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In search of general mechanisms for long-lasting plasticity: *Aplysia* and the hippocampus

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Long-term synaptic plasticity is thought to underlie many forms of long-lasting memory. Long-lasting plasticity has been most extensively studied in the marine snail *Aplysia* and in the mammalian hippocampus, where Bliss and Lømo first described long-term potentiation 30 years ago. The molecular mechanisms of plasticity in these two systems have proven to have many similarities. Here, we briefly describe some of these areas of overlap. We then summarize recent advances in our understanding of the mechanisms of long-lasting synaptic facilitation in *Aplysia* and suggest that these may prove fruitful areas for future investigation in the mammalian hippocampus and at other synapses in the mammalian brain.

Keywords: long-term potentiation; facilitation; hippocampus; *Aplysia*; long-term plasticity

1. INTRODUCTION

Memory formation requires the long-term storage of information in the brain, and long-lasting synaptic plasticity is thought to be a principal mechanism by which this information is stored. Learning-related synaptic plasticity has been most thoroughly studied in the marine snail *Aplysia* and in the mammalian hippocampus, where Bliss & Lømo (1973) first described the phenomenon of LTP 30 years ago. The relationship between behavioural change and plasticity at a particular set of synapses is necessarily complex in a neural system as complicated as the mammalian brain, especially in hippocampus-based explicit memory. In spatial memory involving the hippocampus, the synaptic changes that contribute to a given behavioural change are likely to be distributed across many synapses. Even an apparently unitary behavioural change may require changes in different populations of synapses, such as the different synapses in the hippocampal circuit and elsewhere in the medial temporal lobe. Finally, several dissociable forms of plasticity can coexist at the same synapse, potentially obscuring the relationship between learning and experimentally observed synaptic change. Nevertheless, a number of instances in which molecules and pathways involved in LTP have independently been implicated in learning have validated LTP as a useful model of the plasticity underlying hippocampus-dependent memory.

The mechanisms of memory can be divided into two parts: the molecular mechanisms and the systems properties of storage. Study of the molecular mechanisms of plasticity in the hippocampus has been complemented and counterbalanced by studies in simpler model systems, such as the marine snail *Aplysia*. While there will, of course, be aspects of the systems properties of hippocampus-based memory that cannot be recapitulated in a simple invertebrate, these molecular mechanisms are proving to be remarkably well conserved. In many instances, synergy between the two systems has advanced our understanding more quickly than would have been possible with either alone. In a complex area, such as the mechanisms of learning-related synaptic plasticity, results from any single model of plasticity must be accepted with caution. When similar or identical molecules and mechanisms are implicated in two such different model systems, as has been the case in many instances, we can be much more confident of their validity and importance.

Studies of the late phase of synaptic plasticity illustrate this point. Persistence of both memory and plasticity requires regulated gene induction and the production of new proteins. When these processes are blocked pharmacologically in various model systems, both memory (Flexner *et al.* 1965; Agranoff 1967; Castellucci *et al.* 1986; Freeman *et al.* 1995; Bourtchouladze *et al.* 1998) and synaptic plasticity (Castellucci *et al.* 1986; Huang *et al.* 1996) are truncated to a short period of time after training or stimulation. Many behaviourally relevant memories, including those that we hold most dear and that define our individuality, must persist in the long term. Long-lasting, transcription-dependent plasticity therefore merits close investigation. Recently, there have been three major developments in the study of hippocampal L-LTP, all of which have been encountered independently in *Aplysia*.

(i) A consensus is emerging as to the mechanisms of communication between the synapse and the nucleus (Impey *et al.* 1999).
(ii) We are beginning to better understand the transcriptional regulators involved in L-LTP (Barco *et al.* 2002; Pittenger *et al.* 2002).
(iii) The discovery of synaptic capture and synaptic tagging has given us new insight into the targeting of newly synthesized macromolecules to potentiated

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synapses (Frey & Morris 1997; Barco *et al.* 2002; Dudek & Fields 2002).

Aplysia was introduced for the study of learning and memory precisely because it allows a relatively straightforward mapping of learned behaviour onto synaptic change and because it allows a cell and molecular biological analysis of these changes (Kandel 1979; see Antonov *et al.* 2003). Here, we briefly review instances where molecular and cellular mechanisms of long-lasting plasticity in *Aplysia* recapitulate those in the more behaviourally interesting, but more complicated, mammalian brain. In discussing L-LTP we focus on the Schaffer collateral synapse in the rodent hippocampus, because this is where the most detailed work has been done. However, there is no *a priori* reason to believe that results from the *Aplysia* system, or any other model system, should be better recapitulated at the Schaffer collateral synapse than at other plastic synapses in the mammalian brain. We then describe some new developments in the study of *Aplysia* plasticity, with the hope that these may point the way to fruitful areas for investigation of L-LTP and learning in mammals.

2. MOLECULAR MECHANISMS OF MEMORY IN *APLYSIA* AND THE MAMMALIAN HIPPOCAMPUS: PARALLEL LIVES

(a) *Communication between synapses and the nucleus*

For a synaptic trigger to lead to gene induction in the nucleus requires the transmission of a signal along the length of the dendrite—often a considerable distance. In *Aplysia*, synaptic stimulation activates several kinases, which can physically move into the nucleus to act on nuclear substrates. After sufficiently robust and repeated synaptic stimulation with the modulatory transmitter serotonin, the catalytic subunit of the cAMP-dependent PKA moves to the nucleus, where it can participate in late-phase processes (Bacskai *et al.* 1993). PKA activates the p42 MAPK, which can likewise move to the nucleus and phosphorylate nuclear targets (Martin *et al.* 1997*a*). The activation of kinases, by repetitive synaptic stimulation or by the action of modulatory transmitters such as dopamine, also mediates signalling to the nucleus during Schaffer collateral LTP. PKA is involved in L-LTP (Frey *et al.* 1993; Abel *et al.* 1997), but current data more strongly support a role in gating inhibition by phosphatases than a direct role in phosphorylating nuclear substrates (Blitzer *et al.* 1995; Winder *et al.* 1998). The role of the MAPK cascade, however, is clearly conserved; MAPK is activated by robust, repeated synaptic stimulation, is necessary for L-LTP, and appears to gain access to nuclear substrates by physically moving into the nucleus upon activation (English & Sweatt 1996, 1997; Martin *et al.* 1997*a*; Patterson *et al.* 2001). Mammals thus recapitulate at least some aspects of the signalling from synapse to nucleus seen in *Aplysia*.

(b) *Transcriptional regulation for LTP and long-term memory*

Once the inducing signal has been transmitted to the nucleus, regulated transcription factors must be activated. In *Aplysia*, early evidence indicated that the CRE was a critical enhancer for this gene induction (Dash *et al.* 1990). Later cloning of the *Aplysia* CREB gene allowed confirmation that this inducible transcription factor is a central early element of the cascade of gene activation required for the establishment of long-lasting synaptic facilitation (Bartsch *et al.* 1998). In mammals the situation is complicated by the existence of several alternatively spliced and heterodimerizing CREB-like transcription factors, but CREB and CRE-driven transcription appear to have a similarly central role (Bourtchouladze *et al.* 1994; Pittenger *et al.* 2002).

(c) *Inhibitory constraints: memory suppressor genes*

Recent studies in both *Aplysia* and the hippocampus have revealed the importance of memory suppressor genes, genes whose function is to limit synaptic strengthening in the short or the long term. Interference with such molecules enhances synaptic plasticity, and in some cases enhances learning and memory (Abel *et al.* 1998). In *Aplysia*, this was first shown with the inhibitory transcription factor CREB2. Interference with CREB2 enhances long-lasting facilitation, such that weaker synaptic stimulation can lead to long-lasting change (Bartsch *et al.* 1995). In the mouse hippocampus, recent results from our laboratory suggest that interference with ATF4, the mammalian homologue of *Aplysia* CREB2, can likewise enhance LTP and can potentiate hippocampus-dependent learning (Chen 2001; Chen *et al.* 2003).

A better-studied memory suppressor in rodents is the phosphatase calcineurin. Calcineurin (or PP2B) is the first step in a phosphatase cascade that parallels and antagonizes signalling by kinases. These phosphatases remove phosphate groups from various regulatory molecules, including substrates of PKA, and thereby gate communication between the synapse and the nucleus in the induction of lasting potentiation (Blitzer *et al.* 1995; Mansuy *et al.* 1998). Reduction in calcineurin activity enhances LTP *in vitro* and *in vivo* and improves animals' learning in a number of hippocampus-dependent tasks (Malleret *et al.* 2001). The importance of memory suppressor genes in multiple forms of lasting plasticity identifies them as important, and perhaps conserved, regulators.

(d) *Targeting of newly synthesized proteins to activated synapse*

Once genes have been induced and new gene products produced, they must be targeted to the appropriate synapses for long-term plastic processes to take hold. This is a difficult sorting problem, because each mammalian hippocampal neuron has *ca.* 10 000 synapses for its one nucleus. The prevailing model of how this might occur is the 'synaptic tagging' hypothesis. Plasticity-inducing synaptic activity is proposed to initiate three processes: local events that lead to immediate (but labile) synaptic changes; a synaptic mark that tags the synapse as an appropriate target for long-term strengthening; and a signal back to the nucleus, as described in § 2a, to induce the genes that are required for that strengthening to take place. As newly produced proteins and RNAs are transported from the cell body, the synaptic tag controls which synapses they reinforce. This process can be demonstrated, even though we do not yet know the specific nature of the

synaptic tag, through the phenomenon of 'synaptic capture': a synapse tagged with a relatively weak stimulus can 'capture' the products of transcription induced by stronger stimulation at a different synapse, acquiring L-LTP with a stimulation normally only sufficient for E-LTP.

Synaptic capture has been demonstrated in both mammals (Frey & Morris 1997) and *Aplysia* (Martin *et al.* 1997*b*; Casadio *et al.* 1999). The experiments in *Aplysia* revealed a requirement for local synthesis of proteins in the establishment of long-lasting plasticity. While mRNA targeting and local protein synthesis have long been studied in mammals, it is only more recently that their importance in synaptic plasticity has become clear (Steward & Schuman 2001).

3. NEW DIRECTIONS FROM *APLYSIA*

In light of these similarities, other aspects of the mechanisms of long-lasting plasticity in *Aplysia* bear investigation in mammalian systems. The cell biological simplicity of the *Aplysia* system continues to allow levels of analysis that are not yet feasible in mammalian systems; this is particularly true since the development of techniques that allow isolation of two synapses from the same presynaptic neuron (Martin *et al.* 1997*b*). Here, we review several recent findings in *Aplysia* that we hope will illuminate productive avenues of exploration in the hippocampus and other mammalian systems.

(a) *Transcriptional regulators: CREB and its partners*

In *Aplysia*, it has been clear for some time that CREB does not act alone to activate the genes required for long-lasting facilitation. CREB is merely a central component of an interacting group of related transcription factors—both activators and repressors—which together may be better able to orchestrate an appropriate transcriptional response than a single factor could. CREB cooperates with several different categories of regulators. A repressor, ApCREB2, antagonizes CREB's actions; alleviating this repression in cell culture reduces the threshold for producing long-lasting synaptic facilitation (Bartsch *et al.* 1995). (As we will discuss in a moment, CREB2 also has a more active role in regulating long-lasting synaptic depression.) *Aplysia* CREB itself provides a splice variant that also acts as a repressor (ApCREB-1B, which is similar to mammalian ICER (inducible cAMP element repressor, a short splice variant of the CREB gene)) as well as an isoform that acts in the cytoplasm to modulate CREB activity (Bartsch *et al.* 1998). A constitutively expressed activator, ApAF, acts downstream of CREB to contribute to facilitation (Bartsch *et al.* 2000), and another activator, ApC/EBP, also contributes to facilitation; but it is itself induced by CREB and thus represents the next level in a cascade of transcriptional regulators (Alberini *et al.* 1994).

Experiments in cell culture clearly show that CREB cannot by itself achieve all gene induction required for long-lasting facilitation. Injection of phosphorylated CREB can produce facilitation independent of any synaptic stimulation; but this facilitation is only 50% of that achieved through more conventional induction, showing that other contributors are required. Interference with transcription factors that act downstream of CREB can disrupt facilitation despite presumably normal CREB function, showing that these downstream regulators have a similarly critical role (Alberini *et al.* 1994; Bartsch *et al.* 2000). Findings such as these confirm that CREB does not operate as a unitary transcriptional switch but rather as an important component of a more complex machinery.

It will be fascinating to investigate whether similar complexity attends CREB-mediated gene regulation in the hippocampus and other mammalian brain regions; indeed, since the CREB gene itself is so much more complicated in mammals, it would be surprising if attendant factors were not similarly elaborated. Some early data support this prediction. Interference with CREB produces compensation by the related CREM gene in the hippocampus and elsewhere, suggesting that both participate in transcriptional regulation (Hummler *et al.* 1994). C/EBP is upregulated by learning in mice (Taubenfeld *et al.* 2001), and interference with inhibitory isoforms of C/EBP and with ATF4 (the mammalian homologue of *Aplysia* CREB2) leads to improved learning in some hippocampus-dependent behavioural tasks (Chen 2001; Chen *et al.* 2003). These initial findings suggest that in mammals, as in *Aplysia*, CREB cooperates with a constellation of other factors in the consolidation of memory.

(b) *Induced genes*

While a large number of genes have been shown to be regulated by neuronal activity, we have as yet a relatively poor understanding of the downstream genes induced by CREB and other regulators, specifically in the consolidation of memory. Several candidates come from investigations in *Aplysia* and may bear investigation in mammalian systems.

The first induced gene identified in *Aplysia* was C/EBP (Alberini *et al.* 1994). As noted above, mammalian C/EBP has recently been shown to be induced in the hippocampus after learning; this induction correlates with CREB activation, further supporting the notion that at least this aspect of the *Aplysia* machinery is conserved in mammals (Taubenfeld *et al.* 2001).

Induced degradation of the PKA regulatory subunit in *Aplysia* extends PKA's activity and contributes to long-lasting facilitation (Chain *et al.* 1995). This is achieved by CREB-mediated induction of a neuron-specific ubiquitin C-terminal hydrolase (Hegde *et al.* 1997). This novel mechanism for extending an intracellular signalling bears investigation in mammalian systems. Pharmacological inhibition of the proteosome in rat hippocampus can interfere with memory formation (Lopez-Salon *et al.* 2001), suggesting that this mechanism, too, may be preserved.

With the increased power and availability of gene profiling analyses and the sequencing of the mouse genome, our knowledge of the specific genes induced during long-lasting LTP and memory will doubtless increase dramatically in the next few years. It will be exciting to see to what extent the handful of genes known to be upregulated during the induction of long-term facilitation in *Aplysia* are recapitulated in mammals.

(c) *Long-lasting synaptic depression*

LTP is complemented by the capacity of hippocampal synapses to undergo synaptic depression; depression may be as important for information processing and storage as

potentiation. Recently, several studies have demonstrated a long-term, protein synthesis-dependent form of synaptic depression in the hippocampus and elsewhere (e.g. Huber *et al.* 2000; Kauderer & Kandel 2000). This synaptic depression can be captured, at least in organotypic hippocampal cultures (Kauderer & Kandel 2000), indicating mechanisms of synaptic tagging and communication with the nucleus similar to those shown in synaptic potentiation. Long-term, transcription-dependent synaptic depression also occurs in *Aplysia* (Montarolo *et al.* 1988), once again making it an attractive model system for further mechanistic studies.

We have seen above that the induction of long-lasting synaptic plasticity leads to the transmittal of a signal to the nucleus, largely by p40/42 MAPK, and the induction of transcription of specific target genes, in both hippocampus and *Aplysia*. What are the parallel processes in long-lasting depression? In both rodents (Bolshakov *et al.* 2000) and *Aplysia* (Guan *et al.* 2003), p38 MAPK is an important carrier of this signal. In *Aplysia*, p38 MAPK targets and activates the transcription factors CREB2 and ATF2 in the nucleus (Guan *et al.* 2003). These data show that, in addition to being a repressor of CREB-mediated transcription (Bartsch *et al.* 1995), CREB2 has an important role as a transcriptional activator. This suggests that, in the nucleus as well as at the synapse, synaptic potentiation and synaptic depression are both mechanistically and functionally complementary.

(d) *A new logic for long-term synaptic integration: interaction between synaptic events in the nucleus—the role of chromatin modulation*

We have known for decades that different synapses on a single neuron interact nonlinearly in the short term to determine whether and how that neuron responds. Only recently has it become clear that distant synapses on a single neuron also interact in their production of long-lasting, transcription-dependent changes (Kandel 2001). Heroic studies of this issue have been undertaken in mammalian cell culture, leading to some interesting phenomenology (Fitzsimonds *et al.* 1997). However, the power of *Aplysia* as a cellular model of memory makes it an ideal system in which to study these interactions and their mechanisms.

The phenomenon of synaptic capture, described in § 2d, was the first type of these long-term interactions to be described, in both *Aplysia* and mammals. A consequence of this phenomenon is that the effects of stimulation at one synapse on a neuron depend not only on the local environment and the recent history of that synapse, but also on the recent history of all other synapses on the neuron.

Do synaptic potentiation and depression at different synapses on the same neuron interact in a similar fashion? In the short term, these processes are independent. In the long term, however, when long-lasting potentiation is induced at one synapse and long-lasting depression at a distant synapse on the same neuron, depression dominates. The nuclear events accompanying long-lasting synaptic depression in *Aplysia* appear able to suppress those otherwise induced by the induction of potentiation, truncating the potentiation to the transcription-independent short term (Guan *et al.* 2002).

How does this competition in the nucleus occur? We have found a critical role for modulation of chromatin structure at induced genes. The induction of long-lasting facilitation leads to CREB1 activation, the recruitment of the transcription cofactor CBP, a histone acetylase, and acetylation of histones at the induced gene C/EBP. In this process, the basal level of CREB2, which normally competes with CREB1, is reduced at the C/EBP locus, presumably alleviating this competition. When long-lasting depression is induced, CREB2 at this locus increases and CREB1 decreases. CREB2 recruits the histone deacetylase HDAC5, which deacetylates histones and thereby makes the gene inaccessible for induction.

There is thus a competition, at least at the promoter of the C/EBP gene, between CREB1 and CREB2, between CBP and HDAC5, and between histone acetylation and histone deacetylation. When stimuli to induce long-lasting potentiation and long-lasting depression are presented simultaneously to widely separated synapses on the same neuron, the events related to depression outcompete those related to potentiation: CREB2 displaces CREB1, and deacetylation by HDAC5 dominates. As a result of this histone deacetylation, the C/EBP gene remains inaccessible to transcription (Guan *et al.* 2002).

It is much more difficult to study this level of integration in a complex mammalian structure such as the hippocampus. But it will be important to investigate whether chromatin modulation represents a mechanism of signal competition and integration in mammalian systems, too. If so, this mode of signal interaction in the nucleus may prove to be of broad importance. It may provide new insights into long-term integration—a process that appears to be fundamentally different from short-term synaptic integration.

(e) *What is the molecular nature of the synaptic tag?*

Induced genes need to be appropriately targeted to potentiated (or to depressed) synapses. As discussed in § 2d, the phenomenon of synaptic capture suggests that this occurs through the creation of a synaptic 'tag', a marker at potentiated synapses. What is the nature of this synaptic tag? Experiments in *Aplysia* have provided us with some insight.

Using the bifurcated culture system, Martin *et al.* (1997*b*) showed local protein synthesis to be critical for the signal from the stimulated synapse back to the nucleus, but not for the initial phase of synaptic capture, producing facilitation lasting 24 hours. Continuing this analysis, Casadio *et al.* (1999) found that the synaptic tag for capture requires PKA. This second study revealed a role for local protein synthesis in the persistence of captured synaptic facilitation, and therefore in some aspect of the synaptic tag. Capture of long-term facilitation in the absence of local protein synthesis is not maintained; it can produce facilitation and synaptic growth lasting 24 hours but not 72 hours. The required local protein synthesis is sensitive to the drug rapamycin. As rapamycin blocks specific mechanisms of translational induction, this finding provides a first insight into the mechanisms of local protein synthesis in plasticity. Further experiments in our laboratory, as well

as elsewhere, seek to further characterize the local synaptic tags involved in these temporally distinct capture phenomena (Martin & Kosik 2002; K. Si and E. R. Kandel, unpublished data).

Studies in mice and rats (Frey & Morris 1997; Barco *et al.* 2002) have found that synaptic capture also occurs in the hippocampus; the latter study suggests that protein synthesis is required for its full expression. Future experiments must address whether the two-phase nature of capture and the specific molecules involved in it recapitulate those found in *Aplysia*. These will be challenging experiments, as it is difficult to manipulate separate populations of synapses in the hippocampus. It is to be hoped that the more tractable *Aplysia* system will continue to provide mechanistic clues, telling us where to start looking in more complicated organisms.

4. CONCLUSION

The ability to ask an important scientific question in an answerable way often hinges on the choice of model system. As a central purpose of neurobiology is to cast light on the functions of the human brain, mammalian model systems are often preferable. However, the complexity of the mammalian brain encourages parallel work in simpler model systems.

The similarities in some mechanisms of lasting synaptic change in these two evolutionarily disparate contexts invite the suggestion that other synapses in the mammalian brain may use similar mechanisms for long-term plasticity. The amygdala, which is an important locus of the changes underlying learned fear (LeDoux 2000), is an attractive structure in which to study learning and memory, because its circuitry is simpler than that of the hippocampus, and the relationship between specific synaptic changes and specific changes in behaviour is perhaps more clear. The mechanisms of plasticity at synapses in the amygdala, especially long-lasting plasticity, are not yet as well understood as those in the hippocampus. However, similarities to some of the mechanisms outlined in § 2 are already apparent. For example, PKA, MAPK and CREB are activated by synaptic stimuli that induce plasticity (Huang *et al.* 2000), and interference with CREB function can impair amygdala-dependent fear conditioning (Kida *et al.* 2002). Clearly, the mechanisms of lasting plasticity elucidated in *Aplysia* will bear investigation, not only at the Schaffer collateral synapse in the hippocampus, but at all synapses in which lasting potentiation is observed. While the mechanisms of plasticity at different synapses are unlikely to be identical, they appear to be variations on certain shared themes. This allows for a fruitful synergy between different model systems.

This synergistic approach has proven fruitful in the study of learning and memory, with *Aplysia* providing an important example of the relationship between learning and plasticity, as well as elucidation of various mechanisms of plasticity that are conserved across phylogeny. Ideally, different model systems complement each other and converge on conserved mechanisms. In a problem as complex as the nature of synaptic plasticity and its relationship to learning, we can be far more confident of the validity of our findings when they are reproduced in such disparate model systems. There has been a recent spate of work on the details of long-lasting facilitation in *Aplysia*. We propose that some of the mechanisms we have reviewed are likely to be conserved, perhaps with variations or elaborations, at mammalian synapses important for various types of memory storage.

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REFERENCES

Abel, T., Nguyen, P. V., Barad, M., Deuel, T. A., Kandel, E. R. & 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.

Abel, T., Martin, K. C., Bartsch, D. & Kandel, E. R. 1998 Memory suppressor genes, inhibitory constraints on the storage of long-term memory. *Science* **279**, 338–341.

Agranoff, B. W. 1967 Memory and protein synthesis. *Sci. Am.* **216**, 115–122.

Alberini, C. M., Ghirardi, M., Metz, R. & 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.

Antonov, I., Antonova, I., Kandel, E. R. & Hawkins, R. D. 2003 Activity-dependent presynaptic facilitation and Hebbian LTP are both required and interact during classical conditioning in *Aplysia*. *Neuron* **37**, 135–147.

Bacskai, B. J., Hochner, B., Mahaut-Smith, M., Adams, S. R., Kaang, B. K., Kandel, E. R. & Tsien, R. Y. 1993 Spatially resolved dynamics of cAMP and protein kinase A subunits in *Aplysia* sensory neurons. *Science* **260**, 222–226.

Barco, A., Alarcon, J. M. & Kandel, E. R. 2002 Expression of consitutively active CREB protein facilitates the late phase of long-term potentiation by enhancing synaptic capture. *Cell* **108**, 689–703.

Bartsch, D., Ghirardi, M., Skehel, P. A., Karl, K. A., Herder, S. P., Chen, M., Bailey, C. H. & Kandel, E. R. 1995 *Aplysia* CREB2 represses long-term facilitation, relief of repression converts transient facilitation into long-term functional and structural change. *Cell* **83**, 979–992.

Bartsch, D., Casadio, A., Karl, K. A., Serodio, P. & Kandel, E. R. 1998 CREB1 encodes a nuclear activator, a repressor, and a cytoplasmic modulator that form a regulatory unit critical for long-term facilitation. *Cell* **95**, 211–223.

Bartsch, D., Ghirardi, M., Casadio, A., Giustetto, M., Karl, K. A., Zhu, H. & Kandel, E. R. 2000 Enhancement of memory-related long-term facilitation by ApAF, a novel transcription factor that acts downstream from both CREB1 and CREB2. *Cell* **103**, 595–608.

Bliss, T. V. & Lømo, T. 1973 Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path. *J. Physiol.* (*Lond.*) **232**, 331–356.

Blitzer, R. D., Wong, T., Nouranifar, R., Iyengar, R. & Landau, E. M. 1995 Postsynaptic cAMP pathway gates early LTP in hippocampal CA1 region. *Neuron* **15**, 1403–1414.

Bolshakov, V. Y., Carboni, L., Cobb, M. H., Siegelbaum, S. A. & Belardetti, F. 2000 Dual MAP kinase pathways mediate opposing forms of long-term plasticity at CA3-CA1 synapses. *Nature Neurosci.* **3**, 1107–1112.

Bourtchouladze, R., Frenguelli, B., Blendy, J., Cioffi, D., Schutz, G. & 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.

Bourtchouladze, R., Abel, T., Berman, N., Gordon, R., Lapidus, K. & Kandel, E. R. 1998 Different training procedures

recruit either one or two critical period for contextual memory consolidation, each of which requires protein synthesis and PKA. *Learn. Memory* **5**, 365–374.

Casadio, A., Martin, K. C., Giustetto, M., Zhu, H., Chen, M., Bartsch, D., Bailey, C. & Kandel, E. R. 1999 A transient, neuron-wide form of CREB-mediated long-term facilitation can be stabilized at specific synapses by local protein synthesis. *Cell* **99**, 221–237.

Castellucci, V., Frost, W. N., Goelet, P., Montarolo, P. G., Schacher, S., Morgan, J. A., Blumenfeld, H. & Kandel, E. R. 1986 Cell and molecular analysis of long-term sensitization in *Aplysia*. *J. Physiol.* (*Lond.*) **81**, 349–357.

Chain, D. G., Hegde, A. N., Yamamoto, N., Liu-Marsh, B. & Schwartz, J. H. 1995 Persistent activation of cAMP-dependent protein kinase by regulated proteolysis suggests a neuron-specific function of the ubiquitin system in *Aplysia*. *J. Neurosci.* **15**, 7592–7603.

Chen, A. 2001 The role of C/EBP in hippocampal-based neuronal plasticity and memory storage: evidence for inhibitory constraints at the transcriptional level. PhD thesis, Columbia University, NY, USA.

Chen, A., Muzzio, I. A., Malleret, G., Grody, M., Bartsch, D. & Kandel, E. R. 2003 Inducible enhancement of long-term synaptic plasticity and memory storage formation in transgenic mice expressing a dominant-negative of ATF-4 (CREB-2) and other members of the C/EBP family of transcription factors. *Neuron*. (Submitted.)

Dash, P. K., Hochner, B. & Kandel, E. R. 1990 Injection of the cAMP-responsive element into the nucleus of *Aplysia* sensory neurons blocks long-term facilitation. *Nature* **345**, 718–721.

Dudek, S. M. & Fields, R. D. 2002 Somatic action potentials are sufficient for late-phase LTP-related cell signaling. *Proc. Natl Acad. Sci. USA* **99**, 3962–3967.

English, J. D. & Sweatt, J. D. 1996 Activation of p42 mitogen-activated protein kinase in hippocampal long term potentiation. *J. Biol. Chem.* **271**, 24 329–24 332.

English, J. D. & Sweatt, J. D. 1997 A requirement for the mitogen-activated protein kinase cascade in hippocampal long term potentiation. *J. Biol. Chem.* **272**, 19 103–19 106.

Fitzsimonds, R. M., Song, H. J. & Poo, M. M. 1997 Propagation of activity-dependent synaptic depression in simple neural networks. *Nature* **388**, 439–448.

Flexner, L. B., Flexner, J. B., De La Haba, G. & Roberts, R. B. 1965 Loss of memory as related to cerebral protein synthesis. *J. Neurochem.* **12**, 535–541.

Freeman, F. M., Rose, S. P. & Scholey, A. B. 1995 Two time windows of anisomycin-induced amenesia for passive avoidance training in the day-old chick. *Neurobiol. Learn. Mem.* **63**, 291–295.

Frey, U. & Morris, R. G. 1997 Synaptic tagging and long-term potentiation. *Nature* **385**, 533–536.

Frey, U., Huang, Y. Y. & Kandel, E. R. 1993 Effects of cAMP simulate a late stage of LTP in hippocampal CA1 neurons. *Science* **260**, 1661–1664.

Guan, Z., Giustetto, M., Lomvardas, S., Kim, J.-H., Miniaci, M. C., Schwartz, J. H., Thanos, D. & Kandel, E. R. 2002 Integration of long-term-memory-related synaptic plasticity involves bidirectional regulation of gene expression and chromatin structure. *Cell* **111**, 483–493.

Guan, Z., Kim, J.-H., Lomvardas, S., Holick, K., Xu, S., Kandel, E. R. & Schwartz, J. H. 2003 p38 MAP kinase mediates both short-term and long-term synaptic depression in *Aplysia*. *J. Neurosci.* (Submitted.)

Hegde, A. N., Inokuchi, K., Pei, W., Casadio, A., Ghirardi, M., Chain, D. G., Martin, K. C., Kandel, E. R. & Schwartz, J. H. 1997 Ubiquitin C-terminal hydrolase is an immediate-early gene essential for long-term facilitation in *Aplysia*. *Cell* **89**, 115–126.

Huang, Y. Y., Nguyen, P. V., Abel, T. & Kandel, E. R. 1996 Long-lasting forms of synaptic potentiation in the mammalian hippocampus. *Learn. Mem.* **3**, 74–85.

Huang, Y. Y., Martin, K. C. & Kandel, E. R. 2000 Both protein kinase A and mitogen-activated protein kinase are required in the amygdala for the macromolecular synthesis-dependent late phase of long-term potentiation. *J. Neurosci.* **20**, 6317–6325.

Huber, K. M., Kayser, M. S. & Bear, M. F. 2000 Role for rapid dendritic protein synthesis in hippocampal mGluR-dependent long-term depression. *Science* **288**, 1254–1257.

Hummler, E., Cole, T. J., Blendy, J. A., Ganss, R., Aguzzi, A., Schmid, W., Beermann, F. & Schutz, G. 1994 Targeted mutation of the CREB gene, compensation within the CREB/ATF family of transcription factors. *Proc. Natl Acad. Sci. USA* **91**, 5647–5651.

Impey, S., Obrietan, K. & Storm, D. R. 1999 Making new connections: role of ERK/MAP kinase signaling in neuronal plasticity. *Neuron* **23**, 11–14.

Kandel, E. R. 1979 *Behavioral biology of* Aplysia*, a contribution to the comparative study of opisthobranch mollusks*. San Francisco, CA: Freeman.

Kandel, E. R. 2001 The molecular biology of memory storage, a dialogue between genes and synapses. *Science* **294**, 1030–1038.

Kauderer, B. S. & Kandel, E. R. 2000 Capture of a protein synthesis-dependent component of long-term depression. *Proc. Natl Acad. Sci. USA* **97**, 13 342–13 347.

Kida, S., Josselyn, S. A., de Ortiz, S. P., Kogan, J. H., Chevere, I., Masushige, S. & Silva, A. J. 2002 CREB required for the stability of new and reactivated fear memories. *Nature Neurosci.* **5**, 348–355.

LeDoux, J. E. 2000 Emotion circuits in the brain. *A. Rev. Neurosci.* **23**, 155–184.

Lopez-Salon, M., Alonso, M., Vianna, M. R., Viola, H., Mello e Souza, T., Izquierdo, I., Pasquini, J. M. & Medina, J. H. 2001 The ubiquitin-proteosome cascade is required for mammalian long-term memory formation. *Eur. J. Neurosci.* **14**, 1820–1826.

Malleret, G., Haditsch, U., Genoux, D., Jones, M. W., Bliss, T. V., Vanhoose, A. M., Weitlauf, C., Kandel, E. R., Winder, D. G. & Mansuy, I. M. 2001 Inducible and reversible enhancement of learning, memory, and long-term potentiation by genetic inhibition of calcineurin. *Cell* **104**, 675–686.

Mansuy, I. M., Mayford, M., Jacob, B., Kandel, E. R. & 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.

Martin, K. C. & Kosik, K. S. 2002 Synaptic tagging—who's it? *Nature Rev. Neurosci.* **3**, 813–820.

Martin, K. C., Michael, D., Rose, J. C., Barad, M., Casadio, A., Zhu, H. & Kandel, E. R. 1997*a* MAP kinase translocates into the nucleus of the presynaptic cell and is required for long-term facilitation in *Aplysia*. *Neuron* **18**, 899–912.

Martin, K. C., Casadio, A., Zhu, H. E. Y., Rose, J. C., Chen, M., Bailey, C. H. & Kandel, E. R. 1997*b* Synapse-specific, long-term facilitation of *Aplysia* sensory to motor synapses, a function for local protein synthesis in memory storage. *Cell* **91**, 927–938.

Montarolo, P. G., Kandel, E. R. & Schacher, S. 1988 Long-term heterosynaptic inhibition in *Aplysia*. *Nature* **333**, 171–174.

Patterson, S. L., Pittenger, C., Morozov, A., Martin, K. C., Scanlin, H., Drake, C. & Kandel, E. R. 2001 Some forms of cAMP-mediated long-lasting potentiation are associated with release of BDNF and nuclear translocation of phospho-MAP kinase. *Neuron* **32**, 123–140.

Pittenger, C., Huang, Y.-Y., Paletzki, R. F., Bourtchouladze, R., Scanlin, H., Vronskaya, S. & Kandel, E. R. 2002 Reversible inhibition of CREB/ATF transcription factors in region CA1 of the dorsal hippocampus disrupts hippocampus-dependent spatial memory. *Neuron* **34**, 447–462.

Steward, O. & Schuman, E. M. 2001 Protein synthesis at synaptic sites on dendrites. *A. Rev. Neurosci.* **24**, 299–325.

Taubenfeld, S. M., Wiig, K. A., Monti, B., Dolan, B., Pollonini, G. & Alberini, C. M. 2001 Fornix-dependent induction of hippocampal CCAAT enhancer-binding protein β and δ co-localizes with phosphorylated cAMP response element-binding protein and accompanies long-term memory consolidation. *J. Neurosci.* **21**, 84–91.

Winder, D. G., Mansuy, I. M., Osman, M., Moallem, T. M. & Kandel, E. R. 1998 Genetic and pharmacological evidence for a novel, intermediate phase of long-term potentiation suppressed by calcineurin. *Cell* **92**, 25–37.

GLOSSARY

C/EBP: CCAAT-enhancer-binding protein
CRE: cAMP-responsive element
CREB: cAMP-responsive element binding protein
L-LTP: late, transcription-dependent phase of long-term potentiation
LTP: long-term potentiation
MAPK: mitogen-activated protein kinase
PKA: protein kinase A