learning. Brain Res. **606:** 315–318
140 Harvey R. J., McCabe B. J., Solomonia R. O., Horn G. and Darlison M. G. (1998) Expression of the $GABA_A$ receptor γ4-subunit gene: anatomical distribution of the corresponding mRNA in the domestic chick forebrain and the effect of imprinting training. Eur. J. Neurosci. **10:** 3024–3028
141 Solomonia R. O., McCabe B. J., Jackson A. P. and Horn G. (1997) Clathrin proteins and recognition memory. Neurosci. **80:** 59–67
142 Godard R. (1991) Long-term memory of individual neighbours in a migratory songbird. Nature **350:** 228–229
143 Chew S. J., Vicario D. S. and Nottebohm F. (1996) Quantal duration of auditory memories. Science **274:** 1909–1914
144 Mello C. V., Vicario D. S. and Clayton D. F. (1992) Song presentation induces gene expression in the songbird forebrain. Proc. Natl. Acad. Sci. USA **89:** 6818–6822
145 Mello C., Nottebohm F. and Clayton D. (1995) Repeated exposure to one song leads to a rapid and persistent decline in an immediate early gene's response to that song in zebra finch telencephalon. J. Neurosci. **15:** 6919–6925
146 Nastiuk K. L., Mello C. V., George J. M. and Clayton D. F. (1994) Immediate-early gene responses in the avian song control system: cloning and expression analysis of the canary c-*jun* cDNA. Mol. Brain Res. **27:** 299–309
147 Chew S. J., Mello C., Nottebohm F., Jarvis E. and Vicario D. S. (1995) Decrements in auditory responses to a repeated conspecific song are long-lasting and require two periods of protein synthesis in the songbird forebrain. Proc. Natl. Acad. Sci. USA **92:** 3406–3410
148 Vicario D. S. and Nottebohm F. (1998) Quantal memory durations: observations not reproduced. Science **279:** 1437
149 Rose S. P. R. (1991) How chicks make memories: the cellular cascade from c-fos to dendritic remodelling. Trends Neurosci. **14:** 390–397
150 Sojka M., Davies H. A., Harrison E. and Stewart M. G. (1995) Long-term increases in synaptic density in chick CNS after passive avoidance training are blocked by an inhibitor of protein synthesis. Brain Res. **684:** 209–214
151 Stewart M. G. (1991) Changes in dendritic and synaptic structure in chick forebrain consequent on passive avoidance learning. In: Neural and Behavioural Plasticity, pp. 305–328, Andrew R. J. (ed.), Oxford University Press, Oxford
152 Freeman F. M., Rose S. P. R. 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
153 Tiunova A., Anokhin K. V., Rose S. P. R. and Mileusnic R. (1996) Involvement of glutamate receptors, protein kinases and protein synthesis in memory for visual discrimination in the young chick. Neurobiol. Learn. Mem. **65:** 233–243
154 Tiunova A., Anokhin K. V. and Rose S. P. R. (1998) Two critical periods of protein and glycoprotein synthesis in memory consolidation for visual categorization learning in chicks. Learn. Mem. **4:** 401–410
155 Mileusnic R., Rose S. P. R. and Tillson P. (1980) Passive avoidance learning results in region-specific changes in concentration of and incorporation into colchicine-binding proteins in chick forebrain. J. Neurochem. **34:** 1007–1015
156 Sukumar R., Rose S. P. R. and Burgoyne R. D. (1980) Increased incorporation of [^{3}H]fucose into chick brain glycoproteins following training on a passive avoidance task. J. Neurochem. **34:** 1000–1006
157 Bullock S., Rose S. P. R. and Zamani R. (1992) Characterisation and regional localisation of pre- and postsynaptic glycoproteins of the chick forebrain showing changed fucose incorporation following passive avoidance training. J. Neurochem. **58:** 2145–2154
158 Scholey A. B., Rose S. P. R., Zamani M. R., Bock E. and Schachner M. (1993) A role for the neural cell adhesion molecule in a late, consolidating phase of glycoprotein synthesis six hours following passive avoidance training of the young chick. Neurosci. **55:** 499–509
159 Scholey A. B., Mileusnic R., Schachner M. and Rose S. P. R. (1995) A role for a chicken homolog of the neural cell adhesion molecule L1 in consolidation of memory for a passive avoidance task in the chick. Learn. Mem. **2:** 17–25
160 Sandi C. and Rose S. P. R. (1992) Protein synthesis- and fucosylation-dependent mechanisms in corticosterone facilitation of long-term memory in the chick. Behav. Neurosci. **111:** 1098–1104
161 Rose S. P. R. and Jork R. (1987) Long-term memory in the chick is blocked by 2-deoxygalactose, a fucose analogue. Behav. Neural Biol. **48:** 246–258
162 Bourne R. C., Davies D. C., Stewart M. G., Csillag A. and Cooper M. (1991) Cerebral glycoprotein synthesis and long-term memory formation in chick (*Gallus domesticus*) following passive avoidance training depends on the nature of the aversive stimulus. Europ. J. Neurosci. **3:** 243–248
163 Quatermain D. and McEwen B. S. (1970) Temporal characteristics of amnesia induced by protein synthesis inhibitor: determination by shock level. Nature **228:** 677–678
164 Quinton E. E. and Kramarcy N. R. (1977) Memory impairment correlates closely with cycloheximide dose and degree of inhibition of protein synthesis. Brain Res. **131:** 184–190
165 Stäubli U., Faraday R. and Lynch G. (1985) Pharmacological dissociation of memory: anisomycin, a protein synthesis inhibitor, and leupeptin, a protease inhibitor, block different learning tasks. Behav. Neural Biol. **43:** 287–297
166 Lee E. H. Y., Hung H. C., Lu K. T., Chen W. H. and Chen H. Y. (1992) Protein synthesis in the hippocampus associated with memory facilitation by corticotropin-releasing factor in rats. Peptides **13:** 927–937
167 Berman R. F., Kesner R. P. and Partlow L. M. (1978) Passive avoidance impairment in rats following cycloheximide injection into the amygdala. Brain Res. **158:** 171–188
168 Flood J. F., Bennett E. L., Orme A. E. and Rosenzweig M. R. (1975) Effects of protein synthesis inhibition on memory for active avoidance. Physiol. Behav. **14:** 177–184
169 Flood J. F., Jarvik M. E., Bennett E. L., Orme A. E. and Rosenzweig M. R. (1977) Protein synthesis inhibition and memory for pole jump active avoidance and extinction. Pharmacol. Biochem. Behav. **7:** 71–77
170 Nikolaev E., Kaminska B., Tischmeyer W., Matthies H. and Kaczmarek L. (1992) Induction of expression of genes encoding transcription factors in the rat brain elicited by behavioral training. Brain Res. Bull. **28:** 479–484
171 Squire L. R. and Barondes S. H. (1974) Anisomycin, like other inhibitors of cerebral protein synthesis, impairs 'long-term' memory of a discrimination task. Brain Res. **66:** 301–308
172 Malefant S. A., Barry M. and Fleming A. S. (1991) Effects of cycloheximide on the retention of olfactory learning and maternal experience effects in postpartum rats. Physiol. Behav. **49:** 289–294
173 Grecksch G. and Matthies H. (1980) Two sensitive periods for the amnesic effect of anisomycin. Pharmacol. Biochem. Behav. **12:** 663–665
174 Grimm R. and Tischmeyer W. (1997) Complex patterns of immediate early gene induction in rat brain following brightness discrimination training and pseudotraining. Behav. Brain Res. **84:** 109–116
175 Popov N., Schulzeck S., Pohle W. and Matthies H. (1980) Changes in the incorporation of [^{3}H]fucose into rat hippocampus after acquisition of a brightness discrimination reaction. An electrophoretic study. Neurosci. **5:** 161–167
176 Meiri N. and Rosenblum K. (1998) Lateral ventricle injection of the protein synthesis inhibitor anisomycin impairs long-term memory in a spatial memory task. Brain Res. **789:** 48–55
177 Guzowski J. F. and McGaugh J. L. (1997) Antisense ologideoxynucleotide-mediated disruption of hippocampal cAMP response element binding protein levels impairs con-

solidation of memory for water maze training. Proc. Natl. Acad. Sci. USA **94:** 2693–2698
178 Paylor R., Johnson R., Papaioannou V., Spiegelman B. M. and Wehner J. M. (1994) Behavioral assessment of c-fos mutant mice. Brain Res **651:** 275–282
179 Cavallaro S., Meiri N., Yi C.-L., Musco S., Ma W., Goldberg J. et al. (1997) Late memory-related genes in the hippocampus revealed by RNA fingerprinting. Proc. Natl. Acad. Sci. USA **94:** 9669–9673
180 Murphy K. J., O'Connell A. W. and Regan C. M. (1996) Repetitive and transient increases in hippocampal neural cell adhesion molecule polysialylation state following multitrial spatial training. J. Neurochem. **67:** 1268–1274
181 O'Connell A. W., Fox G. B., Barry T., Murphy K. J., Fichera G., Foley A. G. et al. (1997) Spatial learning activates neural cell adhesion molecule polysialylation in a corticohippocampal pathway within the medial temporal lobe. J. Neurochem. **68:** 2538–2546
182 Davis S., Rodger J., Hicks A., Mallet J. and Laroche S. (1996) Brain structure and task-specific increases in expression of the gene encoding syntaxin 1B during learning in the rat: a potential molecular marker for learning-induced synaptic plasticity in neural networks. Eur. J. Neurosci. **8:** 2068–2074
183 Schmaltz G. and Marcant P. (1983) Transient aversion and long-lasting amnesia following cycloheximide injection in the rat. Physiol. Behav. **30:** 845–852
184 Kogan J. H., Frankland P. W., Blendy J. A., Coblentz J., Marowitz Z., Schutz G. et al. (1997) Spaced training induces normal long-term memory in CREB mutant mice. Curr. Biol. **7:** 1–11
185 Milanovic S., Radulovic J., Laban O., Stiedl O., Henn F. and Spiess J. (1998) Production of the Fos protein after contextual fear conditioning of C57BL/6N mice. Brain Res. **784:** 37–47
186 Rosen J. B., Fanselow M., Young S. L., Sitcoske M. and Maren S. (1998) Immediate-early gene expression in the amygdala following footshock stress and contextual fear conditioning. Brain Res. **796:** 132–142
187 Sterneck E., Paylor R., Jackson-Lewis V., Libbey M., Przedborski S., Tessarollo L. et al. (1998) Selectively enhanced contextual fear conditioning in mice lacking the transcriptional regulator CCAAT/enhancer binding protein delta. Proc. Natl. Acad. Sci. USA **95:** 10908–10913
188 Tucker A. and Gibbs M. (1976) Cycloheximide-induced amnesia for taste aversion memory in rats. Pharmacol. Biochem. Behav. **4:** 181–184
189 Rosenblum K., Meiri N. and Dudai Y. (1993) Taste memory: the role of protein synthesis in gustatory cortex. Behav. Neural Biol. **59:** 49–56
190 Bartsch D. G. M., Skehel P. A., Karl K. A., Herder S. P., Chen M., Bailey C. H. et al. (1995) *Aplysia* CREB2 represses long-term facilitation: relief of repression converts transient facilitation into long-term functional and structural change. Cell **83:** 979–992
191 Kandel E. R. and O'Dell T. J. (1992) Are adult learning mechanisms also used for development? Science **258:** 243–245
192 Castrén E., Zafra F., Thoenen H. and Lindholm D. (1992) Light regulates expression of brain-derived neurotrophic factor mRNA in rat visual cortex. Proc. Natl. Acad. Sci. USA **89:** 9444–9448
193 Rocamora N., Welker E., Pascual M. and Soriano E. (1996) Upregulation of BDNF mRNA expression in the barrel cortex of adult mice after sensory stimulation. J. Neurosci. **16:** 4411–4419
194 Tiunova A., Anokhin K. V., Schachner M. and Rose S. P. R. (1998) Three time windows for amnestic effect of antibodies to cell adhesion molecule L1 in chicks. Neuroreport **9:** 1645–1648
195 Sheng M. and Greenberg M. E. (1990) The regulation and function of c-fos and other immediate early genes in the nervous system. Neuron **4:** 477–485
196 Lapchak P. A., Araujo D. and Hefti F. (1993) BDNF and trkB mRNA expression in the rat hippocampus following entorhinal cortex lesions. Neuroreport **4:** 191–194
197 Falkenberg T., Mohammed A. K., Henriksson B., Persson H., Winblad B. and Lindefors N. (1992) Increased expression of brain-derived neurotrophic factor mRNA in rat hippocampus is associated with improved spatial memory and enriched environment. Neurosci. Lett. **138:** 153–156
198 Thoenen H. (1995) Neurotrophins and neuronal plasticity. Science **270:** 593–598
199 Tongiorgi E., Righi M. and Cattaneo A. (1997) Activity-dependent dendritic targeting of BDNF and TrkB mRNAs in hippocampal neurons. J. Neurosci. **17:** 9492–9505
200 Sharp F. R., Gonzales M., Hisanaga K., Mobley W. C. and Sagar S. M. (1989) Induction of the c-fos gene product in rat forebrain following cortical lesions and NGF injections. Neurosci. Lett. **100:** 117–122
201 Carnahan J and Nawa H (1995) Regulation of neuropeptide expression in the brain by neurotrophins. Potential role in vivo. Mol. Neurobiol. **10:** 135–139
202 Lüthi A., Laurent S. P., Figurov A., Müller D. and Schachner M. (1994) Hippocampal long-term potentiation and neural cell adhesion molecules L1 and NCAM. Nature **372:** 777–779
203 Cremer H., Lange R., Christoph A., Plomann M., Vopper G., Roes J. et al. (1994) Inactivation of the NCAM gene in mice results in size-reduction of the olfactory bulb and deficts in spatial learning. Nature **367:** 455–459
204 Jucker M., Mondadori C., Mohajeri H., Bartsch U. and Schachner M. (1995) Transient upregulation of NCAM mRNA in astrocytes in response to entorhinal cortex lesions and ischemia. Mol. Brain Res. **28:** 149–156
205 Arami S., Jucker M., Schachner M. and Welzl H. (1996) The effect of continuous intraventricular infusion of L1 and NCAM antibodies on spatial learning in rats. Behav. Brain Res. **81:** 81–87
206 Rutishauser U. and Landmesser L. (1996) Polysialic acid in the vertebrate nervous system: a promoter of plasticity in cell-cell interactions. Trends Neurosci. **19:** 422–427
207 Jacobsson G., Piehl F., Bark I. C., Zhang X. and Meister B. (1996) Differential subcellular localization of SNAP-25a and SNAP-25b RNA transcripts in spinal motoneurons and plasticity in expression after nerve injury. Mol. Brain Res. **37:** 49–62
208 Collet J., Fehrat L., Pollard H., de Pouplana L. R., Charton G., Bernard A. et al. (1997) Developmentally regulated alternative splicing of mRNAs encoding N-terminal tau variants in the rat hippocampus: structural and functional implications. Eur. J. Neurosci. **9:** 2723–2733
209 Yamaguchi S. and Nakanishi S. (1998) Regional expression and regulation of alternative forms of mRNAs derived from two distinct transcription initiation sites of the rat mGluR5 gene. J. Neurochem. **71:** 60–68
210 Fields R. D. and Itoh K. (1996) Neural cell adhesion molecules in activity-dependent development and synaptic plasticity. Trends Neurosci. **19:** 473–480
211 Kashiwabuchi N., Ikeda K., Araki K., Hirano T., Shibuki K., Takayama C. et al. (1995) Impairment of motor coordination, Purkinje cell synapse formation and cerebellar long-term depression in GluR delta 2 mutant mice. Cell **81:** 245–252
212 Mayford M., Baranes D., Podsypanina K. and Kandel E. R. (1994) The 3′-untranslated region of CaMKII alpha is a cis-acting signal for the localization and translation of mRNA in dendrites. Proc. Natl. Acad. Sci. USA **93:** 13250–13255
213 Gelot A., Moreau J., Ben Ari Y. and Pollard H. (1996) Alpha-brain spectrin mRNA belongs to the population of intradendritically transported mRNAs. Neuroreport **8:** 113–116
214 Steward O. (1993) Molecular sorting in neurons: cell biological processes that play a role in synapse growth and plasticity. In: Synaptic Plasticity, pp. 13–43, Baudry M.,

Thompson R. F. and Davis J. L. (eds), Bradford Book, MIT Press, Cambridge, MA

215 Morris B. J. (1997) Stabilization of dendritic mRNAs by nitric oxide allows localized, activity-dependent enhancement of hippocampal protein synthesis. Eur. J. Neurosci. **9:** 2334–2339

216 Martin K. C. and Kandel E. R. (1996) Cell adhesion molecules, CREB and the formation of new synaptic connections. Neuron **17:** 567–570

217 Tully T. (1996) Discovery of genes involved with learning and memory: an experimental synthesis of Hirschian and Benzerian perspectives. Proc. Natl. Acad. Sci. USA **93:** 13460–13467

218 Wehner J. M., Bowers B. and Paylor R. (1996) The use of null mutant mice to study complex learning and memory processes. Behav. Genet. **23:** 301–312

219 Lopatina N. G., Sharagina L. and Chesnokova E. G. (1997) Genetic approaches to the study of memory in insects. Neurosci. Behav. Physiol. **27:** 250–253