Molecular Neurobiology

ISSN0893-7648/92/5/2-3-4/297–314/$3.60

The Role of Immediate Early Genes in the Stabilization of Long-Term Potentiation

Wickliffe C. Abraham,*[1] Michael Dragunow,[2] and Warren P. Tate[3]

[1]*Department of Psychology and the Neuroscience Research Centre, and* [3]*Department of Biochemistry, University of Otago, Dunedin, New Zealand;* [2]*Department of Pharmacology and Clinical Pharmacology, University of Auckland Medical School, Dunedin, New Zealand*

Contents

Abstract

Immediate early genes (IEGs) are a class of genes that show rapid and transient but protein synthesis-independent increases in expression to extracellular signals such as growth factors and neurotransmitters. Many IEGs code for transcription factors that have been suggested to govern the growth and differentiation of many cell types by regulating the expression of other genes. IEGs are expressed in adult neurons both constitutively and in response to afferent activity, and it has been suggested that during learning, IEGs may play a role in the signal cascade, resulting in the expression of genes critical for the consolidation of long-term memory. Long-term potentiation (LTP) is a persistent, activity-dependent form of synaptic plasticity that stands

*Author to whom all correspondence and reprint requests should be addressed.

as a good candidate for the mechanism of associative memory. A number of IEGs coding for transcription factors have been shown to transiently increase transcription in the dentate gyrus of rats following LTP-inducing afferent stimulation. These include *zif/268* (also termed *NGFI-A, Krox-24, TIS-8,* and *egr-1*), *c-fos*-related genes, *c-jun, junB, and junD*. Of these, *zif/268* appears to be the most specifically related to LTP since it is evoked under virtually all LTP-inducing situations and shows a remarkably high correlation with the duration of LTP. There are a number of outstanding questions regarding the role of *zif/268* and other IEGs in LTP, including which second messenger systems are important for activating them, which "late effector" genes are regulated by them, and the exact role these genes play, if any, in the stabilization and maintenance of LTP.

Index Entries: Immediate early gene; long-term memory; long-term potentiation; Northern blot; immunohistochemistry; hippocampus; *N*-methyl-D-aspartate; elongation factor-2; transcription factor.

Introduction

Orderly programs of expression and repression of genes are well-known to be essential for the normal growth and development of all tissues, including the central nervous system (CNS). However, after differentiation, migration, and formation of synaptic contacts, neurons show a dramatic decline in the expression of many genes, in part because mature neurons do not enter the mitotic cycle. Nonetheless, neurons show a substantial repertoire of differential gene expression in response to changes in local extracellular conditions, such as prolonged alterations in the frequency of afferent activity or the removal of afferent fibers (Hendry, 1973; Phillips and Steward, 1990). The question arises, therefore, whether the more subtle patterns of neuronal activity that normally occur during behavior are sufficient to elicit changes in gene expression and whether such changes mediate long-term neural plasticity. If so, could such differential gene expression contribute to the cellular mechanisms of long-term memory?

Immediate Early Genes and Transcription Factors

Gene responses to changes in the extracellular environment require the presence of factors that can regulate gene transcription by binding directly to *cis*-acting elements on DNA. Such transcription factors can be in the form of either repressors preventing the transcription of genes or activators inducing transcription and can themselves be activated in a variety of ways. One class of external signal molecules that can directly activate transcription factors are steroid hormones, which are lipid-soluble and can readily pass through cell membranes and bind to intracellular steroid hormone receptors. These receptors, when activated by the appropriate hormone ligand, regulate gene expression by binding to specific *cis*-acting elements on DNA called hormone-response elements. In contrast, other external signaling molecules such as peptides, neurotransmitters, growth factors, and cytokines do not readily penetrate cell membranes but produce their effects indirectly by binding to cell membrane-bound receptors and activating a cascade of events ultimately leading to differential gene expression. For example, the ligand-receptor complex, via generation of an intracellular second messenger, may activate specific protein kinases that phosphorylate and thereby activate constitutively expressed transcription factors.

The first class of genes induced by an external signal are cellular IEGs. Their induction is rapid and transient and is not dependent on *de novo* protein synthesis. The protein products (IEGPs) of the IEGs have been shown to be expressed in response to a number of different stimuli in a variety of cell types. They were first identified in dividing and differentiating cells, where they were rapidly and transiently induced in response to application of growth factors and cytokines (Greenberg and Ziff, 1984; Kruijer et al., 1984;

Müller et al., 1984; Lau and Nathans, 1987; Tippetts et al., 1988). Here, they may play a major role in cell division and/or differentiation by regulating the expression of a cascade of "late-effector" genes and thus are likely to play a crucial role in regulating the genetic program induced by growth factors.

The first studies to suggest that IEGPs play a role in regulating neuronal gene expression were performed on fully differentiated PC12 cells in culture. In these pioneering studies, it was found that nerve growth factor (NGF; Curran and Morgan, 1985) and depolarizing agents, e.g., KCl, calcium, and muscarinic receptor agonists, (Greenberg et al., 1986; Morgan and Curran, 1986) induce the IEG c-*fos*. Induction by depolarizing agents was dependent on the influx of calcium ions (Greenberg et al., 1986; Morgan and Curran, 1986). Since these initial studies, a number of IEGs have been described, including c-*fos*, *Fos*-related genes (*FosR*), *Fos-B, FRA-1, FRA-2, c-jun, junB, junD, Krox-20, and Krox-24* (Doucet et al., 1990; Morgan and Curran, 1991).

Krox-24 (Lemaire et al., 1988), also known as *NGFI-A* (Changelian et al., 1989), *TIS-8* (Tippetts et al., 1988), *zif/268* (the term we shall use in this article; Christy et al., 1988), and *egr-1* (Sukhatme et al., 1988) codes for a zinc-finger-containing transcription factor that is induced in mammalian neurons after seizures (Saffen et al., 1988) and is also expressed constitutively in certain brain areas (Herdegen et al., 1990, Mack et al., 1990; Schlingensiepen et al., 1991). Members of the *Fos* and *Jun* families of transcription factors form homo- (*Jun* family only) and hetero-dimers using a leucine-zipper motif (Chiu et al., 1988; Nakabeppu et al., 1988; Ryder and Nathans, 1988), and form part of the AP-1 transcription factor complex, which binds to a specific response element with varying affinity, depending on the protein combination. These molecules regulate their own transcription in addition to that of other genes. IP-1, a factor that blocks binding by AP-1 to DNA following protein kinase A activation, has recently been discovered (Auwerx and Sassone-Corsi, 1991).

Fos-like molecules were first observed in adult brain neurons under basal conditions using immunohistochemical methods (Dragunow et al., 1987). This was followed by demonstrations that *Fos* and *FosRs* are induced throughout the CNS by brain seizures (Dragunow and Robertson, 1987; Morgan et al., 1987; White and Gall, 1987) and in the spinal cord by noxious stimulation (Hunt et al., 1987). Other IEGs (c-*jun, junB, zif/268*) were subsequently found to be induced in adult brain neurons after seizures as well (Saffen et al., 1988). The suggestion arose that IEGPs, as transcription factors rapidly induced by neural activity, may play a role in long-lasting neuronal plasticity and thus, possibly, in long-term memory (Goelet et al., 1986; Curran and Morgan, 1987).

Immediate Early Genes and LTP

IEGs Are Induced Following LTP

One popular experimental model of synaptic plasticity in the mammalian CNS is LTP, a lasting enhancement of synaptic efficacy following brief high-frequency electrical stimulation of afferent pathways (Bliss and Lømo, 1973). LTP has been shown to persist from days to months (Bliss and Gardner-Medwin, 1973; Douglas and Goddard, 1975; Racine et al., 1983), a property that, in conjunction with several others, makes it an attractive model of the mechanisms underlying long-term memory (Teyler and Discenna, 1984). The prolonged time course of LTP decay has raised the possibility that gene expression changes may play a role in the maintenance of LTP, and IEGs have been among the first genes studied. IEGs now known to be induced in dentate gyrus granule cells following LTP induction by perforant path stimulation are *zif/268*, c-*Jun, JunB, JunD, and FosR* (Fig. 1; Cole et al., 1989; Dragunow et al., 1989; Jeffery et al., 1990; Wisden et al., 1990; Richardson et al., in press; Dragunow et al., in preparation).

An increase in immunostaining for Raf-1, a serine/threonine-specific protein kinase, has also

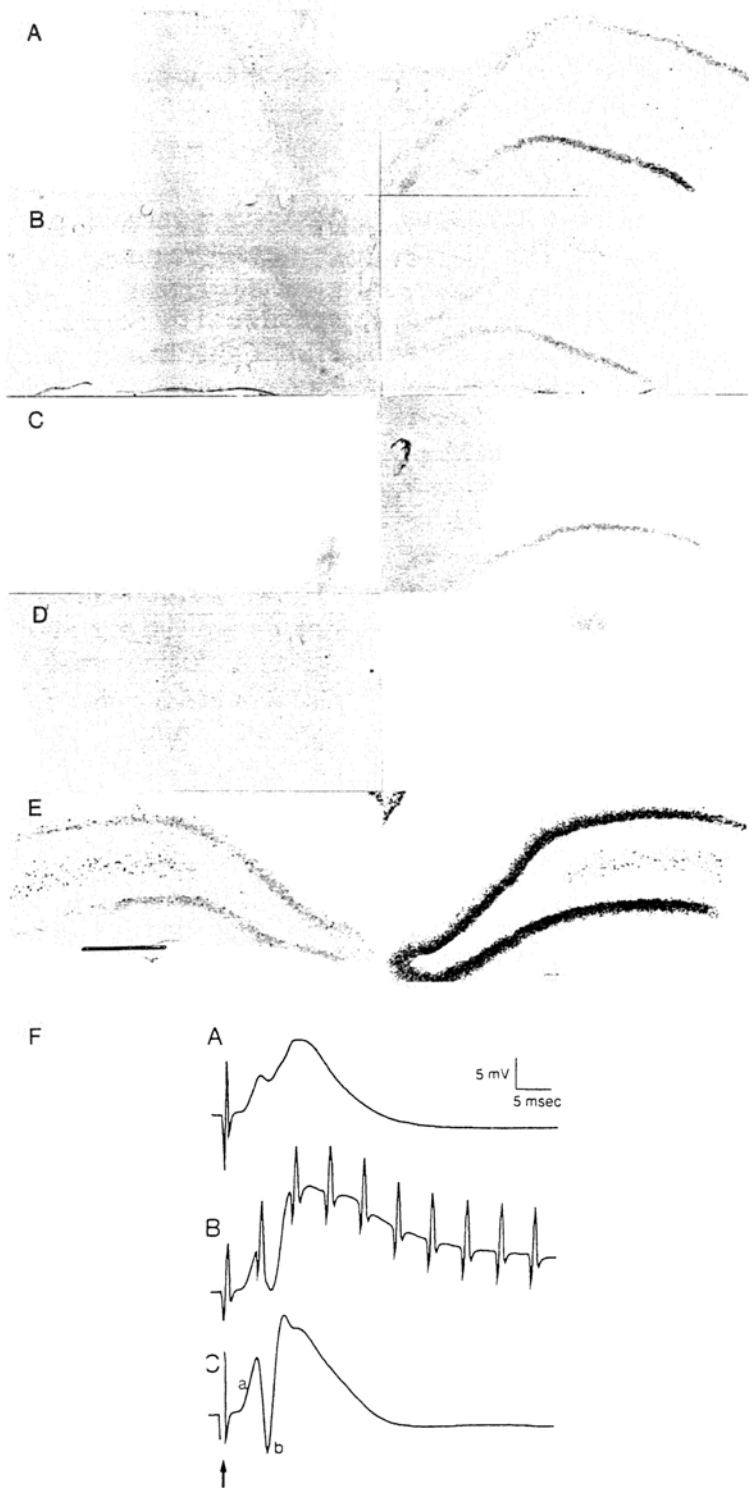

Fig. 1. Photomicrographs showing *FosR*-like (**A**), c-*jun*-like (B), *junB*-like (**C**), *junD*-like (**D**) and *zif/268*-like (**E**) immunoreactivity in ipsilateral (right) and contralateral (left) dentate granule cells after LTP induction in awake rats, i.e., LTP on right side. Bar = 400 μm. LTP was induced by 50 stimulus trains delivered in a burst type paradigm (explained in Fig. 2 caption). LTP is measured as a change in the slope of the rising phase of the EPSP (**a**) and the height of the population (**b**) recorded extracellularly in the dentate hilus to test pulses in the perforant path. Note the increase in these waveform components (lower part of **f**) recorded 30 min posttetanization (middle part of **f**), relative to the baseline pretetanization recording (upper part of **f**).

been reported to occur shortly after LTP induction (Mihaly et al., 1990). The extent of IEG induction depends considerably on the tetanization protocols employed and whether anesthetic agents are used *(see below)*. Genes that apparently do not respond to LTP-inducing stimulation include c-*fos*, NGFI-B, *fos-B*, *PC4*, and *SRF* (Cole et al., 1989; Wisden et al., 1990; Dragunow et al., in preparation). Early reports that c-*fos* induction occurred with LTP were apparently attributable to the use of non-selective antibodies and cDNA probes, resulting in crossreactivity and possibly crosshybridization with *FosR* (Dragunow et al., 1989; Matthies, 1989). It is also possible, however, that c-*fos* mRNA is induced by LTP (in awake rats) but the protein is not (Morgan and Curran, 1991).

All of the responding genes show a time course of induction typical of IEGs but with slight variations between genes. We have studied the various time courses in awake animals using immunohistochemical techniques most extensively (Fig. 2). *FosR* is induced within 20 min of stimulation, is maximal at 1 h, and returns to baseline after 4–8 h. *JunB and* c-*jun* are induced within 20 min, are maximal between 20 min and 1 h, and are back to baseline by 4 h. *zif/268* is also induced within 20 min, is maximal between 1 and 2 h, and is back to baseline after 8 h. JunD is induced at 2 h, is maximal at 4 h, and is back to baseline between 8 and 24 h (Dragunow et al., 1989; Richardson et al., in press; Dragunow et al., in preparation). *In situ* hybridization and Northern blot analyses demonstrated that the mRNA levels of *zif/268* and *junB* maximize 20–30 min posttetanization and are back to baseline by 2–3 h (Cole et al., 1989; Wisden et al., 1990; Richardson et al., in press). The induction of *FosR* and to a lesser extent, the Jun proteins, occurs most strongly in the outer portion of the lower blade of the dentate gyrus.

Our impression from the immunoreactivity analyses is that *zif/268* induction is more dramatic and widespread in the dentate gyrus than for the other IEGPs (Cole et al., 1989; Wisden et al., 1990). However, restricted sites of induction laterally and in the ventral blade of the dentate gyrus also occur for *zif/268* under weak stimulation conditions and at early and late times after LTP induction (when levels were not maximal). This may reflect the positioning of the stimulating electrodes to maximize activation of this area of the dentate gyrus or as-yet-uncharacterized regional variations in granule cell molecular properties.

It is important to note that so far, all of the responsive genes have been induced in the dentate gyrus granule cells (and perhaps interneurons), postsynaptic to the perforant path fibers being stimulated. No gene induction or increase in immunoreactivity has been detected in the entorhinal cortex, which contains the cell bodies of origin for the perforant path axons. This postsynaptic gene response is consistent with the requirement for postsynaptic depolarization and NMDA receptor activation in order to induce LTP (Collingridge et al., 1983; Wigström et al., 1986; Gustafsson et al., 1987). These data do not bear on the issue of whether LTP is finally expressed pre- or postsynaptically, however, as retrograde messengers to the presynaptic terminal have been hypothesized to be released by the postsynaptic neurons (Bliss and Lynch, 1988). On the other hand, it is possible that gene induction postsynaptically reflects downstream changes at the synaptic contacts made by granule cells onto the pyramidal cells of CA3. Such changes, however, have not yet been reported.

Relation of IEG Expression to the Induction of LTP

Having established that IEGs are induced by afferent activity sufficient to elicit LTP, the next question is how well the induction of various IEGs correlates with the induction of LTP. In experiments where LTP is blocked or not induced, IEG responses are rarely observed. The *N*-methyl-D-aspartate (NMDA) receptor antagonists CPP, MK-801, and APV have been shown to dramatically reduce or block IEG responses and LTP following high-frequency stimulation (Cole et al., 1989; Dragunow et al., 1989; Wisden et al., 1990). Similarly, stimulation of the inhibitory commis-

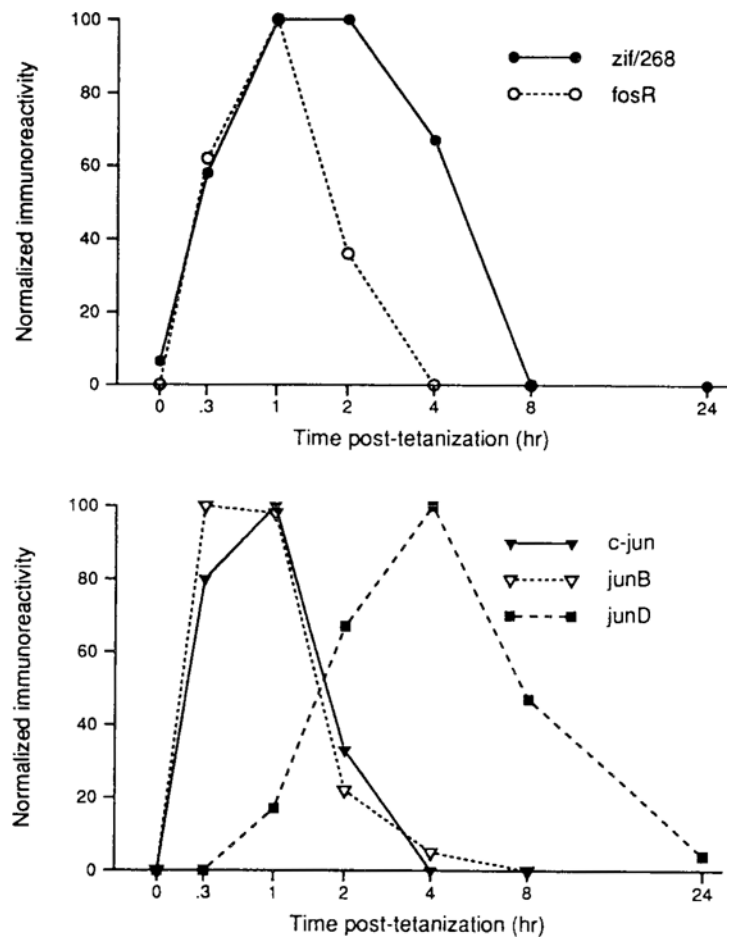

Fig. 2. Time course of induction of IEGPs following LTP-inducing tetanization in awake rats using 50 trains of stimulation in a burst paradigm, i.e., in bursts of 5 400-Hz trains spaced 1 s apart with 1 min between bursts. The degree of immunostaining has been rated on a six point scale, corrected for the degree of staining in the contralateral control hemisphere and averaged across 3–6 rats at each time point. The mean value at each time point has then been normalized relative to the maximum response for each IEGP, which has been given a value of 100%. Time is plotted on a log scale. Data are taken from Richardson et al., (in press) and Dragunow et al. (in preparation.)

sural input to the dentate gyrus, which blocks perforant path-induced LTP, also blocks IEG responses in the dentate granule cells (Cole et al., 1989; Wisden et al., 1990). Finally, low-frequency or sub-threshold high-frequency stimulation, which does not induce LTP, does not elicit an IEG response (Cole et al., 1989; Dragunow et al., 1989).

There are, however, differences between genes in their response to LTP-inducing stimulation. Of the IEGs studied so far, *zif/268* expression shows the greatest correlation with LTP induction, although some discrepancies have been noted. For example, Cole et al. (1989) reported that 10 out of 13 animals showed *zif/268* and LTP induction, but that in 2 of 13 animals, LTP was induced without *zif/268* induction; and in another animal, the reverse was true. When Richardson et al. (in press) compared average LTP induction with *zif/268* mRNA and protein induction from three different stimulation protocols, the correlation coefficients were high (0.65–0.86) but not statistically significant (Fig. 3A). In contrast to *zif/268*,

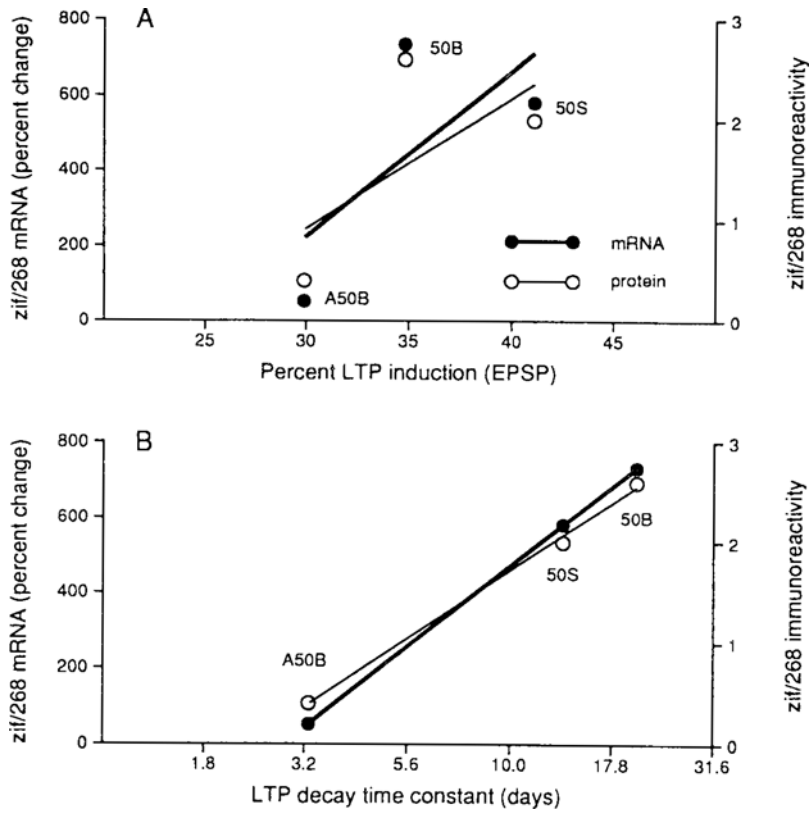

Fig. 3. Correlation between *zif/268* (mRNA and protein) induction and LTP induction (**A**) and decay (**B**). Messenger RNA data are taken from densitometric analysis of Northern blots and presented as a percent change from unstimulated control hemispheres; immunoreactivity data are presented as in Fig. 2 but not normalized. The decay data are averaged across EPSP and population spike data, as these measures show similar decay rates (Jeffery et al., 1990). The correlation between the changes in *zif/268* mRNA and protein levels are extremely high across the three stimulus conditions: 50B, 50 trains in a burst paradigm as described in Fig. 2; A50B, 50 trains in a burst paradigm while the animal is under pentobarbital anesthesia (60 mg/kg); 50S, 50 trains equally spaced 20 s apart (spaced paradigm). Linear regression lines for gene and LTP comparisons are plotted. The correlation between gene induction and LTP induction is high (**A**), but it is even higher when the comparison is with LTP decay (**B**). The LTP decay correlations were made using the log of the decay rate obtained from single negative exponential curve fitting. The decay data are plotted on the log (decay rate) scale, with decay time constant values (1/decay rate) used on the x-axis for clarity. Data are taken from Richardson et al. (in press) and Jeffery et al. (1990). Numbers of animals are 3–6 for immunochemistry, 8–20 for Northern blots, and 5–7 for the LTP decay data.

the Fos*R*, c-*jun*, *junB*, and *junD* genes showed little and in many cases no induction in anesthetized animals, even though LTP is readily elicited (Douglas et al., 1988; Cole et al., 1989; Wisden et al., 1990).

The induction of these genes is more reliable in awake animals; nonetheless, their correlation with LTP induction is still relatively low when compared across a variety of stimulation conditions. FosR, for example, shows much higher induction when the stimulation trains are spaced 20 s apart (spaced paradigm) than when the same number of trains are delivered in bursts of 5 trains spaced only 1 s apart (burst paradigm), even though the two stimulation paradigms elicit nearly equivalent LTP (Fig. 4A; Jeffery et al., 1990; Dragunow et al., in preparation).

The above data clearly suggest that new synthesis of IEG transcription factors is neither nec-

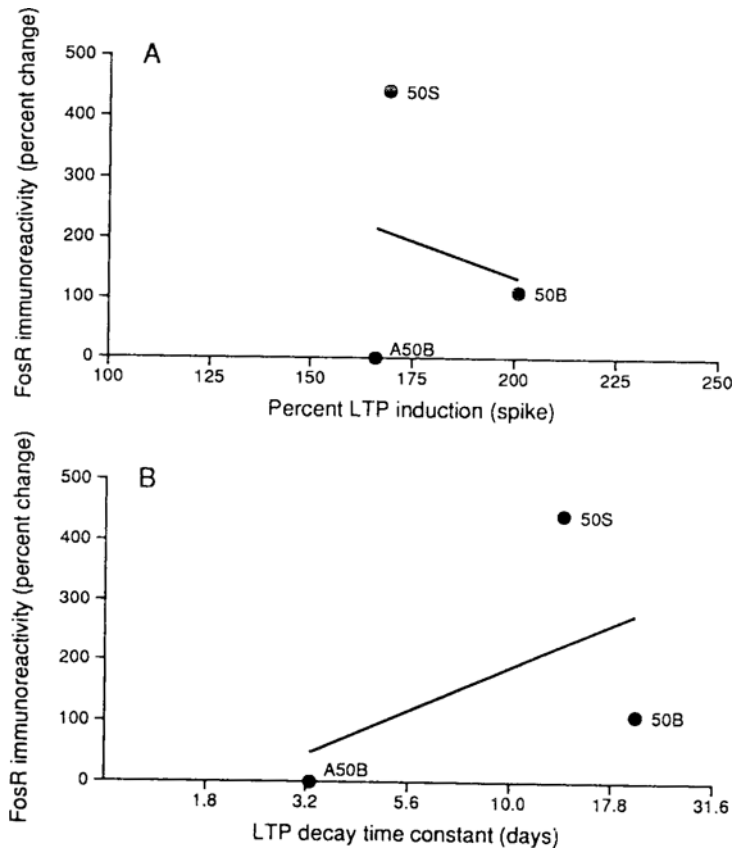

Fig. 4. Correlation between FosR-like immunoreactivity and LTP induction (**A**) and decay (**B**). Data are presented as in Fig. 3, but immunoreactivity has been quantified based on counts of immunoreactive cells/100 um^2. The correlation between FosR-like immunoreactivity changes and the degree of LTP induction is very low, regardless of whether EPSP or population spike measures are used for LTP induction. The correlation is improved when FosR induction is correlated with LTP decay, but this is still substantially lower than for *Zif/268* (*see* Fig. 3). Data are taken from Jeffery et al. (1990).

essary nor sufficient for the initial induction of LTP. This is consistent with the facts that new IEGPs begin to appear some minutes following LTP induction and their levels peak one or more hours after tetanization (*see above*). It is also consistent with the fact that LTP can be induced in the presence of protein synthesis inhibitors (Krug et al., 1984; Deadwyler et al., 1987; Otani et al., 1989) and even in the absence of cell bodies (Frey et al., 1989).

In any event, the degree of LTP peaks within a few minutes after tetanization (Gustafsson et al., 1989), a time when the transcription factors are only just beginning to be synthesized. It could be argued that existing IEGPs are necessary for LTP induction, but if so, this must happen without additional transcription or translation, since protein synthesis is not obligatory. We consider it most likely, therefore, that initial LTP induction occurs distally at the affected synapses and that if differential gene expression is indeed critical, it becomes involved during later stages of LTP.

IEG Responses and the Persistence of LTP

Since IEG responses to LTP-inducing stimulation are transient, lasting 2–8 h, these genes can-

not be directly involved in processes that govern the maintenance of LTP lasting days to weeks. However, through the production of transcription factors, IEGs may regulate the response of other effector genes, which subsequently govern the longevity of LTP. Thus, IEGs may be postulated to play a role in "stabilizing" LTP. Unfortunately, this hypothesis has not been directly tested since we cannot yet manipulate the expression of specific genes while measuring the persistence of LTP. An alternative approach can be used, however, whereby one correlates, in separate groups of animals, IEG induction shortly after tetanization with LTP persistence, as observed following various tetanization protocols. For this reason, we have characterized LTP decay rates across a large number of tetanization conditions (Jeffery et al., 1990) in which LTP decays with a time constant of a number of days (termed LTP2) or of 2–3 wk (termed LTP3). The LTP1, LTP2, and LTP3 nomenclature has been developed through consideration of all published LTP decay rates (Jeffery et al., 1990; Abraham and Otani, 1991) and follows from the terminology introduced by Racine et al. (1983).

The first gene(s) studied with respect to LTP persistence was FosR (Jeffery et al., 1990). Initially, the decay of LTP in awake behaving animals was monitored over weeks following a number of tetanization protocols in which train pattern (spaced vs burst paradigm; *see above*), number of trains (10–50), days of stimulation (1–5), and the presence of pentobarbital during tetanization were manipulated. A single negative exponential function was fitted to the data for each animal studied. On average, LTP decayed with a time constant of 2–3 wk (LTP3) following 50 trains of stimulation, regardless of the pattern or number of days of stimulation; but it decayed with a time constant of only a few days (LTP2) when just 10 trains were delivered or when 50 trains were given in the presence of pentobarbital.

FosR immunoreactivity was then studied in separate groups of animals given the same tetanization treatments. The main point of correlation was that 50 trains of stimulation in awake animals, producing LTP3, elicited a much stronger *FosR* response than the same stimulation in anesthetized animals, when LTP2 was produced. On the other hand, the correlation across the other tetanization conditions was extremely poor. For example, *FosR* induction was three- to fourfold higher following spaced stimulation than following burst stimulation, even though they each elicited equivalent LTP induction and decay (Fig. 4B). Furthermore, multiple days of stimulation progressively lengthened LTP persistence without inducing additional *FosR* after the first day of stimulation. Overall, it is evident that *FosR* levels do not correlate well with either LTP induction or decay, although we cannot rule out the possibility that *FosR* plays some role, perhaps in concert with other second messengers or IEGs, e.g., as part of *fos/jun* dimers.

More recently, we have been examining *zif/268* in detail, combining Northern blot analysis of mRNA levels 20 min posttetanization with immunohistochemistry analysis of protein levels 2 h after tetanization. Comparison of the *zif/268* response with LTP persistence across the various tetanization protocols previously mentioned revealed extremely high correlations (Fig. 3B; Richardson et al., in press). The *zif/268* response was low following conditions giving LTP2 and correspondingly higher under conditions associated with LTP3. Indeed, the correlations between *zif/268* levels and LTP persistence were substantially higher than the moderately strong correlations between *zif/268* and LTP induction, even though the latter were made within-animal.

Preliminary analysis indicates that the Jun family genes that increase their expression following LTP show moderate correlations with LTP persistence, but these are not as strong as for *zif/268* (Dragunow and Abraham, unpublished observations). However, the correlations need to be examined in more detail and across more stimulation conditions for these genes. Nonetheless, what is clear at present is that the increased immunoreactivity for Jun IEGPs, like that for *zif/268* and *FosR*, is reduced or blocked by anesthetics (the responses for a number of genes are compared

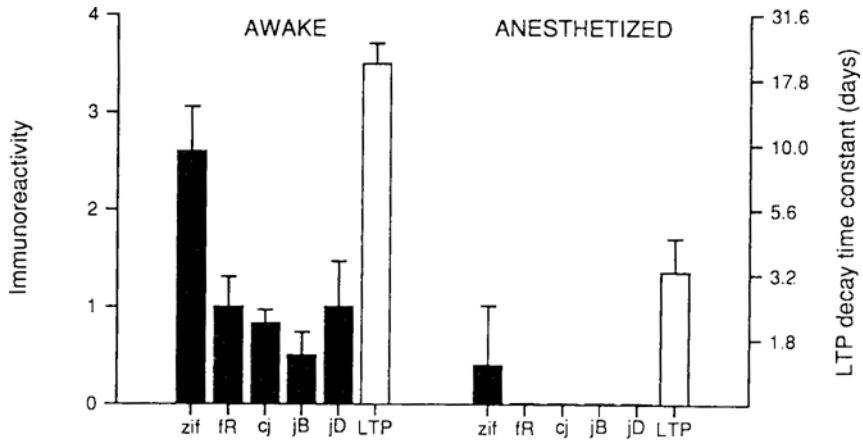

Fig. 5. Immunoreactivity changes for several IEGPs 2 h following LTP-inducing tetanization (data as in Fig. 2 but not normalized). Immunostaining is dramatically decreased for all IEGPs when tetanization is given in the presence of pentobarbital (60 mg/kg), as is the LTP decay time constant (as measured in other groups of animals from Jeffery et al., 1990). Zif, *zif/268*-like; fR, *FosR*-like; cj, c-*jun*-like; jB, *junB*-like; jD, *junD*-like.

in Fig. 5). Since LTP persistence is dramatically shortened (from LTP3 to LTP2) by the presence of the anesthetic pentobarbital during tetanization, it is possible that a coordinate action of IEGs is required for the generation of LTP lasting a period of weeks (LTP3).

Mechanisms of IEG Action in LTP

Triggers for IEG Induction in LTP

The precise biochemical mechanisms that lead to IEGP expression in neurons are presently unclear. It is likely that different mechanisms are operative in different neuronal populations and that different IEGs are linked to different first and second messenger systems. More complicated patterns of response are also possible. For example, Bartel et al. (1989) have shown in PC12 cells that growth factors and membrane depolarization will induce an overlapping set of IEGs but that each stimulus shows preferential activation of a subset of the commonly induced genes.

As previously mentioned, the LTP-associated induction of IEGPs (*FosR*, *zif/268*, and *jun*) in dentate granule cells is blocked by NMDA antagonists. The same is true for trauma-induced IEGP induction in the dentate gyrus, neocortex, hypothalamus, and striatum (Dragunow et al., 1990; Olenik et al., 1991). These results suggest that under certain conditions, IEGPs are induced via an NMDA receptor-mediated signal transduction pathway, although NMDA-independent induction has also been described (Dragunow and Faull, 1990).

If NMDA receptor activation induces IEGs, what intracellular mechanisms might mediate the induction cascade? Calcium is a leading candidate second messenger since it is known to enter through NMDA receptor-linked channels (MacDermott et al., 1986) and because calcium influx has been shown to be a potent inducer of c-*fos* in PC12 cells (Greenberg et al., 1986; Morgan and Curran, 1986). Elevated levels of intracellular free calcium may then result in activation of calcium/calmodulin (CaM) protein kinases II and III, Ca^{++}/phospholipid protein kinase C, and phospholipase C, the latter providing positive feedback through production of inositol triphosphate (IP_3), which releases intracellular stores of calcium. Other potential pathways of IEGP induction include activation of phospholipase A_2, leading to the generation of arachidonic acid and

lipoxygenase second messengers, thought to play roles in LTP process (Williams et al., 1989; Mayer and Miller, 1990), and activation of nitric oxide and cyclic GMP (East and Garthwaite, 1991). A role for serum response factors (SRF) is also possible because they induce IEGs in cell culture systems and may be expressed constitutively in dentate granule cells (Wisden et al., 1990).

It is interesting that there is growing evidence that kinases may directly enter the nucleus and act by phosphorylating transcription factors, with the consequence of either activating or derepressing genes. For example, it has been known for some time that cAMP can stimulate transcription without new protein synthesis in tissue culture cells, suggesting the covalent modification of existing proteins rather than *de novo* synthesis of specific nuclear factors (Gonzalez and Montminy, 1989). More recent studies suggest that the gene expression is mediated through phosphorylation of a factor, most probably CREB (the cAMP-responsive element-binding protein), by the catalytic subunit of the cAMP-dependent kinase (Mellon et al., 1989). The implication of this is that the protein kinases may translocate from the cytoplasm to the nucleus to mediate this regulatory role, thereby making up an active component of the second (or third) messenger system. Indeed, it has been shown that immunoreactivity for the cAMP kinase catalytic subunit appears in the nucleus after treatment of bovine cells with cAMP (Nigg et al., 1985). A connection between these events and long-term facilitation in *Aplysia* has been made since injection of the cAMP-responsive element (TGACGTCA—to which CREB binds) into the nucleus of sensory neurons blocks long-term facilitation, presumably by competing for CREB with the natural gene sequence (Dash et al., 1990)

Whether CREB plays a role in LTP is not clear. CREB has not yet been observed in dentate granule cells, although adenylate cyclase is found in these neurons. Injection of the phosphodiesterase inhibitor rolipram to rats, which leads to accumulation of cAMP in the brain, does not induce expression of *fos*, *jun*, or *zif/268* in dentate neurons, although other regions and cell types in the brain express increased levels of these IEGPs (Dragunow and Faull, 1989). Thus, it seems unlikely that cAMP is involved in IEG expression in granule cells, but it is still possible that calcium-dependent activation of CREB may be involved.

Another kinase that could be directly responsible for IEG induction is Ca^{++}/CaM protein kinase III, which phosphorylates the protein synthesis factor EF-2, a 100-kDa protein involved in the elongation of the polypeptide chain (Ryazanov, 1987). This factor is inactivated in its phosphorylated form, an event that occurs when a cell moves from a quiescent state into a proliferative state (Ryazanov and Spirin, 1990). Transcriptional activation of IEGs may then occur by shutting down translation of short-lived IEG repressors. This effect can be simulated by cycloheximide, an inhibitor of elongation during protein synthesis. Because phosphorylation of EF-2 has the same inhibitory effect on translation, Ryazanov and Spirin (1990) have suggested that this may be the critical factor allowing the activation of IEGs in cells during transition out of the quiescent state. In LTP, this mechanism could provide a link between NMDA receptor activation, the subsequent increase in intracellular Ca^{++}, and the activation of a cascade of gene responses relevant to the long-term maintenance of LTP. We are currently investigating the phosphorylation status of EF-2 in the dentate gyrus and how this changes during the induction and stabilization of LTP.

Role of IEG Trancription Factors in the Stabilization of LTP

The time course for induction of the IEG transcription factors, i.e., occuring with some delay yet lasting for a period of hours, indicates that they are not directly governing either initial LTP induction (occuring within minutes) or LTP persistence (lasting days and weeks). It is presumed, therefore, that IEG responses are linked to LTP persistence via IEGP transcription factors regu-

lating the expression of other, effector genes. These other genes, as yet uncharacterized, must then generate proteins that are transported to the potentiated synapses and interact with structural or functional changes already there, such that these synaptic changes become resistant to decay.

Two general forms of interaction at the synapses, occuring separately or together, can be suggested. First, the effector proteins may directly support the LTP expression mechanisms, and LTP will last as long as these effector proteins remain in elevated concentrations. One example is a long-term increase in protein kinase C (PKC) concentrations, resulting in a lasting enhancement of the phosphorylation state of substrate proteins. Indeed, a correlation has been reported between the phosphorylation state of a PKC substrate protein, F1/B50/GAP-43, and the degree of LTP present 3 d after induction (Lovinger et al., 1985).

Alternatively, the effector genes may only transiently increase (or decrease) their expression and thus play a role only in the stabilization of LTP. Effector proteins would facilitate the generation of specific changes in synaptic structure or function, which would then decay (actively or passively) independently of further instructions from the nucleus. Given that IEGs are highly expressed during development, it is plausible that IEG responses after LTP induction may also relate to changes in the size, shape, or number of synapses (Lee et al., 1980; Desmond and Levy, 1983; Chang and Greenough, 1984), all correlates of LTP expression. Gene involvement in this growth response would be consistent with the idea of effector proteins stabilizing but not directly maintaining LTP.

In association with the need to know how effector proteins serve to stabilize or maintain LTP, it is also important to know which effector genes are being regulated by the pool of IEGPs induced by NMDA-receptor activation. *Fos* and *jun* family heterodimers and *jun* family homodimers bind to the AP-1 response element (TGACTCA). Thus, genes containing an AP-1-like sequence, e.g., proenkephalin, are potential targets for these transcription factors (White and Gall, 1987; Sonnenberg et al., 1989); and indeed, there is evidence that proenkephalin mRNA levels change after LTP (Morris et al., 1988). The consensus DNA sequence that *zif/268* binds to is a high-affinity binding site on DNA: GCGTGGGGCG (Christy and Nathans, 1989). *zif/268* is a zinc-finger protein such that finger 1 binds to the last three nucleotides (GCG), finger 2 binds near the center (GGG), and finger 3 binds near the 5' end (GCG) (Pavletich and Pabo, 1991). Genes containing this sequence in their upstream elements may be potential targets of *zif/268* regulation following LTP.

The fact that the expression of some IEGPs, such as *FosR* and *junB*, do not correlate well with either the induction or persistence of LTP raises some question about their role in this model of synaptic plasticity. It is possible, for example, that such genes are involved in stabilizing long-term depression of synaptic efficacy, which can occur at synapses not activated by the LTP-inducing stimulation (Lynch et al., 1977; Levy and Steward, 1979; Abraham and Goddard, 1983). Heterosynaptic long-term depression is also NMDA receptor-dependent in the hippocampus (Abraham and Wickens, 1991; Christie and Abraham, 1991), but it is not yet known how long this form of plasticity persists.

A second possibility is that these genes have nothing to do with synaptic plasticity as such but are involved in global adaptive metabolic responses to high activity rates or prolonged depolarization. On the other hand, we cannot yet rule out that *FosR* or *jun* proteins perform some important function with respect to LTP. These proteins combine with others to make up the actual transcription factors, e.g., AP-1, and it is the levels of such dimers that are critical for determining the relation of these IEGs to LTP processes. Such measurements have yet to be made.

Relation of IEGs and Behavior

Although nonseizure-producing, LTP-inducing stimulation causes an unusually synchronous

activation of neurons not likely to occur in normal behavioral situations. Whereas there is good reason to believe that LTP is nevertheless naturally occuring (Abraham, 1988; Barnes, 1988), the question remains whether IEGs would correspondingly be induced and implicitly, whether any such LTP would persist over days or weeks.

The findings that IEGs show constitutive expression suggest that their expression may be relevant behaviorally. For example, there is strong basal expression of *zif/268* in the neocortex, septal nuclei, subiculum, and CA1 neurons in the hippocampus (Herdegen et al., 1990; Mack et al., 1990; Schlingensiepen et al., 1991; Schreiber et al., 1991; Richardson et al., in press), although basal expression of *zif/268* is low in the dentate gyrus. Furthermore, we have found that 4 h of anesthesia (pentobarbital) greatly reduces basal *zif/268* expression throughout these areas (Richardson et al., in press). This shows that turnover of basal *zif/268* occurs with a time course similar to the LTP-inducible expression in dentate granule cells and suggests that basal *zif/268* expression is maintained by some aspect of the awake state.

Although the fact that *zif/268* is basally expressed supports its possible role in LTP even under relatively mild induction conditions, it seems implausible that all basal expression is related to synaptic plasticity. It should be kept in mind, therefore, that there probably exist multiple intracellular pathways governing *zif/268* expression, e.g., growth factor-induced vs depolarization-induced (Bartel et al., 1989), which may reflect different functional roles for the protein depending on the level of expression of other genes, including IEGs. It is also possible that there is a family of *zif/268* molecules, either mRNA or protein, that has yet to be resolved and that may be mediating different signal cascades.

There is growing interest in the possibility that IEGs can be induced by sensory or behavioral manipulations. An early study by Hunt et al. (1987) showed that c-*fos*-like immunoreactivity is induced in spinal cord neurons by noxious heat or chemical stimulation to the skin. The induction was specific to particular laminae, and the pattern and intensity of staining varied according the particular type of stimulation. Subsequently, this group demonstrated that a variety of other IEGs are also induced in the spinal cord by thermal stimulation of the skin (Wisden et al., 1990). A number of other studies have now appeared demonstrating the induction of c-*fos* in various sensor systems following appropriate stimulation (Bullitt, 1989; Sharp et al., 1989; Carter, 1990; Abe et al., 1991).

Initial attempts to show learning-specific IEG induction were less successful. Neither rats trained in the Morris water maze (a spatial memory task) nor motor activity controls showed an increase in c-*fos* or *zif/268* expression in the dentate gyrus relative to naive animals (Wisden et al., 1990). Acquisition of a footshock-motivated brightness discrimination task was accompanied by an increase in hippocampal c-*fos* mRNA, but this also occured after pseudotraining and thus was probably related to the footshock stimulation rather than to learning *per se* (Tischmeyer et al., 1990). This conclusion is supported by Schreiber et al. (1991), who showed that restraint plus tail shock induces c-*fos* and *zif/268* in the neocortex and hippocampus. On the other hand, recent experiments in chicks showed increases in both c-*fos* and c-*jun* mRNA shortly after discrimination training. The increases were not owing to motor activity, since previously trained chicks exhibited smaller changes after performing the task, nor to stress or arousal, since monocular occlusion led to unilateral changes in c-*fos* (Anokhin and Rose, 1990; Anokhin et al., 1991). These data support the hypothesis that IEGs may play a role in long-term memory consolidation although, as in LTP, a causal role for IEGs in the consolidation or stabilization processes remains to be conclusively demonstrated.

Summary

There is now good evidence that both mRNA and protein levels of various IEGs are increased in the hippocampal LTP model of synaptic plas-

ticity. The increases occur postsynaptically, are rapid and transient (characteristic of IEGs in other cell systems), are linked to NMDA receptor activation, and are sensitive to pentobarbital anesthesia. The degree of IEG induction correlates better with the persistence of LTP than with its initial induction, with *zif/268* showing the best correlations so far. The second messenger systems involved in initiating IEG transcription are unknown, although it is suggested that phosphorylation of elongation factor-2 may play a role by inhibiting the synthesis of rapidly turning over gene repressors. The mechanisms by which IEG induction subsequently leads to LTP stabilization are also uncertain. The key to this may be the identification of the putative "late effector" genes, whose expression is affected by IEGPs.

The central question of whether altered gene expression is an essential part of the maintenance of LTP and thereby neural plasticity remains to be answered. It seems likely, however, given that a "classical" signaling pathway seems to be operating in the establishment of LTP, that differential gene expression will be an important feature of the event. Furthermore, since IEGs are constitutively expressed in awake animals and respond to sensory and behavioral manipulations as well as LTP induction, the suggestion that IEGs may play a role in long-term memory consolidation continues to remain tenable.

Acknowledgments

The authors are grateful to T. Curran for the *fos* antibody, R. Bravo for the *zif/268* (Krox-24) antibody, and D. Nathans for the *zif/268* cDNA probe that we have used for our experiments. We thank K. Jeffery, A. Jellie, P. Lawlor, S. E. Mason, C. Richardson, and J. Williams for their contributions to our experiments as described here. This research and the preparation of this manuscript were supported by grants from the N. Z. Medical Research Council and the Human Frontiers in Science Program to W. C. Abraham and W. P. Tate and from the N. Z. Medical Research Council, the Auckland Medical Research Foundation and the NZ Lottery Board to M. Dragunow.

References

Abe H., Rusak B., and Robertson H. A. (1991) Photic induction of *fos* protein in the suprachiasmatic nucleus is inhibited by the NMDA receptor antagonist MK-801. *Neurosci. Lett.* **127,** 9–12.

Abraham W. C. (1988) Long-term potentiation as a possible associative memory mechanism in the brain. *N. Z. J. Psych.* **17,** 49–58.

Abraham W. C. and Goddard G. V. (1983) Asymmetric relations between homosynaptic long-term potentiation and heterosynaptic long-term depression. *Nature* **305,** 717–719.

Abraham W. C. and Otani S. (1991) Macromolecules and the maintenance of long-term potentiation. *Kindling and Synaptic Plasticity.* Morrell F., ed., Birkhauser, Cambridge, MA.

Abraham W. C. and Wickens J. R. (1991) Heterosynaptic long-term depression is facilitated by blockade of inhibition in area CA1 of the hippocampus. *Brain Res.* **546,** 336–340.

Anokhin K.V. and Rose S. P. R. (1990) Learning-induced increase of immediate early gene messenger RNA in the chick forebrain. *Eur. J. Neurosci.* **3,** 162–167.

Anokhin K. V., Mileusnic R., Shamkina I.Y., and Rose S. P. R. (1991) Effects of early experience on *c-fos* gene expression in the chick forebrain. *Brain Res.* **544,** 101–107.

Auwerx J. and Sassone-Corsi P. (1991) IP-1: A dominant inhibitor of *fos/jun* whose activity is modulated by phosphorylation. *Cell* **64,** 983–993.

Barnes C. A. (1988) Spatial learning and memory processes: the search for their neurobiological mechanisms in the rat. *Trends Neurosci.* **11,** 163–169.

Bartel D. P., Sheng M., Lau L. F., and Greenberg M. E. (1989) Growth factors and membrane depolarization activate distinct programs of early response gene expression: dissociation of *fos* and jun induction. *Genes Dev.* **3,** 304–313.

Bliss T. V. P. and Gardner-Medwin A. R. (1973) Long-lasting potentiation of synaptic transmission in the dentate area of the unanaesthetised rabbit following stimulation of the perforant path. *J. Physiol.* **232,** 357–374.

Bliss T. V. P. and Lømo T. (1973) Long-lasting poten-

tiation of synaptic transmission in the dentate area of the anaesthetised rabbit following stimulation of the perforant path. *J. Physiol.* **232,** 331–356.

Bliss T. V. P. and Lynch M. A. (1988) Long-term potentiation of synaptic transmission in the hippocampus: properties and mechanisms. *Long-term Potentiation: From Biophysics to Behavior.* Deadwyler S. and Landfield P., eds., Liss, New York, pp. 3-72.

Bullitt E. (1989) Induction of c-*fos*-like protein within the lumbar spinal cord and thalamus of the rat following peripheral stimulation. *Brain Res.* **493,** 391–397.

Carter D. A. (1990) Temporally defined induction of c-*fos* in the rat pineal. *Biochem. Biophys. Res. Comm.* **166,** 589–594.

Chang F.-L. and Greenough W. T. (1984) Transient and enduring morphological correlates of synaptic activity and efficacy change in rat hippocampal slice. *Brain Res.* **309,** 35–46.

Changelian P. S., Feng P., King T. C., and 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. USA* **86,** 377–381.

Chiu R., Boyle W. J., Meek J., Smeal T., Hunter T., and Karin M. (1988) The c-*fos* protein interacts with c-*jun*/AP-1 to stimulate transcription of AP-1 responsive genes. *Cell* **54,** 541–552.

Christie B. R. and Abraham W. C. (in press) NMDA-dependent heterosynaptic long-term depression in the dentate gyrus of anaesthetized rats. *Synapse.*

Christy B. and Nathans D. (1989) DNA binding site of the growth factor-inducible protein Zif268. *Proc. Natl. Acad. Sci. USA* **86,** 8737–8741.

Christy B. A., Lau L. F., and Nathans D. (1988) A gene activated in mouse 3T3 cells by serum growth factors encodes a protein with "zinc finger" sequences. *Proc. Natl. Acad. Sci. USA* **85,** 7857–7861.

Cole A. J., Saffen D. W., Baraban J. M., and 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.

Collingridge G. L., Kehl S. J., and McLennan H. (1983) Excitatory amino acids in synaptic transmission in the Schaffer collateral-commissural pathway of the rat hippocampus. *J. Physiol.* **334,** 33–46.

Curran T. and Morgan J. I. (1985) Superinduction of c-*fos* by nerve growth factor in the presence of peripherally active benzodiazepines. *Science* **229,** 1265–1268.

Curran T. and Morgan J. I. (1987) Memories of *fos*. *BioEssays* **7,** 255–258.

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

Deadwyler S. A., Dunwiddie T., and Lynch G. (1987) A critical level of protein synthesis is required for long-term potentiation. *Synapse* **1,** 90–95.

Desmond N. L. and Levy W. B. (1983) Synaptic correlates of associative potentiation/depression: an ultrastructural study in the hippocampus. *Brain Res.* **265,** 21–30.

Doucet J. P., Squinto S. P., and Bazan N. G. (1990) Fos-Jun and the primary genomic response in the nervous system. *Mol. Neurobiol.* **2,** 27–55.

Douglas R. M. and Goddard G. V. (1975) Long-term potentiation of the perforant path-granule cell synapse in the rat hippocampus. *Brain Res.* **86,** 205–215.

Douglas R. M., Dragunow M., and Robertson H. A. (1988) High-frequency discharge of dentate granule cells, but not long-term potentiation, induces c-*fos* protein. *Mol. Brain Res.* **4,** 259–262.

Dragunow M., Abraham W. C., Goulding M., Mason S. E., Robertson H. A., and Faull R. L. M. (1989) Long-term potentiation and the induction of c-*fos* mRNA and proteins in the dentate gyrus of unanesthetized rats. *Neurosci. Lett.* **101,** 274–280.

Dragunow M. and Faull R. L. M. (1989) Rolipram induces c-*fos* protein-like immunoreactivity in ependymal and glial-like cells in adult rat brain. *Brain Res.* **501,** 382–388.

Dragunow M. and Faull R. L. M. (1990) MK801 induces c-*fos* protein in thalamic and neocortical neurons of rat brain. *Neurosci. Lett.* **113,** 144–150.

Dragunow M., Goulding M., Faull R. L. M., Ralph R., Mee E., and Frith R. (1990) Induction of c-*fos* mRNA and protein in neurons and glia after traumatic brain injury: pharmacological characterization. *Exp. Neurol.* **107,** 236–248.

Dragunow M., Peterson M. R., and Robertson H. A. (1987) Presence of c-*fos*-like immunoreactivity in the adult rat brain. *Eur. J. Pharmacol.* **135,** 113,114.

Dragunow M. and Robertson H. A. (1987) Kindling stimulation induces c-*fos* protein(s) in granule cells of the rat dentate gyrus. *Nature* **329,** 441,442.

East S. J. and Garthwaite J. (1991) NMDA receptor activation in rat hippocampus induces cyclic GMP through the L-arginine nitric oxide pathway. *Neurosci. Lett.* **123,** 17–19.

Frey U., Krug M., Brödemann R., Reymann K., and

Matthies H. (1989) Long-term potentiation induced in dendrites separated from rat's CA1 pyramidal somata does not establish a late phase. *Neurosci. Lett.* **97,** 135–139.

Goelet P., Castellucci V. F., Schacher S., and Kandel E. R. (1986) The long and the short of long-term memory—a molecular framework. *Nature* **322,** 419–422.

Gonzalez G. A. and Montminy M. R. (1989) Cyclic AMP stimulates somatostatin gene transcription by phosphorylation of CREB at serine 133. *Cell* **59,** 675–680.

Greenberg M. E. and Ziff L. (1984) Stimulation of 3T3 cells induces transcription of the c-*fos* proto-oncogene. *Nature* **331,** 433–437.

Greenberg M. E., Ziff E. B., and Greene L. A. (1986) Stimulation of neuronal acetylcholine receptors induces rapid gene transcription. *Science* **234,** 80–83.

Gustafsson B., Wigström H., Abraham W. C., Huang Y.-Y. (1987) Long-term potentiation in the hippocampus using depolarizing current pulses as the conditioning stimulus to single volley synaptic potentials. *J. Neurosci.* **7,** 774–780.

Gustafsson B., Asztely F., Hanse E., and Wigström H. (1989) Onset characteristics of long-term potentiation in the guinea-pig hippocampal CA1 region in vitro. *Eur. J. Neurosci.* **1,** 382–394.

Hendry I. A. (1973) Trans-synaptic regulation of tyrosine hydroxylase activity in a developing mouse sympathetic ganglion: effects of nerve growth factor (NGF), antiserum and pempidine. *Brain Res.* **56,** 313–320.

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

Hunt S. P., Pini A., and Evan G. (1987) Induction of c-*fos*-like protein in spinal cord neurons following sensory stimulation. *Nature* **328,** 632–634.

Jeffery K. J., Abraham W. C., Dragunow M., and Mason S. E. (1990) Induction of *fos*-like immunoreactivity and the maintenance of long-term potentiation in the dentate gyrus of unanesthetized rats. *Mol. Brain Res.* **8,** 267–274.

Krug M., Lössner B., and 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.

Kruijer W., Cooper J. A., Hunter T., and Verma I. M. (1984) Platelet-derived growth factor induces rapid but transient expression of the c-*fos* gene and protein. *Nature* **312,** 711–716.

Lau L. F. and 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. USA* **84,** 1182–1186.

Lee K. S., Schottler F., Oliver M., and Lynch G. (1980) Brief bursts of high-frequency stimulation produce two types of structural change in rat hippocampus. *J. Neurophysiol.* **44,** 247–258.

Lemaire P., Revelant O., Bravo R., and Charnay P. (1988) Two mouse genes encoding potential transcriptional factors with identical DNA-binding domains are activated by growth factors in cultured cells. *Proc. Natl. Acad. Sci. USA* **85,** 4691–4695.

Levy W. B. and Steward O. (1979) Synapses as associative memory elements in the hippocampal formation. *Brain Res.* **175,** 233–245.

Lovinger D. M., Akers R. F., Nelson R. B., Barnes C. A., McNaughton B. L., and Routtenberg A. (1985) A selective increase in phosphorylation of protein F1, a protein kinase C substrate, directly related to three day growth of long-term synaptic enhancement. *Brain Res.* **343,** 137–143.

Lynch G. S., Dunwiddie T., and Gribkoff V. (1977) Heterosynaptic depression: a post-synaptic correlate of long-term potentiation. *Nature* **266,** 737–739.

MacDermott A. B., Mayer M. L., Westbrook G. L., Smith S. J., and Barker J. L. (1986) NMDA-receptor activation increases cytoplasmic calcium concentration in cultured spinal cord neurones. *Nature* **321,** 519–522.

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

Matthies H. (1989) In search of cellular mechanisms of memory. *Prog. Neurobiol.* **32,** 277–349.

Mayer M. L. and Miller R. J. (1990) Excitatory amino acid receptors, second messengers and regulation of intracellular Ca^{2+} in mammalian neurons. *Trends Pharmacol. Sci.* **11,** 254–260.

Mellon P. L., Clegg C. H., Correll L. A., and McKnight G. S. (1989) Regulation of transcription by cyclic AMP-dependent protein kinase. *Proc. Natl. Acad. Sci. USA* **86,** 4887–4891.

Mihaly A., Olah Z., Krug M., Kuhnt U., Matthies H., Rapp U. R., and Joo F. (1990) Transient increase of *raf* protein kinase-like immunoreactivity in the rat dentate gyrus during long-term potentiation. *Neurosci. Lett.* **116,** 45–50.

Morgan J. I., Cohen D. R., Hempstead J. L., and Curran

T. (1987) Mapping patterns of c-*fos* expression in the central nervous system after seizure. *Science* **237,** 192–197.

Morgan J. I. and Curran T. (1986) Role of ion flux in the control of c-*fos* expression. *Nature* **322,** 552–555.

Morgan J. I. and Curran T. (1991) Stimulus-transcription coupling in the nervous system: involvement of the inducible proto-oncogenes *fos and jun*. *Ann. Rev. Neurosci.* **14,** 421–451.

Morris B. J., Feasey K. J., ten Bruggencate G., Herz A., and Hollt V. (1988) Electrical stimulation *in vivo* increases the expression of proenkephalin mRNA and decreases the expression of prodynorphin mRNA in rat hippocampal granule cells. *Proc. Natl. Acad. Sci. USA* **85,** 3226–3230.

Müller R., Bravo R., Burckhardt J., and Curran T. (1984) Induction of c-*fos* gene and protein by growth factors precedes activation of c-*myc*. *Nature* **312,** 716–720.

Nakabeppu Y., Ryder K., and Nathans D. (1988) DNA binding activities of three murine jun proteins: stimulation by *fos*. *Cell* **55,** 907–915.

Nigg E. A., Hilz H., Eppenberger H. M., and Dutly F. (1985) Rapid and reversible translocation of the catalytic subunit of cAMP-dependent protein kinase type II from the Golgi complex to the nucleus. *EMBO J.* **86,** 4887–4891.

Olenik C., Lais A., and Meyer D.K. (1991) Effects of unilateral cortex lesions on gene expression of rat cortical cholecystokinin neurons. *Mol. Brain Res.* **10,** 259–265.

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

Pavletich N. P. and Pabo C. O. (1991) Zinc finger-DNA recognition: crystal structure of a *zif268*-DNA complex at 2.1 A°. *Science* **252,** 809–817.

Phillips L. L. and Steward O. (1990) Increases in mRNA for cytoskeletal proteins in the denervated neuropil of the dentate gyrus: an *in situ* hybridization study using riboprobes for *B*-actin and *B*-tubulin. *Mol. Brain Res.* **8,** 249–257.

Racine R. J., Milgram N.W., and Hafner S. (1983) Long-term potentiation phenomena in the rat limbic forebrain. *Brain Res.* **260,** 217–232.

Richardson C. L., Tate W. P., Mason S. E., Lawlor P. A., Dragunow M., and Abraham W. C. (in press) Correlation between the induction of an immediate early gene, *zif/268,* and long-term potentiation in the dentate gyrus. *Mol. Brain Res.*

Ryazanov A. G. (1987) Ca^{2+}/calmodulin-dependent phosphorylation of elongation factor 2. *FEBS Lett.* **214,** 331–334.

Ryazanov A. G. and Spirin A. S. (1990) Phosphorylation of elongation factor 2: a key mechanism regulating gene expression in vertebrates. *New Biol.* **2,** 843–850.

Ryder K. and Nathans D. (1988) Induction of proto-oncogene c-*fos* by serum growth factors. *Proc. Natl. Acad. Sci. USA* **85,** 8464–8467.

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

Schlingensiepen K. -H., Lüno K., and Brysch W. (1991) High basal expression of the *zif/268* immediate early gene in cortical layers IV and VI, in CA1 and in the corpus striatum—an *in situ* hybridization study. *Neurosci. Lett.* **122,** 67–70.

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

Sharp F. R., Gonzalez M. F., Sharp J. W., and Sagar S. M. (1989) c-*fos* expression and (^{14}C) 2-deoxyglucose uptake in the caudal cerebellum of the rat during motor/sensory cortex stimulation. *J. Comp. Neurol.* **284,** 621–636.

Sonnenberg J. L., Rauscher III F. J., Morgan J. I., and Curran T. (1989) Regulation of proenkephalin by *fos and jun*. *Science* **246,** 1622–1625.

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

Teyler T. J. and Discenna P. (1984) Long-term potentiation as a candidate mnemonic device. *Brain Res. Rev.* **7,** 15–28.

Tippetts M. T., Varnum B. C., Lim R. W., and Herschman H. R. (1988) Tumor promoter-inducible genes are differentially expressed in the developing mouse. *Mol. Cell. Biol.* **8,** 4570–4572.

Tischmeyer W., Kaczmarek L., Strauss M., Jork R., and Matthies H. (1990) Accumulation of c-*fos* mRNA in rat hippocampus during acquisition of a brightness discrimination. *Behav. Neural Biol.* **54,** 165–171.

White J. D. and Gall C. M. (1987) Differential regulation of neuropeptide and proto-oncogene mRNA

content in the hippocampus following recurrent seizures. *Mol. Brain Res.* **3,** 21–29.

Wigström H., Gustafsson B., Huang Y.-Y., and Abraham W. C. (1986) Hippocampal long-term potentiation is induced by pairing single afferent volleys with intracellularly injected current pulses. *Acta Physiol. Scand.* **126,** 317–319.

Williams J. H., Errington M. L., Lynch M. A., and Bliss T. V. P. (1989) Arachidonic acid induces a long-term activity-dependent enhancement of synaptic transmission in the hippocampus. *Nature* **341,** 739–742.

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