

# MAPK, CREB and *zif268* are all required for the consolidation of recognition memory

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There has been nearly a century of interest in the idea that encoding and storage of information in the brain requires changes in the efficacy of synaptic connections between neurons that are activated during learning. Recent research into the molecular mechanisms of long-term potentiation (LTP) has brought about new knowledge that has provided valuable insights into the neural mechanisms of memory storage. The evidence indicates that rapid activation of the genetic machinery can be a key mechanism underlying the enduring modification of neural networks required for the stability of memories. In recent years, a wealth of experimental data has highlighted the importance of mitogen-activated protein kinase/extracellular signal-regulated kinase (MAPK/ERK) signalling in the regulation of gene transcription in neurons. Here, we briefly review experiments that have shown MAPK/ERK, cAMP response element-binding protein (CREB) and the immediate early gene (IEG) *zif268* are essential components of a signalling cascade required for the expression of late phase LTP and of certain forms of long-term memory. We also present experiments in which we have assessed the role of these three molecules in recognition memory. We show that pharmacological blockade of MAPK/ERK phosphorylation, functional inactivation of CREB in an inducible transgenic mouse and inactivation of *zif268* in a mutant mouse result in a similar deficit in long-term recognition memory. In the continuing debate about the role of LTP mechanisms in memory, these findings provide an important complement to the suggestion that synaptic changes brought about by LTP and memory consolidation and storage share, at least in part, common underlying molecular mechanisms.

**Keywords:** learning; long-term potentiation; synaptic plasticity; hippocampus; gene expression; transgenic mice

## 1. INTRODUCTION

In 1973, when Tim Bliss and Terje Lømo published their research and discovery of LTP (Bliss & Lømo 1973), they warned that 'whether or not the intact animal makes use in real life of a property which has been revealed by synchronous, repetitive volley to a population of fibres the normal rate and pattern of activity along which are unknown, is another matter'; the central, simple question being: does LTP have a role to play in the laying down and/or readout of memories? Since then, as some of the key molecular biological mechanisms underlying the induction and expression of LTP were being elucidated, many groups were striving to characterize the function of this particular property of brain synapses in the intact behaving animal. The results of the past 30 years of research have firmly established that the type of synaptic

change that is brought about by LTP is a key player in memory function and dysfunction, even if there are still issues and questions about the role of LTP that are the subject of intensive debate. All along, a more profound, paradigmatic shift has been set in motion; no sooner was LTP discovered and a wealth of molecular information on its underlying mechanisms made available, than the beginnings of a new era in the cellular and molecular exploration of memory under both normal conditions and in disease states were framed. This has been driven by the increasing knowledge about LTP mechanisms, and also by the development of novel technologies specifically designed to exploit these molecular data. The net result has been a closer interaction between the fields of molecular, cellular, system and cognitive neuroscience that culminated in the past decade in the birth of the field of molecular and cellular cognition.

Among the many important advances that have been made in uncovering some of the mechanisms of LTP, one of the most intriguing is the realization that the mechanisms underlying the longer-lasting phases of LTP engage the genetic programme of neurons and result in

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One contribution of 30 to a Theme Issue 'Long-term potentiation: enhancing neuroscience for 30 years'.

the synthesis of new proteins. The evidence came from two main sources: the fact that inhibitors of either protein synthesis (Krug *et al.* 1984; Otani & Abraham 1989; Frey & Morris 1997) or transcription (Nguyen *et al.* 1994) affect the duration of LTP; and the finding that LTP itself induces transcriptional regulation of a variety of genes, including inducible transcription factors (Cole *et al.* 1989; Wisden *et al.* 1990; Abraham *et al.* 1993; Worley *et al.* 1993; French *et al.* 2001) and genes encoding synaptic proteins (e.g. Smirnova *et al.* 1993; Thomas *et al.* 1994, 1996; Link *et al.* 1995; Lyford *et al.* 1995; Bramham *et al.* 1996; Hicks *et al.* 1997; Génin *et al.* 2001). Remarkably, experimental evidence also indicates that the expression of long-term memories shares many characteristics with LTP, including similar molecular mechanisms, a requirement for protein synthesis (Davis & Squire 1984; Meiri & Rosenblum 1998) and, in specific areas of the brain, the regulated transcription of a variety of genes (Nikolaev *et al.* 1992; Davis *et al.* 1996, 1998; Okuno & Miyashita 1996; Guzowski *et al.* 1999, 2001; Tischmeyer & Grimm 1999; Hall *et al.* 2000; Zhao *et al.* 2000; Cavallaro *et al.* 2001). Many genes have been shown to be up- and downregulated in a finely tuned and coordinated manner and the full genomic response of neurons associated with specific aspects of LTP and memory processes is currently the subject of intensive investigation using newly available large-scale screening methods.

An important issue in this context is to understand how synaptic events signal gene regulation. Again, studies of synapse-to-nuclear signalling in LTP have provided important inroads for exploring memory mechanisms and in the past 10 years several important operating molecules have been identified. In LTP, as in many other aspects of cell function involving regulated gene transcription, two important steps appear to be critical: the activation of protein kinases and of constitutively expressed transcription factors and shortly after, the expression of a class of IEGs encoding regulatory transcription factors which interact with promoter regulatory elements of a host of downstream effector genes. To illustrate this process, and although not intended as a comprehensive overview of the topic, this article focuses on three key molecules, the ERK family of MAPK, the transcription factor CREB and the IEG *zif268*. We begin with a brief review of recent findings suggesting these three molecules are components of a signalling system from the synapse to the nucleus that is crucial for the long-term stabilization of neural plasticity and for the consolidation of certain forms of memories. We also report experiments evaluating their role in recognition memory, a form of memory based on the ability to discriminate between novel and familiar objects which in humans, monkeys and rodents is affected by damage to structures of the medial temporal lobe, including the hippocampus and adjacent entorhinal and parahippocampal cortices (reviewed in Clark *et al.* 2000; Zola *et al.* 2000). We have assessed the role of these three molecules in trial-unique recognition memory by using three different strategies: pharmacological blockade of MAPK/ERK activation in rats, functional inactivation of CREB in inducible transgenic mice and inactivation of the *zif268* gene in a null mutant mouse. Our results indicate that the MAPK/ERK-CREB-*zif268* signalling pathway is essential for long-term recognition memory.

## 2. THE IEG *zif268*, LTP AND MEMORY CONSOLIDATION

*zif268*, also known as *Egr-1*, *Krox-24*, *NGFI-A* or *Zenk*, is an IEG encoding a zinc finger transcription factor originally identified as a nerve growth factor response gene product in PC12 cells and an immediate-early serum response gene product in fibroblasts (Milbrandt 1987; Christy *et al.* 1988; Lemaire *et al.* 1988; Sukhatme *et al.* 1988). The gene encodes a three zinc finger protein which binds to cognate GC-rich response elements in the DNA to regulate downstream expression of late-response genes (Christy & Nathans 1989; Swirnoff & Milbrandt 1995). The mRNA and protein are expressed in several areas of the neocortex, hippocampus, entorhinal cortex, amygdala, striatum and cerebellum (Christy *et al.* 1988; Mack *et al.* 1990; Worley *et al.* 1991). In the hippocampus, its expression gradually increases in the second week after birth and remains elevated in the CA1/CA3 regions, but it is only transient in the dentate gyrus where its constitutive expression fades after three weeks (Watson & Milbrandt 1990; Herms *et al.* 1994). *zif268* was found to be rapidly and transiently turned on by a variety of pharmacological and physiological stimuli including neurotransmitters, growth factors, peptides, depolarization, seizures, ischemia and brain injury or cellular stress (reviewed in Gashler & Sukhatme 1995; Beckmann & Wilce 1997; O'Donovan *et al.* 1999). Further emphasizing the role of *zif268* in cellular physiology, two independent groups showed rapid and robust activation of *zif268* in the dentate gyrus after the induction of LTP (Cole *et al.* 1989; Wisden *et al.* 1990). Even though tens of distinct IEGs, including other members of the *Egr* family, can be induced by synaptic activity in an *N*-methyl-D-aspartate receptor-dependent manner, *zif268* has attracted much attention because its regulated transcription in the dentate gyrus is both reliably associated with the expression of the protein synthesis-dependent late phase of LTP and appears to correlate with the persistence of LTP (Abraham *et al.* 1991, 1993; Richardson *et al.* 1992; Worley *et al.* 1993). Recently, the analysis of *zif268* DNA binding activity using gel-shift assays revealed that the increase in *zif268* protein following induction of LTP is associated with increased binding of the protein to its response element (Williams *et al.* 2000), indicating functional activation of downstream genes containing *zif268* response elements.

Behavioural studies also provided evidence that the expression of *zif268* is sensitive to natural environmental stimuli following exposure of rats to novel environments (Hall *et al.* 2000) or in a learning context (Tischmeyer & Grimm 1999). The induction occurs rapidly and is transient, indicating a role in the transition from short- to long-term memory. For example, Okuno & Miyashita (1996) found that *zif268*, but not c-Fos or JunD, is activated in the infero-temporal cortex of macaque monkeys trained in a form of explicit memory task, visual paired associate learning. Learning-related increases in *zif268* expression have also been observed in the hippocampus after active avoidance learning (Nikolaev *et al.* 1992), brightness discrimination (Grimm & Tischmeyer 1997), recall of contextual fear memory (Hall *et al.* 2001) or spatial learning (Guzowski *et al.* 2001), and in the amygdala after

acquisition (Malkani & Rosen 2000) or recall (Hall *et al.* 2001) of contextual fear conditioning.

In recent experiments in collaboration with Tim Bliss and colleagues, we were able to investigate the role of *zif268* in LTP and learning using mutant mice (Jones *et al.* 2001). In these experiments, we found that basal synaptic transmission, cell excitability, forms of short-term plasticity such as paired-pulse facilitation and paired-pulse depression were normal in the dentate gyrus of *zif268* mutant mice; however, LTP, which was normal for the first hour, was not maintained over 24 h in awake animals, showing that the *zif268* gene is necessary for the expression of the later phases of LTP. The construct used to inactivate *zif268* in the mutant mice (Topilko *et al.* 1998) involved the insertion of a *LacZ* cassette downstream of the *zif268* promoter; we found that in the mutant mice the constitutive and LTP-inducible expression of the *LacZ* gene was comparable to that of *zif268* in WT mice, indicating that signalling events upstream of *zif268* transcription were not affected by the mutation. We examine short-term and long-term memory in these mice, by using a variety of behavioural tasks that make use of single or repeated training, different types of reinforcement, and the processing of spatial or non-spatial information. The results showed that short-term memory is intact in *zif268* mutant mice. Analysis of the mutants revealed normal levels of spontaneous alternation in a T-maze, a readily observed pattern of behaviour that relies upon spatial working memory, normal short-term retention for odours as tested in a social transmission of food preference task, and normal short-term memory for object recognition. In contrast, we found that long-term memory in *zif268* mutant mice was severely impaired in several tasks including social transmission of food preference, object recognition, conditioned taste aversion and a spatial navigation task in the water-maze (Jones *et al.* 2001). Thus, these experiments establish that *zif268* is essential for the expression of late LTP in the dentate gyrus and for the expression of long-term memories.

### 3. THE MAPK/ERK SIGNALLING PATHWAY, *zif268* TRANSCRIPTION AND LTP

What are the intracellular signalling mechanisms that are responsible for the induction and regulation of *zif268* in synaptic plasticity and learning? A crucial event in signal transduction leading to gene regulation in neurons is the activation of protein kinases. Although several kinases, including PKC, PKA,  $\alpha$ CaMKII are known to play an important role in LTP (see Soderling & Derkach (2000) for a review), recent work has highlighted the potential role of the MAPK/ERK cascade as a critical trigger to initiate gene transcription after synaptic activation (see Sweatt (2001) for a review). Moreover, cross-talk between kinase pathways indicates that MAPK/ERK may be a point of convergence integrating PKC, PKA and CaMK signals (Impey *et al.* 1998a; Roberson *et al.* 1999; Vanhoutte *et al.* 1999), in addition to the activation of other kinase-selective target substrates. In cell lines, experimental evidence indicates that MAPK/ERK, once activated, translocates from the cytosol to the nucleus where it can regulate transcriptional activity of many IEGs (reviewed in Treisman 1996). This is likely to be

accomplished via two prime nuclear targets of activated MAPK/ERK, the transcription factors CREB and Elk-1. CREB is transactivated by MAPK/ERK via the CREB kinase ribosomal protein S6 kinase (Rsk2), and homo or heterodimers of the CREB/ATF family that can bind to CRE sites in the upstream regulatory region of several IEGs (see Lonze & Ginty (2002) for a review). Elk-1 is directly activated by MAPK/ERK and plays a pivotal role in IEG induction by various extracellular signals (Marais *et al.* 1993; Hipskind *et al.* 1994) via a ternary complex assembled on the SRE, another DNA sequence motif also present within the upstream regulatory region of many IEGs (Wasylyk *et al.* 1998). The upstream promoter region of the *zif268* gene contains two putative CRE sites and a series of six SREs in the 5'-flanking region (reviewed in Beckmann & Wilce 1997). The expression of *zif268* is therefore likely to be strongly controlled by the MAPK/ERK-CREB pathway targeting CRE and the MAPK/ERK-Elk pathway targeting SRE.

Several studies have shown that MAPK/ERK is rapidly phosphorylated after LTP and that this activation is essential for the expression of LTP in CA1 *in vitro* (English & Sweatt 1996, 1997; Patterson *et al.* 2001) and in the dentate gyrus *in vivo* (Davis *et al.* 2000; Rosenblum *et al.* 2000). CREB is also critical for hippocampal LTP (Bourtchuladze *et al.* 1994) and studies in CRE-*LacZ* transgenic mice indicate activation of CRE-mediated transcription in CA1 by the induction of LTP (Impey *et al.* 1996). Furthermore, LTP is enhanced in transgenic mice expressing a constitutively active form of CREB (Barco *et al.* 2002). In a recent study, we examined whether MAPK/ERK, CREB and Elk-1 were activated and required for LTP-induced transcriptional regulation of *zif268* *in vivo* (Davis *et al.* 2000). We found that LTP in the dentate gyrus leads to rapid phosphorylation and nuclear translocation of MAPK/ERK and a subsequent coordinated phosphorylation of both CREB and Elk-1. Inhibition of MAPK/ERK phosphorylation by an MEK inhibitor was shown to block phosphorylation of both CREB and Elk-1, and also to block LTP-dependent transcriptional activation of *zif268* in dentate granule cells, resulting in a rapidly decaying LTP. Thus, these results show that MAPK/ERK controls *zif268* expression in LTP and that this is mediated by two parallel and possibly cooperating signalling pathways, one targeting CRE-mediated transcription via CREB and the second targeting SRE-mediated transcription via Elk-1 (Davis *et al.* 2000). This cascade is likely to control the transcriptional activation of many other IEGs under the control of CRE and SRE elements, as has been shown for example for *c-fos* (Sgambato *et al.* 1998) or *arg3.1/arc* (Waltereit *et al.* 2001).

The identification of the crucial role of the MAPK/ERK signalling pathway in LTP precipitated an intensive search for its role in learning and memory. Within a few years, several groups had shown that MAPK/ERK is activated in the hippocampus and is required for a variety of hippocampal-dependent forms of learning (reviewed in Impey *et al.* 1999; Sweatt 2001). For example, inhibition of the upstream kinase MEK produces deficits in memory for cued and contextual fear associations (Atkins *et al.* 1998) and in spatial memory (Blum *et al.* 1999; Selcher *et al.* 1999). Similar results have been obtained in the lateral

nucleus of the amygdala in the consolidation of fear memory (Schafe *et al.* 2000) where *zif268* is also activated (Malkani & Rosen 2000; Hall *et al.* 2001). Moreover, coordinated activation of MAPK/ERK, CREB and Elk-1 has also been reported in the hippocampus after one-trial avoidance learning (Cammarota *et al.* 2000) and in the insular cortex. LTP in the insular cortex is associated with *zif268* expression and blocked by inhibition of MAPK/ERK (Jones *et al.* 1999). The insular cortex is also associated with long-term storage of taste memory (Berman *et al.* 1998).

Several studies have shown that CREB is also strongly implicated in memory formation in different paradigms. Deficits in long-term, but not short-term, memory have been observed in fear conditioning and spatial learning in mice with a targeted disruption of the  $\alpha\Delta$ CREB isoforms (Bourtchuladze *et al.* 1994). More recent experiments have shown deficits in spatial long-term memory after functional inactivation of CREB in the hippocampus using antisense oligodeoxynucleotides (Guzowski & McGaugh 1997), in memory for taste aversion after functional inactivation of CREB in the amygdala (Lamprecht *et al.* 1997), or in the memory for fear associations after induction of an inducible CREB repressor in transgenic mice (Kida *et al.* 2002). Conversely, a gain-of-function study by Josselyn *et al.* (2001) showed that overexpression of CREB in the amygdala via viral vector-mediated gene transfer enhances fear memory. Finally, Impey *et al.* (1998*b*), using CRE-*LacZ* transgenic mice, provided support for the recruitment of CRE-mediated transcription in CA1 during contextual fear conditioning. A more recent report suggests this is associated with MAPK/ERK activation and essential for long-term contextual memory (Athos *et al.* 2002). Thus, together with the functional implication of *zif268* as we have seen above, these findings indicate that the MAPK/ERK-CREB/Elk-1-*zif268* signalling pathway is one important route for inducing molecular changes underlying the type of synaptic plasticity required for formation of long-term memories.

#### 4. REQUIREMENT OF MAPK/ERK ACTIVATION FOR RECOGNITION MEMORY

To assess the role of MAPK/ERK in recognition memory, we used an object recognition task; this is a task based on the natural preference of rodents for novelty and their ability to discriminate the familiarity of previously encountered objects. In the standard task, rodents are placed in a small arena and briefly exposed to two objects that they can explore freely. Then, after a variable delay interval, one object is replaced by a new one. Normal animals prefer to explore the novel rather than the familiar object, thus demonstrating they remember the two objects they had previously had experience with. If the memory of the familiar objects has faded, however, they would spend equal time exploring the two objects.

In the present experiment, adult male Sprague-Dawley rats were allowed to explore and become familiarized to an empty circular open-field arena (five sessions of 20 min) before training. A single training session was then given where in the arena were placed two different complex-shaped three-dimensional objects constructed with Lego pieces that rats were allowed to explore freely (three 5 min periods separated by a 5 min interval). Retention

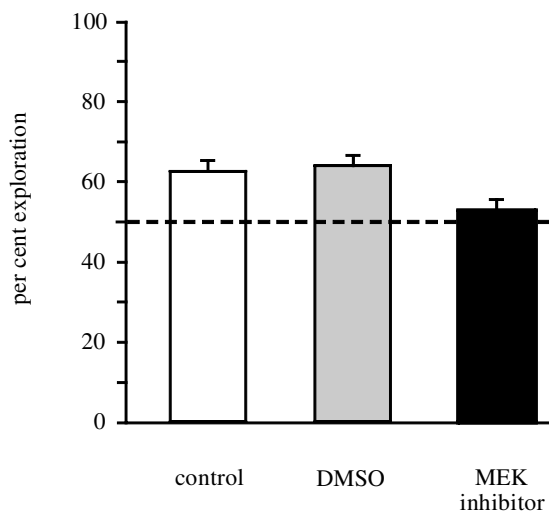


Figure 1. Long-term recognition memory is impaired by injection of the MEK inhibitor. Control rats (white bar) and rats with an implanted cannula receiving either the MEK inhibitor U0126 (black bar) or the vehicle DMSO (grey bar) were exposed to two objects (sample phase) and memory was tested 24 h later with one of the previously encountered objects replaced by a novel object. DMSO or U0126 were injected 40 min before the sample phase. The histograms represent the time spent exploring the novel object during the test session at a 24 h delay, expressed as a per cent of the total time of exploration of objects. Control rats and DMSO-injected rats spent significantly more time exploring the novel object, indicating good recognition memory. Rats injected with the MEK inhibitor showed no preference for the novel object.

was tested 24 h later in a single session (5 min) in which one of the two objects was replaced by a novel-shaped object and the time spent exploring each object was recorded. Objects were cleaned thoroughly between trials to ensure the absence of olfactory cues. We tested in this protocol normal rats ( $n = 8$ ) and rats implanted with a cannula in the lateral ventricle with which we injected an inhibitor of the upstream MAPK/ERK kinase MEK. The MEK inhibitor, U0126 ( $4 \mu\text{l}$ ,  $1 \mu\text{g} \mu\text{l}^{-1}$ , injected at a rate of  $1 \mu\text{l} \text{min}^{-1}$ ;  $n = 6$ ) or the vehicle, DMSO ( $n = 7$ ) were injected 40 min before the training session. We found that both normal rats and rats injected with DMSO explored the novel object more than the familiar object (figure 1). Exploration of the novel object was significantly above chance ( $p < 0.01$  in each case). In contrast, rats injected with the MEK inhibitor just before the training session did not show preference for the novel object ( $p > 0.05$ ) during the retention test 24 h later and explored the novel and familiar objects equally (figure 1). The performance of these rats was significantly different from both the non-injected and DMSO-injected rats ( $p < 0.05$  in each case). Inhibition of MAPK/ERK did not affect exploration during the training phase, and short-term memory tested 10 min after the initial exposure. By contrast, 24 h after training, memory was impaired by the MEK inhibitor (figure 1), indicating that blocking MAPK/ERK function does not impair behavioural exploration of novel objects, the encoding of a new experience or short-term memory for objects, but impairs the consolidation or expression of long-term recognition memory.

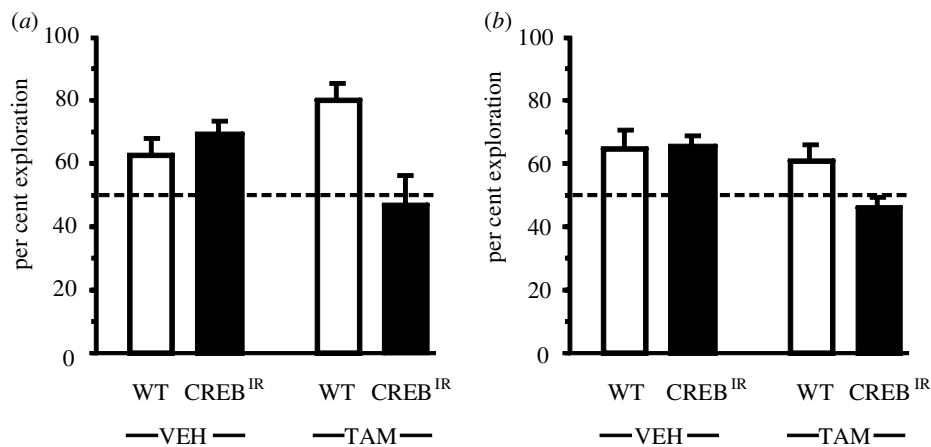


Figure 2. Long-term recognition memory is impaired after activation of the CREB repressor in transgenic mice. WT and CREB<sup>IR</sup> mice were exposed to three objects (sample phase) and memory was tested 24 h later with either (a) one familiar object replaced by a novel object or (b) one familiar object moved to a new location in the arena. The histograms represent the time spent exploring the novel object or the displaced object during the test phase at a 24 h delay, expressed as a per cent of the total time of exploration of objects (mean time for the two familiar/undisplaced objects plus time for novel or displaced objects). When the vehicle was injected (VEH), both WT mice (white bars) and CREB<sup>IR</sup> mice (black bars) spent significantly more time exploring the novel object or the displaced object. Activating CREB<sup>IR</sup> with an injection of tamoxifen (TAM) 6 h before the sample phase disrupted recognition memory in both the novel object task and the spatial change task, whereas it had no effect in WT mice.

## 5. REQUIREMENT OF CREB FOR RECOGNITION MEMORY

To test the role of CREB in recognition memory, we used a transgenic mouse line with a brain-specific and inducible CREB repressor (Kida *et al.* 2002). In these mice, the CREB repressor, a Ser<sup>133</sup> mutated  $\alpha$ CREB isoform, is fused to a LBD of a human oestrogen receptor with a G521R mutation, allowing induction of the CREB repressor by the drug 4-hydroxytamoxifen (tamoxifen). Injection of tamoxifen disrupts CRE-mediated transcription in transgenic mice expressing this inducible CREB repressor (LBD-CREB<sup>IR</sup>) under the control of the  $\alpha$ CaMKII promoter (Kida *et al.* 2002). With this inducible, spatially restricted CREB transgenic mouse, it is possible to switch CREB function off. Inducing the activation of the transgenic CREB repressor with tamoxifen 6 h before training in a cued or a contextual fear conditioning paradigm had no effect on short-term memory, but resulted in impaired long-term memory for both cued and contextual fear associations (Kida *et al.* 2002). Injection of tamoxifen 6 h before training produced the largest deficit in fear memory.

To test the impact of CREB inactivation in recognition memory in these mice, we opted for a more complex recognition task in which we could distinguish between spatial and non-spatial recognition memory in the same paradigm. To this end, we modified the object recognition task to assess separately memory for objects and memory for the spatial configuration of objects, based on the discrimination between novel and familiar objects (figure 2a) or between a novel and a familiar spatial location of an object (figure 2b). In this protocol, three distinct objects were used instead of two during the training phase, and a cue card was placed above one of the side walls of the open field (58 cm in length and 35 cm in height) to aid the mapping of the location of each object in space. The mice were habituated to the empty open field (two ses-

sions of 20 min) before training. On the first day of the experiment, mice were exposed to three novel objects that they were allowed to explore for two 10 min sessions with a 10 min interval. On the second day, one object was displaced to a new position and mice were given a 10 min session of exploration, thus testing the spatial version of the recognition memory task. On the third day, mice were exposed to three new objects and given the same exploration protocol as that on day 1, and then tested 24 h later (day 4) during a 10 min session in which one of the three objects was replaced by a novel object, testing the non-spatial version of the task. The time spent exploring each object was recorded and preference for the novel object or the displaced object was expressed as a per cent exploration of these objects over the total exploration time of objects.

Groups of CREB<sup>IR</sup> mice and WT litter-mate mice were tested twice in this protocol, with new sets of objects each time and a few days rest between sets. In one condition, CREB<sup>IR</sup> and WT mice were injected with tamoxifen (16 mg kg<sup>-1</sup>, i.p.) 6 h before the training phase, whereas in the other condition they were injected with the peanut oil vehicle solution. No difference in basic locomotor activity or in the time spent exploring objects during the training phase was observed, indicating tamoxifen injection or inducing the CREB repressor does not impair motor activity or the spontaneous tendency of mice to explore novelty. In the absence of tamoxifen, all mice, CREB<sup>IR</sup> ( $n = 8$ ) and WT ( $n = 10$ ), displayed strong preference for the novel object 24 h after training (figure 2a). There was no difference between groups ( $p > 0.05$ ) and exploration of the novel object was significantly greater than chance (50%) for both groups ( $p < 0.05$ ). When one of the familiar objects was moved to a new position, again both CREB<sup>IR</sup> ( $n = 8$ ) and WT ( $n = 6$ ) mice injected with the vehicle solution showed significantly greater exploration of the displaced object (figure 2b;  $p < 0.05$ ) with

no significant difference between groups ( $p > 0.05$ ). Similarly, when tamoxifen was injected in the WT mice, these mice displayed a strong preference for the novel (figure 2a) or the displaced (figure 2b) object. By contrast, when the CREB repressor was turned on by injecting tamoxifen 6 h before training in CREB<sup>IR</sup> mice, retention performance fell to chance levels in both the non-spatial (figure 2a) and spatial (figure 2b) version of the task. There was no preferential exploration of the novel object or of the object placed in a new position ( $p > 0.05$  in each case) and performance of the tamoxifen-injected CREB<sup>IR</sup> mice was significantly different from that of the tamoxifen-injected WT mice ( $p < 0.05$ ). Thus, these results indicate that disruption of CREB function impairs both memory for objects and memory for spatial location of objects at the 24 h delay.

## 6. REQUIREMENT OF THE IEG *zif268* FOR RECOGNITION MEMORY

To test the role of the IEG *zif268* in recognition memory, we used mice with a null mutation in the *zif268* gene generated by Patrick Charnay's group (Topilko *et al.* 1998). The targeted inactivation of the *zif268* gene was obtained by insertion of a *LacZ-neo* cassette between the promoter and coding region, and the addition of a frame-shift mutation upstream of the DNA-binding domain, resulting in a complete absence of constitutive and regulated *zif268* expression. In our first study we found these mice have profound deficits in several types of long-term memory (Jones *et al.* 2001). We tested recognition memory in *zif268* mutant mice in this study by using a standard two-object task and found that *zif268* inactivation spares short-term, but impairs long-term, object recognition memory. We next examined performance of the *zif268* mutant mice in the more complex three-object task and tested their ability to remember objects and the spatial location of objects using an identical protocol to that used with the CREB<sup>IR</sup> mutant mice (Bozon *et al.* 2002). As shown in figure 3, we found that *zif268* mutant mice were severely impaired in the novel object recognition task 24 h after the initial exposure to the objects (figure 3a). *Zif268* mutant mice ( $n = 11$ ) explored all three objects equally ( $p > 0.05$ ), compared with their WT litter-mates ( $n = 11$ ) that explored the novel object significantly more than the two familiar objects ( $p < 0.05$ ). When we tested the same mice in the spatial version of the task, a similar impairment was found: WT mice displayed significantly greater exploration of the displaced object ( $p < 0.05$ ), whereas *zif268* mutant mice spent an equal amount of time exploring each of the objects ( $p > 0.05$ ; figure 3b). Thus, in complement to the previous findings that inactivation of *zif268* results in long-term memory deficits in several types of task, these results show that *zif268* mutant mice are unable to consolidate or recall information about the characteristics of objects or the spatial location of the objects in these tasks.

In all, these data indicate that MAPK/ERK, CREB and the transcription factor *zif268* are essential components of a signalling cascade required for the expression of long-term recognition memory. Furthermore, our data are also consistent with recent findings showing, in another transgenic mouse, a deficit in long-term object recognition

memory in a two-object task when an inhibitor of CREB, cAMP-responsive element modulator and ATF1 isoforms is expressed in the CA1 area of the hippocampus (Pittenger *et al.* 2002). Interestingly, genetic inhibition of calcineurin phosphatase activity (Malleret *et al.* 2001) or of protein phosphatase 1 improves object recognition memory and in the latter case, the mutation also increased training-induced activation of CRE-mediated gene transcription (Genoux *et al.* 2002).

## 7. CONCLUSION

The laying-down of a stable memory trace is thought to require a molecular consolidation cascade. A central concept in most neurobiological models of memory is that this process involves activity-dependent changes in gene and protein expression, which result in long-lasting alterations in the strength of synaptic connectivity and remodelling of neural networks activated during the encoding of experience. Over the last few decades, research into the mechanisms underlying LTP has helped exploring the mechanisms of memory consolidation and storage, based on the idea that LTP and the formation of memories share, at least in part, common mechanistic properties at the neuronal level. Molecular accounts of consolidation have identified the requirement of regulated gene expression and synthesis of new proteins and insights have been gained into some of the signal transduction mechanisms that convey the signal from cell surface receptors to the nucleus to control the genomic response of synaptically activated neurons.

One critical mediator in this process is the activation of a class of inducible IEGs encoding nuclear transcription factors that can regulate the expression of downstream late-response genes. Here, as an example of this approach, we have focused on one such IEG, *zif268*, and on two molecules, the MAPK/ERK and CREB, known to be implicated in activity-dependent activation of *zif268* in a variety of cell processes. We have examined the role of these molecules in a fundamental memory ability: recognition memory. Using three different approaches, our results show that blocking MAPK/ERK activation, turning CREB function off or inactivating *zif268* all resulted in the same deficit in long-term recognition memory. In the continuing debate about the role of LTP mechanisms in memory, these findings provide an important complement to the suggestion that synaptic changes brought about by LTP and during memory consolidation and storage share, at least in part, common underlying molecular mechanisms. Together with a wealth of experimental data showing that each of these molecules plays important roles in mediating long-term changes in neuronal function, our results are consistent with the view that a MAPK/ERK–CREB pathway targeting CRE, and possibly a MAPK/ERK–Elk pathway targeting SRE and the subsequent transcriptional regulation of *zif268* and other IEGs, is an important molecular mechanism recruited in neurons during the establishment of long-term recognition memory.

Apart from the MAPK/ERK/CRE–Elk/*zif268* pathway, it is probable that there are many other interacting molecules and pathways mediating the full genomic response of neurons required for memory formation.

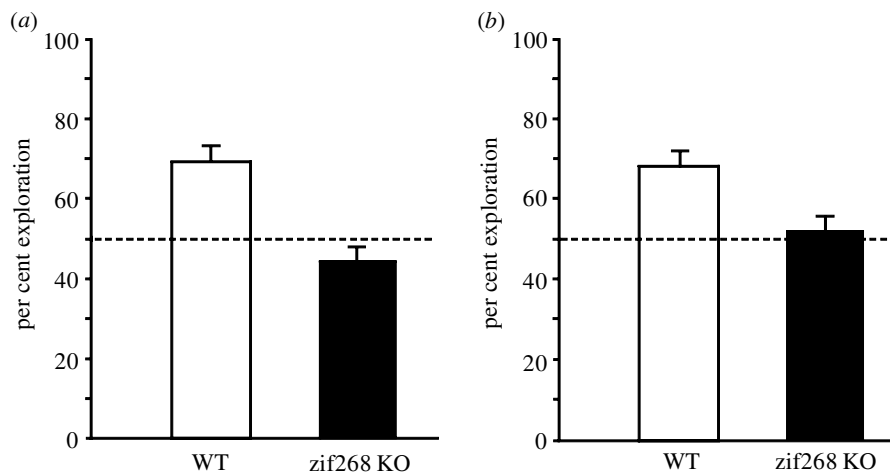


Figure 3. Long-term recognition memory is impaired in *zif268* mutant mice. WT and mutant mice (*zif268* KO) were exposed to three objects (sample phase) and memory was tested 24 hours later with either (a) one familiar object replaced by a novel object or (b) one familiar object moved to a new location in the arena. The histograms represent the time spent exploring the novel object or displaced object (see legend to figure 2). WT mice (white bars) spent significantly more time exploring the novel or the displaced object, showing long-term recognition memory for objects and for location of objects, whereas *zif268* mutant mice (black bars), showed no preference for the novel or the displaced object.

Unravelling these is a great challenge for future research. It will be important to define precisely how a selective set of kinases, phosphatases, transcriptional activators and regulators are put into motion in a coordinated manner to provide neurons with an orchestrated gene response underlying the expression of selective types of plasticity; in what manner any specific type of genomic response corresponds to certain conditions, phases or processes of memory; how it translates into changes in neurons and network properties; and which structures and pathways express these mechanisms in relation to the laying down and/or recall of distinct categories of memories. Armed with ever more specific and powerful technologies, such as those used here, the field of molecular and cellular cognition is starting to make inroads into the core of mechanisms underlying learning and memory.

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## REFERENCES

- Abraham, W. C., Dragunow, M. & Tate, W. P. 1991 The role of immediate early genes in the stabilization of long-term potentiation. *Mol. Neurobiol.* **5**, 297–314.
- Abraham, W. C., Mason, S. E., Demmer, J., Williams, J. M., Richardson, C. L., Tate, W. P., Lawlor, P. A. & Dragunow, M. 1993 Correlations between immediate early gene induction and the persistence of long-term potentiation. *Neuroscience* **56**, 717–727.
- Athos, J., Impey, S., Pineda, V. V., Chen, X. & Storm, D. R. 2002 Hippocampal CRE-mediated gene expression is required for contextual memory formation. *Nature Neurosci.* **5**, 1119–1120.
- Atkins, C. M., Selcher, J. C., Petraitis, J. J., Tzaskos, J. M. & Sweatt, J. D. 1998 The MAPK cascade is required for mammalian associative learning. *Nature Neurosci.* **1**, 602–609.
- Barco, A., Alarcon, J. M. & Kandel, E. R. 2002 Expression of constitutively active CREB protein facilitates the late phase of long-term potentiation by enhancing synaptic capture. *Cell* **108**, 689–703.
- Beckmann, A. M. & Wilce, P. A. 1997 Egr transcription factors in the nervous system. *Neurochem. Int.* **31**, 477–510.
- Berman, D. E., Hazvi, S., Rosenblum, K., Seger, R. & Dudai, Y. 1998 Specific and differential activation of mitogen-activated protein kinase cascades by unfamiliar taste in the insular cortex of the behaving rat. *J. Neurosci.* **18**, 10 037–10 044.
- Bliss, T. V. P. & Lomo, 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.
- Blum, S., Moore, A. N., Adams, F. & Dash, P. K. 1999 A mitogen-activated protein kinase cascade in the CA1/CA2 subfield of the dorsal hippocampus is essential for long-term spatial learning. *J. Neurosci.* **19**, 3535–3544.
- Bourtchuladze, 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.
- Bozon, B., Davis, S. & Laroche, S. 2002 Regulated transcription of the immediate early gene *Zif268*: mechanisms and gene dosage-dependent function in synaptic plasticity and memory formation. *Hippocampus* **12**, 570–577.
- Bramham, C. R., Southard, T., Sarvey, J. M., Herkenham, M. & Brady, L. S. 1996 Unilateral LTP triggers bilateral increases in hippocampal neurotrophin and trk receptor mRNA expression in behaving rats: evidence for interhemispheric communication. *J. Comp. Neurol.* **368**, 371–382.
- Cammarota, M., Bevilacqua, L. R., Ardenghi, P., Paratcha, G., Levi de Stein, M., Izquierdo, I. & Medina, J. H. 2000 Learning-associated activation of nuclear MAPK, CREB and Elk-1, along with Fos production, in the rat hippocampus after a one-trial avoidance learning: abolition by NMDA receptor blockade. *Mol. Brain Res.* **76**, 36–46.
- Cavallaro, S., Schreurs, B. G., Zhao, W., D'Agata, V. & Alkon, D. L. 2001 Gene expression profiles during long-term memory consolidation. *Eur. J. Neurosci.* **13**, 1809–1815.
- Christy, B. A. & Nathans, D. 1989 DNA binding site of the growth factor-inducible protein Zif268. *Proc. Natl Acad. Sci. USA* **86**, 8737–8741.

- Christy, B. A., Lau, L. F. & Nathans, D. 1988 A gene activated in mouse 3T3 cells by serum growth factors encodes a protein with 'zinc finger' sequences. *Proc. Natl Acad. Sci. USA* **85**, 7857–7861.
- Clark, R. E., Zola, S. M. & Squire, L. R. 2000 Impaired recognition memory in rats after damage to the hippocampus. *J. Neurosci.* **20**, 8853–8860.
- Cole, A. J., Saffen, D. W., Baraban, J. M. & Worley, P. F. 1989 Rapid increase of an immediate early gene messenger RNA in hippocampal neurons by synaptic NMDA receptor activation. *Nature* **340**, 474–476.
- Davis, H. P. & Squire, L. R. 1984 Protein synthesis and memory: a review. *Psychol. Bull.* **96**, 518–559.
- Davis, S., Rodger, J., Hicks, A., Mallet, J. & Laroche, S. 1996 Brain structure and task-specific increase in the expression of the gene encoding syntaxin 1B during learning in the rat: a potential molecular marker for learning-induced synaptic plasticity in neural networks. *Eur. J. Neurosci.* **8**, 2068–2074.
- Davis, S., Rodger, J., Stéphan, A., Hicks, A., Mallet, J. & Laroche, S. 1998 Increase in syntaxin 1B mRNA in hippocampal and cortical circuits during spatial learning reflects a mechanism of transsynaptic plasticity involved in establishing a memory trace. *Learn. Mem.* **5**, 375–390.
- Davis, S., Vanhoutte, P., Pages, C., Caboche, J. & Laroche, S. 2000 The MAPK/ERK cascade targets both Elk-1 and cAMP response element-binding protein to control long-term potentiation-dependent gene expression in the dentate gyrus *in vivo*. *J. Neurosci.* **20**, 4563–4572.
- 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 Requirement for the mitogen-activated protein kinase cascade in hippocampal long-term potentiation. *J. Biol. Chem.* **272**, 19 103–19 106.
- French, P. J. (and 12 others) 2001 Subfield-specific immediate early gene expression associated with hippocampal long-term potentiation *in vivo*. *Eur. J. Neurosci.* **13**, 968–976.
- Frey, U. & Morris, R. G. M. 1997 Synaptic tagging and long-term potentiation. *Nature* **385**, 533–536.
- Gashler, A. & Sukhatme, V. P. 1995 Early growth response protein 1 (Egr-1): prototype of a zinc-finger family of transcription factors. *Progr. Nucleic Acid Res. Mol. Biol.* **50**, 191–224.
- Génin, A., Davis, S., Meziane, H., Doyère, V., Jeromin, A., Roder, J., Mallet, J. & Laroche, S. 2001 Regulated expression of the neuronal calcium sensor-1 gene during long-term potentiation in the dentate gyrus *in vivo*. *Neuroscience* **106**, 571–577.
- Genoux, D., Haditsch, U., Knobloch, M., Michalon, A., Storm, D. & Mansuy, I. M. 2002 Protein phosphatase 1 is a molecular constraint on learning and memory. *Nature* **418**, 970–975.
- Grimm, R. & Tischmeyer, W. 1997 Complex patterns of immediate early gene induction in rat brain following brightness discrimination training and pseudotraining. *Behav. Brain Res.* **84**, 109–116.
- Guzowski, J. F. & McGaugh, J. L. 1997 Antisense oligodeoxynucleotide-mediated disruption of hippocampal cAMP response element binding protein levels impairs consolidation of memory for water maze training. *Proc. Natl Acad. Sci. USA* **94**, 2693–2698.
- Guzowski, J. F., McNaughton, B. L., Barnes, C. A. & Worley, P. 1999 Environment-specific expression of the immediate-early gene *Arc* in hippocampal neuronal ensembles. *Nature Neurosci.* **2**, 1120–1124.
- Guzowski, J. F., Setlow, B., Wagner, E. K. & McGaugh, J. L. 2001 Experience-dependent gene expression in the rat hippocampus after spatial learning: a comparison of the immediate-early genes *Arc*, *c-fos*, and *zif268*. *J. Neurosci.* **21**, 5089–5098.
- Hall, J., Thomas, K. L. & Everitt, B. J. 2000 Rapid and selective induction of BDNF expression in the hippocampus during contextual learning. *Nature Neurosci.* **3**, 533–535.
- Hall, J., Thomas, K. L. & Everitt, B. J. 2001 Cellular imaging of *zif268* expression in the hippocampus and amygdala during contextual and cued fear memory retrieval: selective activation of hippocampal CA1 neurons during recall of contextual memories. *J. Neurosci.* **21**, 2186–2193.
- Hermes, J., Zurmohle, U., Schlingensiepen, R., Brysch, W. & Schlingensiepen, K. H. 1994 Developmental expression of the transcription factor *zif268* in rat brain. *Neurosci. Lett.* **165**, 171–174.
- Hicks, A., Davis, S., Rodger, J., Helme-Guizon, A., Laroche, S. & Mallet, J. 1997 Synapsin I and syntaxin 1B: key elements in the control of neurotransmitter release are regulated by neuronal activation and long-term potentiation *in vivo*. *Neuroscience* **79**, 329–340.
- Hipskind, R. A., Baccarini, M. & Nordheim, A. 1994 Transient activation of RAF-1, MEK and ERK2 coincides kinetically with ternary complex factor phosphorylation and immediate-early gene promoter activity *in vivo*. *Mol. Cell Biol.* **14**, 6219–6231.
- Impey, S., Mark, M., Villacres, E. C., Chavkin, C. & Storm, D. R. 1996 Induction of CRE-mediated gene expression by stimuli that generate long-lasting LTP in area CA1 of the hippocampus. *Neuron* **16**, 973–982.
- Impey, S., Obrietan, K., Wong, S. T., Poser, S., Yano, S., Wayman, G., Deloulme, J. C., Chan, G. & Storm, D. R. 1998a Cross talk between ERK and PKA is required for Ca<sup>2+</sup> stimulation of CREB-dependent transcription and ERK nuclear translocation. *Neuron* **21**, 869–883.
- Impey, S., Smith, D. M., Obrietan, K., Donahue, R., Wade, C. & Storm, D. R. 1998b Stimulation of cAMP response element (CRE)-mediated transcription during contextual learning. *Nature Neurosci.* **1**, 595–601.
- Impey, S., Obrietan, K. & Storm, D. R. 1999 Making new connections: role of ERK/MAP kinase signaling in neuronal plasticity. *Neuron* **23**, 11–14.
- Jones, M. W., French, P. J., Bliss, T. V. P. & Rosenblum, K. 1999 Molecular mechanisms of LTP in the insular cortex *in vivo*. *J. Neurosci.* **19**, 1–8.
- Jones, M. W., Errington, M. L., French, P. J., Fine, A., Bliss, T. V. P., Garel, S., Charnay, P., Bozon, B., Laroche, S. & Davis, S. 2001 A requirement for the immediate early gene *Zif268* in the expression of late LTP and the consolidation of long-term memories. *Nature Neurosci.* **4**, 289–296.
- Josselyn, S. A., Shi, C., Carlezon Jr, W. A., Neve, R. L., Nestler, E. J. & Davis, M. 2001 Long-term memory is facilitated by cAMP response element-binding protein overexpression in the amygdala. *J. Neurosci.* **21**, 2404–2412.
- 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.
- Krug, M., Lossner, B. & Ott, T. 1984 Anisomycin blocks the late phase of long-term potentiation in the dentate gyrus of freely moving rats. *Brain Res. Bull.* **13**, 39–42.
- Lamprecht, R., Hazvi, S. & Dudai, Y. 1997 cAMP response element-binding protein in the amygdala is required for long- but not short-term conditioned taste aversion memory. *J. Neurosci.* **17**, 8443–8450.
- Lemaire, P., Revelant, O., Bravo, R. & Charnay, P. 1988 Two mouse genes encoding potential transcription factors with identical DNA-binding domains are activated by growth factors in cultured cells. *Proc. Natl Acad. Sci. USA* **85**, 4691–4695.



- Link, W., Konietzko, U., Kauselmann, G., Krug, M., Schwanke, B., Frey, U. & Kuhl, D. 1995 Somatodendritic expression of an immediate early gene is regulated by synaptic activity. *Proc. Natl Acad. Sci. USA* **92**, 5734–5738.
- Lonze, B. E. & Ginty, D. D. 2002 Function and regulation of CREB family transcription factors in the nervous system. *Neuron* **35**, 605–623.
- Lyford, G. L., Yamagata, K., Kaufmann, W. E., Barnes, C. A., Sanders, L. K., Copeland, N. G., Gilbert, D. J., Jenkins, N. A., Lanahan, A. A. & Worley, P. F. 1995 Arc, a growth factor and activity-regulated gene, encodes a novel cytoskeleton-associated protein that is enriched in neuronal dendrites. *Neuron* **14**, 433–445.
- Mack, K., Day, M., Milbrandt, J. & Gottlieb, D. I. 1990 Localization of the NGFI-A protein in the rat brain. *Mol. Brain Res.* **8**, 177–180.
- Malkani, S. & Rosen, J. B. 2000 Specific induction of early growth response gene 1 in the lateral nucleus of the amygdala following contextual fear conditioning in rats. *Neuroscience* **97**, 693–702.
- 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.
- Marais, R., Wynne, J. & Treisman, R. 1993 The SRF accessory protein ELK-1 contains a growth factor-regulated transcriptional activation domain. *Cell* **73**, 381–393.
- Meiri, N. & Rosenblum, K. 1998 Lateral ventricle injection of the protein synthesis inhibitor anisomycin impairs long-term memory in a spatial memory task. *Brain Res.* **789**, 48–55.
- Milbrandt, J. 1987 A nerve growth factor-induced gene encodes a possible transcriptional regulatory factor. *Science* **238**, 797–799.
- Nguyen, P. V., Abel, T. & Kandel, E. R. 1994 Requirements of a critical period of transcription for induction of a late phase of LTP. *Science* **265**, 1104–1107.
- Nikolaev, E., Werka, T. & Kaczmarek, L. 1992 C-fos proto-oncogene expression in rat brain after long-term training of two-way avoidance reaction. *Behav. Brain Res.* **48**, 91–94.
- O'Donovan, K. J., Tourtellotte, W. G., Milbrandt, J. & Baraban, J. M. 1999 The EGR family of transcription-regulatory factors: progress at the interface of molecular and systems neuroscience. *Trends Neurosci.* **22**, 167–173.
- Okuno, H. & Miyashita, Y. 1996 Expression of the transcription factor Zif268 in the temporal cortex of monkeys during visual paired associate learning. *Eur. J. Neurosci.* **8**, 2118–2128.
- Otani, S. & Abraham, W. C. 1989 Inhibition of protein synthesis in the dentate gyrus, but not the entorhinal cortex, blocks the maintenance of long-term potentiation in rats. *Neurosci. Lett.* **106**, 175–180.
- 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., Bourchouladze, 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.
- Richardson, C. L., Tate, W. P., Mason, S. E., Lawlor, P. A., Dragunow, M. & Abraham, W. C. 1992 Correlation between the induction of an immediate early gene, zif268, and long-term potentiation in the dentate gyrus. *Brain Res.* **580**, 147–154.
- Roberson, E. D., English, J. D., Adams, J. P., Selcher, J. C., Kondratyck, C. & Sweatt, J. D. 1999 The mitogen-activated protein kinase cascade couples PKA and PKC to cAMP response element binding protein phosphorylation in area CA1 of the hippocampus. *J. Neurosci.* **19**, 4337–4348.
- Rosenblum, K., Futter, M., Jones, M., Hulme, E. C. & Bliss, T. V. P. 2000 ERK1/II regulation by the muscarinic acetylcholine receptors in neurons. *J. Neurosci.* **20**, 977–985.
- Schafe, G. E., Atkins, C. M., Swank, M. W., Bauer, E. P., Sweatt, J. D. & LeDoux, J. E. 2000 Activation of ERK/MAP kinase in the amygdala is required for memory consolidation of pavlovian fear conditioning. *J. Neurosci.* **20**, 8177–8187.
- Selcher, J. C., Atkins, C. M., Trzaskos, J. M., Paylor, R. & Sweatt, J. D. 1999 A necessity for MAP kinase activation in mammalian spatial learning. *Learn. Mem.* **6**, 478–490.
- Sgambato, V., Pagès, C., Rogard, M., Besson, M. J. & Caboche, J. 1998 Extracellular signal-regulated kinase (ERK) controls immediate early gene induction on corticostriatal stimulation. *J. Neurosci.* **18**, 8814–8825.
- Smirnova, T., Laroche, S., Errington, M. L., Hicks, A. A., Bliss, T. V. P. & Mallet, J. 1993 Transsynaptic expression of a presynaptic glutamate receptor during hippocampal long-term potentiation. *Science* **262**, 433–436.
- Soderling, T. R. & Derkach, V. A. 2000 Postsynaptic protein phosphorylation and LTP. *Trends Neurosci.* **23**, 75–80.
- Sukhatme, V. P. (and 11 others) 1988 A zinc finger-encoding gene coregulated with c-fos during growth and differentiation, and after cellular depolarization. *Cell* **53**, 37–43.
- Sweatt, J. D. 2001 The neuronal MAP kinase cascade: a biochemical signal integration system subserving synaptic plasticity and memory. *J. Neurochem.* **76**, 1–10.
- Swirnoff, A. H. & Milbrandt, J. 1995 DNA-binding specificity of NGFI-A and related zinc finger transcription factors. *Mol. Cell Biol.* **15**, 2275–2287.
- Thomas, K. L., Laroche, S., Errington, M. L., Bliss, T. V. P. & Hunt, S. P. 1994 Spatial and temporal changes in signal transduction pathways during LTP. *Neuron* **13**, 737–745.
- Thomas, K. L., Davis, S., Hunt, S. P. & Laroche, S. 1996 Alterations in the expression of specific glutamate receptor subunits following hippocampal LTP *in vivo*. *Learn. Mem.* **3**, 197–208.
- Tischmeyer, W. & Grimm, R. 1999 Activation of immediate early genes and memory formation. *Cell. Mol. Life Sci.* **55**, 564–574.
- Topilko, P., Schneider-Maunoury, S., Levi, G., Trembleau, A., Gourdji, D., Driancourt, M. A., Rao, C. V. & Charnay, P. 1998 Multiple pituitary and ovarian defects in Krox-24 (NGFI-A, Egr-1)-targeted mice. *Mol. Endocrinol.* **12**, 107–122.
- Treisman, R. 1996 Regulation of transcription by MAP kinase cascade. *Curr. Opin. Cell Biol.* **8**, 205–215.
- Vanhoutte, P., Barnier, J. V., Guibert, B., Pagès, C., Besson, M. J., Hipskind, R. A. & Caboche, J. 1999 Glutamate induces phosphorylation of Elk-1 and CREB, along with c-fos activation via an extracellular signal-regulated kinase-dependent pathway in brain slices. *Mol. Cell Biol.* **19**, 136–146.
- Waltereit, R., Dammermann, B., Wulff, P., Scafidi, J., Staubli, U., Kauselmann, G., Bundman, M. & Kuhl, D. 2001 Arg3.1/Arc mRNA induction by Ca<sup>2+</sup> and cAMP requires protein kinase A and mitogen-activated protein kinase/extracellular regulated kinase activation. *J. Neurosci.* **21**, 5484–5493.
- Wasylyk, B., Hagman, J. & Gutierrez-Hartmann, A. 1998 Ets transcription factors/nuclear effectors of the Ras-MAP-kinase signaling pathway. *Trends Biol. Sci.* **23**, 213–216.

- Watson, M. A. & Milbrandt, J. 1990 Expression of the nerve growth factor-regulated NGFI-A and NGFI-B genes in the developing rat. *Development* **110**, 173–183.
- Williams, J. M., Beckmann, A. M., Mason-Parker, S. E., Abraham, W. C., Wilce, P. A. & Tate, W. P. 2000 Sequential increase in Egr-1 and AP-1 DNA binding activity in the dentate gyrus following the induction of long-term potentiation. *Mol. Brain Res.* **77**, 258–266.
- Wisden, W., Errington, M. L., Williams, S., Dunnett, S. B., Waters, C., Hitchcock, D., Evan, D., Bliss, T. V. P. & Hunt, S. P. 1990 Differential expression of immediate early gene in the hippocampus and spinal cord. *Neuron* **4**, 603–614.
- Worley, P. F., Christy, B. A., Nakabeppu, Y., Bhat, R. V., Cole, A. J. & Baraban, J. M. 1991 Constitutive expression of *zif268* in neocortex is regulated by synaptic activity. *Proc. Natl Acad. Sci. USA* **88**, 5106–5110.
- Worley, P. F., Bhat, R. V., Baraban, J. M., Erickson, C. A., McNaughton, B. L. & Barnes, C. A. 1993 Threshold for synaptic activation of transcription factors in hippocampus: correlation with long-term enhancement. *J. Neurosci.* **13**, 4776–4786.
- Zhao, W., Meiri, N., Xu, H., Cavallaro, S., Quattrone, A., Zhang, L. & Alkon, D. L. 2000 Spatial learning induced changes in expression of the ryanodine type II receptor in the rat hippocampus. *FASEB J.* **14**, 290–300.
- Zola, S. M., Squire, L. R., Teng, E., Stefanacci, L., Buffalo, E. A. & Clark, R. E. 2000 Impaired recognition memory in monkeys after damage limited to the hippocampal region. *J. Neurosci.* **20**, 451–463.

## GLOSSARY

- CaMK: calcium-calmodulin kinase  
CRE: cAMP response element  
CREB: cAMP response element-binding protein  
CREB/ATF: cAMP response element-binding protein/activating transcription factor  
DMSO: dimethylsulphoxide  
Elk: Ets-like protein  
ERK: extracellular signal-regulated kinase  
IEG: immediate early gene  
LBD: ligand-binding domain  
LTP: long-term potentiation  
MAPK: mitogen-activated protein kinase  
MEK: MAPK/ERK kinase  
PKA: cAMP-dependent protein kinase A  
PKC: protein kinase C  
SRE: serum response element  
WT: wild-type