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Full-length review

Sensory regulation of immediate–early gene expression in mammalian visual cortex: implications for functional mapping and neural plasticity

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Abstract

The expression of immediate–early genes that code for transcription factors has been extensively studied in the brain with regard to imaging functional activity. The components of the AP-1 transcription factor—in particular, c-Fos—and Zif268 have been widely used for this purpose. However, the precise details by which they are induced after synaptic stimulation remain unknown. Furthermore, the roles of these two proteins in neurons remains speculative and include such varied functions as short-term maintenance of cellular homeostasis to long-term changes that guide cortical plasticity. Current efforts at elucidating the physiological roles of AP-1 and Zif268 rely on assessing their expression in response to different conditions of sensory and pharmacological stimulation. In this review, we have examined the expression patterns of these transcription factors in the mammalian visual cortex under different conditions, with particular emphasis on the constitutive levels and how they change after visual deprivation and stimulation. A synthesis of this information offers further insight into their likely functions and the extent to which transcription factors may represent patterns of neural activity as a possible prelude to plastic events.

Keywords: Transcription factor; AP-1; c-Fos; Zif268; Neocortex; Development

Contents

Abbreviations: EMSA electrophoretic mobility shift assay; Fos-LI Fos-like immunoreactivity; ICC immunocytochemistry; IEG immediate–early gene; ISHH in situ hybridization histochemistry; ITF inducible transcription factor; LGN lateral geniculate nucleus; LK leukotrienes; NMDA *N*-methyl-D-aspartate; PD postnatal day; PG prostaglandins; TF transcription factor; TTX tetrodotoxin; VSCC voltage sensitive calcium channel

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1. Introduction

The immediate early genes (IEGs) c-*fos* and *zif268* [1] have become popular neurobiological tools for mapping functional activity [17,36,64,127,131,136,137]. We now know that the expression of these genes is strongly, though not exclusively, linked to synaptic stimulation and that their products may be involved in key aspects of normal cellular function. Although much progress has been made toward understanding the intracellular processes that guide the expression of these genes, the precise physiological roles of the proteins encoded by them remain largely unknown [64]. Members of the IEG family in general are activated shortly after cell stimulation and without the requirement for de novo protein synthesis. The products of many of these genes have been observed in a wide range of tissues and under a variety of stimulation conditions [58,107,109].

In the brain, IEGs that are linked to neural activity and which have been used in mapping applications generally encode proteins that serve as transcription factors (TF). The TF category includes many different products, most of which are not IEGs even if they are inducible by such modifications as phosphorylation and translocation from the cytoplasm to the nucleus. In this review, we use the common term ITF (inducible transcription factor) with the understanding that induciblity in this context refers to gene expression. While it is known that ITFs serve as regulators of gene expression, much of our present knowledge regarding their induction is largely based on correlating expression patterns to various types of stimulation. Indeed, the correlative approach presently provides the best means to deduce the functions of various ITFs in brain physiology. Intervention approaches-such as gene knockout and antisense knockdown-are potentially more powerful in revealing their function [13,49,135,154]. However, these approaches are fraught with technical difficulties at present. Furthermore, compensatory mechanisms may be initiated after gene knockout that can obscure the results [28,29,43,73,90,139,146,153].

To interpret ITF functional maps of the brain, it is necessary to understand the physiological context within which the various genes in this category may be operative. The purpose of this review is to assimilate the known data on the expression patterns of ITFs under different conditions of brain activity. Since the physiological, and even molecular, meaning of such labeling remains elusive, such a synthesis of information may offer clues into their possible functions. Indeed, one of the vexing issues in this field is whether ITF labeling exclusively represents patterns of neural activity within the cortex. For example, the influence of various hormones, neurotrophins, and possibly even hyperpolarizing signals may have an effect on ITF expression as well. Conversely, neural activity in certain regions when visualized by 2-deoxyglucose accumulation and electrophysiology are not necessarily accompanied by elevated ITF expression [36,131].

Given the complex expression patterns of various ITFs in different tissues and under different paradigms, it is now timely to sort out the details within a specific system. For this, we have chosen to restrict our survey to a system that has been intensely studied—the mammalian visual cortex.

[1] Specific genes such as c-*fos* or *zif 268* are designated by italics, while the proteins encoded by the genes (in this case, c-Fos and Zif 268) are written in plain type with one capital letter, and DNA-binding activities of the transcription factors (AP-1, ZIF 268) are presented in the upper case [76].

The three issues that we wish to explore in detail in this review are the basal levels of expression, down-regulation following visual deprivation, and up-regulation or induction. For all of these situations, comparisons will be made between adult and developing cortex.

1.1. Cortical development and the critical period

One of the most striking facets of ITF expression is within the context of postnatal development. Cortical development shortly after birth involves extensive changes in neuronal connectivity and responsiveness [34,40,48,63,74,118,132,148]. The modifiability of the cortical tissue at this stage is believed to be driven by sensory input. Most of the experiments to date concern the development of the visual cortex, where the existence of a critical period for the plasticity of thalamocortical axon terminations and for modifications of ocular dominance columns has been well documented. Although the temporal boundaries of the critical period cannot be sharply defined, the most malleable period for thalamocortical connections extends 20–35 days postnatally in the rat, 4–7 weeks in the cat, and from birth to about 8 weeks in the monkey [34,38,40,62,95]. During this period, blocking sensory input from one eye results in a shift of the responsiveness of cortical neurons toward the open eye. Furthermore, the duration of the critical period can be prolonged by dark-rearing after birth. Together, these findings have fortified the notion that ocular dominance plasticity is largely guided by sensory input.

While it was evident from early studies that the neural processes underlying such plasticity are particularly robust during post-natal development of the nervous system, it is now clear that even in the adult such mechanisms are operative and can have a profound influence upon the neural circuits that mediate a number of different cortical operations [41,45–47,78,103,104,118,122,123,157]. At the neuronal level, these changes are believed to be initiated by the interaction of extracellular signaling molecules, such as neurotrophins and neurotransmitters, acting upon specific receptor systems. The NMDA receptor-Ca^{2+} ionophore complex has been widely studied in this regard and repeatedly implicated in neuronal plasticity [25,27,35,39,40,85]. However, details of the manner in which intracellular molecular processes are transformed into a plastic event are largely unknown. It is believed though that the coordinate effects of these processes ultimately converge upon the regulation of gene expression. The discovery that neuronal activation provokes expression of ITFs has thus focused attention on their role in regulating long-term changes in brain function [30,31,56,64,70,71,88,108–110,125,133].

1.2. AP-1 and ZIF268 transcription factors

The two main transcription factors investigated in this context are AP-1 and ZIF268. These products belong to separate classes and are characterized by different molecular structures. AP-1 belongs to the leucine-zipper class of transcription factors in which protein dimers are formed from heterogeneous groups that are linked by a leucine-zipper [109]. Both the Fos and Jun family of nuclear phosphoproteins may participate in forming homo- or heterodimeric AP-1 complexes that bind to a DNA consensus sequence (TGAC/GTCA [109]) that is known to be present in numerous gene promoters. Upon binding, AP-1 may either activate or repress the candidate gene [3,86], although no firm evidence has yet emerged for AP-1 driving specific genes in the brain. The Fos and Jun families include several known members: c-Fos, FosB, Fra-1, Fra-2 and c-Jun, JunB, JunD [109]. It has been shown recently that the content of AP-1 may vary under different physiological situations and even closely related members of the same family may contribute to quite distinct biological phenomena [61,75,83].

ZIF268 belongs to the zinc-finger class of transcription factors. The gene encoding this ITF has been cloned independently by several groups and is therefore recognized by different names—NGFI-A, Krox-24, Egr-1, TIS-8, ZENK [15,16,23,24,68,91,96,105,142]. The protein contains finger-like protrusions that are stabilized by ionic zinc and which act directly on the regulatory sequence GCGG/TGGGCG [23,91]. The presence of ZIF268 at the promoter site serves to modulate the expression of the target gene. As with AP-1, the genes that are affected by ZIF268 in the neocortex are largely unknown.

Studies on cultured neurons, including those from cerebral cortex, have shown that glutamate binding on NMDA receptors is capable of inducing both c-*fos* and *zif268*

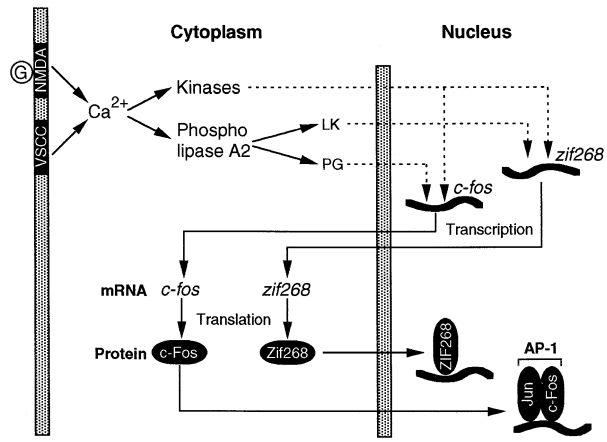

Fig. 1. Molecular pathways involved in c-*fos* and *zif268* induction. NMDA receptor activation by glutamate (G) or opening of voltage sensitive calcium channels (VSCC) after depolarization produce Ca^{2+} influx that initiates a cascade of events that are guided by effector enzymes (kinases, phospholipase A2). Ultimately, this results in increased transcription of both c-*fos* and *zif268* (dotted lines). After translation in the cytoplasm, the Zif268 protein migrates into the nucleus where it serves as a transcription factor (ZIF268). The protein c-Fos forms a dimer with a member of the Jun family to produce the AP-1 transcription factor in the nucleus.

[4,5,26,60,72,87,92,143–145,151] (Fig. 1). Similar results have been obtained by direct treatment of animals with NMDA [32,69,72,111,138]. However, it has been reported that c-*fos* expression may also be triggered by the opening of voltage sensitive calcium channels (VSCC) that may occur after activation of AMPA-kainate receptors by glutamate [4,44,94,113,149]. The elevation of cytosolic Ca^{2+} after stimulation by either means has been implicated as the major mediator of induction of both genes. However, it now appears that there is divergence in the intracellular pathways by which this information is subsequently conveyed to the nucleus to initiate transcription of c-*fos* and *zif268* [93] (Fig. 1). The same excitatory amino acid neurotransmitter may therefore produce its effects through different receptors, whose effects converge upon a single second messenger (Ca^{2+}) and which then proceed along divergent signal transduction pathways to induce two different genes.

2. Experimental approaches to studying ITF expression

2.1. Conditions that affect ITF levels

In this review, we will examine how the prevailing sensory input affects the nature of ITF expression. The main parameters that need to be considered in this regard are the standard laboratory environment, visual deprivation, and stimulation. The standard condition allows an estimation of the basal level of gene expression. Sensory deprivation reveals whether the basal level of the gene product was dependent upon the prevailing sensory input, and if so, provides an estimate of the degree to which this product may be down-regulated by its removal. In some instances, removal of afferent input may also cause up-regulation of the product and this too may be estimated. The stimulation condition reveals the extent to which the gene product is induced by sensory input. However, this does not replicate the basal condition since the neurons have undergone a period of quiescence prior to activation. Whereas the basal condition reveals gene expression due to ongoing sensory input, the stimulation condition provides a measure of the response to a change in the sensory environment. This is of critical importance since in some cases ITFs are normally expressed at low basal levels but rapidly and transiently induced by changes in environmental conditions. This may be studied by simple exposure to light after a period of visual deprivation or by more elaborate behavioral settings where visual cues may be included as part of a conditioned stimulus.

Once the experimental situation is defined, the next parameter to consider is the duration of sensory manipulation. This issue is largely driven by the manner in which the detection of the gene product is to take place. The molecular scenario for typical ITF expression is the following. Extracellular stimulation initiates a cascade of signal transduction mechanisms leading to the activation of transcription within minutes. This leads to rapid accumulation of a specific mRNA species within the cytoplasm, reaching a peak at about one half hour, and persisting with a very short half-life ranging in minutes. The mRNAs then serve as templates for translation into protein products. In most cases the accumulation of proteins in the nucleus peaks at 1–2 h after the initial stimulation [14,22,97,152,156,158].

Much of our knowledge on this matter is derived from studies of cultured cells, including cortical neurons, that had been stimulated by specific agonists (e.g., NMDA) [26,60,72,151]. It is much more difficult to obtain the details of this scenario at the cellular level in the brain because of its complex circuitry. In general, there are several factors that may play a role in guiding the kinetics of ITF expression and persistence. The expression of some genes may be delayed and/or prolonged after stimulation is initiated, the kinetics of posttranslational modification (e.g., phosphorylation) may differ, and there may be different rates of degradation. These factors may either individually or collectively contribute to the temporal patterns of specific ITF expression.

The interactive nature of the cortical circuitry imposes a further complication in defining the routes by which ITF induction may occur (Fig. 2). The laminar composition of primary visual cortex (also known as area 17, V1, or striate cortex) can be partitioned into three general divisions-thalamorecipient, cortical integration, and subcortical output layers [33,74]. The input from the lateral geniculate nucleus (LGN) arrives into layer IVC (granular layer), from which it is transmitted to layers II/III. These layers send and receive connections to homotypical cortical areas

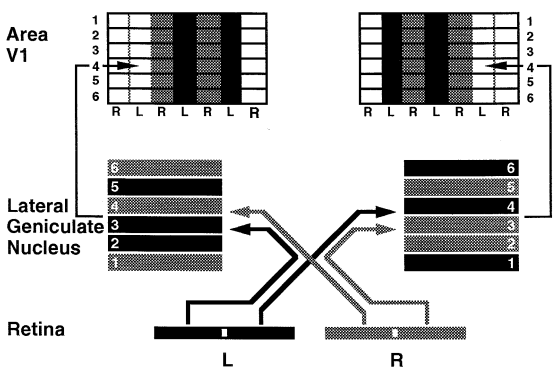

Fig. 2. Simplified diagram of early connections in primate visual cortex. Visual stimulation of the retina is conveyed to the lateral geniculate nucelus (LGN) into specific layers representing only one eye. Each LGN receives information from the nasal portion of the contralateral retina and the temporal portion of the ipsilateral retina. The LGN afferents containing monocular signals arrive into layer 4C (granular layer) of the primary visual cortex (area V1) where they remain segregated. This segregation is largely maintained throughout the other layers to produce vertically oriented ocular dominance columns. Although the basic retina-LGN-primary visual cortex connections are maintained in the rat and cat brain, specific aspects of this organization are different for each species.

in the opposite hemisphere and other cortical areas of the same hemisphere. From these supragranular layers, sensory signals are transmitted to layers V and VI and then back to layer IV. In all cases, the excitatory connections are believed to be mediated by glutamate [74]. The anatomical crosstalk among different layers generates uncertainty as to the source of the signal that is driving gene expression. For example, ITF expression in layer IVC neurons may be the result of excitatory input from a thalamic source or from intracortical ones. The reliance of c-*fos* and *zif268* expression upon NMDA receptor activation suggests the latter since the thalamo-recipient connections of layer IV are largely devoid of functional NMDA receptors [74]. If so, then ITF expression in these neurons is largely influenced by higher order neurons and not just by a direct feedforward pathway from retina to cortex.

2.2. Methodological considerations in ITF detection

There are several technical approaches to the study of ITF expression in the brain, the choice being largely guided by the nature of the inquiry. If ITF expression is being used for mapping purposes then either in situ hybridization histochemistry (ISHH) or immunohistochemistry (ICC) may be employed to label the mRNA and protein products respectively. The transient nature of IEG expression and the temporal differences between mRNA and protein accumulation are critical factors that affect ITF labeling. If ITFs expression is being used to study their biological function, then it is important to focus on the functional form of their products. This is not a trivial point because increased transcription of these genes may not necessarily be accompanied by translation under some conditions [60,161]. Even if the proteins are available in the cell, their functionality is not assured since this may rely on post-translational modification, such as phosphorylation and interaction with other partner proteins [12,67,82,109,147]. This is especially important for the AP-1 products.

To obtain veridical labeling of a particular gene product, it is necessary to study expression at the mRNA level. Although ISHH provides considerable confidence for such detection, the dominant procedure for visualizing the signal relies on autoradiography. This procedure suffers from poor spatial resolution and therefore cannot provide a direct display at the cellular level. Continuing developments in the non-radioactive ISHH technique should resolve this problem. Northern blotting gives the most reliable identification of mRNA as well as quantitative assessment of it levels. However, this is not a mapping tool since its use relies on homogenized tissue as the starting material.

For mapping purposes, the procedural simplicity and cellular-level resolution that is offered by ICC has led to its widespread use. However, ICC applications have sometimes been complicated by the close homologies of proteins belonging to the same class. This has been especially problematic for the Fos family of proteins and was previously an obstacle to evaluating the expression of specific AP-1 members since the specificity of the antigenic reaction was unclear. Thus, often in the case of c-Fos, a generalized term—Fos-LI (Fos-like immunoreactivity)—has been used. The recent availability of antibodies that recognize epitopes specific to individual AP-1 components has largely resolved this problem (see, e.g., [53,76]).

While ICC staining provides good qualitative results, quantitative analysis is difficult because of the non-linear nature of substrate amplification and technical problems of accurately determining the signal strength. The principal advantage of ICC labeling of ITFs is that staining is confined to the nucleus. This provides a punctate visual display of immunostained neurons that actually extends the resolution even beyond the cellular level. An additional advantage of the nuclear locus of the immunostained product is that double-labeling procedures can be employed by counterstaining the same tissue section for various products that are confined to the cytoplasm. This allows one to correlate the expression of a particular ITF with endogenous products that may have physiologic or morphologic specificity and thereby reveal a functional association [19,21].

An estimate of the functional form of a particular ITF can be obtained by analysis of its DNA-binding activity and is usually determined by electrophoretic mobility shift assay (EMSA), also known as gel shift assay [76,89]. This technique is based on in vitro interaction between purified protein extract and a radiolabeled gene regulatory (consensus) sequence. The binding of proteins that recognize this sequence produces a shifted band during gel electrophoresis. Protein composition within the transcriptional complex can be revealed by preincubation of the protein extract with specific antibodies, resulting in the appearance of a further ‘supershifted’ band (EMSA-supershift) [76]. This technique is of particular importance in assessing AP-1 levels because in addition to revealing the DNA-binding activity, it provides information on protein composition under specific situations.

3. Basal levels of ITF expression in the adult brain

3.1. The fos family

The presence of c-*fos* mRNA and protein has been investigated in various mammalian species and found to be detectable at very low levels throughout the neocortex [6,37,56,100,121,163,167]. Northern analysis has shown that c-*fos* mRNA levels in rat cerebral cortex oscillate in a circadian manner, reaching higher values during the dark phase of the diurnal cycle—i.e., the period of enhanced behavioral activity [50]. The visual cortex of the rat generally contains weak basal levels of c-*fos* mRNA [116,163].

Furthermore, ICC analysis with a c-Fos specific antibody has revealed very little staining [1], although a few neurons in layers II, IV, and VI were labeled [54]. Treatment of animals with MK-801—a non-competitive NMDA receptor antagonist—at doses ranging from 0.3 mg/kg to 8.0 mg/kg does not reduce c-*fos* mRNA, protein, or AP-1 levels appreciably [37,42,162]. Rather, such treatment produces striking c-Fos induction in the neocortex, most notably in layer IV and, to a lesser extent, in layers V and VI [37,42].

ISHH staining in visual cortex of adult cats has shown a weak presence of c-*fos* mRNA in layer VI of areas 17 and 18 [165]. In other areas of visual cortex, c-*fos* levels were even lower and often no distinct laminar pattern was discernible [165]. In vervet monkey (*Cercopithecus aethiops*) primary visual cortex, we have observed a weak basal presence of c-*fos* mRNA and protein [20].

The basal expression of FosB has been found to be restricted to a few scattered neurons in layers II, IV, V and VI of adult rat neocortex [42,54]. Kaminska et al. [76] have found that the low AP-1 levels under basal conditions in the rat contain FosB as the major Fos component. Alcantara and Greenough [1] have reported weak immunostaining in rat neocortex with an antibody that recognized c-*fos* as well as other fos-related antigens, including FosB. As with c-Fos, treatment of rats with both low and high doses of MK-801 resulted in increased FosB expression, mostly in layer IV and somewhat less in layers V and VI [42].

3.2. The jun family

In the rat, expression of all three *jun* mRNAs has been observed in neocortex with *c-jun* being the least abundant [101]. The ISHH results showed that *c-jun* mRNA labeling was confined to layers II/III and V/VI. Unlike c-*fos*, the basal level of *c-jun* did not appear to depend on the circadian cycle [102]. ICC experiments with c-Jun specific antibodies showed sparse labeling of neurons in layers II, III and VI. No effect of MK-801 treatment was observed on c-Jun levels in the neocortex [42]. In the cat, *c-jun* ISHH has revealed labeling of superficial and deep layers giving a bilaminar appearance in both primary visual complex as well as high order visual cortical areas [165]. We have also observed a similar bilaminar appearance in vervet monkey primary visual cortex treated to ISHH (unpublished observations).

In rat cerebral cortex, *junB* mRNA was reported to be highly expressed in layers II/III and V/VI and with no expression in layer IV [101]. In general, *junB* mRNA levels in rat cortex oscillate in a circadian manner, reaching higher values during the dark phase of the diurnal cycle [102]. ICC with a JunB specific antibody revealed sparse labeling of layer II during the light phase [54]. Treatment of animals with MK-801 at either 0.3 or 3.0 mg/kg significantly elevated JunB immunoreactivity, especially in layer IV [42].

Uniform, non-laminar *junD* mRNA distribution has been reported in rat cerebral cortex [101]. A similar labeling pattern was also seen with a JunD specific antibody [54]. The expression of JunD in the neocortex appears to be unaffected by treatment with MK-801 [42]. An investigation into AP-1 composition in rat visual cortex under basal conditions has revealed that JunD is the major Jun family member involved in its DNA-binding activity [76]. In cat visual cortex, *junD* mRNA levels were higher in superficial and deep cortical layers then in the layer IV of the area 17, but no clear lamination was observed in other regions [165].

3.3. zif268

High basal levels of *zif268* mRNA and Zif268 protein have been repeatedly observed in the visual cortex of various mammalian species. In the rat, both mRNA and protein levels are elevated in layers II, IV, and VI whereas layers III and V stain less intensely [54,55,98,129]. Worley et al. [162] reported that approximately 30% of cells in layer IV of rat and mouse were Zif268 immunopositive. The basal levels of *zif268* mRNA, protein and DNA-binding activity in adult rat cortex were markedly down-regulated by treating animals with MK-801 [42,162–164]. However, the doses applied (equal or higher than 0.3 mg/kg) are known to affect the behavioral state of the animal [51,134,159]. This may obscure the MK-801 results since it is unclear if the effect was produced by antagonism of NMDA receptors belonging to neurons expressing Zif268 or at earlier sites in the visual pathway. Basal expression of *zif268* has also been found to be down-regulated by blocking noradrenergic input [8].

In cat primary visual cortex, *zif268* mRNA levels are also high, particularly in the superficial and deep layers. One noteworthy finding in cat visual cortex is that *zif268* mRNA levels are lower in higher order areas where the laminar pattern also becomes less discernible [165]. Kaplan et al. [80,81] have also reported that Zif268 protein is expressed at high levels in layers II, III and VI. However, they noted a narrow band of staining in layer IVb at border with layer V.

In the adult monkey, both *zif268* mRNA and protein levels are expressed at high levels in layers II/III, parts of layer IV, and VI of primary visual cortex [17,19]. Within layer IV, much of the basal expression is restricted to layer IVCβ. There is less expression in layers IVCα and IVA. Layer IVB is the least stained and usually contains only a few labeled cells. As in the cat, Zif268 immunopositive cells are generally more numerous in primary visual cortex than in the extrastriate visual areas. Double-labeling of Zif268-positive neurons with MAP2 and SMI-32, both markers of pyramidal neurons, showed coincidental staining in all layers whereas such co-staining was absent if markers of inhibitory neurons were used, e.g., parvalbumin and calbindin D28K. Furthermore, Zif268 immunopositive

cells consistently showed pyramidal morphology when filled with lucifer yellow. Together, these results showed that Zif268 is largely expressed within a subpopulation of excitatory neurons in monkey visual cortex [19].

3.4. Summary

The basal level of c-*fos* expression is very weak in adult visual cortex, independent of the animal species, though it may be dependent on the behavioral state of the animal. Given the high levels of neural activity normally present in the visual cortex, and especially in the granular layer, it would seem that neuronal activity alone is not sufficient to drive c-*fos* expression. The basal expression of other members of the *fos* family also appears to be low, but nevertheless clearly higher than c-*fos*. Indeed, the weak AP-1 DNA-binding activity that prevails under basal conditions may be largely mediated by FosB in conjunction with a member of the Jun family.

The role of various receptors in regulating c-*fos* expression remains controversial. This is underscored by the MK-801 results which, given their antagonistic properties on NMDA receptors, would have been expected to block c-*fos* expression rather than inducing it. The possible explanations for this paradoxical result may include the involvement of other types of receptors in basal c-*fos* expression, the possibility that MK-801 at high doses may interact with NMDA receptors in an unknown manner, and indirect stimulatory effects mediated by cortical circuitry (see, e.g., [52,119]).

It appears that cortical expression of *jun* family members is quite heterogeneous in respect to laminar distribution, dependence on the behavioral state of the animal, and to NMDA receptor activation and blocking. The expression pattern of *junB* in all of these conditions bears similarities to c-*fos* expression. The absolute levels of all AP-1 components, whether they belong to the Fos or Jun families, are very low under basal conditions. Nevertheless, the data on mRNA, protein, and DNA-binding activities taken together suggest that the low level of AP-1 under this condition is composed of JunD (and possibly c-Jun) and FosB proteins.

There is striking contrast between expression of *zif268* and the AP-1 components under basal conditions. Whereas the AP-1 proteins of the Fos and Jun families were largely expressed at negligible to low levels, Zif268 has been found to be present at rather high levels. This suggests that *zif268* expression is driven by constitutive synaptic activity and corroborates the findings in cultured cortical neurons [113,114].

One aspect of the Zif268 immunostaining results in the thalamorecepient layers, may have a functional implication. The heavy Zif268 labeling found in layer IVb of the cat and IVCβ of the monkey may be due to the specific nature of the LGN input. Layer IVb in the cat receives an exclusive input from X-cells of the LGN whereas layer IVCβ in the monkey is driven by the parvocellular LGN layers [11,65,66,84,160]. Both of the these thalamic sources are known to be composed of cells that discharge in a sustained manner and whose firing generally coincides with the duration of visual stimulation [10,79]. Thus, the tonic firing characteristics of LGN neurons that project to these layers may account for the high constitutive expression of Zif268. In contrast, layers IVa in the cat and IVCα in the monkey receive mixed 'sustained' and 'transient' thalamic input [11,65]. This may account for the relatively reduced expression of Zif268 in these layers because the phasic discharge of at least a portion of the input abbreviates the temporal span of synaptic stimulation.

The assumption that maintained afferent activity may explain differences in Zif268 constitutive expression in visual cortex is also supported by the generally higher levels of immunostaining that is found in striate cortex when compared to other extrastriate visual areas. An alternative interpretation of these results, however, is that Zif268 expression specifically—and ITF expression in general—is attributable to particular classes of neurons whose distribution is heterogeneous among laminae and across cortical areas. For example, Zif268 appears to be restricted to cortical excitatory neurons, and even then, possibly to a restricted set within that group [19]. Thus, if the distribution of this subpopulation of neurons shows characteristic laminar and areal features, then Zif268 expression will also be faithful to that distribution.

4. Basal levels of ITF expression in the developing brain

4.1. Rat

The developmental expression of c-Fos and the fos-related antigens (Fras) has been studied in the rat [1]. In general, c-Fos specific immunostaining throughout neocortex has been found to be negligible during the entire developmental period. However, restricted expression of Fras has been observed upto postnatal day 9 (PD9) in the deep layers of visual cortex. A second peak of Fras expression is evident throughout all layers at PD12 and PD15. The authors interpret this rise as being associated with eye opening which occurs around this time. Kaminska et al. [77] have examined AP-1 levels during development of the rat visual cortex and found an increase in DNA-binding activity between PD14 and PD21, followed by a decrease at PD42. EMSA-supershift assay revealed the presence of Fra-2 and FosB in the AP-1 DNA-binding complex. In addition, a small supershift indicating the presence of c-Fos protein was detected in the gels.

Mellstroem et al. [101] compared *c-jun*, *junB* and *junD* mRNA levels in developing (PD15 and PD20) and adult rat brains. *c-jun* expression was found to be highest at PD15 and then declined to adult levels. The data presented

by them also reveal that *c-jun* expression in occipital cortex at PD15 is considerably higher than in the surrounding non-occipital regions. This pattern was exactly the opposite to that seen with *junB* mRNA staining where the occipital component was reduced in comparison to neighboring regions. *junD* mRNA expression was found to be ubiquitous throughout neocortex at PD15. EMSA-supershift experiments on PD21 rat visual cortex also showed the presence of JunB and JunD within the AP-1 DNA-binding complex [77]. The antibody against Jun-D supershifted the majority of the AP-1 activity, suggesting that Jun-D is the major protein component of this complex.

Worley et al. [163] have reported that *zif268* mRNA and protein levels rose sharply in rat visual cortex between PD11 and PD16, reaching peak values by PD21. An ISHH study of *zif268* mRNA expression during rat development also showed a marked rise between PD 10 and PD12, and even further increase at PD14 [57]. The elevation in *zif268* mRNA levels at this time was attributed to eye opening.

4.2. Cat

McCormack et al. [100] have reported a significant increase in c-*fos* and *zif268* mRNA levels in kitten visual cortex between the 1st and 5th postnatal weeks. The elevated levels of both gene products continued throughout development and began to decrease at the 20th postnatal week to reach adult levels. *junB* mRNA levels rose steadily between 1 and 10 weeks and then gradually declined to adult levels. There was no clear developmental alteration in *c-jun* mRNA levels. Beaver et al. [6] have reported that Fos-LI could be detected in the primary visual cortex of 30-day-old kittens but not in adult animals. The immunopositive cells were most abundant in layers II/III, VI and upper half of layer IV.

Kaplan et al. [80,81] have examined the developmentally regulated expression pattern of Zif268. In 0.5-week-old animals, the protein expression was localized almost exclusively in layer VI/subplate. At 1 week, immunopositive cells spread out from a dense band in deep layers to more superficial cortical layers, being distributed principally in two distinct bands, one in layer VI/subplate, and another in the lower part of the cortical plate (destined to be lower layer IV in mature cortex). In 2.5-week-old animals, Zif268 immunopositive cells were more widely distributed across cortical layers IV–VI, with no expression in layers I–III. In kittens of both 1 and 2.5 weeks of age an intensely stained band of cells was noted in the lowest portion of layer IV where it bordered layer V. By 5 weeks of age, Zif268 was observed to be highly expressed in neurons in all cortical layers, with reduced immunolabeling in layers I and V. In 10-week-old animals, the staining was essentially the same as at the age of 5 weeks except for reduced intensity in layer IV. In cats of 20 weeks of age, the staining in layer IV was further decreased and as in adults, the Zif268 immunopositive cells were located almost exclusively above and below layer IV.

4.3. Monkey

The developmental expression of AP-1 proteins has not yet been reported in the monkey. However, we have found preliminary evidence for an intense band of c-Fos expression that was localized to layer IVCβ in the vervet monkey. This band was visible as early as PD0 and remained for the first 6 weeks of development. Our recent work on Zif268 immunodetection has shown only minor differences at various points during the critical period in comparison to the adult laminar profile (Chaudhuri and Kaczmarek, unpublished observations). The only notable difference was that Zif268 levels in the superficial layers are weak at birth but become elevated by PD6. The characteristic adult Zif268 laminar profile—high in layers II/III, IVC, and VI—is evident by PD6 and do not appear to deviate during the remainder of the critical period.

4.4. Summary

The developmental scenario of AP-1 and Zif268 expression shows certain temporal patterns that coincide with important events in development. One of these is the initiation of visual input at the time of eye opening when Zif268 and certain components of AP-1 are up-regulated in the rat and cat. Since monkeys are born with their eyes open, elevated Zif268 immunostaining is apparent even at PD0. By contrast, the basal levels of c-*fos* products are generally low throughout development, though somewhat higher than in the adult. The available data suggest that other members of the fos family, such as FosB and Fras, may be involved in AP-1 formation under basal conditions during development. Among the *jun* family, all three members are present and may therefore pair with each other or with the Fos-related antigens to produce a functional AP-1 complex.

A second issue concerns the roles that ITFs may play in the developmental maturation of visual cortex. One manner in which this may be assessed is by comparing the relative abundance of the various proteins throughout the critical period. It appears that in some instances such expression is guided by afferent input and may be triggered by eye opening. However, the expression of *junB* appears unrelated to this event alone since the peak levels of its products are reached in the later stages of the critical period. In general, the temporal correlations and laminar details of basal expression of various ITFs suggest that they are important participants in the development of neocortical tissue, though their precise roles remain unknown.

5. Effects of sensory deprivation on ITF expression

The effect of sensory deprivation on ITF expression has been approached in several ways. The easiest manipulation

is to block light input into the eyes by either lid suturing, placing an eye patch, or dark-rearing/adapting the animals for a period of time. In general, we will refer to an extended period of deprivation—usually in the order of days to weeks—as *dark-rearing*. On the other hand, *dark-adaptation* will refer to animals being deprived of vision on the scale of minutes to hours. In either case, the procedure would show the results that follow from removal of light input but without abolishing spontaneous neuronal activity. This, however, can be achieved by intravitreal injection of tetrodotoxin (TTX), a reversible sodium channel blocker [124,141]. The functional blindness that TTX administration causes may remain upto several days, depending upon the amount and concentration. A more severe form of visual deprivation involves removal of the eyes (enucleation). This is not used for short term deprivations because of the associated trauma and possible upstream degenerative complications that may follow severing of the optic nerve.

The choice of a visual deprivation procedure may be influenced by the animal species that is chosen for the study. The monkey is an especially good candidate for studying monocular deprivation effects because of the existence of a precise set of ocular dominance columns. Monocular paradigms can be employed in rodent models because the visual pathways cross over to a large extent at the optic decussation. This leaves each visual cortex with an extensive input from the contralateral eye, allowing the other hemisphere to be used as a control. Furthermore, the presence of a narrow cortical strip that receives input from both eyes provides an opportunity to examine binocular interactions in this species.

5.1. AP-1 components

The components of the basal AP-1 complex are largely unaffected by TTX treatment. The expression of c-*fos*, *c-jun*, and *junB* mRNA and protein products has been shown by ISHH and ICC staining to remain unaltered in adult rats [162,163]. Furthermore, visual deprivation did not significantly affect AP-1 DNA-binding activity. During development, visual deprivation by dark-rearing (PD21 to PD42) also does not seem to affect c-*fos* and *junB* mRNA levels significantly (Konopka et al., unpublished observations). However, as noted before, the levels of AP-1 products are low to begin with in control animals.

In the cat, unilateral visual deafferentation by surgery resulted in a time-dependent decrease of c-*fos* mRNA levels in the affected hemisphere, as shown by ISHH [166]. Three days after the surgery, a bilaminar pattern was present, though at a lower intensity than in normal animals, and completely disappeared after 30 days. On the contrary, levels of *c-jun* mRNA apparently increased by the deafferentation procedure [166]. Rosen et al. [126] have reported that dark-rearing of kittens from birth significantly decreased *junB* mRNA levels after 20 weeks deprivation. No significant effect of dark-rearing on c-*fos* mRNA was observed. The results of dark-rearing for 1 week on c-Fos protein levels in visual cortex of kittens and adult cats are consistent with the results obtained at the mRNA level. Dark-rearing had only marginal effects on the low basal levels of c-Fos [81].

5.2. zif268

Worley et al. [162,163] have examined the effects of monocular deprivation on *zif268* levels in the rat. Intravitreal injection of tetrodotoxin (TTX) with 2–4 h survival selectively reduced *zif268* mRNA and protein levels in the contralateral primary visual cortex. This effect was most pronounced in layer IV and, to a lesser extent, in layers II/III and VI. Further experiments involving dark-rearing showed reductions in *zif268* mRNA and protein levels after deprivation periods of 4 days or longer. Although these changes were less dramatic than those found with TTX injection, they were anatomically more widespread and involved both primary visual cortex and adjacent association cortex. A reduction in ZIF268 DNA-binding activity has been shown by EMSA experiments after 1 week of dark-rearing [76]. Dark rearing of rats from birth to PD12 also showed low *zif268* mRNA levels that were similar to normally reared rats of the same age. However, extending the period of dark-rearing to PD21 caused *zif268* mRNA and protein levels to be significantly down-regulated in comparison to control animals of the same age [163].

In the cat, unilateral visual deafferentation by surgery produced a time-dependent decrease of *zif268* mRNA levels in the affected hemisphere, as shown by ISHH [166]. A bilaminar pattern was still present 3 days after surgery, though at a lower intensity that in normal animals, and completely disappeared after 30 days. The effects of dark-rearing for 1 week on Zif268 protein expression in visual cortex of kittens and adult cats confirm results obtained at the mRNA level [81]. There was a marked reduction in Zif268 levels throughout the cortical thickness, although a laminar profile similar to that in control animals was still discernible. This effect was observed in both young and adult cats. In both cases, an intense band of staining persisted in the lower part of layer IV. Kittens that were dark-reared for longer periods—from birth to either 5 or 20 weeks—also showed significant reductions of *zif268* mRNA levels at both ages in the visual but not frontal cortex [126].

In the vervet monkey, visual deprivation has been studied by both monocular and binocular visual removal [17,19]. Dark-rearing for 24 h showed significant down-regulation of Zif268 in all layers of area V1 except layer II where a very dense band of immunostaining was observed. Monocular deprivation revealed a series of ocular dominance columns containing elevated levels of Zif268 interdigitated between columns of low expression. Monocular

deprivation by lid suturing produced columns of the lowest contrast whereas both TTX injection and enucleation provided a sharp, well-delineated set of columns throughout area V1.

The columns of high Zif268 expression were shown to correspond to the open eye because they were spatially coincident with ocular dominance columns obtained by cytochrome oxidase staining. There was sufficient down-regulation of Zif268 to make the columns visible within as little as 3 h after monocular lid suture. A shorter period of monocular deprivation—around 30 min—caused sufficient down-regulation of *zif268* mRNA in the deprived eye columns. Although such down-regulation was evident in all layers, it was weakest in layer IV. A band of labeling punctuated by weak columnar discontinuities persisted in layer IVCβ regardless of the nature or duration of monocular visual deprivation. Columns of high contrast were visible in layers II/III and VI [19].

5.3. Summary

The differential effects of visual deprivation on AP-1 products and *zif268* in the visual cortex are striking. Much of these are attributable to the low basal presence of the AP-1 products in general. If AP-1 components are at low levels to begin with, then visual deprivation would only reduce it further. If the magnitude of this change is small, it would likely remain undetectable by conventional labeling techniques. However, another interpretation of this data is that basal levels of AP-1 proteins are not regulated by sensory input and thus removal of it has negligible effects.

The Zif268 results are the most striking in the deprivation paradigm. One interpretation of these results is that the high basal expression normally seen in primary visual cortex is due to continuous sensory input. Removal of such input, either to one or both eyes, results in a sudden and dramatic reduction in the levels of *zif268* products. This is further strengthened by the relatively weak down-regulation that is seen after eyelid suturing in the monkey when compared to TTX injection or enucleation. The latter two treatments completely abolish afferent input whereas some light always penetrates through a closed eyelid which, combined with the presence of spontaneous activity in the retina, may drive *zif268* expression in visual cortex to a modest degree.

There are two intriguing aspects of Zif268 expression following visual deprivation in the monkey. First, the intense band of immunostaining in layer II that is evident after dark-rearing for 24 h may be driven by intracortical feedback that is known to reach this layer. If so, then the sensory input from higher visual areas is occurring in the total absence of any photic stimulation. This may simply reflect residual activity in extrastriate areas of the visual cortex or the signals may originate from cross-modal interactions that are known to occur in the higher centers. And second, the ocular dominance columns being sharpest in all layers except layer IVC is surprising since the LGN input into this layer is known to be well segregated. However, given that NMDA receptors apparently are the major mediator of *zif268* expression and that thalamocortical connections have been shown in other species to be non-NMDA receptor driven [74], it is likely that *zif268* expression in layer IVC is driven by intracortical circuits. The sensory input that drives *zif268* expression in this layer may therefore contain a mixture of monocular signals or an integrated binocular signal and stimulate either a separate population of neurons with functional NMDA receptors or thalamorecepient neurons in which such receptors are stimulated only by intracortical afferents.

6. Effects of sensory stimulation on ITF expression

Stimulated expression or induction studies offer a further opportunity to examine how the expression of various ITFs is linked to sensory activation. The experiments generally begin with some sort of dark-adaptation or rearing followed by light stimulation in one of several ways. The transition from a quiescent period to intense synaptic stimulation affects the expression of ITFs. Whereas basal expression shows how these ITFs respond to ongoing activation, induction experiments reveal the extent to which these genes are up-regulated in response to onset of synaptic stimulation. This is particularly important, as seen below, with regard to the AP-1 products whose basal expression is generally low but which are nevertheless inducible.

There are several parameters that require careful attention in induction experiments. First, the length of visual deprivation prior to stimulation needs to be sufficiently long so as to provide adequate quiescence for the visual neurons. However, if the animals are in the critical period of their development, plastic changes in visual cortex may obscure the spatial pattern of ITF induction if the length of deprivation is too long. Second, the nature of the stimulation can be either a short pulse or ongoing stimulation during the entire induction period. And third, the period after stimulation onset must be sufficient to allow for expression of the gene products. In general, mRNA induction can occur within a few minutes whereas proteins begin to accumulate later and peak at about 1–2 h after stimulation onset. Nevertheless, the different genes in this category may follow a slightly different time course of expression.

6.1. AP-1 components

The induction of AP-1 components has been studied in rats by various procedures. At the mRNA level, Worley et al. [163] have reported c-*fos* and *junB* induction to be robust in 3-week-old rats that were dark-reared from birth and then exposed to light stimulation. However, such increases were not as apparent in adult rats. Konopka et al.

(unpublished observations) have observed marked levels of c-*fos* and *junB* mRNA accumulation in two dark-reared groups—PD21 to PD42 and PD35 to PD42—that were exposed to light. Kaminska et al. [76] investigated AP-1 DNA-binding activities in visual cortex of adult rats that were dark-reared for 1 week and then exposed to light for various times. There was a marked increase of AP-1 DNA-binding activity in the visual (but not frontal) cortex at 2 and 6 h after the light exposure. EMSA-supershift assay revealed that the major components of the AP-1 complex was c-Fos, JunB, and to a lesser extent, c-Jun. This contrasts with the basal condition where AP-1 is composed of FosB and JunD. ICC analysis with antibodies specific for c-Fos further confirmed its inducibility and moreover provided evidence that this expression was limited to the visual cortex only [76].

Induction of c-Fos protein in the rat visual cortex has been investigated by exposing animals to localized patterned stimuli after dark adaptation [106]. High levels of c-Fos expression were evident in the contralateral primary and association cortices upon exposure of the right eye to moving stimuli. Immunopositive cells within primary visual cortex were predominantly distributed in layers IV and VI, with a few labeled cells in layers II/III. In extrastriate visual cortex, the pattern of c-Fos immunoreactivity was similar with the exception that more cells were present in layer V. In contrast to the results obtained with moving patterns, stationary stimuli induced c-Fos expression in retinotopically corresponding regions of visual area V1 but not in other extrastriate visual areas [106]. No c-Fos immunopositive cells were observed in the visual cortex ipsilateral to the light-exposed eye.

Induction of c-Fos protein in rat visual cortex has also been observed after exposure to ultraviolet light [2]. Wistar rats were dark-reared for 24 h and then stimulated with a low intensity UV light pulse (λ_{max} 360 nm). A 30 min exposure induced c-Fos expression throughout the rostrocaudal extent of visual cortex in all major areas (17, 18, 18a). The most intense c-Fos immunostaining was noted in layer IV whereas layers II/III and VI were moderately labeled. Although maximal immunostaining was observed after 30 min stimulation, a very short UV pulse (30 s) was sufficient to induce some c-Fos expression. c-Fos induction by UV stimulation was observed during both subjective night and subjective day after extended dark rearing, indicating that it was unaffected by the circadian cycle.

In the cat, dark-rearing from birth to 5 weeks age followed by light stimulation produced changes in the mRNA levels of various AP-1 products [126]. A high degree of c-*fos* and *junB* mRNA induction (2.3–3.9 fold) was observed in visual cortex after 1 h of stimulation. However, light exposure for 6 h per day for 2 successive days after dark-rearing resulted in returning the mRNA levels for both genes to the values observed in dark-reared controls. No effect on *c-jun* mRNA was observed under these conditions. In addition, adult animals placed in darkness for 2 weeks and then given 1 h light exposure were also investigated. This treatment produced increased c-*fos* and *junB* mRNA induction though at a lower magnitude (1.4–2.0-fold).

In a separate study, the effects of light exposure on c-Fos protein induction was examined in visual cortex of dark-reared kittens [6]. The animals were maintained in darkness from birth to 30 days age and then binocularly exposed to light for periods of 0.5, 1, 2, 4 or 6 h. Control animals included dark-reared littermates, including those handled and engaged actively in play for 2 h. Visual exposure produced an increase in c-Fos expression that was restricted to the visual cortical areas, with maximum induction after 2 h of stimulation. Layers II/III, upper half of layer IV, and VI were the most densely labeled. In addition, a strip of immunopositive cells was also seen at the very bottom of layer IV. Prolonged light-exposure times resulted in decreased c-Fos immunoreactivity. At 6 h light exposure, this was found mainly in layers II and VI. c-Fos expression was very weak in visual cortex of control animals.

Mower et al. [81,112] compared the induction of c-Fos protein in 5-week-old kittens and adult cats. The animals were dark-reared for 1 week and then exposed to light for 1 or 4 h. An accumulation of c-Fos was observed in all layers of kitten primary visual cortex, though with somewhat lower levels in layer V, after 1 h visual stimulation. In adults, however, immunoreactive cells were concentrated in layers II/III and VI with only faint labeling in layers IV and V. Furthermore, c-Fos protein expression could be detected in kittens using lower antibody concentrations than in adults, suggesting that its levels were in general higher in kittens. The main difference between adult and kitten c-Fos expression is then evident in layer IV where adults show negligible induction and kittens robust induction.

In the vervet monkey, c-*fos* mRNA and protein induction has been observed in both adult animals and at various time points during the critical period ([20], and Kaczmarek, Chaudhuri, unpublished observations). The animals were treated to a reverse occlusion procedure involving monocular eye patch for 3 h followed by reversing the patch to cover the other eye for 30 min or 2 h. The shorter period of reverse occlusion revealed c-*fos* mRNA induction in ocular dominance columns representing the second eye. Immunostained columns were evident after both periods and again represented the second eye. Layers II/III, IVC, and VI showed the highest levels of mRNA and protein induction in both the adult and developing animals. Indeed, there were no major differences in either the magnitude or laminar profile of c-*fos* induction between these two groups of animals.

6.2. zif268

A significant increase in *zif268* mRNA and protein levels is evident in adult rats that are exposed to light after

a period of dark-rearing [115,162,163]. As with c-*fos* and *junB*, there was a more robust response of *zif268* mRNA in young rats compared to adult animals. The induction of *zif268* by light stimulation has also been noted in EMSA experiments where ZIF268 DNA-binding activity was shown to be increased within 45 min of visual stimulation [76]. However, the peak level of induction was reached after 2 h followed by a return to basal levels after 6 h. Immunocytochemical staining for Zif268 confirmed that the light-dependent alterations in its expression was limited to the visual cortex.

In the cat, *zif268* mRNA expression is also elevated by light stimulation following dark-rearing for 5 weeks after birth [126]. Exposure to light for 1 h resulted in marked accumulation of *zif268* mRNA whereas exposure for 6 h each day for 2 successive days resulted in a return to basal levels that are ordinarily seen in dark-reared kittens. Adult cats placed in darkness for 2 weeks and then given 1 h light exposure showed elevated *zif268* mRNA expression, although at a lower magnitude than in kittens under the same condition.

Using the same paradigm as described above for c-Fos, Kaplan et al. [81] examined Zif268 immunoreactivity in the visual cortex of young and adult cats. Both groups of animals showed a sustained increase in Zif268 immunoreactivity with 1 h of light exposure following 1 week of dark-rearing. Immunostained cells were evident in all layers in kitten primary visual cortex, though at a reduced intensity in layers I and V. In adults, in addition to the poor labeling in layers I and V, layer IV also remained weakly stained except for a narrow band of positive cells at the very bottom of the layer. This pattern of expression persisted even after 4 h of stimulation and was essentially similar to that observed with light-adapted control animals.

In the vervet monkey, *zif268* induction has been examined at both the mRNA and protein levels. In the first set of experiments, adult animals were dark-reared for 24 h and then monocularly exposed to ambient light for 2 h and 5 h [19]. In both cases, ocular dominance columns of high and low Zif268 protein expression were observed. Although the columns were faint after 2 h, there was clear induction in all layers. One intriguing aspect of the staining profile was that layer IV columns were actually sharper after 2 h of stimulation than at the 5-h time point whereas the opposite was true for the other layers.

In the second set of experiments, both adult animals and those at various points during the critical period were treated to a reverse occlusion procedure involving monocular eye patch for 3 h followed by reversing the patch to cover the other eye for 30 min [18,20]. In general, there was little difference in the patterns of *zif268* induction between the adult and developing animals. Combined ISHH and ICC staining in both animal groups showed two separate sets of columns, one defined by elevated Zif268 protein levels and the other by elevated mRNA levels. The protein-defined columns were shown to represent the eye that was open for the initial 3 h, i.e., continued basal expression in these columns was contrasted by down-regulation in adjacent columns representing the closed eye. However, the mRNA-defined columns were ones in which *zif268* induction occurred through the open eye during the terminal 30 min. These results were interpreted in terms of a functional mapping utility since they show that a dual-labeling approach may be used to stain different neuronal clusters in response to two different stimulation conditions.

6.3. Summary

Several lines of evidence show that both c-*fos* and *junB* expression is elevated in mammalian visual cortex by light stimulation after a period of dark-rearing. These include mRNA detection by northern blotting, slot blotting, and ISHH staining, protein detection by ICC staining, and DNA-binding activity by EMSA-supershift experiments. Furthermore, the results from rat, cat, and monkey are generally consistent. EMSA experiments confirmed that both AP-1 components were indeed inducible in adult rat visual cortex. The high constitutive levels of *zif268* are rapidly re-established by visual stimulation after dark-rearing in all three species examined here.

Although many of the experiments relied on visual deprivation for several days prior to stimulation, it was also seen that a shorter period of dark adaptation was sufficient to allow c-*fos* and *zif268* induction. This was especially apparent in the rat and monkey where several hours of monocular or binocular deprivation produced detectable changes in both mRNA and protein levels. Monocular stimulation experiments in monkeys had shown that as little as 3 h of deprivation was sufficient for subsequent induction of either c-*fos* or *zif268*. Furthermore, brief visual stimulation-indeed as little as 30 s in the rat-was capable of inducing c-*fos*. This shows that sufficient ITF induction occurs after a brief pulse to produce a detectable presence, although the cumulative effect of ongoing stimulation leads to greater levels and thus more intense staining of either the mRNA or protein product.

The differences in ITF induction between adult and developing brains is of interest because of the potential link between these proteins and experience-dependent plasticity. Although there were few dramatic differences in ITF induction between these two groups of animals, the results in cat layer IV stand out because of its well-defined critical period and the special role it plays as the thalamorecepient layer in primary visual cortex. The diminished c-*fos* induction in this layer in adults was taken as further evidence of the lack of plasticity here. The developmental implications of these results are complicated by the findings in monkeys where such differential expression was not observed. Indeed, layer IV induction of c-*fos* was equally robust in primary visual cortices of both adult and developing monkeys. This discrepancy may be understood in terms of NMDA receptor distributions in the two animals. In layer

IV, the NMDA receptor abundance in adult cats is low. However, in monkeys, there is significant expression of this receptor complex in adult layer IVC. Thus c-*fos* expression appears to follow the level of NMDA receptors in the two species.

7. Complex environmental, behavioral and multimodal stimulation

Several research groups have investigated the effects of behavioral training with complex stimuli on ITF gene expression. A single training session of 2-way active avoidance behavior with darkness being used as a conditioned stimulus (CS) resulted in a massive accumulation of c-*fos* and *zif268* mRNA levels in various brain regions, including the visual cortex [116]. Long term training with darkness as CS for 9 days (one session a day) up to an asymptotic level of performance produced negligible c-*fos* activation. However, the addition of an auditory stimulus to create a compound CS, produced c-*fos* mRNA expression in sensory neocortex [117] and improved performance levels. Further evidence for increased c-*fos* expression in the same brain region has been found with sexual training of male rats to acquire copulatory proficiency [9]. Tischmeyer et al. [150] reported that behavioral training of brightness discrimination resulted in increases in *junB* and *c-jun* expression in the cerebral cortex of the rat.

Hess et al. [59] have compared c-*fos* mRNA levels in three groups of rats: (i) home cage controls; (ii) animals well trained for four sessions to a nose poke task; and (iii) animals, who after completion of the nose poke task, were required to learn an odor discrimination task in a single session. The authors found marked accumulation of c-*fos* mRNA in the visual cortex in both groups of trained rats, when compared to home cage controls. This appears to be a surprising result, given that a nose-poke group performed a well trained task during the test session prior to collection of the brains for ISHH analysis. However, there was a notable difference in the test session when compared to the training sessions—namely, the test session lasted twice as long thus providing a significant component of novelty to the experimental situation. This temporal difference between test and training situations may cause different levels of arousal, perception, stress, etc.

A significant increase in c-Fos immunostaining has been observed in sensory neocortex after rats were exposed to novel objects [168]. Different groups of animals were exposed to either novel objects, familiar objects, or to the same pattern of illumination without any objects being shown. c-Fos expression was elevated when animals were exposed only to novel objects. An enhancing effect of exposure to a novel environment on c-Fos and c-Jun immunoreactivity has also been observed [120]. Beck et al. [7] have also observed elevated c-Fos expression in the visual cortex of animals exposed to footshocks for 3 days and then sacrificed after a test session in the same apparatus where the shock were previously delivered but without any shock. Although the induction of c-*fos* in this situation may be the result of conditioned fear, as the authors claim, there was a clear component of novelty in the test situation when compared to the training procedure.

Wallace et al. [155] analyzed *zif268* mRNA levels by in situ hybridization in rats exposed for 2–4 days to various environmental conditions: (i) group housing in a complex environment (EC); (ii) individual housing with daily handling (HIC); and (iii) individual housing without handling (IC). Quantitative analysis of autoradiograms revealed that EC rats had significantly higher levels of *zif268* mRNA than IC rats in visual cortex. HIC group produced intermediate results. Furthermore, keeping the EC group in a complex environment for 30 days resulted in still higher *zif268* expression.

In summary, behavioral experiments suggest that c-*fos* inducibility in visual cortex may occur even in the absence of any previous sensory deprivation. It appears though that novelty associated with the experimental situation is a prerequisite for c-*fos* activation. Behaviorally driven *junB* expression has also been observed, reinforcing the notion that coincidental c-*fos* and *junB* expression occurs under various conditions. Accumulation of *zif268* mRNA in animals reared in an enriched environment may reflect a higher level of neuronal activity that correlates with plastic changes of the sensory cortex.

8. Summary and perspectives

The preceding review has shown that each of the ITFs have characteristic expression patterns under various conditions of visual manipulation. Nevertheless, certain trends have emerged that now allow us to classify the gene responses into three categories, as outlined below. It should be noted that in these studies the expression of ITFs appears to be restricted to neurons. Furthermore, the limited information available for other neocortical sensory areas generally support the conclusions that were drawn from studies of the visual system [99,121,128,130,140,169].

The expression of c-*fos* and *junB* is consistently low under basal conditions in adult animals, slightly but significantly increased during the critical period of development, and markedly elevated by sensory stimulation. The expression of members of a second category that includes *fos*B, *c-jun*, and *junD* was somewhat higher under basal conditions and generally unalterable by sensory manipulation. Indeed, there is evidence that FosB and JunD are the main components of AP-1 DNA-binding activity under basal conditions. c-Jun may also participate in this complex, however, the evidence for this is not as strong. The final category includes *zif268* which shows a high basal level that is reduced by sensory deprivation and rapidly restored by stimulation.

It is apparent that with regard to sensory responsivity, the first and third ITF categories are particularly interesting. Since most of our knowledge to date is based on expression profiles of c-*fos* and *zif268* in the primary visual cortex of three species—rat, cat, and vervet monkey, we have summarized this information in Fig. 3. To facilitate comparison of the responses of these two genes, we have separated the information into two broad categories—critical period and adult—within which the basal, deprivation, and induction data are provided. For each condition, the expression levels for rat, cat, and monkey are shown for the three major strata—layer IV (granular layer), supragranular, and infragranular layers. These profiles represent combined staining patterns obtained from various ISHH and ICC studies in these species. The intensity of staining in the different layers was qualitatively judged to be at one of three levels—light, moderate, or heavy—and is represented accordingly by the intensity of the stippling pattern in this figure. Although northern blotting and EMSA results are not included here, they were generally consistent with the expression levels obtained by ISHH and ICC staining.

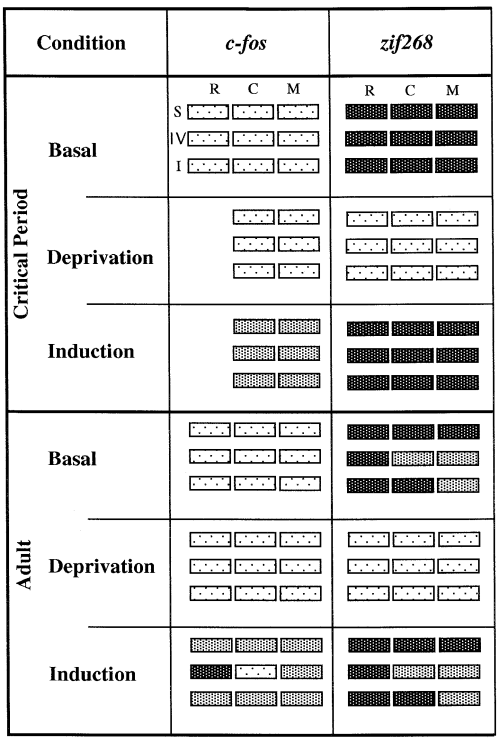

Fig. 3. Comparison of c-*fos* and *zif268* expression under different sensory conditions in rat, cat, and vervet monkey visual cortex. The information presented here was compiled from laminar expression profiles of the mRNA and protein products of both genes. In situ hybridization or immunohistochemical staining levels were assigned one of three levels (low, moderate or heavy) based on the original histological data and descriptions, as cited in the text. These three qualitative levels of staining are represented accordingly by the intensity of the stippling pattern. Each cell in this figure depicts the staining intensity separately for the thalamorecepient (IV), supragranular (S), and infragranular (I) layers in the rat (R), cat (C), and vervet monkey (M). In most instances, we took layer VI to represent the infragranular layer because of the consistently poor staining that has been observed in layer V. The laminar expression profiles for all three species are shown for three sensory conditions — basal, visual deprivation, and visual stimulation — in adult animals and during the critical period of development.

8.1. Differential expression of c-fos and zif268: implications for functional mapping

The most striking aspect of the data presented in Fig. 3 is the difference in basal expression of c-*fos* versus *zif268*. In general, c-*fos* levels are very low whereas *zif268* levels are very high. Given the results on basal expression of these genes, a picture emerges whereby the high constitutive expression of *zif268* is strongly down-regulated by removal of visual input, but nevertheless is capable of a rapid return to basal levels by stimulation. In contrast, the low constitutive levels of c-*fos* remain relatively unaffected by removal of visual input and therefore differences in the levels of c-*fos* between light-adapted and dark-reared animals are negligible. However, these levels are rapidly elevated by stimulation. This leads to the notion that *zif268* expression is linked to ongoing synaptic activity whereas c-*fos* expression is reliant upon activity being triggered only after a period of quiescence or after exposure to a novel situation.

These results have certain implications regarding the utility of these ITFs in functional mapping. A role for these products in labeling neural activity was proposed when it was evident that c-*fos* is rapidly and transiently induced by synaptic stimulation [36,127]. Numerous functional mapping studies in different brain compartments have since employed ITF probes to reveal neuronal ensembles activated under specific stimulation conditions. Activity labeling, however, may be actualized by two different approaches. In the down-regulation paradigm, the stimulus that is of interest is withheld from the animal and therefore the brain regions that are affected may be identified because they fail to express the ITF. With regard to information content, this approach offers limited utility. Alternatively, an up-regulation approach may be used whereby only the stimulus of interest is given to the animal and thus brain areas that are selective to it are identifiable by the presence of positive staining.

The low constitutive expression of the c-*fos* gene makes it an unwise candidate for down-regulation studies because there is little room for the basal levels to accommodate a downward shift in expression. Rather, *zif268* is ideally suited for this and, indeed, has been used successfully to document cortical columns that differ in activity from surrounding ones [17,19]. The transient nature of c-*fos* induction make it a suitable candidate for up-regulation approaches. Brain regions that are positively stained are likely to have been specifically driven by the stimulus because of the low constitutive levels of c-*fos*.

8.2. Regulation of ITF expression: implications for information processing

One of the features of c-*fos* and *zif268* is that they are triggered shortly after sensory stimulation and, in the case of c-*fos*, the expression is of a transient nature that mimics the expression kinetics observed in cultured neurons. This implies that the brief presence of c-Fos is sufficient to exercise its biological function. Nevertheless, given that c-Fos is a component of the AP-1 transcription factor, it may have a prolonged impact on neuronal physiology by initiating the expression of targeted (effector) genes.

The expression of the *zif268* gene, on the other hand, is maintained at relatively high levels with ongoing stimulation. However, its rapid down-regulation by sensory deprivation, together with cell culture results that show very short half-lives of *zif268* mRNA and protein, suggest that such expression under basal conditions may be dynamic. For example, the levels of *zif268* in individual neurons may go through asynchronous increments and decrements. If so, then the expression at the population level may reflect the integration of such transient modulations in expression. One intriguing aspect of c-*fos* and *zif268* expression in primary visual cortex is that they may be predominantly, if not exclusively, driven by intracortical circuits. Although this is obvious for the supragranular and infragranular layers of monkey area V1, the data in the thalamorecepient layer can also been interpreted in such a manner.

The laminar profile of c-*fos* and *zif268* expression and NMDA receptor distribution for both developing animals and adults is highly correlated. However, no direct evidence has yet emerged that sensory-driven c-*fos* expression may indeed be mediated by these receptors. The evidence for such involvement in activation of *zif268* expression is still equivocal, given that the high doses of NMDA receptor antagonists that were applied to down regulate its expression had numerous side-effects including perturbed behavior.

8.3. Patterns of ITF expression: implications for homeostasis and plasticity

A major issue that needs to be resolved is the functional implication of ITF expression in sensory cortex. To answer this, it is important to be mindful of the fact that transcription factors regulate gene expression. There are three plausible functions of neuronal gene expression that may be considered [73]. First, there is a continuous need for homeostatic *maintenance* and ITFs may exercise their control of the genome to fulfill this purpose. The transcription factors that are involved in such operations should be ubiquitously expressed, however, their levels may be modulated according to neuronal activity. The qualities of the *zif268* gene make it well suited for this function.

The second role for gene expression is to control the *replenishment* machinery. Although this may be considered to be an aspect of homeostasis, it can be viewed separately to reflect the sudden needs of a neuron under conditions of intense neural activity. As an example, consider the stimulation condition where a prior period of quiescence was often required as a necessary step. The sudden burst of spiking activity following this quiescence may result in rapid use of neuronal components (e.g., synaptic release machinery, metabolic enzymes, etc.) and thus a need arises to replace these exhausted elements. An analogous situation is present when a novel behavioral situation is applied. The neuronal activity that accompanies this may result in the same consequences of neural fatigue and therefore the necessity for replenishment. c-*fos* expression appears to correlate with replenishment phenomena and may indeed be involved in their regulation.

The third possible role for ITFs can be thought of in terms of *information integration* [70,71]. The gene regulatory regions contain sites for binding multiple transcription factors and their interaction is believed to be mandatory to drive gene expression. Thus, the regulatory regions may act as coincidence detectors allowing a convergence of information provided by various transcription factors activated by different signals of behavioral value, such as sensory information, arousal, motivation, reward, etc. The molecular meaning of such coincidence detection may be to elaborate a long-term plastic change. Thus, an additional facet of c-*fos* expression, when driven by sensory stimulation, may be to transduce the visual signal into a genomic response. Indeed, the developmental and behaviorally driven response of c-*fos* is consistent with a possible role in guiding plastic changes.

8.4. Future directions

Our understanding of the physiological functions of ITF expression at present is limited and thus opens up numerous opportunities for future work. It is still unknown what conditions guide c-*fos* (and *junB*) activation. In particular, the exact role of visual deprivation remains unclear since it may provide either a period of neural quiescence or it may allow the visual stimulus to be presented within a novel (arousing) behavioral context. Resolving this issue would have practical implications for mapping purposes by establishing the prerequisites that are required to optimize c-*fos* (and *junB*) expression.

At the molecular level, it is critical to establish the precise role of NMDA and/or other receptors in driving ITF expression. Given that even NMDA receptors alone can trigger various transduction pathways in cultured neurons, it is also necessary to elucidate the second messenger systems that are responsible for transferring the information from the membrane to the nucleus. This would reveal the molecular features of ITF control and provide insight into differences in their expression patterns that are evident under specific conditions. And finally, it is necessary to understand the interactions between the various ITFs, such

as the components of AP-1 and between AP-1 and ZIF268. This is reliant upon identifying the genes that are regulated by them.

At a neuroanatomical level, understanding the role that cortical circuitry imposes on ITF expression would help in interpreting the expression profiles that are evident either under basal conditions or following stimulation. For example, understanding whether c-*fos* and *zif268* expression in the geniculorecepient layer is driven by thalamic sources, by intracortical circuits, or both would simplify the interpretation of such labeling under various conditions of visual stimulation.

Continued efforts at resolving each of these major issues would help in answering one of the most important questions on ITF expression, i.e., is it only a response to sensory activation or does it also play a role in guiding experience-driven cortical plasticity.

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