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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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; Gézin 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 Znk, 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; Swirnow & 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, αCaMKII 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 (Sambato 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 αΔCREB 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, Impoy et al. (1998b), 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 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 μl, 1 μg μl–1; injected at a rate of 1 μl 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 CREBIR 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 CREBIR mice (black bars) spent significantly more time exploring the novel object or the displaced object. Activating CREBIR 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 Ser133 mutated αCREB 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–CREBIR) under the control of the αCaMKII 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 sessions 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 CREBIR 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, CREBIR and WT mice were injected with tamoxifen (16 mg kg\(^{-1}\), 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, CREBIR \((n = 8)\) 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 CREBIR \((n = 8)\) 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$^{IR}$ 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$^{IR}$ 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 frameshift 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$^{IR}$ 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 transgeneric 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 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 \textit{zif268} mutant mice. WT and mutant mice (\textit{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 \textit{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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**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