ELSEVIER

Progress in Neurobiology 74 (2004) 183–211

progress in Neurobiology

www.elsevier.com/locate/pneurobio

A gene for neuronal plasticity in the mammalian brain: Zif268/Egr-1/NGFI-A/Krox-24/TIS8/ZENK?

Ewelina Knapska[a], Leszek Kaczmarek[b,*]

[a]*Department of Neurophysiology, Nencki Institute, Pasteura 3, 02-093 Warsaw, Poland*
[b]*Department of Molecular and Cellular Neurobiology, Nencki Institute, Pasteura 3, 02-093 Warsaw, Poland*

Received 23 May 2003; accepted 26 May 2004

Abstract

Zif268 is a transcription regulatory protein, the product of an immediate early gene. Zif268 was originally described as inducible in cell cultures; however, it was later shown to be activated by a variety of stimuli, including ongoing synaptic activity in the adult brain. Recently, mice with experimentally mutated *zif268* gene have been obtained and employed in neurobiological research. In this review we present a critical overview of Zif268 expression patterns in the naive brain and following neuronal stimulation as well as functional data with Zif268 mutants. In conclusion, we suggest that Zif268 expression and function should be considered in a context of neuronal activity that is tightly linked to neuronal plasticity.

Contents

* Corresponding author. Tel.: +48 22 659 3001; fax: +48 22 822 5342.
E-mail address: l.kaczmarek@nencki.gov.pl (L. Kaczmarek).

0301-0082/$ – see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.pneurobio.2004.05.007

1. Introduction

The gene named *zif268*[1] (zinc finger binding protein clone 268) is also called early growth response gene 1 (Egr-1), nerve growth factor-induced gene A (NGFI-A), gene containing sequences homologous to the *Drosophila* Kr finger probe (Krox-24) and tetradecanoyl phorbol acetate-induced sequence 8 (TIS8). The ambiguity in the nomenclature results from the fact that this gene has been independently identified in different laboratories. Thus, a term ZENK (the acronym of the previous four names) has been coined, and it is used nowadays in parallel with all the other names.

zif268 was initially identified by Lau and Nathans (1987) in mouse fibroblasts, where it was induced by serum and growth factors. At the same time Milbrandt (1987) identified NGFI-A in a screening strategy that aimed at detecting genes induced by NGF (nerve growth factor) in rat PC12 cells.[2] These discoveries were quickly followed by several independent descriptions of similar gene sequences from mice, rats and humans (Almendral et al., 1988; Christy et al., 1988; Lemaire et al., 1988; Sukhatme et al., 1988; Tsai-Morris et al., 1988; Arenander et al., 1989; Changelian et al., 1989; Janssen-Timmen et al., 1989; Cao et al., 1990; Lemaire et al., 1990; Suggs et al., 1990).

zif268 belongs to the category of immediate early genes[3] (IEG) since it is activated in the absence of de novo protein synthesis (Milbrandt, 1987; Lemaire et al., 1988; Arenander et al., 1989). It codes for a transcription factor protein (Cao et al., 1990; Lemaire et al., 1990; Waters et al., 1990), which has a distinct pattern of expression in the brain (Milbrandt, 1987; Mack et al., 1990; Herdegen et al., 1990; Waters et al., 1990). Acting as a transcription factor, Zif268 directly controls expression of other genes, which makes this protein an important object of studies aimed at understanding the orchestration of neuronal responses to a variety of stimuli. In addition, mice with genetic ablation of the gene have recently become available, which provides novel tools to complement previous studies on gene and protein expression patterns.

In this review, we will present the results of the studies that have documented plasticity-related expression of *zif268* in the mammalian brain, with a special emphasis on long-term potentiation and behavioral training, in which the evidence for plasticity-linked function of this gene appears to be particularly strong. We have collected the data on patterns of the expression of *zif268* in the brain under different conditions of behavioral, electrophysiological and pharmacological stimulation in a hope that such a synthesis of information may offer clues to its possible physiological function, which still remains elusive. We also consider the recent data on *zif268* products' expression and function in cortical plasticity. For an extensive coverage of the previous literature, the reader is referred to the earlier reviews

[1] Throughout this review, we use the standard convention of denoting genes and their mRNA products in italics (*zif268*) and the protein encoded by them with a single capital letter (Zif268).

[2] Neuron-like cells that are derived from a malignant neural crest tumor (a pheochromocytoma); when treated with NGF, they acquire a phenotype resembling symphatetic neurons, including the extension of neurites, cessation of cell division, acquisition of an action potential and expression of genes encoding specific neuronal markers.

[3] The genes, whose expression rises markedly and transiently in the cell shortly after the stimulation and in the absence of de novo protein synthesis, have been termed immediate-early genes (IEG) or primary response genes.

(Kaczmarek and Chaudhuri, 1997; Tischmeyer and Grimm, 1999; Clayton, 2000; Guzowski, 2002; Leah and Wilce, 2002; Davis et al., 2003; Bozon et al., 2003). It is also worth mentioning that much elaborated cause for a role of this gene has been made in the avian brain in the context of song learning. This issue has been excellently covered by Mello's recent review (Mello, 2002). In this section, we shall discuss the most general features of the gene, the protein structure, and regulation as well as expression pattern in the naive brain, which will allow a better understanding of the relation between *zif268* expression and neuronal plasticity. A detailed discussion of the complicated mechanisms controlling *zif268* expression, many possible interactions between Zif268 and other transcription factors and, finally, a variety of different late-response genes whose expression could be regulated by Zif268 allows us to consider various potential functions of Zif268.

2. Characteristics of *zif268* and its protein

Following the original observation of *zif268* as NGF-inducible and thus possibly related to neuronal functions (Milbrandt, 1987), it was noted that this response could also be triggered by either neurotransmitters or depolarization, which suggested a potential function of Zif268 in the mature nervous system (Christy et al., 1988; Lemaire et al., 1988; Sukhatme et al., 1988; Arenander et al., 1989; Ito et al., 1990). A more convincing argument was given by Sukhatme et al. (1988), who confirmed that *zif268* is expressed in mature neurons in the adult nervous system in vivo. Furthermore, it has been demonstrated that seizure activity or intense stimulation of spinal-cord afferents elicited *zif268* response (Saffen et al., 1988; Cole et al., 1990; Wisden et al., 1990). For the 15 years following the discovery, numerous investigations demonstrated the expression of *zif268* in many different behavioral paradigms (which are described below in detail). However, the role of this gene in the neuronal cells still remains unclear.

2.1. Structure of the *zif268* gene

The structure of the *zif268* gene has been reviewed in detail (Beckmann and Wilce, 1997; Herdegen and Leah, 1998; O'Donovan et al., 1999). The coding region of the gene spans about 3.8 kb and consists of two exons and one intron. The 3′ exon includes three zinc-finger DNA-binding domains (Tsai-Morris et al., 1988; Changelian et al., 1989). The structure of the promoter of the *zif268* gene is described in Table 1. Christy and Nathans (1989b) demonstrated that each of the SREs conferred inducibility of *zif268* by serum, platelet-derived growth factor and phorbol 12-myristate 13-acetate in 3T3 (embryonal mouse fibroblasts) cells. DeFranco et al. (1993) defined three elements (serum responsive factor, SRE1, SRE2 and activator protein, AP-1-like) that contributed to induction of *zif268* in PC12 cells by NGF (nerve growth factor), serum, as well as phorbol 12-myristate 13-acetate. McMahon and Monroe (1995) showed that two SREs with the adjacent Ets motifs are necessary for *zif268* induction following antigen receptor cross-linking in B lymphocytes. Moreover, Aicher et al. (1999) demonstrated that *zif268* transcription in fibroblasts was mainly activated by a single SRE, whereas other transcription factor binding sites played a minor role. Thus, it seems that the SREs' elements play the main role in the coordinated induction of *zif268*, whereas proteins binding to the other regulatory sites modulate *zif268* transcription. Notably, since the gene contains the ERE (egr-1 responsive element), *zif268* can potentially auto-regulate its own expression.

Table 1
The structure of the promoter of the *zif268* gene in different species

Regulatory sequence	Species (no. of sequences)	Reference
Serum response element (SRE)	Rat (4)	Changelian et al. (1989)
	Mouse (5)	Christy et al. (1988), Tsai-Morris et al. (1988), Janssen-Timmen et al. (1989) and Christy and Nathans (1989a)
	Human (5)	Sakamoto et al. (1991) and Schwachtgen et al. (2000)
Specificity protein 1 element (Sp1)	Rat (1)	Changelian et al. (1989)
	Mouse (4)	Tsai-Morris et al. (1988)
	Human (2)	Sakamoto et al. (1991) and Schwachtgen et al. (2000)
Activator protein 1 element (AP-1)	Rat (1)	DeFranco et al. (1993)
	Mouse (2)	Tsai-Morris et al. (1988)
	Human (1)	Schwachtgen et al. (2000)
Calcium/cAMP responsive element (CRE)	Rat (1)	Changelian et al. (1989)
	Mouse (2)	Tsai-Morris et al. (1988)
	Human (2)	Sakamoto et al. (1991) and Schwachtgen et al. (2000)
Egr/Zif268 response element (ERE)	Human (1)	Sakamoto et al. (1991) and Schwachtgen et al. (2000)
Potential estrogen response element	Rat (1)	Slade and Carter (2000)
NFκB — similar element (nuclear factor kappa B)	Human (1)	Aicher et al. (1999)

2.2. Characteristics of the Zif268 protein

Two protein species of molecular weights 82 and 88 kDa encoded by *zif268* were observed due to different initiation points of translation (Lemaire et al., 1990). Zif268 contains a highly conserved DNA-binding domain composed of three Cys_2His_2 zinc finger motifs. The zinc fingers recognize the 9-bp segment of DNA 5′-GCGC/GGGGCG-3′ in the promoters of target genes (Christy and Nathans, 1989a; Cao et al., 1990; Lemaire et al., 1990; Pavletich and Pabo, 1991). The DNA binding zinc fingers act in a sequence-specific manner with each finger spanning three nucleotides. However, there is some variability in the possible sequences of binding sites of Zif268 (see Swirnoff and Milbrandt, 1995), which can extend the known list of its target genes.

The protein was localized to the cell nucleus (Cao et al., 1990; Waters et al., 1990). The nuclear localization of Zif268 is provided by a bipartite signal in the DNA-binding domain (the second or third zinc finger) and in the basic flanking sequences (Gashler et al., 1993). Zif268 protein was found to be phosphorylated on serine residues and glycosylated (Cao et al., 1990; Lemaire et al., 1990). Therefore, given that the activation potential of Zif268 is distributed over an extensive serine- and threonine-rich N-terminal domain (Gashler et al., 1993), the phosphorylation state or other modifications can affect the DNA binding properties. Zif268, whose expression is linked to neural activity, is one of the inducible transcription factors (ITF) in the neuronal cells. Zif268 can act in concert with other transcription factors to modulate (activate or repress) the expression of the target genes by binding to their promoters. The details of these mechanisms are yet to be clearly identified.

The regulatory sequence for Zif268 binding is present within the promoters of a host of different, late-response genes, thereby enabling Zif268 to exert a commanding influence on long-term cellular homeostasis by regulating the expression of such genes (cf. Table 2). Although several genes, whose expression in the brain can be controlled by Zif268, have been identified, most of them probably still remain unknown. Owing to the great diversity of possible target genes, different potential functions of Zif268 can be expected. It may be engaged in cell growth and differentiation, as well as in structural and metabolic changes in the mature neural cell. Even though discovering the target genes of Zif268 is a valuable effort, it will be difficult to uncover its role without the knowledge of the mechanisms by which Zif268 interacts with other transcription factors, such as those listed in the Table 3. Given a lot of possibilities of such interactions in the cell, it is obvious that these mechanisms create a very efficient and sensitive, but potentially very complicated, regulatory system. For instance, mutagenesis of *zif268* identified an inhibitory domain mapped to the 5′ of the zinc fingers, named R1, which, when deleted, resulted in the enhanced Zif268 transcriptional activity (Gashler et al., 1993; Russo et al., 1993). Next, a repressor named NGFI-A binding protein (NAB1), which negatively regulates the transcriptional activity of Zif268, was identified (Russo et al., 1995). NAB1 is an active repressor that works by a direct mechanism and its repression is not specific to particular activators (Swirnoff et al., 1998). Subsequent work identified another protein, NAB2, that shared two large regions of homology with NAB1 and was also capable of repressing Zif268. The NAB2 expression was regulated by some of the stimuli that also induced Zif268 expression (Svaren et al., 1996). Thus, NAB2 could serve as a negative-feedback mechanism that causes a decrease in the transcription initiated by Zif268.

The discovery of *zif268* mRNA in developing dendrites (Crino et al., 1998) suggested the possibility of an additional

Table 2
Genes identified as regulated by Zif268

Gene name	Reference
Synapsin I and II	Thiel et al. (1994) and Petersohn et al. (1995)
Glutamate dehydrogenase	Das et al. (1993)
Neurofilament light chain	Pospelov et al. (1994)
Nicotinic acetylocholine receptor (α7 subunit)	Carrasco-Serrano et al. (2000)
Adenosine deaminase	Ackerman et al. (1991)
Glutamic acid decarboxylase	Szabo et al. (1996) and Yanagawa et al. (1997)
Thymidine kinase	Molnar et al. (1994)
Acetylocholinesterase	Li et al. (1993)
Phenylethanolamine *N*-methyltransferase	Morita and Wong (1996)
Apolipoprotein A1	Ge et al. (1994) and Kilbourne et al. (1995)
Desmin	Li and Paulin (1993)
p75 nerve growth factor (p75 NGF) receptor	Nikam et al. (1995)
Transforming growth factor α1 (TGF-α1)	Kim et al. (1994)
Platelet-derived growth factor A	Wang and Deuel (1992)
Neuropeptide Y	Wernersson et al. (1998)
Neuroserpin	Berger et al. (1999)
Human low density lipoprotein receptor	Zhang et al. (2003)
Thymus-expressed chemokine (TECK)	Fu et al. (2003)
IP-30	Fu et al. (2003)
TNFα-related apoptosis inducing ligand (TRAIL)	Fu et al. (2003)

Table 3
Transcription factors interacting with Zif268

Transcription factor	Reference
cAMP responsive element binding protein binding protein (CBP/p300)	Silverman et al. (1998)
c-Fos	Gius et al. (1990) and Dragunow et al. (1994)
JunD	de Groot et al. (1991)
Nerve growth factor-induced gene B, nur77 (NGFI-B)	Williams and Lau (1993)
NF-κB	Cogswell et al. (1997) and Chapman and Perkins (2000)
c/EBPβ	Zhang et al. (2003)

signaling pathway between the distal dendrite and the nucleus. One could expect the protein corresponding to the dendritically localized mRNA to be synthesized, posttranslationally modified and transported to the nucleus, where it modulates gene transcription. Then the gene expression during development and plastic changes could be controlled by distant cellular events.

2.3. Signal transduction pathways involved in controlling *zif268* expression

Zif268 is induced in neurons after extracellular stimulation with neurotransmitters or trophic substances (Fig. 1). The elevation of cytosolic Ca^{2+} has been shown to mediate the induction of *zif268* (see Ghosh et al., 1994). Moreover, it has been shown that glutamate, as well as more specific agonists activating various classes of glutamate receptors, can stimulate expression of *zif268*. For instance, in neuronal cultures, Condorelli et al. (1994) have observed that kainate was the most potent, followed by glutamate, *N*-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazole-propionate (AMPA), *trans*-1-aminocyclopentane-1,3-dicarboxylic acid (*t*-ACPD) and quisqualate (the last two agonists activate metabotropic glutamate receptors). These results suggest an important role of cytoplasmic calcium in regulation of zif268 expression as has also been shown by Murphy et al. (1991).

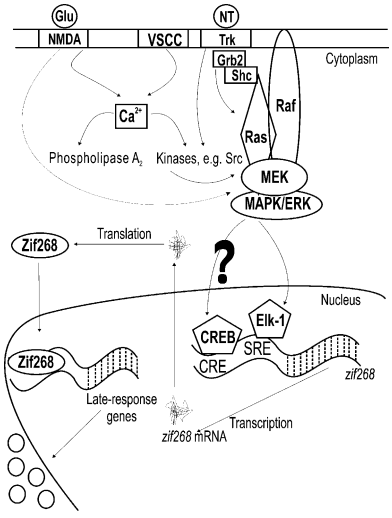

Fig. 1. Schematic representation of possible signal transduction pathways involved in controlling *zif268* induction.

As far as the in vivo conditions are concerned, Beckmann et al. (1997) observed an increase of Zif268 activity after administration of NMDA and kainate in the cerebral cortex and hippocampus of rats using EMSA method. Moreover, Gass et al. (1993b) noted that pretreatment with MK-801 (dizocilpine maleate, an uncompetitive NMDA receptor antagonist) did not affect kainate-induced Zif268 expression in the limbic system but abolished its expression in the somatosensory cortex and striatum. These data suggest that the involvement of various classes of glutamate receptors in Zif268 activation may depend on the brain structure. Furthermore, it was observed that MK-801 itself could increase Zif268 expression in layer III of the posterior cingulate and retrosplenial cortex and in subcortical areas, such as the hypothalamus and thalamus (Gass et al., 1993a). However, neurons in these regions had previously been shown to be the principal target of NMDA receptor antagonist toxicity, and therefore MK-801-elicited expression of Zif268 seems to identify reversibly injured neurons (Olney et al., 1989; Hughes et al., 1993; Hetman et al., 1997).

In response to increased cytosolic calcium level, several downstream signal transduction pathways are activated and many of them have been implicated in *zif268* activation. Sgambato et al. (1998) observed a transient activation of mitogen-activated protein kinase/extracellular signal-regulated kinase (MAPK/ERK), spatially coincident with the onset of *zif268* expression in the lateral striatum, following in vivo stimulation of the glutamatergic corticostriatal pathway. In addition, Elk-1 (one of ternary complex factors binding to SRE) and CREB transcription factors were activated (phosphorylated) simultaneously with ERK and *zif268* induction. Zif268 induction as well as Elk-1 and CREB phosphorylation was abolished by the inhibition of ERK activation with an inhibitor which exerts an effect on MEK1 and MEK2 — acting upstream MAPK/ERK kinase. Furthermore, the induction of long-term potentiation (LTP, see Section 8) in the dentate gyrus in vivo caused phosphorylation and nuclear translocation of MAPK/ERK. In addition to CREB and ERK activation, phosphorylation of Elk-1 and subsequent up-regulation of *zif268* transcription were observed. The inhibition of MAPK/ERK cascade by the MAPK kinase inhibitor prevented CREB and Elk-1 phosphorylation, *zif268* induction and resulted in rapidly decaying LTP (Davis et al., 2000). The above-mentioned studies showed that a transient activation of the MAPK/ERK signaling pathway targeted both CREB and

Elk-1 transcription factors and, as a result, induced *zif268* expression via CRE and SRE transcriptional regulation sites. However, Walton et al. (1999) did not observe any increase in the level of either unmodified or phosphorylated CREB associated with *zif268* induction in dentate granule cells after rat perforant-path-stimulated LTP. The authors obtained analogous results after exposing animals to a novel environment (Walton et al., 1999).

Besides in vivo studies on *zif268* regulation by signaling pathways, there are numerous investigations carried on cell cultures. Greenwood and Dragunow (2002) observed that the treatment of the SK-N-SH neuroblastoma cells with a cholinergic agonist, carbachol, led to an increased Zif268 expression, preceded by the phosphorylation of CREB. However, Zif268 stimulation was dependent on activation of ERK1/2, as it was blocked by the MEK inhibitor, in contrast to CREB phosphorylation induced by carbachol, which was relatively unaffected by MAPK kinase inhibition. In PC12 cells, the activation of protein-tyrosine kinase (such as Fps, Src) led to *zif268* expression mediated by Ras (a small G-protein) and Raf (a serine/threonine protein kinase) (Qureshi et al., 1991; Alexandropoulos et al., 1992). In PC12 cells treated with NGF, *zif268* induction was also mediated by Src, Ras and Raf (D'Arcangelo and Halegoua, 1993; Wood et al., 1993). Calcium influx through voltage-dependent calcium channels into PC12 cells caused Src activation, formation of an Shc/Grb2 complex (an adaptor protein/growth-factor-receptor-binding protein 2), leading to Ras and MAPK activation and subsequent induction of *zif268* (Rusanescu et al., 1995).

In studies carried out by Kumahara et al. (1999) in PC12 cells treated with NGF, *zif268* expression was mediated by MAPK, with a possible involvement of JNK (Jun N-terminal kinases) as well as by PI 3-kinase (phosphatidylinositol 3-kinase). Lerea et al. (1995) observed an NMDA induction of *zif268* in dentate granule neurons, which was dependent on phospholipase A2 and lipoxygenase. Furthermore, in addition to ERK, two other types of MAP kinases, JNK (SAPK) and p38 (see Johnson and Lapadat, 2002), increased *zif268* expression through Elk-1 in response to environmental stress (Lim et al., 1998). These data suggest that the induction of *zif268* by different factors, such as growth factors and stress may be mediated through different MAP kinases. The induction of *zif268* also depends on the activation of protein kinase A (PKA) and protein kinase C (PKC) (Mechta et al., 1989; Ginty et al., 1991; Vaccarino et al., 1992; Ferhat et al., 1993; Simpson and Morris, 1995). Altogether the aforementioned data suggest that CREB may not play the major role in *zif268* activation, in contrast to Elk-1. Possible signal transduction pathways involved in controlling *zif268* expression are depicted in Fig. 1.

2.4. Transient nature of zif268 mRNA and protein expression

The expression of *zif268* mRNA and protein exhibits well-defined time dependence. The knowledge of the time-course of *zif268* expression is a key component to any hypothesis explaining its role. Lau and Nathans (1987) described a time-course of *zif268* expression in BALB/c 3T3 cells. *zif268* mRNA appeared within 20 min, reached the maximum level in about 60 min and declined within a few hours following serum, fibroblast growth factor and platelet-derived growth factor stimulation. Sukhatme et al. (1988) observed an increase of *zif268* mRNA expression in PC12 cells 30–60 min after addition of NGF and K^+ depolarization.

Zangenehpour and Chaudhuri (2002) provided a complete up- and down-regulation profile of *zif268* resulting from light stimulation and deprivation with the use of a fluorescence-based approach for the simultaneous detection of *zif268* mRNA and protein. To determine the up-regulation profile in rat visual cortex the authors applied three days of dark adaptation followed by 30, 60, 90, 120 or 360 min of light stimulation. The level of *zif268* and its protein expression after dark adaptation was assumed to be the baseline. *zif268* mRNA reached a peak of expression after 30 min of light stimulation and this level was maintained after longer continuous light stimulation. The level of *zif268* expression was similar to that in normal animals exposed for 12 h of light per day, suggesting that prolonged stimulation provoked high basal level of *zif268* expression. In order to examine the down-regulation profile the rats were subjected to dark adaptation for three days, then exposed to continuous light stimulation for 120 min followed by another dark-adaptation lasting 30, 60, 90, 120 or 360 min. *zif268* mRNA reached the baseline level between 30 and 60 min of dark adaptation.

zif268 mRNA is induced rapidly and transiently also after a pharmacological stimulation. In the studies of Lanaud et al. (1993), *zif268* reached the maximal level in 30 min and decreased to the basal level within 3 h following seizures evoked focally from the deep prepiriform cortex in rat. Similar results were also obtained in behavioral paradigms, e.g., Malkani and Rosen (2000b) observed the peak of *zif268* induction in the lateral nucleus of the rat amygdala in 30 min after contextual fear conditioning. Williams et al. (2000) observed a peak of Zif268 activity 1 h after the induction of long-term potentiation (see Section 8) in the rat dentate gyrus. Gass et al. (1992) detected the peak level of Zif268 expression in the rat hippocampus 2 h after bicuculline-induced seizures. Whisker stimulation by tactile exploration of an enriched environment caused an elevated Zif268 expression in the barrel cortex (see Section 6.3) with the peak level at 1 h (Bisler et al., 2002) and visual stimulation after dark rearing increased DNA-binding activity of Zif268 to the highest level within 2 h (Kaminska et al., 1996). Herdegen et al. (1991) observed the highest level of Zif268 expression in the dorsal horn 1 h after electrical stimulation of the sciatic nerve.

In contrast to the aforementioned very rapid and transient expression of *zif268* mRNA and proteins in most instances, Richter-Levin et al. (1998) reported an enhanced expression

of *zif268* in the potentiated rat dentate gyrus 3 h after the induction of LTP. Notably, okadaic acid and calyculin A, which are specific inhibitors of protein serine/threonine phosphatases 1 and 2A, stimulated a sustained induction of *zif268* expression in the mouse fibroblasts (Cao et al., 1992). Okadaic-acid-induced *zif268* expression has been reported to be significantly more stable — the half-life of serum-induced *zif268* mRNA was estimated to be 12 min, compared with the half-life of 2 h for okadaic-acid-induced *zif268* mRNA. Interestingly, the treatment with okadaic acid and calyculin A also induced the synthesis of Zif268 protein, which was much more phosphorylated than Zif268 protein in the quiescent cells.

An increased level of mRNA encoding MAPK phosphatase (MKP-1) at the time point when MAPK/ERK phosphorylation had returned to basal level may suggest a negative feedback loop regulating deactivation of MAPK/ERK and subsequently *zif268* expression (Sgambato et al., 1998; Davis et al., 2000). Therefore, it is possible that *zif268* expression is additionally regulated by phosphatases.

3. Learning-related gene expression

Keeping in mind complex mechanisms controlling *zif268* expression, many possible interactions between Zif268 and other transcription factors, a variety of different late-response genes whose expression could be regulated by Zif268, and the time-course of its expression, we can consider various potential functions of Zif268. In this section, we would like to present possible functions of regulatory genes in general, which will allow us to discuss the specific role of Zif268 in the neuronal cell in the next sections.

There are several possible functions of the neuronal genes that could be considered in the context of neuronal plasticity and learning (for details, see Kaczmarek, 2000, 2002). Essentially, learning is a complex phenomenon that includes numerous events. The influence of various inputs, such as sensory information processing, arousal, motivation, emotion, stress responses, etc. are in fact inseparable from learning. Notably, although these components of learning processes have to be integrated in the memory formation, the genes whose expression is activated by them are not necessarily involved in plastic changes. This potential influence should be taken into consideration, as one analyzes possible functions of the genes that are up-regulated in different behavioral paradigms.

The first, simplest hypothesis explaining the role of genes in the neuronal cell is *homeostatic maintenance of neural functioning*, e.g., making up for the proteins that are lost during physiological metabolic turnover. The second possible explanation for the function of the regulatory gene in the neuronal cell is given by *the replenishment hypothesis*. The concept of replenishment implies that the neuronal activity-evoked immediate early gene expression is involved in the metabolic recovery triggered by depletion of key cellular components (e.g., synaptic release machinery, metabolic enzymes, etc.) during neuronal activation and thus serves to reinstate the same situation as before the training. The third potential role of gene expression in the neuron is *maintenance of plastic changes*, e.g., when they serve to produce the proteins whose function is to maintain the plastically reorganized neuronal connections. These proteins should be targeted to specific synapses to support their newly gained functions. The fourth possibility is *information integration*. This hypothesis assumes that the regulatory regions of the genes encoding the proteins directly subserving synaptic reorganization may act as ‘‘coincidence detectors,’’ thereby allowing convergence of information provided by various transcription factors, activated by different signaling pathways of behavioral relevance, such as sensory information, arousal, motivation, etc. (Kaczmarek, 1993, 1995). Below, the *zif268* gene expression will be presented in the context of the aforementioned gene functions in neurons in order to elucidate the role played by Zif268 transcription factor.

4. Basal expression of *zif268* mRNA and protein in the brain

In the brain, Zif268 has a distinct basal expression, i.e., the one maintained by normal ongoing synaptic or neurohormonal/neurotrophic activity (Worley et al., 1991; Herdegen and Leah, 1998; Beckmann and Wilce, 1997). This feature is probably vital for Zif268 functions since it allows for either increasing or decreasing the level of its expression.

High basal levels of *zif268* and its protein have repeatedly been observed in the visual cortex of various mammalian species (for details, see Kaczmarek and Chaudhuri, 1997 and reference therein). In the rat somatosensory cortex, the high levels of *zif268* and its protein expression were also observed with a two-peak pattern with maximal expression in layer IV and deep layer V/layer VI (Mack and Mack, 1992; Steiner and Gerfen, 1994; Melzer and Steiner, 1997; Bisler et al., 2002).

Hughes et al. (1992) observed a high basal Zif268 level in the CA1-subiculum region of the rat hippocampus. Similarly, Desjardins et al. (1997) demonstrated that Zif268 was highly expressed in the CA1 pyramidal cells of the dorsal hippocampus. Cullinan et al. (1995) also showed a subregion-specific pattern of *zif268* basal expression in the rat hippocampal formation, with the highest expression within the subiculum and the CA1–CA2 regions, and low levels in the CA3 area and in the dentate gyrus. Okuno et al. (1995) described subdivision-specific Zif268 expression in the hippocampal formation of the macaque monkey. The large number of Zif268-expressing neurons was observed in the presubiculum and layer II of the rostral part of the enthorinal cortex. A moderate number of

immunopositive neurons was observed in the parasubiculum, and the smallest one in the subiculum proper (among the three subicular subdivisions). The caudal part of the enthorinal cortex contained immunopositive neurons mainly in layer VI and a weak expression of Zif268 was seen in the dentate gyrus and in the CA1–CA3 areas of the hippocampus proper.

Cullinan et al. (1995) detected high *zif268* expression also in the anterior olfactory nucleus, olfactory tubercle, nucleus of the lateral olfactory tract, tenia tecta, dorsal and endopiriform nucleus. They noted a moderate expression of *zif268* in a number of subcortical regions, such as the nucleus accumbens, lateral septal nucleus, caudate putamen, several subnuclei of amygdala (lateral, basolateral and basomedial), thalamus and also in brainstem areas, including the pontine nuclei, nucleus ambiguous and in the cerebellum. Zif268 protein was present in rats in the striatum, cerebellum, nucleus raphe magnus, colliculi, periaqueductal gray, hypothalamus, geniculate nuclei, caudate putamen, amygdala, hippocampus, lateral septal nucleus, olfactory tubercle and the cortex (Herdegen et al., 1990, 1995).

The NMDA receptor appears to play the major role in driving high basal levels of zif268 in several brain regions. The basal levels of *zif268* mRNA, protein and DNA-binding activity in adult rat in the visual and piriform cortices, as well as in the hippocampus, were found to be markedly down-regulated by treating animals with MK-801 (Worley et al., 1990, 1991; Gass et al., 1993a). However, the MK-801 doses applied (0.3 mg/kg and more) are known to affect the behavioral state of the animal (Whishaw and Auer, 1989; Hargreaves and Cain, 1992; Sierocinska et al., 1991). This may obscure the MK-801 results, since it is unclear if the effect was produced by the antagonism of NMDA receptors in the neurons expressing Zif268 or at earlier sites in the pathway. Gao et al. (1998) observed an early (after 1 h) activation of *zif268* mRNA and its delayed (after 48 h) suppression following PCP (phencyclidyne, a competitive NMDA antagonist) and MK-801 treatments in the neocortical areas. The changes, which were particularly prominent in the cingulate and auditory cortices, showed that the effects of NMDA antagonists could also be time-dependent. Furthermore, the basal expression of *zif268* was also found to be down-regulated by blocking noradrenergic input (Bhat and Baraban, 1992).

The abovementioned results demonstrate that the basal expression of *zif268* and its protein depends on the structure of the brain. Interestingly, there are also apparent differences in the basal expression of *zif268* among different mammalian species. The basal expression of Zif268 in the mice brain is shown in the Fig. 2.

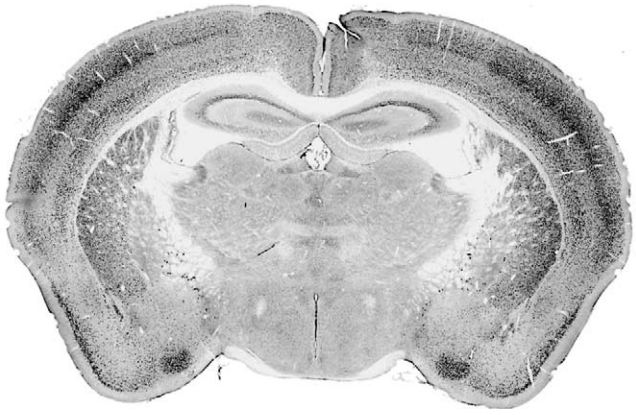

Fig. 2. Basal expression of Zif268 in mouse brain. Coronal section of the brain was immunostained with Zif268 specific antibody visualized by the peroxidase-DAB reaction (see Filipkowski et al., 2001 for the staining procedure details).

5. Stress and expression of *zif268*

Stress appears to be an indissociable component of learning processes and therefore it is very important to keep in mind that *zif268* expression may at least partly result from stressful aspects of learning situation. On the other hand, all the stress experiments described below involve also aversive learning (context conditioning) of the stressful situation. Thus, they are not fundamentally different from, e.g., classical fear conditioning.

Schreiber et al. (1991b) observed an induction of *zif268* in the rat neocortex (particularly in layers IV, V and VI) and in the CA1 region of the hippocampus after 1 h of restraint and an intermittent tail shock. Moreover, Cullinan et al. (1995) described an expression of *zif268* in response to acute swim and restraint stress in rats. The pattern of induction of *zif268* following both types of stress was similar. An increase of *zif268* expression was seen in neocortical areas (the most prominent in layer IV, but also observable in layers II, V and VI), in the hippocampal formation (apart from the CA2 area), lateral septal nucleus, caudate putamen, amygdala (in particular in the cortical nuclei), thalamic and hypothalamic regions (the largest induction in the paraventricular nucleus) and in many other subcortical regions. The highest level of *zif268* expression was observed after 30 and 60 min and the expression returned to the basal level within 120 min post-stress.

Watanabe et al. (1994) noted an activation of *zif268* by the acute restraint stress and the acute shaking stress in the paraventricular nucleus of the rat hypothalamus within 1 h after the onset of stress stimulation. Repeated restraint stress (for 14 days, 1 h a day) resulted in the habituation to the acute restraint stress, but not to the acute shaking stress, i.e., there was no cross-habituation of the *zif268* response. In contrast, Umemoto et al. (1997) observed that repeated immobilization (for six days, 2 h a day), as well as chronic glucocorticoid administration, did not attenuate an increase of *zif268* expression in the paraventricular nuclei of rats in response to subsequent acute immobilization stress. They noted prolonged expression of zif268 following the challenge immobilization — at 90 min after the beginning of the challenge immobilization the level of *zif268* in the

repeated stress group was significantly higher than in the acute stress group and remained increased after 120 min, but decreased after 240 min. As the authors point out, these data may indicate that the adaptation to repeated stress requires Zif268 expression, which may be involved in the establishment or maintenance of synaptic re-organization. However, it is possible that *zif268* induction in response to a stressful event is sensitive to the neuronal activity, and it needs more time to habituate.

The stress, an inseparable component of learning processes, evokes the expression of Zif268, which may create a formidable obstacle in uncovering the role of Zif268 in learning (with the use of the correlative approach). The solution to circumventing this problem seems to be the application of the appropriate inter-subject comparisons (cf. Guzowski et al., 1999; Tokuyama et al., 2002).

6. Expression patterns of *zif268* products in sensory cortex in response to change in environmental conditions — possible involvement in cortical plasticity

6.1. Visual cortex

The expression of *zif268* products in neurons is maintained at a relatively high level by ongoing synaptic stimulation. However, numerous physiological and pharmacological stimuli can induce a rapid up-regulation of Zif268 in specific areas of the brain. On the other hand, the level of Zif268 expression can be markedly decreased after sensory deprivation. The expression patterns of *zif268* and its protein in the visual cortex in response to changing visual conditions have been extensively covered previously (Kaczmarek and Chaudhuri, 1997). The main conclusions from this analysis were: (i) there is a high constitutive expression of *zif268* in the adult primary visual cortex of various mammalian species; (ii) this expression is strongly down-regulated by removal of visual input; (iii) however, it is capable of a rapid return to basal levels by stimulation; (iv) there is a tight developmental regulation of the gene expression with peak levels during the critical periods. Thus, *zif268* expression in the adult visual cortex is linked to ongoing synaptic activity and the developmental profile of the expression implies its role in synaptic plasticity (Kaczmarek and Chaudhuri, 1997). Recent studies on *zif268* expression patterns in the visual cortex additionally support the aforementioned notions (Kaczmarek et al., 1999; Yamada et al., 1999; Heynen and Bear, 2001; Mataga et al., 2001; Zangenehpour and Chaudhuri, 2001; Pinaud et al., 2003).

Surprisingly, in contrast to this unequivocal demonstration of the robust expression of *zif268* in the visual cortex, functional studies on Zif268 mutant mice (created by Lee et al., 1995, see Section 8.3) have not revealed any marked abnormality of cortical functioning (Mataga et al., 2001). Furthermore, when the mutants were deprived of the use of one eye during the developmental critical period, extracellular in vivo single-unit recording from the binocular zone of the visual cortex showed that visual responses developed normally. Moreover, similar shift of responsiveness in favor of the open eye was produced in both mutant and wild mice by either brief or long-term occlusion of one eye.

6.2. Somatosensory cortex

The vibrissae comprise an important sense organ for nocturnal rodents. The most prominent of them are the so-called mystacial vibrissae, which are arranged in five longitudinal rows on the snout. Additional smaller vibrissae are present along the lips and around the nose. Sensory input from the whisker mechanoreceptors caused by an angular deflection of a vibrissa is relayed by the trigeminal ganglion cells to the ipsilateral brainstem (trigeminal nuclei). Then, the signal is conveyed to the contralateral ventrobasal thalamus and further to the clusters of closely packed neurons in the layer IV of the primary somatosensory cortex. These clusters, called barrels, are functional columns, which extend through all the cortical layers. The entire vibrissae-to-barrel system is somatotopically organized, and there is one-to-one correspondence between each barrel and each vibrissa. A tactile stimulation of the vibrissae results in turning behavior towards the side of the stimulus; unilateral clipping leads to turning towards the side opposite to vibrissae removal and lateralized facial scanning. Hence, the vibrissae system offers a unique possibility to study functional changes in the brain caused by non-invasive experimental manipulations and allow designing precise and well-controlled experiments (for a review, see Filipkowski, 2000).

After clipping the vibrissae the level of *zif268* mRNA expression decreased in the corresponding parts of the contralateral barrel fields (Steiner and Gerfen, 1994). The expression of Zif268 protein in the rat barrel cortex after clipping mystacial vibrissae was analyzed by Filipkowski et al. (2001). Rats were habituated to the clipping procedure for 20 days, then the mystacial vibrissae were clipped on one side of the snout and rats were sacrificed one and one-half hours later. The clipping procedure alone led to a reduction in Zif268 expression in layers II/III, IV and V/VI on the deprived side.

The induction of Zif268 by a physiological stimulus was described for the first time by Mack and Mack (1992). They used a paint brush to stroke rat's whiskers on one side of the snout for 15 min (the rats were lightly anesthetized during this procedure). The induction of Zif268 in the hemisphere contralateral to the side of the stimulation was compared to the induction in the ipsilateral hemisphere. The increment in Zif268 expression after 2 h following the stimulation was most prominent in layer IV, but it was also observed in layers II, III and VI. In these layers, the intensity of staining and the number of stained cells increased.

Apomorphine, a dopamine receptor agonist that acts at both D_1 and D_2 receptors, elicits motor activity with emphasis on the orofacial behavior (sniffing, whisking, licking and snout contact fixation — a continuous contact with the snout to surfaces) leading to the activation of vibrissae representation in the barrel cortex. Steiner and Gerfen (1994) observed the influence of tactile sensory input on the basal and the apomorphine-induced *zif268* expression in the rat barrel cortex. They clipped the mystacial vibrissae on the left side of the snout (rats were habituated to the clipping procedure for the preceding four days). Four hours after vibrissae removal, the rats were sacrificed or injected with apomorphine and killed 1 h later. Steiner and Gerfen detected high basal level of *zif268* expression in the somatosensory cortex with the maximal expression in layer IV and deep layer V/layer VI and a reduced *zif268* expression in the part of the contralateral barrel field corresponding to the clipped mystacial vibrissae. The decrement was seen in all layers of the barrel cortex (stronger in layers V/VI than in layer IV). On the other hand, the injection of apomorphine caused an increased expression of *zif268* in the cortex and the striatum. In the somatosensory cortex, the expression of *zif268* showed a laminar pattern similar to that of the basal expression, with the additional peak in deep layer VI bordering the white matter. This apomorphine-induced increment was blocked in the sensory-deprived somatosensory cortex. Deprivation effects were seen in all layers, but the reduction in *zif268* expression in layer IV was similar or even bigger than in layers V/VI. The level of *zif268* was very low in the thalamic nuclei, either under basal conditions or after apomorphine treatment.

The expression of Zif268 in the rat barrel cortex following apomorphine-evoked whisking behavior was investigated by Filipkowski et al. (2001). Rats were habituated to the clipping procedure and to the injections for 20 and 14 days, respectively. Then the mystacial vibrissae were clipped on one side of the snout. Four hours after the clipping the rats received an injection of apomorphine, MK-801, MK-801 and apomorphine or saline (control group) and were sacrificed one and one-half hours later. The apomorphine treatment evoked an insignificant increase in the Zif268 immunoreactivity in the postero-medial barrel subfield (representing the vibrissae of the mystacial pad), except for layer VIb, where there was a statistically significant Zif268 elevation. No effect of MK-801 pre-injection (0.1 mg/kg) was noted.

It may appear that the immunocytochemical results of Filipkowski et al. (2001) are not in perfect agreement with the Steiner and Gerfen (1994) results obtained with mRNA. However, this discrepancy may be due to some differences between the experimental paradigms. Furthermore, it cannot be excluded that differential regulation of mRNA and protein expression, which was observed in other situations (Worley et al., 1993), is responsible for this inconsistency.

Melzer and Steiner (1997) examined the effects of whisker stimulation in freely moving rats on the expression of *zif268* in the barrel cortex. Whiskers equipped with metal filaments were stimulated for 5–15 min with a pulsating magnetic field. Melzer and Steiner showed that the expression of *zif268* was largely restricted to the radial columns across the barrels representing the stimulated whiskers, with the magnitude of expression being proportional to the intensity of stimulation. The maximal expression was observed in layer IV.

Staiger et al. (2000) subjected adult rats to clipping of different sets of whiskers and subsequently placed them overnight in a novel, enriched environment. This stimulation led to a specific and significant increment in the expression of Zif268 in the barrel cortex corresponding to the stimulated vibrissae. The cortical columns related to the clipped vibrissae showed a decline in the immunostaining. The response displayed a layer-specific pattern with the most intense staining in layers IV and VI. No induction of Zif268 was observed in the subcortical stations of the whisker-to-barrel pathway, i.e., the trigeminal nuclei and the ventrobasal complex.

Summing up, the basal *zif268* and its protein expressions in neurons of the somatosensory cortex seem to depend on sensory input (Mack and Mack, 1992; Steiner and Gerfen, 1994). However, Filipkowski et al. (2001) did not observe the down-regulatory effect of sensory deprivation on the Zif268 protein expression in cortical layer VIb, and the apomorphine-evoked induction was also more pronounced in this layer. A similar pattern of the *zif268* mRNA expression in the somatosensory cortex was observed by Steiner and Gerfen (1994). These data suggest that the prevailing signal leading to changes in Zif268 expression in this sub-layer is non-sensory. Notably, it is known that the effects of apomorphine can result from a sensory stimulation but also from a direct stimulation of dopamine D_1 receptors (Cole et al., 1992; Jung and Bennett, 1996). Thus, it appears that the sensory stimulation provides a major but not the only component to the apomorphine-evoked barrel cortex *zif268* activation. Vibrissae removal produces acute sensorimotor deficits, which are followed by recovery of the impaired function. Therefore, the changes in *zif268* expression may reflect early stages of adaptive mechanisms, but they can also result from reduced neuronal activity in some barrel columns.

6.3. Expression of zif268 mRNA and protein following exposure to an enriched environment

Enriched environment usually means that experimental animals are placed in a cage filled with toys, tubes and food rewards, which encourage them to engage in the exploratory behavior. They may be exposed to such a stimulating cage individually or in a group.

Wallace et al. (1995) analyzed *zif268* mRNA level by in situ hybridization in the brains of periadolescent rats exposed for 2–4 days to various environmental conditions: group housing in a complex environment (EC), individual

housing with daily handling (HIC), and individual housing without handling (IC). Quantitative analysis of autoradiograms revealed that EC rats had significantly higher level of *zif268* mRNA in the visual cortex than IC rats. HIC group produced intermediate results. Furthermore, keeping the EC group in a complex environment for 30 days resulted in still higher *zif268* expression.

The effects of tactile exploration of an enriched environment on Zif268 expression in the whisker-to-barrel pathway of rats were examined by Bisler et al. (2002). They clipped all but two whiskers on the right side of the anesthetized rats and placed each animal in a stimulatory box. Rats were kept in the enriched environment for different periods of time (from 10 min to five days) and sacrificed. The animals of the control groups were taken from their home cages or were given the anesthetic procedure 1 h before perfusion. Bissler et al. observed the Zif268 induction throughout the barrel cortex, with intense staining in layers IV and VI, peaking at 1 h. In conclusion, the research concerning expression patterns of *zif268* products in the sensory cortex in response to changes in the environmental conditions suggests that Zif268 is engaged in functioning of neurons, but the obtained results do not allow the conclusion that Zif268 is involved in neuronal plasticity (for review see also Pinaud, 2004).

7. Seizure-induced expression of *zif268* products in the hippocampus

Both *zif268* mRNA and its protein expressions have been demonstrated to be induced in the hippocampus in response to a variety of seizure-inducing stimuli. Saffen et al. (1988), as well as Yount et al. (1994), noted that the administration of the convulsant pentylenetetrazole (aka Metrazole) caused a rapid and transient increase of *zif268* in the rat hippocampus. Moreover, both single and repeated electrically induced seizures evoked an increase in *zif268* mRNA expression, which was most prominent in the granule cells of the hippocampus (Cole et al., 1990). This response was blocked by an infusion of tetrodoxin (TTX, a highly specific, reversible Na^+ channel blocker in excitable tissues) without blocking seizures. In contrast, the pretreatment with anticonvulsants or selective NMDA antagonists, which reduced seizure intensity, did not affect *zif268* expression. These data indicate that the electrical excitation rather than seizure spreading is responsible for *zif268* induction (Cole et al., 1990).

Maximal electroconvulsive shock (MECS) is a very brief but intense electrical stimulation, which can evoke behavioral seizures. Barnes et al. (1994) observed Zif268 activation throughout the entire hippocampus, following MECS. Gass et al. (1992) demonstrated that bicuculline-induced seizures resulted in a rapid rise of Zif268 expression in the rat dentate gyrus granule cells and later in the CA1 and CA3 areas of the hippocampus. Correspondingly, Dragunow et al. (1992) observed an increase in Zif268 expression in the dentate gyrus granule cells as well as in the somatostatin- and parvalbumin-containing interneurons in the rat hippocampus, following electrically induced hippocampal seizures. Moreover, Lanaud et al. (1993) showed an increment in *zif268* mRNA expression in the rat hippocampus following seizures evoked focally from the deep prepiriform cortex. Similarly, after kainic-acid-induced limbic seizures, an increase of Zif268 expression was noted in the dentate gyrus, as well as in the CA1 and CA3 areas of the rat hippocampus (Gass et al., 1993b). The results described above may indicate that Zif268 is engaged in neuronal plasticity caused by electrical stimulation. However, an alternative conjecture that the expression simply mirrors strong neuronal activity evoked by electrical stimulation cannot be ruled out.

7.1. Kindling and associated mossy fibre sprouting

Kindling is an animal model of epileptogenesis, in which repeated administration of a low-intensity focal non-convulsive electrical stimulation leads to development of prolonged, widespread electrical seizures with generalized motor seizures. Kindling is also accompanied by the long-lasting reorganization of synaptic connections observable as the sprouting of the mossy fibres, i.e., axons of the hippocampal dentate granule cells and the aberrant synapses, which they form. Therefore, kindling provides a model of neuronal plasticity in the hippocampus.

Burazin and Gundlach (1996) investigated the time course of *zif268* induction in the rat forebrain in the kindling model of epilepsy. Kindling was produced by daily unilateral stimulation of the amygdala. The authors observed a bilateral increase in *zif268* expression in the dentate gyrus after 30 min to 1 h following the stimulation. However, no long-term changes in *zif268* expression were detected beyond 2 h and up to eight weeks after the last seizure. Zheng et al. (1998) investigated kindling and associated mossy fibre sprouting in mice with disrupted *zif268* gene. They used mice created by Lee et al. (1995) (see Section 8.3). Zheng et al. observed that neither the rate of kindling (measured by electrophysiological and behavioral indicators) nor kindling-induced granule cell axonal sprouting into the supragranular regions of the dentate gyrus was altered in *zif268* deficient mice. Moreover, NMDA receptor blockade with a dose of MK-801 that significantly retards the development of amygdala kindling failed to attenuate *zif268* expression (Hughes et al., 1994). These results may indicate that neither constitutive nor seizure-induced expression of Zif268 is required for kindling development and kindling-induced synaptic plasticity of mossy fibres. On the other hand, these results do not exclude the possibility of a functional compensation due to the products of other genes.

Nahm and Noebels (1998) examined axonal sprouting in the dentate granule cells of stargazer mice. The stargazer mice are mutants with a salient epileptic phenotype. They exhibit spontaneous, prolonged, generalized spike-wave

cortical, hippocampal and thalamic discharges (Noebels et al., 1990), which are associated with mossy fibres sprouting without any signs of cell death, gliosis or cellular injury. The stargazer mice showed no Zif268 induction in the hippocampus before or during mossy fibres growth. However, Nahm and Noebels observed that kainate (a convulsant known to induce seizures preceding mossy fibres synaptic reorganization in the hippocampus) induced an increase in Zif268 expression in these mice.

8. *zif268* products in long-term potentiation

The term *long-term potentiation* (LTP) refers to the phenomenon of the activity-dependent increase in synaptic efficacy, which serves as an important model for the mechanisms responsible for modification of the responses in the brain evoked by experience. There are several forms of LTP which differ as far as their location, stimulation paradigms and the pharmacological properties are concerned (see Bliss and Collingridge, 1993). The original description involved awake rabbits in which the electrodes implanted in the perforant path were used to evoke responses in the granule cell layer of the hippocampal dentate gyrus. Brief and repeated (so-called tetanic) stimulation resulted in an enhancement of the response to subsequent stimuli with respect to the response to the same stimuli before tetanization. Such an enhancement of synaptic efficacy was maintained for weeks (Bliss and Lomo, 1973). Further studies, carried out mainly on rats, involved a variety of the stimulation protocols and allowed identifying three major categories of LTP with respect to its duration: LTP1 with an average decay constant of approximately 2 h, LTP2 with a time constant of approximately four days and LTP3 with an average decay constant of approximately 23 days (the latter two are often collectively called late- or L-LTP in contrast to early- or E-LTP) (Racine et al., 1983; Abraham and Otani, 1991). Recently, stable LTP lasting months or even one year for both medial and lateral perforant path synapses has been obtained (Abraham et al., 2002). The induction of such a long-term stable LTP was sensitive to high-frequency stimulation variables and its maintenance was also experience-dependent.

Extensive studies on the dentate gyrus LTP and, especially, on the LTP evoked in hippocampal slices by stimulation of Schaffer collaterals projecting from pyramidal neurons of CA3 to CA1 revealed distinct cellular and molecular mechanisms that underlie different temporal phases of hippocampal LTP. The induction phase, which involves combined activation of Src family tyrosine kinases, protein kinase A, protein kinase C, MAPK and, in particular, Ca^{2+}/calmodulin-dependent protein kinase II, results in phosphorylation of several synaptic proteins, e.g., glutamate receptor-gated ion channels, and the enhancement of the subsequent postsynaptic current (see Soderling and Derkach, 2000). Longer-lasting forms of LTP, i.e., LTP2 and LTP3, but not LTP1, require gene transcription and protein synthesis (Krug et al., 1984; Otani et al., 1989).

8.1. Activation of *zif268* products in response to LTP-evoking stimuli

zif268 has been reported as the first gene whose expression was consistently augmented in the rat dentate gyrus following the induction of long-lasting LTP in awake rats (Cole et al., 1989). This original report, as well as several others, demonstrated the increase in the expression of either *zif268* mRNA or the protein in the dentate gyrus that appeared after brief high frequency stimulation of the perforant path, which resulted in the induction of LTP. This effect was not observed after continuous low-frequency stimulation (Cole et al., 1989; Richardson et al., 1992; Worley et al., 1993). The increment in expression of *zif268* and its protein was found to be ipsilateral (on the side of high-frequency stimulation) and more intense in the dorsal than the ventral hippocampus (Cole et al., 1989; Richardson et al., 1992; Abraham et al., 1993). The thresholds for the induction of LTP and *zif268* expression were found to be similar (Worley et al., 1993). Furthermore, various NMDA receptor antagonists, which block LTP induction, also blocked *zif268* expression, normally evoked by high-frequency stimulation (Cole et al., 1989; Wisden et al., 1990; Worley et al., 1993; Salin et al., 2002). Moreover, Zif268 DNA binding activity increased in a NMDA-receptor-dependent way after LTP induction, as demonstrated by Williams et al. (2000).

Abraham et al. (1993) altered the duration of LTP in the dentate gyrus of awake rats by varying the number of stimulus trains delivered. They correlated this factor with the induction of *zif268* and its protein in the distinct groups of animals subjected to identical stimulus regimes. The tetanization consisted of 10–50 trains presented either all on one day or as 10 trains on two, three or five consecutive days. A monotonic increase in the persistence of LTP was observed in the groups subjected to all the stimulus trains on one day. This increase was found to be a monotonic function of the number of trains, growing from a 10- to 50-train group with a saturation occurring in the 40- and 50-train groups. An increment in LTP persistence was also observed when 10 trains were repeatedly applied across a number of days. However, the magnitude of this effect was smaller than in the former regime, in which the trains were delivered repeatedly on one day. The expression of *zif268* and its protein displayed strong augmentation dependent on the number of trains delivered as LTP-inducing stimulation on one day. Different patterns of *zif268* expression occurred when the tetanization consisted of 10 trains repeatedly delivered across a number of days — *zif268* response appeared on the second, third and fifth day of the tetanization, but there was no correlation between the number of trains delivered as the LTP-inducing stimulation and the Zif268 level (Abraham et al., 1993).

If Zif268 induction is a critical step in the orchestration of the expression of the genes necessary for the stabilization of LTP, the relation of Zif268 expression to LTP persistence is far more important than its relation to LTP induction. In order to determine whether *zif268* responses had been related to the persistence of L-LTP (LTP2 and LTP3), Abraham et al. (1993) calculated correlations between the mean LTP decay rates and the mean mRNA and protein responses on the last day of tetanization. Such an analysis for the groups receiving all trains on one day showed very high correlation, whereas analogous correlations for the groups with multiple days of tetanization were relatively low. A better prediction of LTP persistence for the groups with multiple days of the tetanization is given by the cumulative amount of Zif268 (i.e., the sum of Zif268 amounts calculated for each day of stimulation). The study carried out by Richardson et al. (1992) showed a similar correlation — the activation of *zif268* and its protein was found to be more correlated with the duration of LTP than with the magnitude of initial LTP.

In contrast to the above, there are reports suggesting that Zif268 expression can be dissociated from the induction of LTP. It is known that LTP at the perforant path-granule cell synapses can be blocked by a crossed inhibitory input via activation of the contralateral dentate hilus. Cole et al. (1989) demonstrated that both LTP and *zif268* were simultaneously blocked by this procedure. In contrast, bilateral activation of *zif268* was observed by Wisden et al. (1990) after such a treatment, in spite of the lack of LTP induction in the contralateral hemisphere. In addition, Worley et al. (1993) observed, in some preparations, a modest increase in *zif268* expression (relative to the hippocampus of the naive control) in granule cells of the ipsilateral dentate gyrus in response to the low-frequency stimulation. However, the increase was less intense than the increase caused by high-frequency stimulation.

Schreiber et al. (1991a) examined the effects of different temporal patterns of synaptic stimuli and obtained a negative correlation between LTP and *zif268* induction. They observed that the parameters of the stimulation effectively inducing LTP were not associated with *zif268* activation, whereas the stimulation parameters that failed to induce LTP consistently resulted in *zif268* activation. The former fact may be explained by the observation that general anesthesia with sodium pentobarbital (which was used by Schreiber et al.) produces a significant reduction in the response of transcription factors (French et al., 2001; see also Kaczmarek, 1992). Notably, Cole et al. (1989), who reported a high correlation between LTP induction and *zif268* activation in the dentate gyrus, also employed anesthetized rats, but they used chloral hydrate. The observed discrepancies might also result from differences in the stimulation protocols used for LTP induction in the above-mentioned studies. In the study of Schreiber et al. (1991a), the patterned stimulation was applied over a relatively brief interval, whereas Cole et al. (1989) employed longer periods and different intraburst interval frequencies to deliver an equivalent number of pulses. However, Jones et al. (2001) were able to obtain both LTP induction and *zif268* activation using even shorter periods than in the study of Schreiber et al. Irrespective of the reason for these discrepancies, the study of Schreiber et al. (1991a) showed that LTP can be induced without the expression of *zif268*, thereby supporting the conjecture of a double dissociation between the LTP induction and Zif268 expression.

There are very few and rather contradictory data on the association between *zif268* activation and LTP induction in the brain locations different from the perforant path-granule cell synapse. French et al. (2001) did not manage to detect changes in the expression of *zif268* and its protein following the induction of LTP by high-frequency stimulation of the commissural projection to CA1 pyramidal cells. However, they showed that Zif268 could be modulated in CA1 pyramidal cells after electroconvulsive shock, which demonstrated the potential responsiveness of this region of the brain to electrical stimuli. In contrast, the study of Heynen and Bear (2001) demonstrated that patterned stimulation of the dorsal lateral geniculate nucleus induced LTP of the field potentials evoked in primary visual cortex of adult rats. In concert with the LTP induction, the Zif268 activation was also observed. Interestingly, Heynen and Bear (2001) detected an increase in the Zif268 induction under the conditions of sodium pentobarbital anesthesia.

The existence of a correlation between the expression of Zif268 and the duration of LTP (cf. Abraham et al., 1993; Richardson et al., 1992) can also be verified by using the known deficit in the maintenance of LTP in old animals. Worley et al. (1993) found similar LTP- and electroconvulsive shock-induced increases in *zif268* expression in young and old rats, which questions this correlation.

Furthermore, in various studies different levels of *zif268* were observed. Cole et al. (1989) showed that the level of *zif268* expression after the high-frequency stimulation and *zif268* expression following MECS were almost alike. Analogous results were obtained by Worley et al. (1993). In contrast, Barnes et al. (1994) using the same MECS parameters found that *zif268* activation in the dorsal hippocampus in response to the tetanic stimulation was only 60% of the values observed after MECS. All the abovementioned authors employed rats from the same strain and at the same age. Moreover, the study of the kinetics of LTP decay brought further unexpected discrepancies. Worley et al. (1993) observed the identical decay kinetics — about three days — regardless of the pattern of the LTP-inducing electrical stimulation (10 or 50 repetitions of a 20 ms train of pulses delivered at 400 Hz). On the other hand, Abraham et al. (1993) demonstrated with the use of a similar paradigm a monotonic increase in the persistence of LTP from 10 to 50 trains (in the last case LTP lasted over 20 days). This discrepancy makes one of the assumptions of Abraham et al. (1993), namely that the number of stimulus

trains correlates with the persistence of LTP, questionable. A possible explanation is suggested by the results obtained by Cirelli et al. (1996) who showed that Zif268 level is much lower in the sleeping that in the awake brain of a rat. This could result from the differential noradrenergic input of the locus coeruleus in these two different behavioral states. Notably, a dependence on noradrenergic input for light-induced Zif268 expression in rat visual cortex was reported by Pinaud et al. (2000). In light of these two studies, it cannot be excluded that the discussed discrepancies were due to using rats with different basal level of *zif268*. If it was the case and the increase in *zif268* expression corresponded to the strength of the stimulation, the ceiling effect of *zif268* expression might have been observed.

8.2. Functional studies on Zif268 mutant mice

The mice with mutated *zif268* gene were generated independently by Lee et al. (1995) and Topilko et al. (1998). In both cases, mice were obtained with the use of the murine strain 129/SvJ embryonic stem cells, which were injected into murine C57BL/6 blastocysts and backcrossed onto a C57BL/6 background. However, the mice produced by Topilko et al. (1998) had the insertion of the cassette containing the lacZ gene and the G148 resistance gene (neo) under the control of the phosphoglycerate kinase (PGK) promoter 50 bp upstream of the *zif268* initiation codon. Additionally, a frameshift mutation has been introduced upstream of the DNA-binding domain (at the beginning of the region encoding the first zinc finger). Lee et al. (1995) obtained mutant mice by a homologous recombination with the use of the target vectors, which contain a neomycin resistance gene insertion under the control of PGK promoter into the NdeI site, located at the beginning of the region encoding the first zinc finger. Because of different sites of the cassette insertion, the uncontrollable influence on the other genes expression may vary between these mice. In both cases, the animals were born normally and capable of survival to adulthood; however, there were also some differences. Topilko et al. (1998) observed that both male and female mice homozygous for the mutation had a reduced body size and were sterile. Furthermore, the homozygous mutant males did not exhibit an aggressive behavior in the presence of other males and the homozygous mutant females did not show behavioral signs of estrus. In contrast, Lee et al. (1996) did not observe any developmental or behavioral defects and only the homozygous mutant females were infertile.

Jones et al. (2001) used the mice generated by Topilko et al. (1998) to demonstrate that late LTP was absent in the mutants 24 and 48 h after tetanus, whereas the animals exhibited early LTP in the dentate gyrus. LTP decayed at a similar rate in both the heterozygous (with the level of *zif268* about twice smaller than that seen in wild-type mice) and the homozygous mice. Another study on the *zif268* targeted disruption and LTP induction in mouse was conducted by Wei et al. (2000), who used the mice generated by Lee et al. (1995). Neither in long-term depression (LTD) nor in LTP induced by a single tetanic stimulation were any significant differences between wild type and mutant mice noted. Furthermore, there were no differences in the paired-pulse facilitation of the field EPSP at different interpulse intervals and in the other basal synaptic responses to stimulation. However, the late phase of LTP induced by a four-train tetanic stimulation was significantly decreased in mutant mice and the observed defect was not due to changes in the functions of the NMDA receptor or in the inhibitory tone.

The late phase of LTP was impaired in both strains of the knockout mice. In addition, Wei et al. (2000) showed the impairment of LTP in another experimental paradigm. The authors observed that tissue/nerve injury caused a rapid increase in the level of Zif268 in the hippocampal CA1 pyramidal neurons of normal mice that receive nociceptive inputs from the periphery.[4] These changes in Zif268 expression were selective and limited to CA1 region of the hippocampus, whereas only minor changes were detected in the CA3 region and dentate gyrus. The changes in Zif268 expression were also selective for noxious stimuli. In parallel, synaptic potentiation induced by a single tetanic stimulation was significantly bigger and lasted longer after the injury. The conscious experience of pain was necessary for this enhancement. This LTP augmentation was absent in *zif268* knockout mice.

At first sight, these results appear to support the hypothesis that there is a link between the induction of LTP (which is a model of neuronal plasticity) and Zif268 expression. However, the impairment of LTP may be caused by metabolic disturbances due to the absence of Zif268, which does not allow the discrimination between the maintenance of plastic changes and the replenishment hypothesis (see Section 3).

8.3. Zif268 in LTP — summary

Aforementioned results are consistent with the hypothesis about the role of Zif268 in the maintenance of plastic changes, but it is also likely that Zif268 expression is related to stronger neuronal activation, e.g., according to the replenishment hypothesis. A similar level of Zif268 expression detected after each day of 10-trial-stimulation (Abraham et al., 1993) and the fact that Zif268 is also activated throughout the entire hippocampus by MECS (Barnes et al., 1994) suggest correlation with the level of cell activation and may support the replenishment hypothesis. The increase in Zif268 expression following high-frequency stimulation (inducing LTP) is far more selective than this induced by MECS. However, it simply reflects activation of

[4] An increase in Zif268 expression was also observed in the anterior cingulate cortex following the same kind of tissue injury (amputation of the third digit of the hind paw) in the adult rat (Wei et al., 1999).

the perforant path fibres, because most of them terminate ipsilaterally, and the position of the stimulating electrode results in the maximal synaptic potentials in the dorsal hippocampus (Cole et al., 1989). In the perspective of abovementioned facts, the replenishment hypothesis of *zif268* action in the cell cannot be excluded.

9. *Zif268* in learning

9.1. Two-way avoidance training

The first demonstration of the increased level of *zif268* mRNA resulting from behavioral training was provided by Nikolaev et al. (1992) with the use of the training of the two-way avoidance reaction. The two-way (active) avoidance behavior is acquired in a shuttle-box apparatus, which consists of two compartments. Both of them are equipped with a source of a conditioned stimulus (CS) and a gridded floor through which an unconditioned stimulus (US, a footshock) can be delivered. The animal is originally placed in one of the two compartments, then the CS is presented and followed by the US within a few seconds. The session is usually composed of a number of trials. The animal is supposed to learn the signaling value of the CS and to avoid the US by moving to the opposite compartment.

Nikolaev et al. (1992) initially subjected adult rats to a single training session of the active avoidance procedure. In the first experiment, the session involved 40 trials separated by the average intertrial interval (ITI) of 1 min. The CS was composed of an overhead light and a 70 dB tone switched on simultaneously in the compartment opposite to that in which the rat was located. Moving from one to the other compartment within 4 s immediately terminated the CS and precluded the activation of the US (avoidance reaction). If the subject did not move to the opposite compartment, after 4 s a footshock was delivered to the grid floor of the occupied compartment. In the second experiment, a somewhat different design was used: 50 trials per session with the ITI of 0.5 min and the visual CS. A transient marked accumulation of *zif268* (as detected by Northern method) occurred in the hippocampus as well as in the visual cortex of rats following one session of both training procedures. In the following part of the experiment, the authors employed a long-term saturation training, carried on until the animal's performance level reached asymptote. This procedure served as a control, since the components of the learning process that could be responsible for the increase in the gene expression, e.g., footshocks and motor reactions, were still present but there was no learning. Importantly, no accumulation of *zif268* mRNA following the prolonged training was observed in the hippocampus and the visual cortex. Thus, the observed changes in *zif268* mRNA expression were associated with the acquisition phase of the training but not with the performance of the already well-trained task.

9.2. Fear conditioning and fear memory retrieval

The greatest number of studies on *zif268* expression and behavioral training involved fear conditioning. Two major training/testing procedures were employed. (i) *Contextual fear conditioning* is a training paradigm in which the animal is placed for a short time in a chamber where it receives an inescapable footshock or footshocks (US). Then, it is brought back to the home cage and after some delay it is again exposed to the same apparatus and tested without the US. The memory of the situation is examined by measuring the freezing reaction during the immediate post-shock period and the retention test. (ii) The procedure of *cued fear conditioning* is similar, but the original exposure to the US is accompanied by a clear sensory stimulus, e.g., a tone (CS), and the testing is carried out in an experimental cage different from the training chamber, but in the presence of the CS. It is noteworthy that the animals exposed to a footshock immediately upon entering a novel environment do not efficiently acquire a conditioned freezing reaction. The other important phenomenon known as latent inhibition is the reduction of contextual conditioning by a pre-exposure to the training environment.

It has been found, by applying specific brain lesions, that the acquisition of conditioned fear involves the amygdala, and the contextual conditioning also requires an intact hippocampus (see Kim and Fanselow, 1992; Maren and Fanselow, 1996; Rogan and LeDoux, 1996; Frankland et al., 1998). Two subsystems engaged in both the contextual and the cued fear conditioning have been characterized within the amygdala. The basolateral complex of the amygdala (comprised of the lateral, basolateral and basomedial nuclei) is considered as a substrate for sensory convergence from the cortical and subcortical areas and a putative locus for creating CS–US associations during fear conditioning. On the other hand, the central nucleus is regarded to be involved in the generation of fear responses (see Maren and Fanselow, 1996; Rogan and LeDoux, 1996; Goosens and Maren, 2001, but see Cahill et al., 1999). According to other authors, the neural basis for fear conditioning is limited to the lateral and central nuclei of the amygdala (see Schafe et al., 2001; Nader et al., 2001). Recently, Nader et al. (2000) have found, using anisomycin (protein synthesis inhibitor), that fear memories require protein synthesis in the lateral nucleus of the amygdala for reconsolidation after retrieval training. Therefore, the changes in *zif268* expression related to fear conditioning could be expected in these regions of the brain.

9.2.1. Contextual fear conditioning

There are two different lines of results related to *zif268* expression following contextual fear conditioning. The increase in *zif268* expression, demonstrated by Rosen et al. (1998) and by Malkani and Rosen (2000a, 2000b, 2001) was limited exclusively to the lateral nucleus of the amygdala and to the group of rats that exhibited freezing reaction. In contrast, Hall et al. (2000) reported non-specific induction of

zif268 following contextual fear conditioning, i.e., the expression of *zif268* was increased in the pyramidal cells of CA1 and CA3 areas of the hippocampus and in the lateral nucleus of the amygdala not only in the experimental group but also in all control groups exposed to the training chamber relative to naive controls. In the following, we shall argue that these discrepancies might result from different procedures that were used by the authors.

Malkani and Rosen (2000b) used four behavioral conditions: (i) handling-only, (ii) context-no-shock, (iii) immediate-shock and (iv) delayed-shock. The first three groups served as control ones. Rats from the first group were not placed in the training chamber; rats from the second group were placed there and left without any footshock. The immediate-shock group was given a footshock immediately upon the placement into the chamber, which precluded acquisition of the conditioned freezing response. The delayed-shock rats were allowed a three-minute acclimation period, and then the footshock was given. Before the training, all the rats were handled for several minutes every day for five days. During the post-shock period and the retention test, a significant increase in the freezing reaction was detected only in the delayed-shock group. In this group, the expression of *zif268* mRNA in the dorsolateral portion of the lateral nucleus of the amygdala was significantly increased at 15 and 30 min after the footshock in comparison to all other groups. However, *zif268* expression in the immediate-shock group was significantly greater than in the context-no-shock group 15 min after the footshock. There was no specific increment in *zif268* expression in the hippocampus and the neocortex. Notably, a number of investigations, in which a similar procedure was used, brought similar results (Rosen et al., 1998; Malkani and Rosen, 2000a, 2000b, 2001).

In contrast, Hall et al. (2000) trained rats according to the contextual conditioning procedure in which freezing of the animals exposed to a footshock in a novel environment and control animals receiving a footshock in a familiar environment (latent inhibition phenomenon) were compared. Two further control groups were also examined — the first one was exposed to the training chamber to assess the effects of the novelty itself and the second one was taken directly from their home-cages. The expression of *zif268* was increased in pyramidal cells of the CA1 and CA3 areas of the hippocampus and in the lateral nucleus of the amygdala in all the groups exposed to the training chamber with respect to naive controls (home-cage control). However, the authors did not observe any changes in *zif268* expression selectively associated with contextual fear conditioning.

The observed discrepancies in *zif268* expression can be explained by taking stress response into account. The immediate-footshock group used by Malkani and Rosen (2000b) in order to control for the effects exerted by the footshock, which is not paired with the environment and the latent inhibition group in the study of Hall et al. (2000) were employed as the most important controls, since no specific contextual fear conditioning occurred in these groups. However, in the former investigation the influence of the footshock might be quite different than in the latter one. The footshock in the first case was applied as one of a number of novel stimuli on the background of a completely unknown environment. These circumstances should be taken into account, because one can expect here a stronger stress response, which can affect the sensitivity to the footshock. Therefore, a different pattern of *zif268* expression in the brain can be observed.

Another important difference between these two experiments may be due to non-equivalent handled/naive controls that were used. In the investigation of Malkani and Rosen, the rats were handled several minutes per day for five days, whereas in the report of Hall et al. such data were not included. As shown by Wallace et al. (1995), the level of *zif268* mRNA in the rats exposed for 2–4 days to individual housing with daily handling was increased in the visual cortex in comparison to the rats reared in similar conditions, but without handling. Consequently, if there had been differences in the amount of handling in the considered experimental procedures, the basal level of *zif268* to which all the results were compared could have been unequal in the two experiments. This could account for the observed differences in *zif268* expression within the CA3 field of the hippocampus (increased level of *zif268* in the novelty group, but not in the context-no-shock group, though these groups were behaviorally comparable).

Consequently, the differences in the experimental procedures can seriously affect the levels of *zif268*. The discussion of the two abovementioned experiments shows that the increment in *zif268* expression in the regions of the brain associated with contextual fear conditioning may result solely from the stimulation, irrespective of creating a context-footshock association. In addition, Weitemier and Ryabinin (2004) have recently shown the lack of differences between fear conditioned or naive C57BL/6J mice after the training phase in the Zif268 expression within the septum, amygdala, hippocampus and anterior cingulated cortex. In this study, either delay conditioning (the US delivered simultaneously with the CS) or trace conditioning (the US delivered after a temporal gap after the CS, more context dependent than delay conditioning) were applied.

9.2.2. Contextual and cued fear memory retrieval

The retrieval tests not only reactivate the memory, but they also establish new memories for the events occurring during the retrieval tests. The retrieval of the fear memory appears to involve an active, protein synthesis-sensitive component, as shown by Nader et al. (2000). During retrieval trials in contextual fear conditioning, an animal is placed in the context in the absence of shock. This procedure constitutes extinction trials, which result in decreasing of fear evoked by the context, because the animal learns that the context no longer predicts the shock. It has been

demonstrated that the formerly established association remains strong, though the performance in the presence of the extinguished stimulus is decreased (Bouton, 1993). Therefore, the extinction seems to be an active learning process that suppresses rather than removes the traces of former learning. Examination of the gene expression occurring during this process might shed some light on the molecular mechanisms of learning.

Hall et al. (2001) investigated *zif268* expression in the hippocampus and the amygdala during contextual and cued fear memory retrieval. They found a selective increase in *zif268* expression in the hippocampal CA1 neurons as a result of recalling contextual memories three days after the training. However, Malkani and Rosen (2000b) did not record any changes in *zif268* mRNA level in the hippocampus, the neocortex and the amygdala 24 h after the training. Moreover, Weitemier and Ryabinin (2004), who applied either delay fear conditioning (the US delivered simultaneously with the CS) or trace conditioning (the US delivered after a temporal gap after the CS), demonstrated the lack of differences in the Zif268 expression within the CA1 in C57BL/6J mice after reexposure to contextual *versus* tone cues in delay and trace fear conditioning. This dissimilarity may be due to different behavioral procedures used. For instance, in the procedure applied by Hall et al. (2001), rats were habituated to the experimental chambers for three days and the training was rather long — 25 min with five footshocks either paired or not with the clickers. Thus, the rats, including the control ones, were exposed to much more stimuli than during the conditioning in the investigation of Malkani and Rosen (2000b). There were also different backgrounds — familiar or relatively unknown environment, respectively. Although the behavioral results were similar — rats displayed freezing during the retention test — the molecular events related to learning could be different. Interestingly, the patterns of *zif268* expression could differ also in the time course, e.g., Hall et al. (2001) found that in the CA1 field of the hippocampus, the level of *zif268* mRNA was increased in response to a re-exposure to the training context 24 h but not 28 days after training, though in both cases the freezing response could be observed. Recently, Frankland et al. (2004) have extended this observation to show that Zif268 protein was activated within CA1 after re-exposure to the training chamber at day 1 but not day 36 after the training. On the other hand, Zif268 expression in various cortical areas was at higher levels at day 36 than day 1. Further analysis of these cortical regions with a functional inactivation approach has revealed that the anterior cingulate cortex (ACC) plays a critical role in remote memory for contextual fear conditioning in contrast to the prelimbic cortex. Interestingly, Zif268 expression levels were higher in the latter, on the contrary to another IEG protein product, c-Fos, whose expression was much more pronounced in the ACC. It is of note that c-Fos appears to be closely related in its expression pattern to learning and memory formation (Kaczmarek, 1993, 2002).

Thomas et al. (2002) performed further analysis of *zif268* expression in the brains of the animals in which Hall et al. (2001) reported changes in *zif268* expression in the amygdala and hippocampus during the recall of cued and contextual fear memories. Thomas et al. examined the nucleus accumbens core and shell, the anterior cingulate (Cg1) and prelimbic cortices of the medial prefrontal cortex and the orbitofrontal cortex. They showed that the retrieval of the contextual and the cued fear memory differentially increased *zif268* expression in distinct subregions of the nucleus accumbens and frontal cortex. The increase in *zif268* expression in the core of the nucleus accumbens was observed following the retrieval of both contextual and cued fear associations. However, the increments observed in the nucleus accumbens shell and the Cg1 region of the anterior cingulate cortex were selective for the recall of contextual fear memory.

It is of note that certain disparities in *zif268* expression were observed between two repetitions of the experiment described by Hall et al. (2001).[5] The level of *zif268* expression in the CA1 field of the hippocampus in the contextual fear group differed significantly from all other groups that played a control role in the first part of this investigation, but in the second part, in the cued fear group this level was not significantly different from both the contextual fear group and the control groups. This may reveal the influence of various, unidentified and uncontrolled aspects of the training situation on *zif268* expression. These inconsistent results regarding specific *zif268* expression might be another argument suggesting that it may be not directly connected to neuronal plasticity.

9.2.3. *Impaired contextual fear conditioning*

Diazepam (DZ) is a drug which exerts both anxiolytic effects during the post-shock period and amnesic effects during a retention test, when administered before fear conditioning. If Zif268 expression is directly connected to fear conditioning, the administration of DZ should block the increment in Zif268 expression in the brain structures involved in fear learning (e.g., in the lateral nucleus of the amygdala). Malkani and Rosen (2000a) addressed this issue by applying a behavioral schedule very similar to that in the experiment described above (Malkani and Rosen, 2000b). In the behavioral training, there were five groups described in Table 4. Malkani and Rosen (2000a) found that diazepam blocked the fear-conditioning-induced increment in *zif268* expression in the lateral nucleus of the amygdala. There were significant differences between the *cpDZ2.5* and the *cp* group but not between the *cpDZ2.5* and the *c* group. In the *cpDZ5* group, *zif268* expression was significantly lower than in the *c* group. Furthermore, *zif268* expression in the central nucleus of the amygdala in both the *cpDZ2.5* and *cpDZ5*

[5] There were two replications of the experiment in which *zif268* expression in the contextual fear group and the cued fear group, respectively, was compared to the control groups.

Table 4
The description of the groups used in the experiment of Malkani and Rosen (2000a); see Section 9.2 for details; LaDL — lateral nucleus of the amygdala, dorsal part, Ce — central nucleus of the amygdala

Group	Treatment		Dose of diazepam (mg/kg)	Relative expression levels of *zif268* mRNA	
	Context	Footshock		LaDL	Ce
c	+	–	–	++	++
cp	+	+	–	+++	++
cDZ	+	–	2.5		++++
cpDZ2.5	+	+	2.5	++	+++
cpDZ5	+	+	5.0	+	+++++

groups was increased in comparison to the *cp* and the *c* groups. However, *zif268* expression in the central nucleus in the *cDZ* group was much higher than in the *cpDZ2.5* group. Significantly decreased shock sensitivity and sedation were observed in the *cpDZ5* but not in the *cDZ* and *cpDZ2.5* groups.

It is known that the lateral nucleus of the amygdala is involved in encoding the emotional component of memories formed during fear conditioning (see Repa et al., 2001, and Radwanska et al., 2002). Therefore, one could argue for the role of Zif268 in fear conditioning, relying on the results quoted above. However, an alternative explanation should also be considered. The anxiolytic effects of diazepam are mediated through benzodiazepine binding sites on the $GABA_A$ receptor complex by enhancing GABA-induced synaptic inhibition (see File, 2001). Diazepam acting upon GABA receptor on glutamatergic pyramidal cells of the lateral nucleus of the amygdala could decrease their excitability, thereby making fear conditioning impossible and reducing *zif268* expression. Thus, the reduction in *zif268* expression would not be necessarily related to learning but simply to neuronal activity. In the central nucleus of the amygdala, the diazepam-induced increase in *zif268* expression is independent of fear conditioning, because it was also seen in the *cDZ* group. It may be caused by reduced activity of glutamatergic pyramidal cells of the lateral nucleus of the amygdala, which normally affect interneurons that, in turn, inhibit the GABA-ergic neurons in the central nucleus (Collins and Pare, 1999). Disinhibition of the latter ones may be a rationale for higher neuronal activity and higher *zif268* expression in this part of the amygdala. The footshock diminished the diazepam-induced increase in *zif268* expression in the central nucleus, probably through one of the pain pathways. Consequently, ambiguous interpretation of the results obtained by Malkani and Rosen does not produce unequivocal conclusions about the role of Zif268 in fear learning.

DL-2-amino-5-phosphonovalerate (APV), NMDA receptors antagonist, is known to block the acquisition of fear conditioning using auditory, visual or contextual cues as conditioned fear stimuli (Kim et al., 1991; Maren et al., 1996; Lee and Kim, 1998). Therefore, Malkani and Rosen (2001) inspected the influence of APV on *zif268* expression (cf. Section 2.3). In the behavioral training, there were four groups —(*c*) context (without APV), (*cp*) context paired with footshock (without APV), (*cAPV*) context (with APV) and (*cpAPV*) context paired with footshock (with APV). Prior to the experiment, longer time of handling was applied (two weeks), possible consequences of which are discussed in Section 9.2.1. The administration of APV blocked the increase in *zif268* expression associated with fear conditioning (*cpAPV* group) in the lateral nucleus and increased the expression in the central nucleus of the amygdala. The basal expression was defined as that in the *c* and *cAPV* groups. Effects of APV on the basal *zif286* expression were not observed in the lateral nucleus of the amygdala nor in other brain regions (the hippocampus and the neocortex). Post-shock freezing remained intact, whereas fear-conditioned freezing during the retention test was abolished. These data are quite impressive from the perspective of *zif268* involvement in learning, but it cannot be excluded that the changes in *zif268* expression are simply the effect of modifications in neuronal activity. They may be due to reduced response of glutamatergic pyramidal cells of the lateral nucleus of the amygdala to environmental stimuli (this effect was discussed above).

Contextual fear conditioning can also be impaired by a mechanical injury of the brain. Abrous et al. (1999) examined changes in Zif268 expression 3 h after low-pressure fluid percussion-induced brain injury of the right parietal cortex, which resulted in a deficit in the conditioned freezing response to context. They observed an increase in Zif268 expression within the cingulate, piriform, perirhinal and enthorinal cortices, as well as in the CA1 and CA3 areas of the hippocampus, in the dentate gyrus, in the hilus and in the amygdala complex. The observed changes were limited to the hemisphere ipsilateral to the side of injury. The affected regions are known to be sensitive to excitatory amino acids and there is a rapid rise in brain extracellular glutamate concentration which appears after trauma (Hovda et al., 1995). Furthermore, the effects of trauma on conditioned freezing response can be blocked by MK-801 (Hogg et al., 1998). This suggests that observed effects on Zif268 expression may result from rapid increase in neuronal activity in these regions.

Summing up, there are a number of results regarding *zif268* expression during fear conditioning which may suggest a possible vital role of this gene in learning

processes. However, these results can also be well explained by other hypotheses discussed in Section 3.

9.3. Learning of a visual task

Another paradigm used to correlate a behavior with Zif268 expression employed learning of the visual task. Okuno and Miyashita (1996) examined Zif268 expression in the temporal lobe of the macaque monkey, using an interesting model of declarative memory[6] formation. The monkeys were trained to learn a visual pair-association task or a visual discrimination (that served as a control) with the use of a set of computer-generated pictures.

Declarative memory has been well described in a number of neuropsychological studies of amnesic patients. These studies revealed that integrity of the medial temporal lobe and the neocortex is required for declarative memory formation. Okuno and Miyashita observed Zif268-immunopositive neurons accumulated in patches in IV, II/III and V/VI layers of the inferior temporal gyrus (the perirhinal cortex) during learning of the pair-association task. On the contrary, learning of the visual discrimination evoked Zif268 expression mainly in layer IV of the inferior temporal gyrus and did not show a patchy distribution. Analogous results were obtained by Tokuyama et al. (2002), who used split-brain monkeys to inspect the amount of *zif268* and the distribution pattern during learning of the visual pair-association. This technique provided intra-animal comparison, which allowed eliminating animal-to-animal variation in gene expression.

It is known that ablations of the perirhinal/entorhinal cortex produce impairment in learning of the visual pair-association task (Murray et al., 1993), but not in visual pattern discrimination (Suzuki et al., 1993; Zola-Morgan et al., 1993). Therefore, the results described above appear to support the hypothesis about the role of Zif268 in learning processes. However, the alternative hypothesis that Zif268 expression is evoked by neuronal activity cannot be excluded, since learning may cause a very intense neuronal activity in the structures engaged in the formation of associations.

9.4. Spatial learning

The expression of *zif268* following spatial learning was examined mainly in the hippocampus. In most studies, the *Morris water task* and its modifications were employed. The Morris water escape task (the Morris water maze) utilizes a round pool of water in which a platform is submerged beneath the surface. When placed in the maze, the animal is expected to find the platform. The animal's learning can be assessed by the time it takes to find the platform over a number of trials and, once the platform has been removed, the percentage of time spent in the quadrant in which the platform was previously located. In this task, the animal must learn the spatial position of the invisible platform in the surrounding environment. As a control task, the cued (non-spatial) task is employed, in which the platform is visible to the animal.

9.4.1. Studies on zif268 expression pattern

There are a number of studies, in which the existence of the correlation between either the basal or the post-activation expression of *zif268* products and the performance in tasks involving spatial learning has been investigated. Guzowski et al. (2001) studied *zif268* expression in the dorsal hippocampus following spatial and non-spatial water maze tasks. An increase in *zif268* expression was observed after a single session of the spatial (hippocampal-dependent) and the non-spatial (hippocampal-independent) task, relative to the home-caged control. There were no differences in the level of *zif268* expression between rats trained in the spatial and cued water tasks. Guzowski et al. (2001) additionally used a spatial reversal task, in which rats were trained in the spatial water maze for three days with the submerged platform located in a fixed position. On each day, the rats received two training sessions consisting of six trials. On the experimental day, one group was given the training identical to the previous ones (the seven-session group). The second group was trained with the submerged platform moved to a new location in the pool (the reversed group). There was also another group that received only one session of the spatial water task (the one-session group). The level of *zif268* expression was examined in the dorsal hippocampus and the enthorinal and primary visual cortices. The seven-session group performed the familiar task at an asymptotic level of performance. The reversal group learned the new location of the platform in one trial and performed the task at the level similar to the seven-session group for the remaining trials. The one-session group became skilled at this task very rapidly and performed at the level of the other two groups by the fifth and sixth trials. In the dorsal hippocampus, the level of *zif268* expression was the highest in the one-session group. Surprisingly, *zif268* expression did not differ significantly between the seven-session and the reversal groups, contrarily to what can be expected after the introduction of novelty. In the lateral enthorinal cortex and the primary visual cortex, the level of *zif268* did not differ significantly in the trained groups. These results are contradictory to those obtained by Wisden et al. (1990) who did not observe any changes in *zif268* expression following the training in a water maze.

However, it should be noted that the method of RNA detection (RNase protection assay) used by Guzowski et al. (2001) is more quantitative and sensitive than in situ hybridization — the approach employed by Wisden et al. (1990). The lack of an increase in *zif268* expression in the reverse group may be explained by the relatively small amount of new learning required. As Guzowski et al. point out, the rats were already proficient at the task, swam more

[6] The long-term memory about facts and events.

direct paths and were less attentive to the surrounding environment than in the first stages of learning. They just had to learn a new spatial location of the platform.

The relationship between the hippocampal *zif268* expression and the rate of learning was also examined (Guzowski et al., 2001). During the training session, which consisted of six trials, the performance of the rats had been improving in the first three trials and stabilized in the last three ones. Therefore, the performance in the last three trials provided a reliable measure of the ability of spatial learning for individual rats. In the spatial task, the level of *zif268* mRNA expression was negatively correlated with the mean escape latency (the measure of learning) in the last three trials of the training session, but this correlation did not reach statistical significance. There was no such a correlation in the cued task.

Yau et al. (1996) examined *zif268* expression in young (eight months) and aged (22–24 months) rats subjected to the training in the water maze with extra-maze cues. The authors demonstrated a positive correlation between spatial learning and the constitutive expression of *zif268* in the CA1 pyramidal neurons. A significant decrease in *zif268* expression in this hippocampal area with age was observed. The chronic antidepressant treatment (with amitriptyline), which is associated with improved spatial memory in young, but not aged rats (Yau et al., 1995), did not affect *zif268* expression in the aged rats, compared to equivalently treated young rats. A significant decrement in *zif268* expression with age was also observed in the CA2 area of the hippocampus and in the layer IV of the neocortex. However, no significant correlations between *zif268* expression in these regions and spatial memory were found. On the other hand, housing of rats in an enriched environment for 30 days, in contrast to an isolated one, has been shown to improve water maze spatial learning (Mohammed et al., 1990) and increased the level of *zif268* expression in the CA2 area of the hippocampus (Olsson et al., 1994).

The discrepancies between the three correlative approaches may be due to examination of the basal (Yau et al. and Olsson et al.) or induced (Guzowski et al.) *zif268* expression and/or different behavioral conditions, which were used. However, it should be noted that the correlative approach assumes implicitly the linear dependence between *zif268* expression and learning efficacy, which does not have to hold true (for discussion see Section 8.1).

Fordyce et al. (1994) examined the effect of a physical activity regimen (previously shown to improve spatial learning performance) on *zif268* expression in the hippocampus. They used C57BL/6 and DBA/2 mice, the two strains that differed in spatial learning performance. C57BL/6 and DBA/2 mice were subjected to acute (a single 60-min running bout) or chronic (forty 60-min running bouts for eight weeks) physical activity with a subsequent analysis of *zif268* expression. The chronic running is known to improve a spatial learning performance in the Morris task in both C57BL/6 and DBA/2 mice (Fordyce and Wehner, 1993). During the last two weeks of the period of the chronic physical activity, the mice were tested in the Morris water maze task for six days. The control mice remained sedentary in their home cages, except for daily handling. The chronic-run mice and their controls were killed 24 h after the last running. The acute-run mice and their controls were sacrificed immediately, 1, 6 and 24 h after the exercise bout. The basal expression of *zif268* was determined in both strains.

DBA/2 control mice had poor hippocampal-specific learning performance compared to C57BL/6 mice and displayed lower basal levels of *zif268* in the CA1 and CA3 areas of the hippocampus and the overlying cortex. The acute physical activity increased *zif268* levels in the CA1 and CA3 regions of the hippocampus and the overlying cortex of both DBA/2 and C57BL/6 mice. The expression of *zif268* was elevated immediately after the running bout, remained increased for 1 h and decreased to the basal level after 6 h. However, the chronic physical activity suppressed the basal expression of *zif268* in C57BL/6 mice in the CA1 region of the hippocampus and the overlying cortex with respect to the control group. In DBA/2 mice, the level of *zif268* expression after the chronic running was similar to that in control DBA/2 mice.

The differences in learning between the two strains of mice may result from the possible relation between Zif268 expression and the efficacy in learning. However, the chronic-running, which improved the performance of the spatial learning, led to a decrease in the basal level of *zif268* expression in better learners, C57BL/6 mice. The suppression of the basal expression of *zif268* through physical activity may be due to enhanced learning performance also observed in other investigations (e.g., Nikolaev et al., 1992). However, the reduction of the basal level of *zif268* expression associated with the better performance appears contradictory to the hypothesis that the level of the expressed gene is related to the efficacy of learning. In summary, none of the works described above brought a clear conclusion about involvement of Zif268 in learning.

9.4.2. zif268, LTP and spatial learning

Overlapping neural circuits in the hippocampus are exploited in LTP and spatial learning (McNaughton et al., 1986; Castro et al., 1989). Therefore, it would be interesting to discuss whether Zif268 expression caused by one of these procedures affects the other one. Barnes et al. (1994) observed that LTP-inducing, bilateral perforant path stimulation did not saturate capacity of LTP induction in the hippocampus. Such a stimulation produced no deficit in learning the Morris water task but led to a significant deficit in the reversal of a well-learned spatial response on the so-called Barnes circular platform task in the same animals (Barnes et al., 1994). The Barnes circular platform was equipped with 18 equally spaced holes around the periphery. One hole led to a dark escape box, which was fixed with respect to the distal environmental cues. The bright

illumination served as a motivator for escaping to the dark box.

In the same analysis, Barnes et al. (1994) demonstrated that MECS, which led to occlusion of LTP produced by discrete high-frequency stimulation, i.e., provided saturation of LTP at hippocampal synapses, led to deficits in spatial learning in the Morris water task, as well as to a robust *zif268* activation throughout the entire hippocampus. On the other hand, there was no impairment in the cued version of the water task after MECS treatment. The research by Barnes et al. demonstrated that MECS and spatial learning indeed engage overlapping neural circuits in the hippocampus and result in *zif268* expression.

A completely reversed experimental scheme was used by Richter-Levin et al. (1998). The authors compared changes in *zif268* expression following the unilateral induction of LTP in rats previously trained in a water maze and in behaviorally naive animals. The rats in the trained group were subjected to the training in the spatial water maze for five days (six trials per day). The naive rats were not introduced to the water maze. One hour after the last training session (for trained rats) LTP was induced in all animals. The level of *zif268* expression was increased in the potentiated dentate gyrus of naive and trained animals 3 h after the induction of LTP, but there was no difference between the groups.

These results demonstrated dissociation between LTP and spatial learning with respect to expression of *zif268*, which appears contradictory to the work of Barnes et al. (1994) discussed earlier. However, the stimulation with MECS might have been so strong that *zif268* was expressed in the entire hippocampus, thereby constituting a very high background which did not allow determining specific neuronal circuits responsible for spatial learning. Thus, it is possible that LTP and spatial learning are dissociable with respect to expression of Zif268. However, even if a link between LTP, spatial learning and Zif268 expression exists, the observation of *zif268* expression in the structures responsible for spatial learning does not reveal the function of this gene in the cell.

9.4.3. Functional studies

Spatial learning was also examined in the mutant mice with the targeted disruption of *zif268*. Jones et al. (2001) compared the learning of the knock-outs, the heterozygotes and the wild-type mice in the spatial water maze test, which requires long-term memory. After a one-day habituation, the mice were given a massive training (five blocks of five trials, with inter-block interval 15 min) or a distributed training (two blocks of four trials, with inter-block interval 5 h, for 10 days). In the massive-trained group, a 60-s probe trial was given 48 h after the training, while in the distributed-trained group a 90-s probe trial was applied eight days after the training. In the massive-trained group, the performance of the mutant mice and the heterozygous mice was markedly impaired in comparison to the performance of the normal ones. However, when the extended and distributed training in the water maze was applied, all mice showed normal acquisition and long-term recall. Short-term memory remained intact in mutants (it was also examined by testing spontaneous alternation in a T-maze).

Jones et al. (2001) also investigated the Zif268 deficient mice in a hippocampus-dependent novel object recognition test. They allowed mice exploring two objects for 20 min and then, after a 10-min or 24-h delay, one of the familiar objects was replaced with a novel object and the time spent exploring the novel and the familiar objects was measured. After the 10-min delay, when only the short-term memory was required, all genotypes explored the novel object significantly longer than the familiar one. After the 24-h delay, when the long-term memory was engaged, the normal and heterozygous mice still explored the novel object for longer time than the familiar one, in contrast to the homozygous mutants.

Bozon et al. (2002) extended the studies on a task-dependent *zif268* dosage effect in the abovementioned heterozygous mice. These mice showed about 50% reduction in *zif268* expression in comparison to the wild-type mice. Bozon and associates modified the object recognition task in order to distinguish between the spatial and non-spatial component in the same type of learning. They exposed (in two 10-min sessions) the wild-type, heterozygous and mutant mice to three objects, with a cue card placed on one of the sidewalls of the open field. The long-term memory was tested 24 h later in a 10-min session with one of the familiar objects moved to a new location in the arena (the place-change task). On the third day, the mice were given a training session with three new objects and the remaining conditions identical to those used on the first day. On the following day, the mice were tested in a 10-min session with one of the yet familiar objects replaced by a novel object (the object-change task). All mice were tested three times with new arrays of objects each time and with a few days of rest between testing sets. The mutant mice did not express long-term memory of the objects, in contrast to both wild type and heterozygous mice. However, after changing the spatial configuration of the objects the heterozygous mice, as well as the mutant mice, exhibited a severe deficit in the long-term memory in contrast to the wild-type mice.

Because of the impaired acquisition and the severe deficit in long-term spatial memory of the mutant mice (Jones et al., 2001) during massive training, one could believe that Zif268 is required either for memory consolidation or retrieval. However, there are two interpretations of the fact that the mutant mice managed to overcome the deficits in the extended and distributed training. Either this kind of training recruited other unidentified signaling pathways that allow information entering long-term memory store or Zif268 is not necessary for plasticity of the neuronal cell, but for maintaining or replenishing of some aspects of cell homeostasis, also required for the creation of plastic

changes. In this case, other signaling pathways which re-establish homeostasis would be activated. Therefore, the maintaining and replenishment hypotheses cannot be excluded. Since the impairment caused by deficiency in *zif268* expression is restrained to the spatial version of the object recognition task (Bozon et al., 2002), it should be noted that the spatial and non-spatial versions of the object recognition task engage different brain structures; e.g., Mumby et al. (2002) observed that the hippocampal damage impaired memory for contextual and spatial aspects of experience, whereas memory for objects was left intact. The efficacy of compensating processes may vary between different brain structures, thereby accounting for the observed differences.

The augmentation of *zif268* expression following spatial learning was observed in almost all the studies described in Sections 9.4.1–9.4.3. However, neither of them yields an insight into the mechanisms underlying Zif268 action in the cell. The attempts to correlate the level of *zif268* expression and learning efficacy often lead to ambiguous results. Furthermore, the long-term spatial memory in Zif268 deficient mice is impaired, but the molecular mechanisms subserving this profound behavioral effect are still unknown.

9.5. Other learning paradigms resulting in zif268 expression

There exist analyses in which *zif268* expression was observed in learning paradigms different from those discussed above. In the following, we shall briefly describe the results of these studies.

The *conditioned taste aversion* is a test, which involves the long-term memory. In this test, learning is achieved in a single trial. The water-deprived animals are taught to associate a novel taste of sucrose or saccharin with the malaise caused by lithium chloride injected after the onset of drinking of sweetened water. Lamprecht and Dudai (1995) detected elevated *zif268* expression in the nucleus of the solitary tract, the parabrachial nucleus, the hypothalamic paraventricular nucleus and the central nucleus of the amygdala, following either the conditioned taste aversion training or the lithium chloride injection itself. Jones et al. (2001) examined the conditioned taste aversion in the *zif268* deficient mice. Twenty-four and 48 h after the training of the conditioned taste aversion the mice were to choose between water and sucrose solution. The normal mice avoided drinking the sucrose solution at both points of time, but neither heterozygous nor homozygous mutants exhibited significant taste aversion.

The *social transmission of food preference* test is a one-trial, olfactory discrimination task that allows examination of learning both immediately (short-term memory) and after some delay (long-term memory). This test measures the preference of rodents for novel food that has recently been smelled on the breath of another animal. Jones et al. (2001) investigated behavior of mice with a targeted disruption of *zif268* in this test. Food-deprived demonstrator mice were allowed eating cocoa- or coriander-scented food, and then they interacted with observer mice. Subsequently, the observer mice were to choose between cocoa- and coriander-scented food 30 s and 24 h after the interaction. Thirty seconds after the interaction, all genotypes showed the preference of food they had previously smelled on the breath of the demonstrator mouse. However, 24 h later, only the normal mice showed preference for the demonstrated food. No expression data on *zif268* products in this context have been reported.

During 4 h following mating, female mice form *memory of the pheromones* of the males with which they mated. The accessory olfactory bulb has been proposed as the site of synaptic plasticity underlying this type of memory (Brennan et al., 1990). Brennan et al. (1992) observed that the level of *zif268* expression increased in the granule and mitral cells of the accessory olfactory bulb during the period of memory formation. The increase of *zif268* expression required association of mating and pheromonal exposure. Furthermore, similar increment in *zif268* expression in the accessory olfactory bulb was noted after the infusion of bicuculline (GABA receptor blocker) in the absence of mating. This procedure has been shown to result in the formation of nonspecific memory for male pheromones (Brennan and Keverne, 1989). Brennan et al. (1999) also observed an increase in Zif268 expression in the olfactory accessory bulb of female mice in response to putative pheromonal constituents. These results support association between the expression of *zif268* in the accessory olfactory bulb and the formation of an olfactory memory for male pheromones in female mice. However, Polston and Erskine (1995) reported that mating stimulation in female rats is associated with activation of Zif268 in the medial amygdala, preoptic area, bed nucleus of stria terminalis and ventromedial and paraventricular nuclei of the hypothalamus, but not in the accessory olfactory bulb. These results require further investigation.

10. Summary

Struhl (1991) estimated that there are at least hundreds of regulatory transcription factors. These transcription factors (TFs) could potentially regulate an enormous number of different late-response genes. One of these TFs is Zif268, whose expression has been described in many pharmacological, behavioral and electrophysiological paradigms. However, the physiological role of its protein in the neuron still remains elusive.

Most of our present knowledge regarding *zif268* induction is based on correlating expression patterns with various types of stimulation. Extensive review of the literature on this topic suggests that there is a close link between *zif268* mRNA and protein expression with neuronal activity. However, this correlation has been observed in

studies on brain structures that are usually implicated in neuronal plasticity as well. It is conspicuous that very little is known about Zif268 activation in, e.g., thalamus, known to be also engaged in intense neuronal activity during processing of sensory information. Hence, it seems that plasticity-linked neuronal activity is particularly effective in driving expression of Zif268.

At present, the correlative approach allows for drawing the most credible suggestions about Zif268 functions. However, in the light of the possible influence of various inputs, e.g., sensory information processing, arousal, motivation, emotion, stress responses, etc., careful scrutiny of various aspects of patterns of gene expression in a context of learning has to be applied. Such a scrutiny seems to be a necessary step before any learning-related roles could be ascribed to Zif268.

The correlative method does not allow distinguishing between learning and the processes engaged in the homeostatic maintenance of the cell or, especially, in the replenishment (see Section 3). Therefore, it is impossible to conclude about direct or indirect engagement of Zif268 in neuronal plasticity with the use of this approach. The fact that Zif268 recognition element was found in genes coding for such neuronal proteins as synapsins and glutamate dehydrogenase underscores the role of this transcription factors in critical neuronal functions. Identifying of further Zif268-regulated genes should be an important avenue for the following investigations. Recent applications of knock-out mice have also contributed to our knowledge about Zif268 in the brain. However, this approach cannot exclude the possibility that observed learning deficits in *zif268* KO animals results from, e.g., disturbance of basic metabolic functions that is revealed upon strong neuronal stimulation occurring during behavioral training. Thus, ultimately, the full description of Zif268 function in the brain cells will require combination of all the aforementioned approaches as well as use of new transgenic methods allowing for inducible down- and upregulation of the gene.

References

Abraham, W.C., Otani, S., 1991. Macromolecules and the maintenance of long-term potentiation. In: Morrell, F. (Ed.), Kindling and Synaptic Plasticity. Birkhauser, Boston, pp. 92–109.

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.

Abraham, W.C., Logan, B., Greenwood, J.M., Dragunow, M., 2002. Induction and experience-dependent consolidation of stable long-term potentiation lasting months in the hippocampus. J. Neurosci. 22, 9626–9634.

Abrous, D.N., Rodriguez, J., le Moal, M., Moser, P.C., Barneoud, P., 1999. Effects of mild traumatic brain injury on immunoreactivity for the inducible transcription factors c-Fos, c-Jun, JunB, and Krox-24 in cerebral regions associated with conditioned fear responding. Brain Res. 826, 181–192.

Ackerman, S.L., Minden, A.G., Williams, G.T., Bobonis, C., Yeung, C.Y., 1991. Functional significance of an overlapping consensus binding motif for Sp1 and Zif268 in the murine adenosine deaminase gene promoter. Proc. Natl. Acad. Sci. U.S.A. 88, 7523–7527.

Aicher, W.K., Sakamoto, K.M., Hack, A., Eibel, H., 1999. Analysis of functional elements in the human Egr-1 gene promoter. Rheumatol. Int. 18, 207–214.

Alexandropoulos, K., Qureshi, S.A., Bruder, J.T., Rapp, U., Foster, D.A., 1992. The induction of Egr-1 expression by v-Fps is via a protein kinase C- independent intracellular signal that is sequentially dependent upon HaRas and Raf-1. Cell Growth Differ. 3, 731–737.

Almendral, J.M., Sommer, D., Macdonald-Bravo, H., Burckhardt, J., Perera, J., Bravo, R., 1988. Complexity of the early genetic response to growth factors in mouse fibroblasts. Mol. Cell Biol. 8, 2140–2148.

Arenander, A.T., Lim, R.W., Varnum, B.C., Cole, R., de Vellis, J., Herschman, H.R., 1989. TIS gene expression in cultured rat astrocytes: multiple pathways of induction by mitogens. J. Neurosci. Res. 23, 257–265.

Barnes, C.A., Jung, M.W., McNaughton, B.L., Korol, D.L., Andreasson, K., Worley, P.F., 1994. LTP saturation and spatial learning disruption: effects of task variables and saturation levels. J. Neurosci. 14, 5793–5806.

Beckmann, A.M., Wilce, P.A., 1997. Egr transcription factors in the nervous system. Neurochem. Int. 31, 477–510 (discussion 517-6).

Beckmann, A.M., Davidson, M.S., Goodenough, S., Wilce, P.A., 1997. Differential expression of Egr-1-like DNA-binding activities in the naive rat brain and after excitatory stimulation. J. Neurochem. 69, 2227–2237.

Berger, P., Kozlov, S.V., Cinelli, P., Kruger, S.R., Vogt, L., Sonderegger, P., 1999. Neuronal depolarization enhances the transcription of the neuronal serine protease inhibitor neuroserpin. Mol. Cell Neurosci. 14, 455–467.

Bhat, R.V., Baraban, J.M., 1992. High basal expression of zif268 in cortex is dependent on intact noradrenergic system. Eur. J. Pharmacol. 227, 447–448.

Bisler, S., Schleicher, A., Gass, P., Stehle, J.H., Zilles, K., Staiger, J.F., 2002. Expression of c-Fos, ICER, Krox-24 and JunB in the whisker-to-barrel pathway of rats: time course of induction upon whisker stimulation by tactile exploration of an enriched environment. J. Chem. Neuroanat. 23, 187–198.

Bliss, T.V., Collingridge, G.L., 1993. A synaptic model of memory: long-term potentiation in the hippocampus. Nature 361, 31–39.

Bliss, T.V., 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. 232, 331–356.

Bouton, M.E., 1993. Context, time, and memory retrieval in the interference paradigms of Pavlovian learning. Psychol. Bull. 114, 80–99.

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.

Bozon, B., Davis, S., Laroche, S., 2003. A requirement for the immediate early gene zif268 in reconsolidation of recognition memory after retrieval. Neuron 40, 695–701.

Brennan, P.A., Keverne, E.B., 1989. Impairment of olfactory memory by local infusions of non-selective excitatory amino acid receptor antagonists into the accessory olfactory bulb. Neuroscience 33, 463–468.

Brennan, P.A., Kaba, H., Keverne, E.B., 1990. Olfactory recognition: a simple memory system. Science 250, 1223–1226.

Brennan, P.A., Hancock, D., Keverne, E.B., 1992. The expression of the immediate-early genes c-fos, egr-1 and c-jun in the accessory olfactory bulb during the formation of an olfactory memory in mice. Neuroscience 49, 277–284.

Brennan, P.A., Schellinck, H.M., Keverne, E.B., 1999. Patterns of expression of the immediate-early gene egr-1 in the accessory olfactory bulb of female mice exposed to pheromonal constituents of male urine. Neuroscience 90, 1463–1470.

Burazin, T.C., Gundlach, A.L., 1996. Rapid and transient increases in cellular immediate early gene and neuropeptide mRNAs in cortical and limbic areas after amygdaloid kindling seizures in the rat. Epilepsy Res. 26, 281–293.

Cahill, L., Weinberger, N.M., Roozendaal, B., McGaugh, J.L., 1999. Is the amygdala a locus of ''conditioned fear''? Some questions and caveats. Neuron 23, 227–228.

Cao, X.M., Koski, R.A., Gashler, A., McKiernan, M., Morris, C.F., Gaffney, R., Hay, R.V., Sukhatme, V.P., 1990. Identification and characterization of the Egr-1 gene product, a DNA- binding zinc finger protein induced by differentiation and growth signals. Mol. Cell Biol. 10, 1931–1939.

Cao, X., Mahendran, R., Guy, G.R., Tan, Y.H., 1992. Protein phosphatase inhibitors induce the sustained expression of the Egr-1 gene and the hyperphosphorylation of its gene product. J. Biol. Chem. 267, 12991–12997.

Carrasco-Serrano, C., Viniegra, S., Ballesta, J.J., Criado, M., 2000. Phorbol ester activation of the neuronal nicotinic acetylcholine receptor alpha7 subunit gene: involvement of transcription factor Egr-1. J. Neurochem. 74, 932–939.

Castro, C., Silbert, L., McNaughton, B., Barnes, C., 1989. Recovery of spatial learning deficits after decay of electrically induced synaptic enhancement in the hippocampus. Nature 342, 545–548.

Changelian, P.S., Feng, P., King, T.C., Milbrandt, J., 1989. Structure of the NGFI-A gene and detection of upstream sequences responsible for its transcriptional induction by nerve growth factor. Proc. Natl. Acad. Sci. U.S.A. 86, 377–381.

Chapman, N.R., Perkins, N.D., 2000. Inhibition of the RelA(p65) NF-kappaB subunit by Egr-1. J. Biol. Chem. 275, 4719–4725.

Christy, B., Nathans, D., 1989a. DNA binding site of the growth factor-inducible protein Zif268. Proc. Natl. Acad. Sci. U.S.A. 86, 8737–8741.

Christy, B., Nathans, D., 1989b. Functional serum response elements upstream of the growth factor- inducible gene zif268. Mol. Cell Biol. 9, 4889–4895.

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. U.S.A. 85, 7857–7861.

Cirelli, C., Pompeiano, M., Tononi, G., 1996. Neuronal gene expression in the waking state: a role for the locus coeruleus. Science 274, 1211–1215.

Clayton, D., 2000. The genomic action potential. Neurobiol. Learn. Mem. 74, 185–216.

Cogswell, P.C., Mayo, M.W., Baldwin Jr., A.S., 1997. Involvement of Egr-1/RelA synergy in distinguishing T cell activation from tumor necrosis factor-alpha-induced NF-kappa B1 transcription. J. Exp. Med. 185, 491–497.

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.

Cole, A.J., Abu-Shakra, S., Saffen, D.W., Baraban, J.M., Worley, P.F., 1990. Rapid rise in transcription factor mRNAs in rat brain after electroshock-induced seizures. J. Neurochem. 55, 1920–1927.

Cole, A.J., Bhat, R.V., Patt, C., Worley, P.F., Baraban, J.M., 1992. D1 dopamine receptor activation of multiple transcription factor genes in rat striatum. J. Neurochem. 58, 1420–1426.

Collins, D.R., Pare, D., 1999. Reciprocal changes in the firing probability of lateral and central medial amygdala neurons. J. Neurosci. 19, 836–844.

Condorelli, D.F., Dell'Albani, P., Amico, C., Lukasiuk, K., Kaczmarek, L., Giuffrida-Stella, A.M., 1994. Glutamate receptor-driven activation of transcription factors in primary neuronal cultures. Neurochem. Res. 19, 489–499.

Crino, P., Khodakhah, K., Becker, K., Ginsberg, S., Hemby, S., Eberwine, J., 1998. Presence and phosphorylation of transcription factors in developing dendrites. Proc. Natl. Acad. Sci. U.S.A. 95, 2313–2318.

Cullinan, W.E., Herman, J.P., Battaglia, D.F., Akil, H., Watson, S.J., 1995. Pattern and time course of immediate early gene expression in rat brain following acute stress. Neuroscience 64, 477–505.

D'Arcangelo, G., Halegoua, S., 1993. A branched signalling pathway for nerve growth factor is revealed by Src-, Ras-, and Raf-mediated gene inductions. Mol. Cell Biol. 13, 3146–3155.

Das, A.T., Arnberg, A.C., Malingre, H., Moerer, P., Charles, R., Moorman, A.F., Lamers, W.H., 1993. Isolation and characterization of the rat gene encoding glutamate dehydrogenase. Eur. J. Biochem. 211, 795–803.

Davis, S., Bozon, B., Laroche, S., 2003. How necessary is the activation of the immediate early gene zif268 in synaptic plasticity and learning? Behav. Brain Res. 142, 17–30.

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.

de Groot, R.P., Karperien, M., Pals, C., Kruijer, W., 1991. Characterization of the mouse junD promoter—high basal level activity due to an octamer motif. EMBO J. 10, 2523–2532.

DeFranco, C., Damon, D.H., Endoh, M., Wagner, J.A., 1993. Nerve growth factor induces transcription of NGFIA through complex regulatory elements that are also sensitive to serum and phorbol 12-myristate 13-acetate. Mol. Endocrinol. 7, 365–379.

Desjardins, S., Mayo, W., Vallee, M., Hancock, D., Le Moal, M., Simon, H., Abrous, D.N., 1997. Effect of aging on the basal expression of c-Fos, c-Jun, and Egr-1 proteins in the hippocampus. Neurobiol. Aging 18, 37–44.

Dragunow, M., Yamada, N., Bilkey, D.K., Lawlor, P., 1992. Induction of immediate-early gene proteins in dentate granule cells and somatostatin interneurons after hippocampal seizures. Brain Res. Mol. Brain Res. 13, 119–126.

Dragunow, M., Tse, C., Glass, M., Lawlor, P., 1994. c-fos antisense reduces expression of Krox 24 in rat caudate and neocortex. Cell Mol. Neurobiol. 14, 395–405.

Ferhat, L., Khrestchatisky, M., Roisin, M.P., Barbin, G., 1993. Basic fibroblast growth factor-induced increase in zif/268 and c-fos mRNA levels is Ca^{2+} dependent in primary cultures of hippocampal neurons. J. Neurochem. 61, 1105–1112.

File, S.E., 2001. The amygdala: anxiety and benzodiazepines. In: Aggleton, J.P. (Ed.), The Amygdala. Oxford University Press, New York, pp. 195–206.

Filipkowski, R.K., 2000. Inducing gene expression in barrel cortex—focus on immediate early genes. Acta Neurobiol. Exp. 60, 411–418.

Filipkowski, R.K., Rydz, M., Kaczmarek, L., 2001. Expression of c-Fos, Fos B, Jun B, and Zif268 transcription factor proteins in rat barrel cortex following apomorphine-evoked whisking behavior. Neuroscience 106, 679–688.

Fordyce, D.E., Wehner, J.M., 1993. Physical activity enhances spatial learning performance with an associated alteration in hippocampal protein kinase C activity in C57BL/6 and DBA/2 mice. Brain Res. 619, 111–119.

Fordyce, D.E., Bhat, R.V., Baraban, J.M., Wehner, J.M., 1994. Genetic and activity-dependent regulation of zif268 expression: association with spatial learning. Hippocampus 4, 559–568.

Frankland, P.W., Bontempi, B., Talton, L.E., Kaczmarek, L., Silva, A.J., 2004. The involvement of the anterior cingulate cortex in remote contextual fear memory. Science 304, 881–883.

Frankland, P.W., Cestari, V., Filipkowski, R.K., McDonald, R.J., Silva, A.J., 1998. The dorsal hippocampus is essential for context discrimination but not for contextual conditioning. Behav. Neurosci. 112, 863–874.

French, P.J., O'Connor, V., Jones, M.W., Davis, S., Errington, M.L., Voss, K., Truchet, B., Wotjak, C., Stean, T., Doyere, V., Maroun, M., Laroche, S., Bliss, T.V., 2001. Subfield-specific immediate early gene expression associated with hippocampal long-term potentiation in vivo. Eur. J. Neurosci. 13, 968–976.

Fu, M., Zhu, X., Zhang, J., Liang, J., Lin, Y., Zhao, L., Ehrengruber, M.U., Chen, Y.E., 2003. Egr-1 target genes in human endothelial cells identified by microarray analysis. Gene 315, 33–41.

Gao, X.M., Hashimoto, T., Tamminga, C.A., 1998. Phencyclidine (PCP) and dizocilpine (MK801) exert time-dependent effects on the expression of immediate early genes in rat brain. Synapse 29, 14–28.

Gashler, A.L., Swaminathan, S., Sukhatme, V.P., 1993. A novel repression module, an extensive activation domain, and a bipartite nuclear loca-

lization signal defined in the immediate-early transcription factor Egr-1. Mol. Cell Biol. 13, 4556–4571.

Gass, P., Herdegen, T., Bravo, R., Kiessling, M., 1992. Induction of immediate early gene encoded proteins in the rat hippocampus after bicuculline-induced seizures: differential expression of KROX-24, FOS and JUN proteins. Neuroscience 48, 315–324.

Gass, P., Herdegen, T., Bravo, R., Kiessling, M., 1993a. Induction and suppression of immediate early genes in specific rat brain regions by the non-competitive *N*-methyl-D-aspartate receptor antagonist MK-801. Neuroscience 53, 749–758.

Gass, P., Herdegen, T., Bravo, R., Kiessling, M., 1993b. Spatiotemporal induction of immediate early genes in the rat brain after limbic seizures: effects of NMDA receptor antagonist MK-801. Eur. J. Neurosci. 5, 933–943.

Ge, R., Rhee, M., Malik, S., Karathanasis, S.K., 1994. Transcriptional repression of apolipoprotein AI gene expression by orphan receptor ARP-1. J. Biol. Chem. 269, 13185–13192.

Ghosh, A., Ginty, D.D., Bading, H., Greenberg, M.E., 1994. Calcium regulation of gene expression in neuronal cells. J. Neurobiol. 25, 294–303.

Ginty, D.D., Glowacka, D., Bader, D.S., Hidaka, H., Wagner, J.A., 1991. Induction of immediate early genes by Ca^{2+} influx requires cAMP-dependent protein kinase in PC12 cells. J. Biol. Chem. 266, 17454–17458.

Gius, D., Cao, X.M., Rauscher, F.J., 3rd, Cohen, D.R., Curran, T., Sukhatme, V.P., 1990. Transcriptional activation and repression by Fos are independent functions: the C terminus represses immediate-early gene expression via CArG elements. Mol. Cell Biol. 10, 4243–4255.

Goosens, K.A., Maren, S., 2001. Contextual and auditory fear conditioning are mediated by the lateral, basal, and central amygdaloid nuclei in rats. Learn. Mem. 8, 148–155.

Greenwood, J.M., Dragunow, M., 2002. Muscarinic receptor-mediated phosphorylation of cyclic AMP response element binding protein in human neuroblastoma cells. J. Neurochem. 82, 389–397.

Guzowski, J.F., 2002. Insights into immediate-early gene function in hippocampal memory consolidation using antisense oligonucleotide and fluorescent imaging approaches. Hippocampus 12, 86–104.

Guzowski, J.F., McNaughton, B.L., Barnes, C.A., Worley, P.F., 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. Nat. 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 the recall of contextual memories. J. Neurosci. 21, 2186–2193.

Hargreaves, E.L., Cain, D.P., 1992. Hyperactivity, hyper-reactivity, and sensorimotor deficits induced by low doses of the *N*-methyl-D-aspartate non-competitive channel blocker MK801. Behav. Brain Res. 47, 23–33.

Herdegen, T., Leah, J.D., 1998. Inducible and constitutive transcription factors in the mammalian nervous system: control of gene expression by Jun, Fos and Krox, and CREB/ATF proteins. Brain Res. Brain Res. Rev. 28, 370–490.

Herdegen, T., Walker, T., Leah, J.D., Bravo, R., Zimmermann, M., 1990. The KROX-24 protein, a new transcription regulating factor: expression in the rat central nervous system following afferent somatosensory stimulation. Neurosci. Lett. 120, 21–24.

Herdegen, T., Kovary, K., Leah, J., Bravo, R., 1991. Specific temporal and spatial distribution of JUN, FOS, and KROX-24 proteins in spinal neurons following noxious transsynaptic stimulation. J. Comp. Neurol. 313, 178–191.

Herdegen, T., Kovary, K., Buhl, A., Bravo, R., Zimmermann, M., Gass, P., 1995. Basal expression of the inducible transcription factors c-Jun, JunB, JunD, c-Fos, FosB, and Krox-24 in the adult rat brain. J. Comp. Neurol. 354, 39–56.

Hetman, M., Danysz, W., Kaczmarek, L., 1997. Increased expression of cathepsin D in retrosplenial cortex of MK-801- treated rats. Exp. Neurol. 147, 229–237.

Heynen, A.J., Bear, M.F., 2001. Long-term potentiation of thalamocortical transmission in the adult visual cortex in vivo. J. Neurosci. 21, 9801–9813.

Hogg, S., Sanger, D.J., Moser, P.C., 1998. Mild traumatic lesion of the right parietal cortex in the rat: characterisation of a conditioned freezing deficit and its reversal by dizocilpine. Behav. Brain Res. 93, 157–165.

Hovda, D.A., Lee, S.M., Smith, M.L., Von Stuck, S., Bergsneider, M., Kelly, D., Shalmon, E., Martin, N., Caron, M., Mazziotta, J., 1995. The neurochemical and metabolic cascade following brain injury: moving from animal models to man. J. Neurotrauma 12, 903–906.

Hughes, P., Lawlor, P., Dragunow, M., 1992. Basal expression of Fos, Fos-related, Jun, and Krox 24 proteins in rat hippocampus. Brain Res Mol. Brain Res. 13, 355–357.

Hughes, P., Dragunow, M., Beilharz, E., Lawlor, P., Gluckman, P., 1993. MK801 induces immediate-early gene proteins and BDNF mRNA in rat cerebrocortical neurones. NeuroReport 4, 183–186.

Hughes, P., Singleton, K., Dragunow, M., 1994. MK-801 does not attenuate immediate-early gene expression following an amygdala afterdischarge. Exp. Neurol. 128, 276–283.

Ito, E., Nomura, N., Narayanan, R., 1990. Transcriptional regulation of early growth response genes in FOS- expressing PC-12 cells. Cell Regul. 1, 347–357.

Janssen-Timmen, U., Lemaire, P., Mattei, M.G., Revelant, O., Charnay, P., 1989. Structure, chromosome mapping and regulation of the mouse zinc-finger gene Krox-24; evidence for a common regulatory pathway for immediate-early serum-response genes. Gene 80, 325–336.

Johnson, G.L., Lapadat, R., 2002. Mitogen-activated protein kinase pathways mediated by ERK, JNK, and p38 protein kinases. Science 298, 1911–1912.

Jones, M.W., Errington, M.L., French, P.J., Fine, A., Bliss, T.V., 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 long-term memories. Nat. Neurosci. 4, 289–296.

Jung, A.B., Bennett Jr., J.P., 1996. Development of striatal dopaminergic function: II. Dopaminergic regulation of transcription of the immediate early gene zif268 and of D1 (D1a) and D2 (D2a) receptors during pre- and postnatal development. Brain Res. Dev. Brain Res. 94, 121–132.

Kaczmarek, L., 1992. Expression of c-fos and other genes encoding transcription factors in long-term potentiation. Behav. Neural. Biol. 57, 263–266.

Kaczmarek, L., 1993. Molecular biology of vertebrate learning: is c-fos a new beginning? J. Neurosci. Res. 34, 377–381.

Kaczmarek, L., 1995. Towards understanding of the role of transcription factors in learning processes. Acta Biochim. Pol. 42, 221–226.

Kaczmarek, L., 2000. Gene expression in learning processes. Acta Neurobiol. Exp. 60, 419–424.

Kaczmarek, L., 2002. c-Fos in learning: beyond the mapping of neuronal activity. In: Kaczmarek, L., Robertson, H.A. (Eds.), Handbook of Chemical Neuroanatomy: Immediate Early Genes and Inducible Transcription Factors in Mapping of the Central Nervous System Function and Dysfunction, vol. 19. Elsevier, Amsterdam, pp. 189–216.

Kaczmarek, L., Chaudhuri, A., 1997. Sensory regulation of immediate-early gene expression in mammalian visual cortex: implications for functional mapping and neural plasticity. Brain Res. Brain Res. Rev. 23, 237–256.

Kaczmarek, L., Zangenehpour, S., Chaudhuri, A., 1999. Sensory regulation of immediate-early genes c-fos and zif268 in monkey visual cortex at birth and throughout the critical period. Cereb. Cortex 9, 179–187.

Kaminska, B., Kaczmarek, L., Chaudhuri, A., 1996. Visual stimulation regulates the expression of transcription factors and modulates the composition of AP-1 in visual cortex. J. Neurosci. 16, 3968–3978.

Kilbourne, E.J., Widom, R., Harnish, D.C., Malik, S., Karathanasis, S.K., 1995. Involvement of early growth response factor Egr-1 in apolipoprotein AI gene transcription. J. Biol. Chem. 270, 7004–7010.

Kim, J.J., Fanselow, M.S., 1992. Modality-specific retrograde amnesia of fear. Science 256, 675–677.

Kim, J.J., DeCola, J.P., Landeira-Fernandez, J., Fanselow, M.S., 1991. *N*-Methyl-D-aspartate receptor antagonist APV blocks acquisition but not expression of fear conditioning. Behav. Neurosci. 105, 126–133.

Kim, S.J., Park, K., Rudkin, B.B., Dey, B.R., Sporn, M.B., Roberts, A.B., 1994. Nerve growth factor induces transcription of transforming growth factor- beta 1 through a specific promoter element in PC12 cells. J. Biol. Chem. 269, 3739–3744.

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.

Kumahara, E., Ebihara, T., Saffen, D., 1999. Nerve growth factor induces *zif268* gene expression via MAPK-dependent and -independent pathways in PC12D cells. J. Biochem. (Tokyo) 125, 541–553.

Lamprecht, R., Dudai, Y., 1995. Differential modulation of brain immediate early genes by intraperitoneal LiCl. NeuroReport 7, 289–293.

Lanaud, P., Maggio, R., Gale, K., Grayson, D.R., 1993. Temporal and spatial patterns of expression of c-fos, zif/268, c-jun and jun-B mRNAs in rat brain following seizures evoked focally from the deep prepiriform cortex. Exp. Neurol. 119, 20–31.

Lau, L.F., Nathans, D., 1987. Expression of a set of growth-related immediate early genes in BALB/c 3T3 cells: coordinate regulation with c-fos or c-myc. Proc. Natl. Acad. Sci. U.S.A. 84, 1182–1186.

Leah, J., Wilce, P.A., 2002. The Egr transcription factors and their utility in mapping brain functioning. In: Kaczmarek, L., Robertson, H.A. (Eds.), Immediate Early Genes and Inducible Transcription Factors in Mapping of the Central Nervous System Function and Dysfunction, vol. 19. Elsevier, Amsterdam, pp. 309–328.

Lee, H., Kim, J.J., 1998. Amygdalar NMDA receptors are critical for new fear learning in previously fear-conditioned rats. J. Neurosci. 18, 8444–8454.

Lee, S.L., Tourtellotte, L.C., Wesselschmidt, R.L., Milbrandt, J., 1995. Growth and differentiation proceeds normally in cells deficient in the immediate early gene NGFI-A. J. Biol. Chem. 270, 9971–9977.

Lee, S.L., Sadovsky, Y., Swirnoff, A.H., Polish, J.A., Goda, P., Gavrilina, G., Milbrandt, J., 1996. Luteinizing hormone deficiency and female infertility in mice lacking the transcription factor NGFI-A (Egr-1). Science 273, 1219–1221.

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. U.S.A. 85, 4691–4695.

Lemaire, P., Vesque, C., Schmitt, J., Stunnenberg, H., Frank, R., Charnay, P., 1990. The serum-inducible mouse gene Krox-24 encodes a sequence-specific transcriptional activator. Mol. Cell Biol. 10, 3456–3467.

Lerea, L.S., Carlson, N.G., McNamara, J.O., 1995. *N*-Methyl-D-aspartate receptors activate transcription of c-fos and NGFI- A by distinct phospholipase A2-requiring intracellular signalling pathways. Mol. Pharmacol. 47, 1119–1125.

Li, Y., Camp, S., Rachinsky, T.L., Bongiorno, C., Taylor, P., 1993. Promoter elements and transcriptional control of the mouse acetylcholinesterase gene. J. Biol. Chem. 268, 3563–3572.

Li, Z., Paulin, D., 1993. Different factors interact with myoblast-specific and myotube-specific enhancer regions of the human desmin gene. J. Biol. Chem. 268, 10403–10415.

Lim, C.P., Jain, N., Cao, X., 1998. Stress-induced immediate-early gene, egr-1, involves activation of p38/JNK1. Oncogene 16 .

Mack, K.J., Mack, P.A., 1992. Induction of transcription factors in somatosensory cortex after tactile stimulation. Brain Res. Mol. Brain Res. 12, 141–147.

Mack, K., Day, M., Milbrandt, J., Gottlieb, D.I., 1990. Localization of the NGFI-A protein in the rat brain. Brain Res. Mol. Brain Res. 8, 177–180.

Malkani, S., Rosen, J.B., 2000a. Differential expression of EGR-1 mRNA in the amygdala following diazepam in contextual fear conditioning. Brain Res. 860, 53–63.

Malkani, S., Rosen, J.B., 2000b. 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.

Malkani, S., Rosen, J.B., 2001. *N*-Methyl-D-aspartate receptor antagonism blocks contextual fear conditioning and differentially regulates early growth response-1 messenger RNA expression in the amygdala: implications for a functional amygdaloid circuit of fear. Neuroscience 102, 853–861.

Maren, S., Fanselow, M.S., 1996. The amygdala and fear conditioning: has the nut been cracked? Neuron 16, 237–240.

Maren, S., Aharonov, G., Stote, D.L., Fanselow, M.S., 1996. *N*-Methyl-D-aspartate receptors in the basolateral amygdala are required for both acquisition and expression of conditional fear in rats. Behav. Neurosci. 110, 1365–1374.

Mataga, N., Fujishima, S., Condie, B.G., Hensch, T.K., 2001. Experience-dependent plasticity of mouse visual cortex in the absence of the neuronal activity-dependent marker egr1/zif268. J. Neurosci. 21, 9724–9732.

McMahon, S.B., Monroe, J.G., 1995. A ternary complex factor-dependent mechanism mediates induction of egr- 1 through selective serum response elements following antigen receptor cross-linking in B lymphocytes. Mol. Cell Biol. 15, 1086–1093.

McNaughton, B., Barnes, C., Rao, G., Baldwin, J., Rasmussen, M., 1986. Long-term enhancement of hippocampal synaptic transmission and the acquisition of spatial information. J. Neurosci. 6, 563–571.

Mechta, F., Piette, J., Hirai, S.I., Yaniv, M., 1989. Stimulation of protein kinase C or protein kinase A mediated signal transduction pathways shows three modes of response among serum inducible genes. New Biol. 1, 297–304.

Mello, C.V., 2002. Immediate-early gene (IEG) expression mapping of vocal communication areas in the avian brain. In: Kaczmarek, L., Robertson, H.A. (Eds.), Immediate Early Genes and Inducible Transcription Factors in Mapping of the Central Nervous System Function and Dysfunction, vol. 19. Elsevier, Amsterdam, pp. 59–101.

Melzer, P., Steiner, H., 1997. Stimulus-dependent expression of immediate-early genes in rat somatosensory cortex. J. Comp. Neurol. 380, 145–153.

Milbrandt, J., 1987. A nerve growth factor-induced gene encodes a possible transcriptional regulatory factor. Science 238, 797–799.

Mohammed, A.K., Winblad, B., Ebendal, T., Larkfors, L., 1990. Environmental influence on behaviour and nerve growth factor in the brain. Brain Res. 528, 62–72.

Molnar, G., Crozat, A., Pardee, A.B., 1994. The immediate-early gene Egr-1 regulates the activity of the thymidine kinase promoter at the G0-to-G1 transition of the cell cycle. Mol. Cell Biol. 14, 5242–5248.

Morita, K., Wong, D.L., 1996. Role of Egr-1 in cholinergic stimulation of phenylethanolamine *N*- methyltransferase promoter. J. Neurochem. 67, 1344–1351.

Mumby, D.G., Gaskin, S., Glenn, M.J., Schramek, T.E., Lehmann, H., 2002. Hippocampal damage and exploratory preferences in rats: memory for objects, places, and contexts. Learn. Mem. 9, 49–57.

Murphy, T.H., Worley, P.F., Baraban, J.M., 1991. L-type voltage-sensitive calcium channels mediate synaptic activation of immediate early genes. Neuron 7, 625–635.

Murray, E.A., Gaffan, D., Mishkin, M., 1993. Neural substrates of visual stimulus-stimulus association in rhesus monkeys. J. Neurosci. 13, 4549–4561.

Nader, K., Schafe, G.E., Le Doux, J.E., 2000. Fear memories require protein synthesis in the amygdala for reconsolidation after retrieval. Nature 406, 722–726.

Nader, K., Majidishad, P., Amorapanth, P., LeDoux, J.E., 2001. Damage to the lateral and central, but not other, amygdaloid nuclei prevents the acquisition of auditory fear conditioning. Learn. Mem. 8, 156–163.

Nahm, W.K., Noebels, J.L., 1998. Nonobligate role of early or sustained expression of immediate-early gene proteins c-fos, c-jun, and Zif/268 in hippocampal mossy fiber sprouting. J. Neurosci. 18, 9245–9255.

Nikam, S.S., Tennekoon, G.I., Christy, B.A., Yoshino, J.E., Rutkowski, J.L., 1995. The zinc finger transcription factor Zif268/Egr-1 is essential for Schwann cell expression of the p75 NGF receptor. Mol. Cell Neurosci. 6, 337–348.

Nikolaev, E., Kaminska, B., Tischmeyer, W., Matthies, H., Kaczmarek, L., 1992. Induction of expression of genes encoding transcription factors in the rat brain elicited by behavioral training. Brain Res. Bull. 28, 479–484.

Noebels, J.L., Qiao, X., Bronson, R.T., Spencer, C., Davisson, M.T., 1990. Stargazer: a new neurological mutant on chromosome 15 in the mouse with prolonged cortical seizures. Epilepsy Res. 7, 129–135.

O'Donovan, K.J., Tourtellotte, W.G., Millbrandt, 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.

Okuno, H., Saffen, D.W., Miyashita, Y., 1995. Subdivision-specific expression of ZIF268 in the hippocampal formation of the macaque monkey. Neuroscience 66, 829–845.

Olney, J.W., Labruyere, J., Price, M.T., 1989. Pathological changes induced in cerebrocortical neurons by phencyclidine and related drugs. Science 244, 1360–1362.

Olsson, T., Mohammed, A.H., Donaldson, L.F., Henriksson, B.G., Seckl, J.R., 1994. Glucocorticoid receptor and NGFI-A gene expression are induced in the hippocampus after environmental enrichment in adult rats. Brain Res. Mol. Brain Res. 23, 349–353.

Otani, S., Marshall, C.J., Tate, W.P., Goddard, G.V., Abraham, W.C., 1989. Maintenance of long-term potentiation in rat dentate gyrus requires protein synthesis but not messenger RNA synthesis immediately post-tetanization. Neuroscience 28, 519–526.

Pavletich, N.P., Pabo, C.O., 1991. Zinc finger-DNA recognition: crystal structure of a Zif268–DNA complex at 2.1 Å. Science 252, 809–817.

Petersohn, D., Schoch, S., Brinkmann, D.R., Thiel, G., 1995. The human synapsin II gene promoter: Possible role for the transcription factor zif268/egr-1, polyoma enhancer activator 3, and AP2. J. Biol. Chem. 270, 24361–24369.

Pinaud, R., 2004. Experience-dependent immediate early gene expression in the adult central nervous system: evidence from enriched-environment studies. Int. J. Neurosci. 114, 321–333.

Pinaud, R., Tremere, L.A., Penner, M.R., 2000. Light-induced zif268 expression is dependent on noradrenergic input in rat visual cortex. Brain Res. 882, 251–255.

Pinaud, R., Vargas, C.D., Ribeiro, S., Monteiro, M.V., Tremere, L.A., Vianney, P., Delgado, P., Mello, C.V., Rocha-Miranda, C.E., Volchan, E., 2003. Light-induced Egr-1 expression in the striate cortex of the opossum. Brain Res. Bull. 61, 139–146.

Polston, E.K., Erskine, M.S., 1995. Patterns of induction of the immediate-early genes c-fos and egr-1 in the female rat brain following differential amounts of mating stimulation. Neuroendocrinology 62, 370–384.

Pospelov, V.A., Pospelova, T.V., Julien, J.P., 1994. AP-1 and Krox-24 transcription factors activate the neurofilament light gene promoter in P19 embryonal carcinoma cells. Cell Growth Differ. 5, 187–196.

Qureshi, S.A., Rim, M., Bruder, J., Kolch, W., Rapp, U., Sukhatme, V.P., Foster, D.A., 1991. An inhibitory mutant of c-Raf-1 blocks v-Src-induced activation of the Egr-1 promoter. J. Biol. Chem. 266, 20594–20597.

Racine, R.J., Milgram, N.W., Hafner, S., 1983. Long-term potentiation phenomena in the rat limbic forebrain. Brain Res. 260, 217–231.

Radwanska, K., Nikolaev, E., Knapska, E., Kaczmarek, L., 2002. Differential response of two subdivisions of lateral amygdala to aversive conditioning as revealed by c-Fos and P-ERK mapping. NeuroReport 13, 2241–2246.

Repa, J.C., Muller, J., Apergis, J., Desrochers, T.M., Zhou, Y., LeDoux, J.E., 2001. Two different lateral amygdala cell populations contribute to the initiation and storage of memory. Nat. Neurosci. 4, 724–731.

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, zif/268, and long-term potentiation in the dentate gyrus. Brain Res. 580, 147–154.

Richter-Levin, G., Thomas, K.L., Hunt, S.P., Bliss, T.V., 1998. Dissociation between genes activated in long-term potentiation and in spatial learning in the rat. Neurosci. Lett. 251, 41–44.

Rogan, M.T., LeDoux, J.E., 1996. Emotion: systems, cells, synaptic plasticity. Cell 85, 469–475.

Rosen, J.B., Fanselow, M.S., Young, S.L., Sitcoske, M., Maren, S., 1998. Immediate-early gene expression in the amygdala following footshock stress and contextual fear conditioning. Brain Res. 796, 132–142.

Rusanescu, G., Qi, H., Thomas, S.M., Brugge, J.S., Halegoua, S., 1995. Calcium influx induces neurite growth through a Src-Ras signalling cassette. Neuron 15, 1415–1425.

Russo, M.W., Matheny, C., Milbrandt, J., 1993. Transcriptional activity of the zinc finger protein NGFI-A is influenced by its interaction with a cellular factor. Mol. Cell Biol. 13, 6858–6865.

Russo, M.W., Sevetson, B.R., Milbrandt, J., 1995. Identification of NAB1, a repressor of NGFI-A- and Krox20-mediated transcription. Proc. Natl. Acad. Sci. U.S.A. 92, 6873–6877.

Saffen, D.W., Cole, A.J., Worley, P.F., Christy, B.A., Ryder, K., Baraban, J.M., 1988. Convulsant-induced increase in transcription factor messenger RNAs in rat brain. Proc. Natl. Acad. Sci. U.S.A. 85, 7795–7799.

Sakamoto, K.M., Bardeleben, C., Yates, K.E., Raines, M.A., Golde, D.W., Gasson, J.C., 1991. 5′ upstream sequence and genomic structure of the human primary response gene, EGR-1/TIS8. Oncogene 6, 867–871.

Salin, H., Maurin, Y., Davis, S., Laroche, S., Mallet, J., Dumas, S., 2002. Spatio-temporal heterogeneity and cell-specificity of long-term potentiation-induced mRNA expression in the dentate gyrus in vivo. Neuroscience 110, 227–236.

Schafe, G.E., Nader, K., Blair, H.T., LeDoux, J.E., 2001. Memory consolidation of Pavlovian fear conditioning: a cellular and molecular perspective. Trends Neurosci. 24, 540–546.

Schreiber, S.S., Maren, S., Tocco, G., Shors, T.J., Thompson, R.F., 1991a. A negative correlation between the induction of long-term potentiation and activation of immediate early genes. Brain Res. Mol. Brain Res. 11, 89–91.

Schreiber, S.S., Tocco, G., Shors, T.J., Thompson, R.F., 1991b. Activation of immediate early genes after acute stress. NeuroReport 2, 17–20.

Schwachtgen, J.L., Campbell, C.J., Braddock, M., 2000. Full promoter sequence of human early growth response factor-1 (Egr-1): demonstration of a fifth functional serum response element. DNA Seq. 10, 429–432.

Sgambato, V., Pages, 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.

Sierocinska, J., Nikolaev, E., Danysz, W., Kaczmarek, L., 1991. Dextrorphan blocks long- but not short-term memory in a passive avoidance task in rats. Eur. J. Pharmacol. 205, 109–111.

Silverman, E.S., Du, J., Williams, A.J., Wadgaonkar, R., Drazen, J.M., Collins, T., 1998. cAMP-response-element-binding-protein-binding protein (CBP) and p300 are transcriptional co-activators of early growth response factor-1 (Egr-1). Biochem. J. 336, 183–189.

Simpson, C.S., Morris, B.J., 1995. Induction of c-fos and zif/268 gene expression in rat striatal neurons, following stimulation of D1-like dopamine receptors, involves protein kinase A and protein kinase C. Neuroscience 68, 97–106.

Slade, J.P., Carter, D.A., 2000. Cyclical expression of egr-1/NGFI-A in the rat anterior pituitary: a molecular signal for ovulation? J. Neuroendocrinol. 12, 671–676.

Soderling, T.R., Derkach, V.A., 2000. Postsynaptic protein phosphorylation and LTP. Trends Neurosci. 23, 75–80.

Staiger, J.F., Bisler, S., Schleicher, A., Gass, P., Stehle, J.H., Zilles, K., 2000. Exploration of a novel environment leads to the expression of inducible transcription factors in barrel-related columns. Neuroscience 99, 7–16.

Steiner, H., Gerfen, C.R., 1994. Tactile sensory input regulates basal and apomorphine-induced immediate- early gene expression in rat barrel cortex. J. Comp. Neurol. 344, 297–304.

Struhl, K., 1991. Mechanisms for diversity in gene expression patterns. Neuron 7, 177–181.

Suggs, S.V., Katzowitz, J.L., Tsai-Morris, C., Sukhatme, V.P., 1990. cDNA sequence of the human cellular early growth response gene Egr-1. Nucleic Acids Res. 18, 4283.

Sukhatme, V.P., Cao, X.M., Chang, L.C., Tsai-Morris, C.H., Stamenkovich, D., Ferreira, P.C., Cohen, D.R., Edwards, S.A., Shows, T.B., Curran, T., 1988. A zinc finger-encoding gene coregulated with c-fos during growth and differentiation, and after cellular depolarization. Cell 53, 37–43.

Suzuki, W.A., Zola-Morgan, S., Squire, L.R., Amaral, D.G., 1993. Lesions of the perirhinal and parahippocampal cortices in the monkey produce long-lasting memory impairment in the visual and tactual modalities. J. Neurosci. 13, 2430–2451.

Svaren, J., Sevetson, B.R., Apel, E.D., Zimonjic, D.B., Popescu, N.C., Milbrandt, J., 1996. NAB2, a corepressor of NGFI-A (Egr-1) and Krox20, is induced by proliferative and differentiative stimuli. Mol. Cell Biol. 16, 3545–3553.

Swirnoff, A.H., Milbrandt, J., 1995. DNA-binding specificity of NGFI-A and related zinc finger transcription factors. Mol. Cell Biol. 15, 2275–2287.

Swirnoff, A.H., Apel, E.D., Svaren, J., Sevetson, B.R., Zimonjic, D.B., Popescu, N.C., Milbrandt, J., 1998. Nab1, a corepressor of NGFI-A (Egr-1), contains an active transcriptional repression domain. Mol. Cell Biol. 18, 512–524.

Szabo, G., Katarova, Z., Kortvely, E., Greenspan, R.J., Urban, Z., 1996. Structure and the promoter region of the mouse gene encoding the 67-kDa form of glutamic acid decarboxylase. DNA Cell Biol. 15, 1081–1091.

Thiel, G., Schoch, S., Petersohn, D., 1994. Regulation of synapsin I gene expression by the zinc finger transcription factor zif268/egr-1. J. Biol. Chem. 269, 15294–15301.

Thomas, K.L., Hall, J., Everitt, B.J., 2002. Cellular imaging with zif268 expression in the rat nucleus accumbens and frontal cortex further dissociates the neural pathways activated following the retrieval of contextual and cued fear memory. Eur. J. Neurosci. 16, 1789–1796.

Tischmeyer, W., Grimm, R., 1999. Activation of immediate early genes and memory formation. Cell Mol. Life Sci. 55, 564–574.

Tokuyama, W., Okuno, H., Hashimoto, T., Li, Y.X., Miyashita, Y., 2002. Selective zif268 mRNA induction in the perirhinal cortex of macaque monkeys during formation of visual pair-association memory. J. Neurochem. 81, 60–70.

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.

Tsai-Morris, C.H., Cao, X.M., Sukhatme, V.P., 1988. 5′ flanking sequence and genomic structure of Egr-1, a murine mitogen inducible zinc finger encoding gene. Nucleic Acids Res. 16, 8835–8846.

Umemoto, S., Kawai, Y., Ueyama, T., Senba, E., 1997. Chronic glucocorticoid administration as well as repeated stress affects the subsequent acute immobilization stress-induced expression of immediate early genes but not that of NGFI-A. Neuroscience 80, 763–773.

Vaccarino, F.M., Hayward, M.D., Nestler, E.J., Duman, R.S., Tallman, J.F., 1992. Differential induction of immediate early genes by excitatory amino acid receptor types in primary cultures of cortical and striatal neurons. Brain Res. Mol. Brain Res. 12, 233–241.

Wallace, C.S., Withers, G.S., Weiler, I.J., George, J.M., Clayton, D.F., Greenough, W.T., 1995. Correspondence between sites of NGFI-A induction and sites of morphological plasticity following exposure to environmental complexity. Brain Res. Mol. Brain Res. 32, 211–220.

Walton, M., Henderson, C., Mason-Parker, S., Lawlor, P., Abraham, W.C., Bilkey, D., Dragunow, M., 1999. Immediate early gene transcription and synaptic modulation. J. Neurosci. Res. 58, 96–106.

Wang, Z.Y., Deuel, T.F., 1992. An S1 nuclease-sensitive homopurine/homopyrimidine domain in the PDGF A-chain promoter contains a novel binding site for the growth factor- inducible protein EGR-1. Biochem. Biophys. Res. Commun. 188, 433–439.

Watanabe, Y., Stone, E., McEwen, B.S., 1994. Induction and habituation of c-fos and zif/268 by acute and repeated stressors. NeuroReport 5, 1321–1324.

Waters, C.M., Hancock, D.C., Evan, G.I., 1990. Identification and characterisation of the egr-1 gene product as an inducible, short-lived, nuclear phosphoprotein. Oncogene 5, 669–674.

Wei, F., Li, P., Zhuo, M., 1999. Loss of synaptic depression in mammalian anterior cingulate cortex after amputation. J. Neurosci. 19, 9346–9354.

Wei, F., Xu, Z.C., Qu, Z., Milbrandt, J., Zhuo, M., 2000. Role of EGR1 in hippocampal synaptic enhancement induced by tetanic stimulation and amputation. J. Cell Biol. 149, 1325–1334.

Weitemier, A.Z., Ryabinin, A.E., 2004. Subregion-specific differences in hippocampal activity between delay and trace fear conditioning: an immunohistochemical analysis. Brain Res. 995, 55–65.

Wernersson, J., Johansson, I., Larsson, U., Minth-Worby, C., Pahlman, S., Andersson, G., 1998. Activated transcription of the human neuropeptide Y gene in differentiating SH-SY5Y neuroblastoma cells is dependent on transcription factors AP-1, AP-2alpha, and NGFI. J. Neurochem. 70, 1887–1897.

Whishaw, I.Q., Auer, R.N., 1989. Immediate and long-lasting effects of MK-801 on motor activity, spatial navigation in a swimming pool and EEG in the rat. Psychopharmacology 98, 500–507.

Williams, G.T., Lau, L.F., 1993. Activation of the inducible orphan receptor gene nur77 by serum growth factors: dissociation of immediate-early and delayed-early responses. Mol. Cell Biol. 13, 6124–6136.

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. Brain Res. Mol. Brain Res. 77, 258–266.

Wisden, W., Errington, M.L., Williams, S., Dunnett, S.B., Waters, C., Hitchcock, D., Evan, G., Bliss, T.V., Hunt, S.P., 1990. Differential expression of immediate early genes in the hippocampus and spinal cord. Neuron 4, 603–614.

Wood, K.W., Qi, H., D'Arcangelo, G., Armstrong, R.C., Roberts, T.M., Halegoua, S., 1993. The cytoplasmic raf oncogene induces a neuronal phenotype in PC12 cells: a potential role for cellular raf kinases in neuronal growth factor signal transduction. Proc. Natl. Acad. Sci. U.S.A. 90, 5016–5020.

Worley, P.F., Cole, A.J., Murphy, T.H., Christy, B.A., Nakabeppu, Y., Baraban, J.M., 1990. Synaptic regulation of immediate-early genes in brain. Cold Spring Harb. Symp. Quant. Biol. 55, 213–223.

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. U.S.A. 88, 5106–5110.

Worley, P.F., Bhat, R.V., Baraban, J.M., Erickson, C.A., McNaughton, B.L., Barnes, C.A., 1993. Thresholds for synaptic activation of transcription factors in hippocampus: correlation with long-term enhancement. J. Neurosci. 13, 4776–4786.

Yamada, Y., Hada, Y., Imamura, K., Mataga, N., Watanabe, Y., Yamamoto, M., 1999. Differential expression of immediate-early genes, c-fos and zif268, in the visual cortex of young rats: effects of a noradrenergic neurotoxin on their expression. Neuroscience 92, 473–484.

Yanagawa, Y., Kobayashi, T., Kamei, T., Ishii, K., Nishijima, M., Takaku, A., Tamura, S., 1997. Structure and alternative promoters of the mouse glutamic acid decarboxylase 67 gene. Biochem. J. 326, 573–578.

Yau, J.L., Olsson, T., Morris, R.G., Meaney, M.J., Seckl, J.R., 1995. Glucocorticoids, hippocampal corticosteroid receptor gene expression and antidepressant treatment: relationship with spatial learning in young and aged rats. Neuroscience 66, 571–581.

Yau, J.L., Olsson, T., Morris, R.G., Noble, J., Seckl, J.R., 1996. Decreased NGFI-A gene expression in the hippocampus of cognitively impaired aged rats. Brain Res. Mol. Brain Res. 42, 354–357.

Yount, G.L., Ponsalle, P., White, J.D., 1994. Pentylenetetrazole-induced seizures stimulate transcription of early and late response genes. Brain Res. Mol. Brain Res. 21, 219–224.

Zangenehpour, S., Chaudhuri, A., 2001. Neural activity profiles of the neocortex and superior colliculus after bimodal sensory stimulation. Cereb. Cortex 11, 924–935.

Zangenehpour, S., Chaudhuri, A., 2002. Differential induction and decay curves of c-fos and zif268 revealed through dual activity maps. Brain Res. Mol. Brain Res. 109, 221–225.

Zhang, F., Lin, M., Abidi, P., Thiel, G., Liu, J., 2003. Specific interaction of Egr1 and c/EBPbeta leads to the transcriptional activation of the human low density lipoprotein receptor gene. J. Biol. Chem. 278, 44246–44254.

Zheng, D., Butler, L.S., McNamara, J.O., 1998. Kindling and associated mossy fibre sprouting are not affected in mice deficient of NGFI-A/NGFI-B genes. Neuroscience 83, 251–258.

Zola-Morgan, S., Squire, L.R., Clower, R.P., Rempel, N.L., 1993. Damage to the perirhinal cortex exacerbates memory impairment following lesions to the hippocampal formation. J. Neurosci. 13, 251–265.