CREB's control of intrinsic and synaptic plasticity: Implications for CREB-dependent memory models

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Number of Figures, Tables and Boxes: 3 Figures, 1 Table and 3 Boxes

Keywords: CREB, learning and memory, synaptic plasticity, intrinsic plasticity

Abstract

The activation of cAMP-response element binding protein (CREB)-dependent gene expression seems a critical step in the molecular cascade that mediates the formation of long-lasting memories. This view is based both on correlative evidence and on functional assays that demonstrate, through loss- and gain-of-function experiments, the impact of CREB manipulation in memory performance. Mechanistically, CREB's role in memory is thought to be a consequence of its participation in long-term forms of synaptic plasticity. Recent studies demonstrate that CREB, in addition to synaptic plasticity, also modulates the intrinsic excitability of the neuron. This discovery reveals new intriguing connections between intrinsic and synaptic plasticity and is likely to have a significant impact on our understanding of the role of CREB in memory formation.

CREB and memory: still a developing story

A number of landmark articles in the nineties ¹⁻³ gave the cAMP response element binding protein (CREB) the surname of “the memory gene”. Textbooks frequently depict CREB at the convergence of different activity-driven kinase pathways; downstream of different signaling cascades, such as the increase of intracellular cAMP after activation of G protein-coupled receptors, the increase of Ca²⁺ through activation of voltage- or ligand-gated channels, or the activation of receptor tyrosine kinases by growth factors ⁴, and upstream of so-called “plasticity related proteins” (PRPs), such as neurotransmitter receptors, structural proteins, and adhesion and signaling molecules, which regulate synaptic efficacy ^{5, 6}. According to this view, the activation of CREB due to synaptic activity elicited by a behavioral experience would induce the expression of the, still largely unidentified, molecules that are necessary for the stabilization of the structural and functional changes of synaptic strength encoding the memory trace for that experience. This process, sometimes referred to as cellular consolidation of memory, is depicted in Figure 1.

This model is a simplified view; cAMP-dependent gene expression is regulated not only by CREB, but also by other CREB family members that can replace it or modulate its activity ⁷⁻⁹. Other transcription factors and independent transcriptional programs are also likely to intervene in cellular consolidation ¹⁰⁻¹². Also, it should be noted that CREB controls the transcriptional responses of neurons to many extracellular stimuli, not only those relevant to synaptic plasticity (Box 1). Despite controversies in the field ¹³ that arise from the complexity outlined above, the relevance of CREB in synaptic plasticity and memory processes is well supported in the published literature.

A number of recent studies have revealed a novel role for CREB and downstream gene expression in neural plasticity that may alter this classical view: the control of intrinsic excitability (i.e., of the propensity of the neuron to fire action potentials in response to input signals). This new experimental evidence suggests novel mechanisms by which CREB can participate in memory consolidation, and provides new insight into the molecular underpinnings of intrinsic plasticity, a family of processes that, like synaptic plasticity, is thought to play a critical role in learning and memory ¹⁴⁻¹⁶. This review summarizes and discusses the role of CREB in the regulation of both intrinsic and synaptic plasticity, proposes a renewed view of its role in learning a memory, and outlines some of the outstanding questions in the field.

Regulation of intrinsic plasticity by CREB

Despite the large number of studies investigating the role of CREB in synaptic plasticity, research on the influence of CREB in neuronal activity *per se* were surprisingly lacking. Dong and colleagues addressed this question for the first time in a recent study that used recombinant Sindbis pseudovirions to genetically manipulate CREB activity in medium spiny neurons (MSNs) of the nucleus accumbens (NA) ¹⁷. They found that the expression of a constitutively active CREB (ca-CREB) variant enhanced intrinsic excitability in those neurons, whereas the expression of a dominant negative CREB (dn-CREB) mutant reduced it. In particular, current-clamp experiments indicated that CREB influenced the number and/or properties of voltage-gated Na⁺ and K⁺ channels in an opposite manner: CREB overactivation led to an increase in Na⁺ (depolarizing) currents and a decrease in K⁺ (hyperpolarizing) currents, whereas its inhibition had the opposite effect. Modifying CREB activity also

affected the action potential threshold and the minimal current needed to fire a spike, whereas cell morphology and passive membrane properties were unaffected (Table 1).

In a parallel study using recombinant Herpes simplex virus (rHSV) to manipulate CREB function at the *locus ceruleus* (LC), Han and colleagues showed that the expression of a ca-CREB variant in noradrenergic neurons in the LC increased their intrinsic excitability and resulted in a more depolarized resting membrane potential. Conversely, inhibiting CREB activity by expression of a dn-CREB mutant led to a reduction in intrinsic excitability and membrane hyperpolarization. Interestingly, the overexpression of wild-type CREB (wt-CREB) by itself did not affect excitability in the basal condition, but enhanced the effects of forskolin (a stimulator of the cAMP pathway) in excitability, indicating that the cAMP signaling pathway was sensitized after CREB overexpression¹⁸ (Table 1). CREB is an activity-driven transcription factor and needs to be phosphorylated in order to become active; this may explain the lack of effect of wt-CREB overexpression by itself.

Concurrent studies in the hippocampus of bitransgenic mice expressing either a ca-CREB variant, VP16-CREB, or a dominant negative inhibitor of the CREB family of transcription factors, A-CREB, demonstrated that the intrinsic plasticity of CA1 pyramidal neurons is also severely affected by the genetic manipulation of CREB function (Table 1). Enhancing CREB activity inhibited afterhyperpolarization (AHP), a hyperpolarizing current that reduces excitability preventing the same neuron from firing again, reduced spike adaptation and increased neuronal excitability in a dose-dependent manner¹⁹. Both the slow and medium components of the AHP (sAHP and mAHP, respectively) were affected in mice with enhanced CREB activity.

Conversely, CREB inhibition reduced the membrane resistance and the number of action potentials elicited by depolarizing current injections ¹².

Finally, a recent study, again using VP16-CREB bitransgenic mice, showed that the enhancement of CREB activity also produces an increase in spiking frequency and a reduction of AHP in the pyramidal neurons of the basal nucleus of the amygdala ²⁰ (Table 1). This effect was later confirmed by Zhou and colleagues, who demonstrated that the overexpression of wt-CREB in a subset of neurons in the lateral amygdala using a rHSV was sufficient to reduce the spike threshold, increase the number of evoked action potentials and ameliorate spike frequency adaptation and post-burst AHP ²¹.

Overall, these results suggest that the modulation of intrinsic neuronal properties is a well-conserved CREB function throughout different neuronal types in the central nervous system. In fact, similar mechanisms might also be in place in the peripheral nervous system, since recent studies have demonstrated that cAMP signaling and CREB activation correlate with hyperexcitability of enteric after-hyperpolarizing sensory neurons ²².

This model concurs with earlier electrophysiological studies in mollusk and mammalian neurons demonstrating the role of cAMP/PKA signaling in the regulation of intrinsic excitability in different neuronal types ^{14, 23, 24}. Studies in the *Drosophila* memory mutants *dunce* and *rutabaga*, which bear mutations in components of the cAMP signaling pathway, also support a role of the cAMP pathway in the regulation of potassium currents and neuronal excitability ²⁵⁻²⁷. This function, therefore, seems to be highly conserved through evolution.

Importantly, this novel experimental evidence does not preclude the traditional view that CREB could also regulate neuronal plasticity through synaptic mechanisms.

In fact, studies in the NA, in the hippocampus and in the amygdala seem to indicate that CREB modulates the functional output of neurons through both NMDAR-dependent and NMDAR-independent mechanisms. Thus, genetic manipulation of CREB activity in MSNs of the NA increased both the duration of the upstate and action potential firing during the upstate ²⁸, an effect that was likely mediated by modulation of both NMDAR-mediated synaptic currents and surface levels of this receptor. Similarly, in the hippocampus, CREB activation influenced the expression of NMDAR currents, probably by creation of new silent synapses ²⁹, and enhanced long-term potentiation (LTP) through mechanisms that are likely independent of the modulation of intrinsic excitability ^{29, 30}. Interestingly, these studies appear to indicate that the NMDAR-independent mechanisms are more susceptible to bidirectional modulation, whereas the NMDAR-dependent events are very sensitive to gain-of-function approaches, but relatively resistant to CREB inhibition ^{12, 13, 28, 29, 31}.

Putative effector mechanisms

The intrinsic excitability of a neuron can be modulated by modifications in the threshold for firing an action potential, but also by changes in the properties of repeated firing. The specific mechanisms underlying CREB-mediated modulation of intrinsic excitability remain largely unknown, but seem distinct in different cell types. Both changes in the threshold and firing mode have been detected, although the identity of the conductances affected, and whether the changes occur through modulation of existing channels or by insertion or removal of new channels, are questions that should be further explored.

In MSNs of the NA, the contribution of single currents to the increased firing was evaluated individually through the use of specific current inhibitors and revealed

a bidirectional regulation of ionic currents. In particular, the increase in CREB activity correlated with an increase in Na⁺ conductance and a decrease in K⁺ conductance¹⁷. This view is consistent with microarray data indicating that CREB stimulates the transcription of a sodium channel subunit (1β) and reduces the expression of Kv1.4 subunit in that tissue³², and with recent data demonstrating that social isolation upregulates the expression of several types of K⁺ channels and reduces the excitability of MSNs through inhibition of CREB-dependent transcriptional activity³³. Since CREB is a transcriptional activator, this downregulation of K⁺ currents could result from indirect regulatory mechanisms, such as the induction of transcriptional repressors or microRNAs. Alternatively, CREB could also modulate intrinsic excitability through the regulation of enzymatic activities, such as protein kinases or phosphatases that change the gating and conductance properties of preexisting ionic channels through post-translational modifications (Figure 2). This might be the case in the pyramidal neurons at the CA1 subfield of the hippocampus^{12, 19} or at the basolateral nucleus of the amygdala^{20, 21}, where the effects of CREB on spike adaptation after hyperpolarization were explained by regulation of AHP, a known target of the cAMP signaling pathway and PKA activity³⁴⁻³⁷. Although the precise molecules involved in the modulation of the currents underlying AHP remain unidentified³⁸, recent experiments suggest that the cAMP/PKA pathway could mediate both the fast suppression of AHP in response to neuromodulators and the longer-lasting reduction of AHP associated with learning³⁹. CREB activation is downstream of cAMP/PKA signaling, but could also contribute to the modulation of this signaling cascade through transcriptional regulation of specific molecules in that pathway (Figure 2).

CREB modulation of CA1 pyramidal neurons excitability is bidirectional. In agreement with the results described above, the characterization of bitransgenic mice expressing the strong inhibitor of CREB function A-CREB revealed an increase of the AHP, but also a significant increase of the M potassium current, which also contributes to spike frequency adaptation in CA1 pyramidal neurons⁴⁰. Finally, in the case of the change in excitability of LC neurons, no specific molecular mechanism has been proposed¹⁸, but the current observations could be compatible with either changes in the expression or the properties of ion channels.

Can we integrate the genetic programs underlying CREB modulation of synaptic strength and intrinsic excitability into a unitary model explaining the role of CREB in neuronal plasticity? Based on the evidence outlined above, experience-dependent activation of the cAMP signaling cascade could mediate posttranslational modifications (e.g. phosphorylation) of Na⁺ or K⁺ channels, altering their properties and making the neuron more excitable, for example, through inhibition of the AHP. In parallel, but with a slower time-course, cAMP signaling could result in the activation of CREB-dependent gene expression, which could support the maintenance of a more excitable cellular state through regulation of the expression of specific channels subunits or modulators of channel properties. Independently, CREB activation may also drive the expression of the effector molecules necessary for the stable reinforcement of synaptic connections and the outgrowth of new spines, and maybe contribute to presynaptic facilitation through the expression of molecules mediating retrograde signaling, such as BDNF. Overall, the activation of CREB could set the cell in a more responsive state through the regulation of both its electrical properties and its functional synaptic layout (Figure 3).

A novel view on the role of CREB in learning and memory processes

The role of CREB in memory formation and maintenance has been extensively investigated. Seminal studies in *Aplysia* and in *Drosophila* first identified the cAMP signaling pathway as a core component of the molecular switch that converts short- to long-term memory ⁴¹⁻⁴³. The demonstration of a direct role of CREB in memory formation and memory-related synaptic plasticity was provided years later by precise genetic or biochemical manipulation of CREB function in transgenic flies or cultured *Aplysia* neurons ^{1, 3, 44}. Parallel studies on hypomorphic mutant mice also demonstrated a role of CREB in synaptic plasticity and memory in mammals ². These seminal experiments have been followed by numerous studies investigating CREB activation in relation to memory in rats, mice and other organisms, as well as by the detailed behavioral and physiological characterization of diverse strains of genetically modified mice with altered CREB function. Only in rodents, CREB has been shown to be involved in conditioned fear memory (both contextual and cued), conditioned taste aversion, olfactory memory, object and social recognition memory, and different forms of spatial memory. A comprehensive review of the extensive literature on the role of CREB in memory is out of the scope of this article, but there are a number of recent reviews available on that topic (see for example ⁴⁵⁻⁴⁹). Although some studies indicate that CREB itself may be dispensable for certain forms of memory ^{13, 50-52}, likely because of the compensation by other members of the CREB family of transcription factors ⁷⁻⁹, the induction of CRE-driven gene expression seems to be a general requirement for different types of long-term memory, both implicit and explicit. The compensation of function among CREB family members may explain that gain-of-function approaches have been more successful than gene targeting in demonstrating a role for CREB in memory ^{20, 53-57}. Loss-of-function experiments in

which the strategy for CREB repression may have prevented the compensation by other CREB family members also support a role of CREB in memory^{31, 58, 59}.

The discovery that CREB can modulate intrinsic excitability has important implications for our understanding of its function in memory formation and challenges the most conventional view of its role in this process. The physiological modulation of neuronal excitability by CREB can affect learning and memory processes through at least three non-exclusive mechanisms (Figure 3).

First, the suppression of the AHP and the increase in excitability following CREB overactivation^{19, 21} can cause a reduction in the threshold for induction of LTP in the synapses of the sensitized neuron, so that stimuli that would normally elicit a short-lasting synaptic potentiation (early LTP or E-LTP) will now trigger a longer-lasting potentiation (late LTP or L-LTP)^{37, 60-63}. Conversely, the inhibition of CREB and subsequent activation of the AHP would cause an increase of the threshold for induction of LTP. The cross-talk between intrinsic and synaptic plasticity can have behaviorally relevant consequences. For example, the activation of CREB-dependent gene expression in the hippocampus by monoaminergic or dopaminergic inputs might, in this manner, facilitate hippocampal LTP induction and LTP-dependent learning processes during arousal and attention, as well as contribute to the formation of one-trial or flashbulb memory^{64, 65}. This neuromodulatory effect could be also involved in the setting of a *learning mode*, i.e., an state favorable to learning in which, a relatively durable change in neuronal excitability would support the facilitation of the learning of related tasks (rule-learning)⁶⁶. For a recent review on this topic see⁶⁷.

Second, gain-of-function experiments in which CREB activity has been boosted in specific neurons via viral transduction have demonstrated that CREB levels can bias the allocation of a fear memory to specific neurons in the amygdala,

likely through modulation of neuronal excitability^{21, 68, 69}. More excitable neurons would be more likely to be activated during conditioning and therefore are more likely to get engaged in the memory trace. Interestingly, Zhou and colleagues have also proposed that, under given circumstances, the strong activation of CREB could lead to the induction of CREB repressor activities, such as ICER, which would, in turn, have a negative impact on neuronal excitability, thereby preventing different memories from being stored in the same neuronal ensemble²¹. Therefore, the differential level of CREB activity during memory acquisition could have a determinant role in memory allocation. Since the changes in intrinsic excitability are cell-wide, they would contribute to neuronal rather than synaptic selection during learning events⁷⁰. A conceptual caveat of this model is that it does not explain how memory allocation is determined under physiological conditions. The neurons overexpressing CREB after artificial manipulation may be preferentially selected to encode a memory trace^{21, 68, 69}, but still the question remains as to how and why differential levels of CREB activity would be achieved in normal animals prior to the behavioral experience.

Third, if CREB causes a transient, but relatively long-lasting increase of intrinsic excitability, those neurons in which CREB was phosphorylated in response to a particular experience would be more likely to fire again within a time range. This experience-dependent neuronal sensitization could play a major role in the consolidation of memory traces by enabling the selective reactivation of neuronal networks. Those neurons that increased their excitability during training in a memory task would be more likely to be re-activated during post-training resting or sleep, maybe contributing to memory consolidation at the network and system levels^{71, 72}. CREB would contribute to create a “learning state” in which the hyperexcitability of

specific neuronal ensembles would enable the consolidation of the memory trace. This mechanism substantially differs from the conventional view of CREB driving the expression of effector genes that directly stabilize or reinforce synaptic connections, since CREB would mediate circuit or system memory consolidation rather than directly participate in cellular consolidation. According to this view, it would be appropriate to refer to CREB as a *learning gene* (see Glossary Box). We have, therefore, at least two conceptually distinct, but not exclusive, models by which CREB can participate in memory consolidation: directly, through the expression of synaptic effectors (cellular consolidation), and indirectly, through neuronal sensitization. Contrary, to the other two mechanisms discussed above there is no direct experimental evidence supporting this mechanism. It represents, however, an appealing and alternative model to explain the contribution of CREB to memory consolidation.

Although most of the studies investigating the role of CREB in memory have been interpreted in terms of synaptic plasticity, we cannot rule out that excitability changes occurring prior to or at the same time as the synaptic changes could also contribute to the observed behavioral effects. In fact, the review of the overwhelming literature on CREB and memory indicates that most experimental results relating CREB and memory are compatible or could be explained by either mode of action. This is the case for all of the studies assessing memory performance days or months after inducing the genetic manipulation of CREB function, such as the behavioral analyses of different strains of CREB deficient mice and virus-transduced animals (i.e., loss-of-function studies revealing memory impairments and gain-of-function experiments showing enhanced or impaired performance). The studies looking for a correlation between memory acquisition and CREB phosphorylation, the induction of

CREB-downstream genes or a CRE-driven reporter can discern neither these two modes of action.

However, some particularly interesting results, such as the recent report by Han and colleagues referred above demonstrating a role for CREB in neuronal competition during memory formation ⁶⁸, may be better explained considering the sensitization model. This study demonstrated that the overexpression of CREB increased the probability that individual neurons are recruited into a fear memory trace during its formation. The rapid induction of molecular markers of activity, such as *arc*, was facilitated in those neurons in response to training, although their expression was not affected at basal conditions prior to training. These observations are better explained considering the presence of sensitized neurons, in which it would be easier to trigger *arc* expression, than with a late role in cellular consolidation. As a continuation of that study, the same laboratory used sophisticated genetic techniques to co-express CREB and an inducible diphtheria-toxin in a subset of lateral amygdala neurons. In this manner, they could first direct the memory trace formed during a fear conditioning experiment to a specific set of neurons (those overexpressing CREB activity) and then selectively ablate those neurons using the diphtheria toxin and consequently eliminate the created CREB-dependent fear memory ⁶⁹. These findings have been recently reproduced by Zhou et al. using a slightly different system based on the use of a recombinant HSV coexpressing CREB and *Drosophila* allatostatin G protein-coupled receptor (AlstR) ²¹. Their study demonstrates that the selective silencing/inhibition of neurons overexpressing CREB activity disrupted a fear memory 24 h after training, suggesting that the CREB-mediated increase of intrinsic excitability augment the likelihood that an infected neuron will be recruited into the memory trace. Interestingly, CREB-expressing neurons also showed facilitated

synaptic transmission after the learning task, further supporting the idea that those neurons were indeed encoding the memory trace and suggesting that both intrinsic and synaptic changes are relevant in CREB-dependent memory.

In contrast, other experimental results seem more compatible with a direct role of CREB in cellular consolidation. This is the case of the recent work by Viosca and colleagues demonstrating that constitutive CREB activity in fear memory circuits bypassed the requirement for *de novo* gene expression associated with long-term fear memory formation²⁰. In these mice, PRPs necessary for the reinforcement of synaptic connections between specific neurons engaged in encoding the memory trace would be already present at the time of training, thus allowing protein-synthesis-independent consolidation of long-term memories, in agreement with the “synaptic tagging and capture” model³⁰. Similarly, electrophysiological experiments have demonstrated that the expression of ca-CREB facilitated the induction of L-LTP in the Schaffer collateral pathway^{29,30} and that this facilitation was resistant to inhibitors of *de novo* gene expression, such as anisomycin, actinomycin D or 5,6-dichloro-1- β -D-ribofuranosylbenzimidazole (DRB)³⁰. Although the enhanced excitability and inhibition of AHP might contribute to the facilitation of L-LTP and memory consolidation by ca-CREB, the changes in excitability by themselves cannot account for the resistance to gene expression blockers observed in both paradigms. Instead, these results suggest the presence, prior to stimulation, of the PRPs necessary for cellular consolidation.

However, CREB's ability to increase neuronal excitability does not necessarily imply that CREB will favor learning and memory in every behavioral situation. On the contrary, the chronic increase in neuronal excitability observed in ca-CREB mice had some detrimental effects in memory performance. Watermaze

experiments suggested that although the mice were able to acquire spatial information, sustained CREB activation specifically interfered with its retrieval ⁷³. The precise regulation of CREB levels, and therefore CREB-dependent plasticity mechanisms, seems to be required for optimal performance in complex memory tasks, such as spatial navigation in the watermaze.

The putative contribution of non-synaptic plasticity to learning and memory has been well documented ^{14-16, 74}, but the discovery of the participation of CREB in this process represents an important breakthrough. Beyond the seminal experiments described above, a clear dissection of the relative contributions of synaptic versus non-synaptic mechanisms in CREB-mediated memory formation is still lacking and could be difficult to achieve. Intrinsic and synaptic plasticity processes are difficult to discern at their behavioral outputs and may interact at different levels: functionally, because the changes in intrinsic excitability can affect the synaptic properties and *vice versa* ^{60-63, 75}; and molecularly, because the same molecules and second messengers seem to regulate both processes. For historical reasons, most theoretical models on memory capacity in the hippocampus are based on synaptic learning, whereas the contribution of non-synaptic learning mechanisms has raised relatively less attention. As CREB function seems to influence both synaptic and non-synaptic forms of learning simultaneously, future research shall explore the relationship between these two phenomena and their individual relevance in memory formation. Recordings in behaving animals during and after learning tasks in combination with the genetic manipulation of CREB activity and pharmacological treatments (such as inhibitors of gene expression or of specific currents) should help towards this goal.

Certain learning tasks, such as eye blinking conditioning or spatial navigation in the Morris water maze, produce enduring changes in intrinsic excitability by

changing the function or expression of voltage-gated channels and by regulating the size of the postburst AHP ^{76, 77}. The persistence of these changes in neuronal excitability, which may last several days, suggests the participation of molecular mechanisms, such as *de novo* gene expression and protein synthesis, that can in principle support longer lasting changes in molecular function than the covalent posttranslational modification of receptor or channel subunits. Surprisingly, the participation of transcriptional processes and, in particular, of CREB activity (a well known target of PKA) in these enduring changes in intrinsic excitability has only been suggested recently ¹⁹. In agreement with this putative role, a recent detailed histological analysis of CREB phosphorylation during and after learning in the water maze revealed that CREB phosphorylation and downstream gene activation remained sustained in CA1 and CA3 for at least 24 h after extended training, but not during early training, showing a good correlation between the duration of CREB phosphorylation and the animal's performance in the task ⁷⁸. Future research shall assess whether the regulation of neuronal excitability by the cAMP pathway has, like long-term potentiation, a late phase that is dependent on *de novo* protein synthesis and gene expression ^{39, 79}, and the possible involvement of CREB in learning-induced changes in neuronal excitability. Another important aspect for future research is to determine the duration and stability of these changes. Several signaling pathways have been suggested to act on the maintenance of excitability ^{24, 67, 80}, and some negative feedback mechanisms must exist that allow returning to the basal state. The transcriptional loop integrated by CREB/CREM and its negative regulator ICER might represent a first example of such mechanisms.

Misregulation of CREB activity and altered excitability

In addition to the physiological roles outlined above, misregulation of intrinsic excitability by malfunction of CREB signaling may have important pathological consequences. Recent studies exploring the phenotype of various CREB mutant strains have revealed that CREB modulation of neuronal excitability can have important consequences in epileptogenesis. Thus, bitransgenic mice expressing the artificial CREB inhibitor A-CREB were more resistant to pentylenetetrazol (PTZ)-induced kindling and to kainate-induced seizures ¹², two common models of epilepsy, whereas mice with enhanced CREB activity were prone to spontaneous seizures ¹⁹ and showed accelerated PTZ-kindling ¹⁹. Consistently with these results, mutant mice overexpressing ICER, an endogenous, activity-regulated inhibitor of CREB function, showed delayed kindling in response to repeated subthreshold electrical stimulation in the amygdala ⁸¹, another model of epileptogenesis. Correspondingly, ICER knockout mice showed accelerated development of kindling ⁸¹. Also, CREM/ICER null mutants were found to be more prone to spontaneous seizures after pilocarpine-induced status epilepticus ⁸². Together, these results highlight the importance of accurate regulation of intrinsic excitability by CREB in epilepsy.

Misregulation of CREB function and subsequent alterations of neuronal excitability in brain areas other than the hippocampus can have other behaviorally relevant effects, for example in addiction or depression related behavior. Thus, CREB has been shown to exert a bidirectional effect on the intrinsic excitability of MSNs of the NA both under basal and cocaine-addiction conditions ¹⁷. This finding importantly contributes to explain the result of a number of earlier and subsequent behavioral studies showing that CREB inhibition enhanced, rather than attenuated, several behavioral responses to drugs of abuse ^{32, 83-87}. The inhibition of CREB activity and

subsequent reduction in the firing of MSNs would result in decreased GABA and dynorphin-mediated inhibition over VTA-dopaminergic neurons⁸⁸; this reduction of the inhibitory input would, in turn, lead to an increase of the dopaminergic input to the NA core, which could explain the enhanced addictive behavior⁸⁷. The modulation of intrinsic excitability in LC neurons also had relevant behavioral correlates: whereas overexpression of CREB aggravated withdrawal-related behaviors, its inhibition attenuated them¹⁸. Finally, decreased CREB activity and intrinsic excitability in neurons of the NA shell have been shown to trigger anxiety and depression-like behaviors³³.

Conclusion

Current evidence indicates that CREB activity modulates both intrinsic and synaptic plasticity. What is the relative weight of CREB-mediated modulation of intrinsic excitability and synaptic plasticity in memory formation? Is CREB primarily a “learning” gene or a “memory” gene? These questions need further exploration. The schemes illustrating the putative role of CREB in memory should now consider not only the typical pathway leading to consolidation of synaptic plasticity through expression of neurotrophins, adhesion molecules and other synaptic effectors, but also a parallel pathway pointing towards modulation of intrinsic excitability (Figure 3), likely through regulation of the somatic expression of potassium currents. Future experiments shall explore whether these two pathways are equally relevant to explain the role of CREB in memory formation, as well as elucidate the details of their interaction and the identity of specific effector molecules.

Acknowledgements

We thank Eloisa Herrera, Mikel Lopez de Armentia, Jose Lopez-Atalaya, Helene Marie, Matt Nolan and Luis M Valor for critical reading of the manuscript, and members of the Barco's lab for stimulating discussion. Research at AB's lab is supported by the Spanish Ministry of Science and Innovation Grants BFU2008-00611, SAF2008-03194-E and CSD2007-00023, and grants from Fundaci3n Ram3n Areces and Fundaci3n la Marat3 de TV3.

References

1. Yin, J.C., *et al.* (1994) Induction of a dominant negative CREB transgene specifically blocks long-term memory in *Drosophila*. *Cell* 79, 49-58
2. Bourchuladze, R., *et al.* (1994) Deficient long-term memory in mice with a targeted mutation of the cAMP-responsive element-binding protein. *Cell* 79, 59-68
3. Bartsch, D., *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
4. Johannessen, M., *et al.* (2004) What turns CREB on? *Cell Signal* 16, 1211-1227
5. Kandel, E.R. (2001) The molecular biology of memory storage: a dialogue between genes and synapses. *Science* 294, 1030-1038
6. Lonze, B.E., and Ginty, D.D. (2002) Function and regulation of CREB family transcription factors in the nervous system. *Neuron* 35, 605-623
7. Hummler, E., *et al.* (1994) Targeted mutation of the CREB gene: compensation within the CREB/ATF family of transcription factors. *Proc Natl Acad Sci U S A* 91, 5647-5651
8. Blendy, J.A., *et al.* (1996) Targeting of the CREB gene leads to up-regulation of a novel CREB mRNA isoform. *Embo J* 15, 1098-1106
9. Mantamadiotis, T., *et al.* (2002) Disruption of CREB function in brain leads to neurodegeneration. *Nat Genet* 31, 47-54.
10. Ramanan, N., *et al.* (2005) SRF mediates activity-induced gene expression and synaptic plasticity but not neuronal viability. *Nat Neurosci* 8, 759-767
11. Lemberger, T., *et al.* (2008) CREB has a context-dependent role in activity-regulated transcription and maintains neuronal cholesterol homeostasis. *Faseb J* 22, 2872-2879
12. Jancic, D., *et al.* (2009) Inhibition of cAMP Response Element-Binding Protein Reduces Neuronal Excitability and Plasticity, and Triggers Neurodegeneration. *Cereb Cortex* 19, 2535-2547
13. Balschun, D., *et al.* (2003) Does cAMP response element-binding protein have a pivotal role in hippocampal synaptic plasticity and hippocampus-dependent memory? *J Neurosci* 23, 6304-6314
14. Zhang, W., and Linden, D.J. (2003) The other side of the engram: experience-driven changes in neuronal intrinsic excitability. *Nat Rev Neurosci* 4, 885-900
15. Disterhoft, J.F., and Oh, M.M. (2006) Learning, aging and intrinsic neuronal plasticity. *Trends Neurosci* 29, 587-599
16. Mozzachiodi, R., and Byrne, J.H. (2009) More than synaptic plasticity: role of nonsynaptic plasticity in learning and memory. *Trends Neurosci*
17. Dong, Y., *et al.* (2006) CREB modulates excitability of nucleus accumbens neurons. *Nat Neurosci* 9, 475-477
18. Han, M.H., *et al.* (2006) Role of cAMP response element-binding protein in the rat locus ceruleus: regulation of neuronal activity and opiate withdrawal behaviors. *J Neurosci* 26, 4624-4629
19. Lopez de Armentia, M., *et al.* (2007) cAMP response element-binding protein-mediated gene expression increases the intrinsic excitability of CA1 pyramidal neurons. *J Neurosci* 27, 13909-13918
20. Viosca, J., *et al.* (2009) Enhanced CREB-dependent gene expression increases the excitability of neurons in the basal amygdala and primes the consolidation of contextual and cued fear memory. *Learn Mem* 16, 193-197

21. Zhou, Y., *et al.* (2009) CREB regulates excitability and the allocation of memory to subsets of neurons in the amygdala. *Nat Neurosci* 12, 1438-1443
22. Chen, Z., *et al.* (2007) Cyclic AMP signaling contributes to neural plasticity and hyperexcitability in AH sensory neurons following intestinal *Trichinella spiralis*-induced inflammation. *Int J Parasitol* 37, 743-761
23. Conn, P.J., *et al.* (1989) Protein kinase inhibitors selectively block phorbol ester- or forskolin-induced changes in excitability of *Aplysia* neurons. *J Neurosci* 9, 473-479
24. Beck, H., and Yaari, Y. (2008) Plasticity of intrinsic neuronal properties in CNS disorders. *Nat Rev Neurosci* 9, 357-369
25. Alshuaib, W.B., and Mathew, M.V. (1998) Potassium current in *Drosophila* neurons is increased by either *dunce* mutation or cyclic AMP. *J Neurosci Res* 52, 521-529
26. Zhong, Y., and Wu, C.F. (1993) Differential modulation of potassium currents by cAMP and its long-term and short-term effects: *dunce* and *rutabaga* mutants of *Drosophila*. *J Neurogenet* 9, 15-27
27. Zhao, M.L., and Wu, C.F. (1997) Alterations in frequency coding and activity dependence of excitability in cultured neurons of *Drosophila* memory mutants. *J Neurosci* 17, 2187-2199
28. Huang, Y.H., *et al.* (2008) CREB modulates the functional output of nucleus accumbens neurons: a critical role of N-methyl-D-aspartate glutamate receptor (NMDAR) receptors. *J Biol Chem* 283, 2751-2760
29. Marie, H., *et al.* (2005) Generation of silent synapses by acute in vivo expression of CaMKIV and CREB. *Neuron* 45, 741-752
30. Barco, A., *et al.* (2002) Expression of constitutively active CREB protein facilitates the late phase of long-term potentiation by enhancing synaptic capture. *Cell* 108, 689-703
31. Pittenger, C., *et al.* (2002) Reversible inhibition of CREB/ATF transcription factors in region CA1 of the dorsal hippocampus disrupts hippocampus-dependent spatial memory. *Neuron* 34, 447-462.
32. McClung, C.A., and Nestler, E.J. (2003) Regulation of gene expression and cocaine reward by CREB and DeltaFosB. *Nat Neurosci* 6, 1208-1215
33. Wallace, D.L., *et al.* (2009) CREB regulation of nucleus accumbens excitability mediates social isolation-induced behavioral deficits. *Nat Neurosci* 12, 200-209
34. Vogalis, F., *et al.* (2003) PKA-mediated inhibition of a novel K⁺ channel underlies the slow after-hyperpolarization in enteric AH neurons. *J Physiol* 548, 801-814
35. Pedarzani, P., and Storm, J.F. (1993) PKA mediates the effects of monoamine transmitters on the K⁺ current underlying the slow spike frequency adaptation in hippocampal neurons. *Neuron* 11, 1023-1035
36. Pedarzani, P., and Storm, J.F. (1995) Dopamine modulates the slow Ca(2+)-activated K⁺ current IAHP via cyclic AMP-dependent protein kinase in hippocampal neurons. *J Neurophysiol* 74, 2749-2753
37. Haug, T., and Storm, J.F. (2000) Protein kinase A mediates the modulation of the slow Ca(2+)-dependent K(+) current, I(sAHP), by the neuropeptides CRF, VIP, and CGRP in hippocampal pyramidal neurons. *J Neurophysiol* 83, 2071-2079
38. Tzingounis, A.V., and Nicoll, R.A. (2008) Contribution of KCNQ2 and KCNQ3 to the medium and slow afterhyperpolarization currents. *Proc Natl Acad Sci U S A* 105, 19974-19979

39. Oh, M.M., *et al.* (2009) Learning-related postburst afterhyperpolarization reduction in CA1 pyramidal neurons is mediated by protein kinase A. *Proc Natl Acad Sci U S A* 106, 1620-1625
40. Peters, H.C., *et al.* (2005) Conditional transgenic suppression of M channels in mouse brain reveals functions in neuronal excitability, resonance and behavior. *Nat Neurosci* 8, 51-60
41. Brunelli, M., *et al.* (1976) Synaptic facilitation and behavioral sensitization in Aplysia: possible role of serotonin and cyclic AMP. *Science* 194, 1178-1181
42. Byers, D., *et al.* (1981) Defect in cyclic AMP phosphodiesterase due to the dunce mutation of learning in *Drosophila melanogaster*. *Nature* 289, 79-81
43. Dudai, Y., *et al.* (1983) Abnormal activity of adenylate cyclase in the *Drosophila* memory mutant rutabaga. *Neurosci Lett* 42, 207-212
44. Yin, J.C., *et al.* (1995) CREB as a memory modulator: induced expression of a dCREB2 activator isoform enhances long-term memory in *Drosophila*. *Cell* 81, 107-115
45. Silva, A.J., *et al.* (1998) CREB and memory. *Annu Rev Neurosci* 21, 127-148
46. Josselyn, S.A., and Nguyen, P.V. (2005) CREB, synapses and memory disorders: past progress and future challenges. *Curr Drug Targets CNS Neurol Disord* 4, 481-497
47. Cole, C.J., and Josselyn, S.A. (2008) Transcription regulation of memory: CREB, CaMKIV, Fos/Jun, CBP, and SRF. In *Learning and Memory: A comprehensive reference* (Sweatt, J.D., and Byrne, J.H., eds), 547-566, Elsevier
48. Alberini, C.M. (2009) Transcription factors in long-term memory and synaptic plasticity. *Physiological reviews* 89, 121-145
49. Barco, A., *et al.* (2007) CREB-dependent transcription and synaptic plasticity. . In *Regulation of transcription by neuronal activity: To the Nucleus and Back* (Dudek, S., ed), 127-154, Springer Science Publishers
50. Rammes, G., *et al.* (2000) Synaptic plasticity in the basolateral amygdala in transgenic mice expressing dominant-negative cAMP response element-binding protein (CREB) in forebrain. *Eur J Neurosci* 12, 2534-2546
51. Gass, P., *et al.* (1998) Deficits in memory tasks of mice with CREB mutations depend on gene dosage. *Learn Mem* 5, 274-288
52. Graves, L., *et al.* (2002) Behavioral analysis of CREB alphasdelta mutation on a B6/129 F1 hybrid background. *Hippocampus* 12, 18-26
53. Brightwell, J.J., *et al.* (2007) Long-term memory for place learning is facilitated by expression of cAMP response element-binding protein in the dorsal hippocampus. *Learn Mem* 14, 195-199
54. Mouravlev, A., *et al.* (2006) Somatic gene transfer of cAMP response element-binding protein attenuates memory impairment in aging rats. *Proc Natl Acad Sci U S A* 103, 4705-4710
55. Josselyn, S.A., *et al.* (2001) Long-term memory is facilitated by cAMP response element-binding protein overexpression in the amygdala. *J Neurosci* 21, 2404-2412.
56. Wallace, T.L., *et al.* (2004) Effects of cyclic adenosine monophosphate response element binding protein overexpression in the basolateral amygdala on behavioral models of depression and anxiety. *Biol Psychiatry* 56, 151-160
57. Restivo, L., *et al.* (2008) Viral-mediated expression of a constitutively active form of CREB in hippocampal neurons increases memory. *Hippocampus* 19, 228-234
58. Kida, S., *et al.* (2002) CREB required for the stability of new and reactivated fear memories. *Nat Neurosci* 5, 348-355.

59. Josselyn, S.A., *et al.* (2004) Inducible repression of CREB function disrupts amygdala-dependent memory. *Neurobiol Learn Mem* 82, 159-163
60. Sah, P., and Bekkers, J.M. (1996) Apical dendritic location of slow afterhyperpolarization current in hippocampal pyramidal neurons: implications for the integration of long-term potentiation. *J Neurosci* 16, 4537-4542
61. Cohen, A.S., *et al.* (1999) Long-lasting increase in cellular excitability associated with the priming of LTP induction in rat hippocampus. *J Neurophysiol* 82, 3139-3148
62. Faber, E.S., *et al.* (2005) SK channels regulate excitatory synaptic transmission and plasticity in the lateral amygdala. *Nat Neurosci* 8, 635-641
63. Fuenzalida, M., *et al.* (2007) Changes of the EPSP waveform regulate the temporal window for spike-timing-dependent plasticity. *J Neurosci* 27, 11940-11948
64. Reymann, K.G., and Frey, J.U. (2007) The late maintenance of hippocampal LTP: requirements, phases, 'synaptic tagging', 'late-associativity' and implications. *Neuropharmacology* 52, 24-40
65. Barco, A., *et al.* (2008) Synapse-specific stabilization of plasticity processes: The synaptic tagging and capture hypothesis revisited 10 years later. *Neurosci Biobehav Rev* 32, 831-851
66. Zelcer, I., *et al.* (2006) A cellular correlate of learning-induced metaplasticity in the hippocampus. *Cereb Cortex* 16, 460-468
67. Saar, D., and Barkai, E. (2009) Long-lasting maintenance of learning-induced enhanced neuronal excitability: mechanisms and functional significance. *Mol Neurobiol* 39, 171-177
68. Han, J.H., *et al.* (2007) Neuronal competition and selection during memory formation. *Science* 316, 457-460
69. Han, J.H., *et al.* (2009) Selective erasure of a fear memory. *Science* 323, 1492-1496
70. Silva, A.J., *et al.* (2009) Molecular and cellular approaches to memory allocation in neural circuits. *Science* 326, 391-395
71. Marshall, L., and Born, J. (2007) The contribution of sleep to hippocampus-dependent memory consolidation. *Trends Cogn Sci* 11, 442-450
72. Girardeau, G., *et al.* (2009) Selective suppression of hippocampal ripples impairs spatial memory. *Nat Neurosci* 12, 1222-1223
73. Viosca, J., *et al.* (2009) Chronic enhancement of CREB activity in the hippocampus interferes with the retrieval of spatial information. *Learn Mem* 16, 198-209
74. Daoudal, G., and Debanne, D. (2003) Long-term plasticity of intrinsic excitability: learning rules and mechanisms. *Learn Mem* 10, 456-465
75. Li, C.Y., *et al.* (2004) Bidirectional modification of presynaptic neuronal excitability accompanying spike timing-dependent synaptic plasticity. *Neuron* 41, 257-268
76. Moyer, J.R., Jr., *et al.* (2000) Increased excitability of aged rabbit CA1 neurons after trace eyeblink conditioning. *J Neurosci* 20, 5476-5482
77. Oh, M.M., *et al.* (2003) Watermaze learning enhances excitability of CA1 pyramidal neurons. *J Neurophysiol* 90, 2171-2179
78. Porte, Y., *et al.* (2008) Spatial memory in the Morris water maze and activation of cyclic AMP response element-binding (CREB) protein within the mouse hippocampus. *Learn Mem* 15, 885-894
79. Cudmore, R.H., and Turrigiano, G.G. (2004) Long-term potentiation of intrinsic excitability in LV visual cortical neurons. *J Neurophysiol* 92, 341-348

80. Zhang, F.L., and Casey, P.J. (1996) Protein prenylation: molecular mechanisms and functional consequences. *Annu Rev Biochem* 65, 241-269
81. Kojima, N., *et al.* (2008) Inducible cAMP early repressor acts as a negative regulator for kindling epileptogenesis and long-term fear memory. *J Neurosci* 28, 6459-6472
82. Porter, B.E., *et al.* (2008) The role of transcription factors cyclic-AMP responsive element modulator (CREM) and inducible cyclic-AMP early repressor (ICER) in epileptogenesis. *Neuroscience* 152, 829-836
83. Barrot, M., *et al.* (2002) CREB activity in the nucleus accumbens shell controls gating of behavioral responses to emotional stimuli. *Proc Natl Acad Sci U S A* 99, 11435-11440
84. Carlezon, W.A., Jr., *et al.* (1998) Regulation of cocaine reward by CREB. *Science* 282, 2272-2275
85. Green, T.A., *et al.* (2006) Induction of inducible cAMP early repressor expression in nucleus accumbens by stress or amphetamine increases behavioral responses to emotional stimuli. *J Neurosci* 26, 8235-8242
86. Fasano, S., *et al.* (2009) Inhibition of CREB activity in the dorsal portion of the striatum potentiates behavioral responses to drugs of abuse. *Front Behav Neurosci* 3, 29
87. Sanchis-Segura, C., *et al.* (2009) Inhibition of cAMP responsive element binding protein in striatal neurons enhances approach and avoidance responses towards morphine- and morphine withdrawal-related cues. *Front Behav Neurosci* 3, 30
88. Carlezon, W.A., Jr., *et al.* (2005) The many faces of CREB. *Trends Neurosci* 28, 436-445
89. Dudai, Y. (2002) *Memory from A to Z : keywords, concepts, and beyond.* Oxford University Press

Figure legends

Figure 1. The cellular consolidation model: CREB and synaptic plasticity.

Learning and memory storage are thought to depend on long-lasting changes in the strength of synaptic connections. These stable changes may last hours, days or even years, and are known to depend on *de novo* gene expression. This is the case for the late phase of long-term potentiation (LTP) in the mammalian hippocampus or long-term facilitation (LTF) in *Aplysia* sensory neurons, two processes that are blocked by inhibitors of transcription and that have provided a compelling view of how the induction of gene expression can modify synaptic function. The CREB pathway has been identified as a major regulator of both processes. In hippocampal neurons, membrane depolarization results in an increase in intracellular Ca^{2+} concentration via NMDARs (i) which, in turn, leads to activation of the Ca^{2+} sensor calmodulin and its target kinase CamKIV (ii). The activation of G-protein coupled receptors (GPCRs) by modulatory neurotransmitters (iii), such as serotonin or dopamine, increases the intracellular cAMP and activates another important CREB kinase, PKA (iv). Growth factors, neurotrophins, and other stimuli can also play a role in the activation of this pathway (see Box 1). The transcriptional program initiated by CREB activation (v) is thought to provide the building blocks (vi) required for the stabilization of the otherwise transient strengthening of synaptic connections, but its specific composition is still poorly defined.

Figure 2. The cAMP pathway and intrinsic plasticity. The schematic depicts the current understanding of the role of cAMP/PKA-dependent signaling in modulating intrinsic excitability, as well as the putative role of CREB in this process. Following a burst of action potentials, voltage-activated Ca^{2+} channels will open, giving rise to an increase in the intracellular Ca^{2+} concentration, which, in turn, may induce the

liberation of Ca^{2+} from intracellular stores (Ca^{2+} -induced Ca^{2+} release, CICR). This elevation of cytosolic Ca^{2+} concentration activates a series of Ca^{2+} -sensitive K^+ channels that serve to repolarize the cell, and also underlie the AHP. On the other hand, neuromodulatory inputs can result in increased cAMP concentrations, thereby activating the PKA pathway, which inhibits AHP by unknown mechanisms (e.g. phosphorylation of specific channels or accompanying regulatory molecules). The more recent view of this process considers the participation of CREB-dependent gene expression³⁹. Thus, in addition to regulating rapid changes in excitability (short-term), the activation of PKA may induce CREB-dependent changes in the expression of excitability modulators, such as Na^+ or K^+ channels, that may be necessary to sustain the excitability changes over time (long-term, e.g., during learning periods). Since CREB is a transcriptional activator, the downregulation of K^+ currents could result from indirect regulatory mechanisms, such as the induction of transcriptional repressors or microRNAs.

Figure 3. Schematic illustrating the role of CREB in learning and memory.

Neuronal stimulation results in the activation of second messenger signaling cascades (e.g. cAMP or Ca^{2+} -dependent signaling), leading to the modulation of Na^+ and K^+ currents (e.g., regulation of the AHP current) and triggering synaptic potentiation (E-LTP). If a given stimulation threshold is reached, the activation of the same cascades will result in the induction of CREB-dependent gene expression. CREB-regulated genes include both molecules implicated in synaptic remodeling (both structural and functional) and in modulation of intrinsic excitability. Both kinds of changes interact and contribute to learning and memory processes. Whereas the consolidation of synaptic reinforcements is thought to be integral part of the memory trace, the modulation of intrinsic excitability would play a more indirect role: it can contribute

to memory allocation, facilitate the occurrence of the synaptic changes or enable the consolidation of the memory trace at the network or system level by maintaining the neurons in a sensitized state (more details in the text). These three phenomena seem more related to the process of generation of the memory trace (learning) than to its retention over time (memory).

Box 1. Regulation of CREB activation by neuronal activity and subsequent modulation of neuronal function by CREB-dependent gene expression. *Left panel.* CREB, together with CREM and ATF1, is part of the CREB family of transcription factors, a group of structurally related transcription factors characterized by the presence of a highly conserved basic region/leucine zipper (bZIP) domain that bind to a specific DNA sequence called cAMP-responsive-element (CRE) found in one or several copies in the promoters of many genes. Although CREB can bind to CRE sites in the basal condition, it is inactive until it is phosphorylated. Synaptic activity, hormones, growth factors released during development, hypoxia and stress, among other stimuli, can trigger CREB phosphorylation via multiple protein-kinase pathways (green arrows), promoting the recruitment of the RNAPol II complex to CRE-containing promoters, and the subsequent induction of CREB-dependent gene expression. Overall, more than 300 different stimuli have been reported to act through the CREB pathway and many different protein kinases have been found to phosphorylate CREB in vitro ⁴, although the relevance of many of these activities in neuronal function is still unclear. *Right panel.* CREB phosphorylation initiates a transcriptional program that is still poorly understood (purple arrows). The list of CREB target genes is heterogeneous and includes several hundred genes with very different functions, from transcription and metabolism regulation to cell structure or signaling. Some of these genes are themselves transcription factors and their expression may trigger a second wave of gene expression. The activation of CREB-dependent gene expression also results in the activation of repressive feedback mechanisms, for example through the induction of repressor isoforms such as the cAMP early repressor (ICER). In spite of the recent advances in the field, the complete set of genes regulated by CREB activation in a specific cell type or after a

specific stimulus is still not known. Some representative CREB target genes are shown in the figure, but this list is far from exhaustive (left panel modified from Lonze and Ginty, 2002).

(insert here Box 1 Figure)

Glossary Box

- Intrinsic plasticity: Changes in the efficiency of the coupling between excitatory postsynaptic potential and spikes.

- Synaptic plasticity: Changes in the efficacy of excitatory or inhibitory synaptic connections (e.g., *synaptic strength*) between neurons.

- Cellular consolidation of memory: Molecular mechanism underlying the stabilization of functional and/or structural changes in the strength of synaptic connections.

- Neuronal sensitization: Enhancement of the response to a given stimulus or reduction of the threshold that must be surpassed to elicit a given response.

- Synaptic tagging and capture: Conceptual model to explain how the newly synthesized synaptic proteins or mRNAs required in cellular consolidation are specifically targeted to active synapses. It proposes that the persistence of the changes in synaptic strength requires both the generation of a transient local synaptic tag that labels recently activated synapses and the exclusive capture of *de novo* made plasticity-related proteins (PRPs) at those synapses marked with the tag.

- System consolidation of memory: This term is used here to refer, in a broad sense, to the mechanisms for consolidation of the memory trace that acts above the synaptic and cellular levels. This term is most often used to refer in particular to the transfer of the memory trace from the hippocampus to cortical circuits.

- Learning: Process of induction of a lasting alteration in behavior, or in the behavioral potential, caused by the behavioral experience of the individual / Experience-dependent generation of enduring internal representations, or lasting

modifications in such representations (according to the definition in *Memory from A to Z*⁸⁹).

- Learning mode: State that favors the occurrence of learning. The term can be used to refer to both an individual or to single neurons in the brain of that individual. In the context of this review, we refer to the neuronal state.

- Memory: Enduring change in behavior, or in the behavioral potential, that results from the behavioral experience of the individual / Retention over time of experience-dependent internal representations (according to *Memory from A to Z*).

Outstanding questions

- How do intrinsic and synaptic plasticity interact at the molecular and cellular level? And how does this interaction affect the acquisition and maintenance of memory traces?
- Does the regulation of neuronal excitability by the cAMP pathway have, like long-term potentiation, a late phase that is dependent on *de novo* protein synthesis and gene expression? And if so, what is its duration? What are the mechanisms that enable the restoration of basal intrinsic excitability?
- What is the contribution of CREB-controlled intrinsic excitability to memory? Is CREB activity required in learning-induced changes in neuronal excitability? Can CREB activation trigger a relatively durable “learning state” through the regulation of intrinsic excitability?
- Which are the CREB downstream genes involved in intrinsic excitability? And, to which extent are they common with synaptic plasticity-related targets?

Table 1. Modulation of intrinsic and synaptic plasticity by CREB

	CREB overactivation	CREB inhibition
Locus ceruleus	ca-CREB (CREB-VP16) and wt-CREB rHSV transduced mice ¹⁸	dn-CREB (CREB ^{S133A}) rHSV transduced mice ¹⁸
Firing rate	↑ ^{a, 18}	↓ ¹⁸
Resting membrane potential	Depolarized (ca-CREB) ¹⁸	Hyperpolarized ¹⁸
Threshold for eliciting an AP	N.D.	N.D.
Input resistance	N.D.	N.D.
AHP	N.D.	N.D.
Nucleus accumbens	ca-CREB (CREB ^{Y134F}) Sindbis transduced mice ^{17, 28}	dn-CREB (CREB ^{S133A}) Sindbis transduced mice ¹⁷
Firing rate	↑ ¹⁷ ↑ during upstates ²⁸	↓ ¹⁷ ↓ during upstates ²⁸
Resting membrane potential	= ¹⁷	= ¹⁷
Threshold for eliciting an AP	↓ (Na ⁺ spike) ¹⁷	↑ (Na ⁺ spike) ¹⁷
Input resistance	↑ ¹⁷	↓ (trend) ¹⁷
AHP	= ¹⁷	= ¹⁷
Synaptic effects	↑ NMDAR EPSC amplitude ²⁸ ↑ NMDAR surface expression ²⁸	= ²⁸
CA1 subfield	ca-CREB (VP16-CREB) transgenic mice ¹⁹	dn-CREB (A-CREB) transgenic mice ¹²
Firing rate	↑ ¹⁹	↓ ¹²
Resting membrane potential	= ¹⁹	= ¹²
Threshold for eliciting an AP	↑ ¹⁹	↑ (also rheobase) ¹²
Input resistance	= ¹⁹	↓ ¹²
AHP	↓ mAHP and sAHP ¹⁹	↑ mAHP ¹²
Synaptic effects ^b	↑ LTP ^{19, 30}	↓ LTP ¹²
Other	ND	↑ M current ¹²
Amygdala	ca-CREB (VP16-CREB) transgenic mice ²⁰ , wt-CREB rHSV transduced mice ²¹	N.D.
Firing rate	↑ ^{20, 21}	N.D.
Resting membrane potential	= ^{20, 21}	N.D.
Threshold for eliciting an AP	= ²⁰ / ↓ ²¹	N.D.
Input resistance	= ^{20, 21}	N.D.
AHP	↓ ^{20, 21}	N.D.
Synaptic effects	↑ EPSCs ²¹ ↓ PPF ²¹	N.D.

rHSV: recombinant Herpes simplex virus; AP: action potential; AHP: afterhyperpolarization; N.D.: Not determined; EPSCs: excitatory postsynaptic currents; PPF: Paired-pulse facilitation.

^a wt-CREB had no effect on firing at baseline, but enhanced the excitatory effect of forskolin; ca-CREB strongly increased the firing rate.

^b The effects of CREB in synaptic plasticity in the hippocampus have been also examined in CREB knockout and hypomorphic mice with conflicting results ^{2, 13}, in other dn-CREB transgenic strains ³¹, and in animals transduced with recombinant viruses ²⁹.

Figure 1
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Figure 3
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Figure in Box 1
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