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Regulation of Immediate Early Genes in the Visual Cortex

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1. Introduction

Light is the fastest and likely the most complex source of physical energy processed by the mammalian central nervous system (CNS). Throughout evolution, most mammals that rely heavily on vision for their normal behaviors have developed an exquisitely elaborate pattern of connectivity and functionality for harnessing, processing and integrating visual information. This process allowed for remarkable environmental adaptation and ecological success for a number of species. The complexity and behavioral relevance of the visual system, and the relative experimental ease associated with research using this modality in laboratory animal models, has arguably placed research in this sensory system in the forefront of contemporary sensory neuroscience research.

Tremendous gains in our understanding of the anatomical and functional organization of the visual system were obtained with the pioneering experiments of David Hubel and Torsten Wiesel, who employed traditional single-unit electrophysiological recordings to describe orientation and directional selectivity, in addition to several other response properties of neurons in the primary visual cortex (V1) of the anesthetized mammal (Hubel and Wiesel, 1962, 1968, 1970, 1972; Wiesel and Hubel, 1963; Hubel et al., 1977). These experiments paved the way for subsequent research using awake animals (e.g., Evarts, 1968; Wurtz, 1969) and eventually for ensemble recordings in awake, behaving mammals (e.g., Nicolelis et al., 1993, 2003; Fanselow et al., 2001; Gail et al., 2003, 2004).

Despite the large strides obtained through the use of multi-site, multi-electrode recordings in awake animals, one of the main limitations of this methodology is the low spatial resolution and the invasiveness associated with recording both chronically and acutely. Histological methodologies have supplemented data obtained with electrophysiological recordings by allowing for evaluation of the global brain activity patterns that result from experimental manipulations

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(e.g., dark-rearing or light stimulation). With such manipulations, high spatial resolution can be obtained, whereby virtually the entire brain can be mapped for activity, at the cost of low temporal resolution. At the forefront of visual system research, the activity of the mitochondrial enzyme cytochrome oxidase, and of 2-deoxyglucose, have consistently been the most employed histological activity markers, allowing for generation of global response profiles of the visual cortex following sensory stimuli. These methodologies have permitted the successful anatomical delineation of ocular dominance columns in V1, as well as the eye-specific interdigitated organization of retinal projections in the lateral geniculate nucleus (LGN), in primates and cats (reviewed in Sokoloff, 1981; Horton, 1984; Horton and Hedley-Whyte, 1984; Tieman, 1985; Wong-Riley, 1989; Krubitzer and Kaas, 1990; Gattass et al., 2005).

In the late 1980's, activity-dependent immediate early genes (IEGs) began to be employed as a new generation of markers for neuronal activity. The association between neuronal activation and IEG expression has allowed researchers to map the activation of brain networks through the specific probing of the products (both mRNA and protein) that result from gene induction following a number of stimuli, including sensory input (discussed in most chapters in this volume and in Kaczmarek and Chaudhuri, 1997; Herdegen and Leah, 1998; Kaczmarek and Robertson, 2002). Importantly, this methodology allows for the investigation of large-scale activity patterns, with a single-cell level of resolution, and the use of awake animals that have experienced minimal interference with their "natural" behaviors. In addition, high spatial resolution can be achieved with this approach, given that multiple brain areas within the same animal can be studied. One major disadvantage of this methodology is the low temporal resolution, since IEG expression reflects a cumulative effect of activity that occurs in a time-scale ranging from minutes to tens of minutes, depending on the detection methods used (Chapter 1 and Herdegen and Leah, 1998; Kaczmarek and Robertson, 2002; but see Chapter 9 in this volume). In mammals, IEG expression has been successfully used to map neuronal activation within virtually all stations of the ascending visual system, including the retina, the lateral geniculate nucleus (LGN) and lower- and higher-order visual cortical areas, including the V1 and the infero-temporal cortex (IT), respectively, to name a few examples (Chaudhuri and Cynader, 1993; Chaudhuri et al., 1995; Okuno and Miyashita, 1996; Kaczmarek and Chaudhuri, 1997; Okuno et al., 1997; Miyashita et al., 1998; Kaczmarek et al., 1999; Arckens et al., 2000; Pinaud et al., 2002b, 2003a, 2003b; Pinaud, 2004, 2005; Arckens, 2005; Montero, 2005; Pinaud and Tremere, 2005; Soares et al., 2005). This methodology has provided insights into the anatomical and functional properties of visual stations and has proven to be superior to the standard histological markers of activity, as discussed above, given that it offers better spatial and temporal resolution.

The goal of this chapter is to discuss how our understanding of the functional organization of visual cortical circuits was advanced by the use of IEGs as markers for neuronal activation. We will describe and discuss the expression profiles of the most commonly used IEGs in studies of the visual cortex, including the

transcription factors encoded by NGFI-A (also known as *egr-1*, *krox-24*, *zenk* and *zif-268*), *c-fos*, *c-jun*, *junB* and *junD*, as related to the AP-1 transcription factor, and the effector IEG *arc*. These expression profiles will be described from the results of a variety of deprivation and stimulation paradigms. Space constraints do not permit an extensive review of this topic; however, several reviews have recently been published and provide additional resources for the reader (Kaczmarek and Chaudhuri, 1997; Herdegen and Leah, 1998; Montero, 2005; Pinaud, 2005; Pinaud and Tremere, 2005). These reviews also discuss findings on IEG expression obtained from other stations of the ascending visual pathway, including the retina and LGN. Here, however, key scientific works describing the regulation of IEG responses to visual experience in V1 are presented and discussed, as well as the emerging findings of the roles that IEGs play in the normal physiology of visual cortical neurons. Finally, we will compare the similarities and differences in the expression profiles of these genes and will discuss how their protein products may orchestrate experience-dependent changes in the visual cortical circuitry, based on some of our own findings.

2. Immediate Early Gene Expression

The expression of IEGs has been extensively used as a mapping tool for neuronal activation, given that their fast and transient expression is associated with the neuronal depolarization that follows sensory input, without the requirement of *de-novo* protein synthesis. For these reasons, it has been argued that the expression of IEGs provide the first genomic response to cell stimulation (Worley et al., 1991; Kaczmarek and Robertson, 2002; Pinaud, 2004). Research on the biochemical cascades that lead to IEG induction has revealed that, not surprisingly, different genes are activated through mostly different, and often times multiple, intracellular cascades, although in some cases there is a significant degree of overlap. A common requirement for the expression of the most widely used IEGs is the influx of calcium (reviewed in Ginty et al., 1992; Ghosh et al., 1994; Finkbeiner and Greenberg, 1998; Pinaud, 2005). This influx is often associated with the activation of the NMDA-type of glutamatergic receptors, which have been repeatedly implicated in the experience-dependent enhancement of synaptic efficacy (Collingridge and Lester, 1989; Hollmann and Heinemann, 1994; Mayer and Armstrong, 2004), as well as the activation of voltage-sensitive calcium channels (reviewed in Pinaud, 2005). The requirement of calcium for IEG induction potentially places the protein products encoded by this class of genes in the early stages of genomic responses associated with plastic changes in the CNS circuitry, including that of the visual system.

It is important to mention, however, that under certain conditions and in certain CNS regions, IEG expression appears to be dissociated from neuronal excitability. For example, light-driven activity in the LGN is not paralleled by an upregulation of the IEG NGFI-A in a number of mammalian species, including cats, monkeys and opossums (Arckens et al., 2000; Pinaud et al., 2003b; Soares et al., 2005). The specific reasons for such an uncoupling between electrophysiological activity

and IEG expression remain largely unknown but might involve the modulation of critical components of the intracellular cascade that leads to IEG induction. This issue has been discussed in greater detail in other reviews and will not be further extended here (Sharp et al., 1993; Pinaud, 2005). As discussed below, however, the large majority of IEGs reviewed in this chapter are regulated by visual experience in the V1; therefore, they provide a direct read-out of activity patterns that arise following visual input.

In the following paragraphs, we will briefly detail the characteristics, and some of the functional properties of the IEGs discussed in this chapter, followed by a review of the expression profiles of these IEGs in the V1.

2.1. The Fos and Jun Families: AP-1

Members of the Fos family (C-Fos, FosB, Fra-1 and Fra-2) and of the Jun family (c-Jun, JunB and JunD) form homo- or hetero-dimers through "leucine zipper" interactions positioned in the protein's structure; these dimers constitute the AP-1 (activator protein-1) transcriptional regulator (Morgan and Curran, 1991; Herdegen and Leah, 1998). AP-1 exhibits specificity and binds to a well-characterized DNA motif located in the promoter of a number of CNS genes, thereby regulating their expression. The AP-1 transcription factor exerts bidirectional regulatory effects (either up- or down-regulation) of target genes (Angel and Karin, 1991; Kobierski et al., 1991) and its specific effect on target gene regulation has been shown to be influenced by the identity of the members of the Fos and Jun proteins that compose the dimer. For instance, FosB/JunB heterodimers tend to suppress transcriptional activity of target genes given that this dimer binds to its specific cis-element but is inactive. Coversely, Fos/JunD heterodimers display high positive regulatory capabilities upon binding to its regulatory DNA motif (Hughes and Dragunow, 1995; Kaminska et al., 2000; Hess et al., 2004). Subsequent post-translational modifications, such as phosphorylation, are able to promote additional modifications in the transcriptional properties of the AP-1 (Herdegen and Leah, 1998).

2.2. NGFI-A

The protein encoded by the IEG NGFI-A is a transcription factor of the zinc-finger class (Milbrandt, 1987). Finger-like bulges in the structure of the NGFI-A protein are stabilized by zinc and exhibit high affinity for a specific DNA consensus that has been detected in the promoter region of numerous genes expressed in the mammalian nervous system, thereby potentially exerting regulatory effects on their expression. The identities of late-response genes (LGs) regulated by NGFI-A have been under extensive scrutiny and some targets have emerged from studies primarily conducted in *in-vitro* preparations. These assays have demonstrated, for example, that NGFI-A regulates the expression of the synapsin I, synapsin II and synaptobrevin genes that encode pre-synaptic proteins involved in the regulation of the size of the readily releasable pool of neurotransmitters and in neurotransmitter release (Thiel et al., 1994; Petersohn et al., 1995;

Petersohn and Thiel, 1996). NGFI-A also appears to regulate the expression of ligand-gated ion channels, such as the nicotinic acetylcholine receptor (Carrasco-Serrano et al., 2000), metabolic enzymes, such as monoamine oxidase B (Wong et al., 2002), as well as genes involved in neuronal structural stability, such as neurofilament (Pospelov et al., 1994). It is therefore clear that the transcription factor NGFI-A is well positioned to integrate activity-driven changes on the cell surface, with the genomic machinery that mediates a large variety of cellular processes that range from neuronal excitability and neurotransmitter release to long-term metabolic changes and neurite remodeling, as part of experience-dependent network rewiring (Pinaud, 2004, 2005; Pinaud and Tremere, 2005).

2.3. Arc

Unlike NGFI-A, Fos and Jun members, the protein product encoded by the IEG *arc* does not act as a transcription factor, but rather is a growth factor (Lyford et al., 1995). One of the most interesting features of *arc* is that the mRNA that results from its expression is rapidly translocated to dendrites and translated locally, in polyribosomal complexes often located at the base of dendritic spines, in an activity-dependent manner (Lyford et al., 1995; Steward et al., 1998; Steward and Worley, 2001a, b). These properties have spurred significant interest in this IEG, as it is well positioned to mediate fast, activity-driven modifications in synaptic configuration, especially as it relates to dendritic architecture (e.g., retraction and elongation). These types of modifications have been shown to occur in association with alterations in the patterns of sensory input, as in the case of lesions or an animal's exposure to enhanced sensory drive (Grutzendler et al., 2002; Nimchinsky et al., 2002; Trachtenberg et al., 2002; Pinaud, 2004; Guzowski et al., 2005).

3. IEG Regulation by Visual Deprivation and Stimulation

Below we will review the main findings of the effects of light deprivation and stimulation on IEG expression. Most of the studies on visual deprivation have focused on paradigms of either reversible or permanent light deprivation, including dark isolation, monocular and binocular enucleations, as well as retinal lesions. Subsequently, the impact of sensory stimulation on IEG expression in the visual cortex will be discussed.

Unlike *c-fos* and *c-jun*, that are expressed at relatively low basal levels in the rodent, cat and monkey V1, and *arc*, that is expressed at moderate baseline levels, the basal activity of the IEG NGFI-A has been repeatedly reported to be high in these experimental models as a result of ongoing neuronal activity (Worley et al., 1991; Beaver et al., 1993; Zhang et al., 1995; Kaplan et al., 1996; Kaczmarek and Chaudhuri, 1997; Pinaud et al., 2001). This expression property has provided the grounds for extensive use of NGFI-A in investigations of the effects of light deprivation throughout the mammalian visual system, while the low basal expression of *c-fos*, *c-jun*, *junB* and *arc* has largely prevented the use of these IEGs for this purpose.

3.1. Rodent

Pioneering experiments by Paul Worley and colleagues have demonstrated that NGFI-A mRNA and protein levels are dramatically decreased following intraocular infusion of tetrodotoxin (TTX) and dark adaptation in the rodent visual cortex, suggesting that the basal expression of this IEG is regulated by ongoing sensory input (Worley et al., 1991). These results were corroborated and expanded upon by subsequent work in the rat V1 using monocular eyelid suture, as well as intra-vitreal TTX infusion (Caleo et al., 1999). In these experiments, a marked suppression of NGFI-A basal levels was detected in both monocular and binocular V1, contralateral to the deprived eye. In addition, a minor decrease in NGFI-A expression in the ipsilateral supragranular layers of the binocular region of V1 was reported (Caleo et al., 1999).

While visual deprivation decreased basal NGFI-A expression levels, subsequent exposure of animals to light has been repeatedly shown to rapidly recover the normal, baseline levels of this IEG in the rodent visual cortex (Worley et al., 1991; Kaczmarek and Chaudhuri, 1997; Pinaud et al., 2000). Robust light-induced NGFI-A induction has been detected throughout all layers of the rodent V1 both in immature (during the critical period) as well as in adult animals (Worley et al., 1991; Nedivi et al., 1996; Kaczmarek and Chaudhuri, 1997; Pinaud et al., 2000) (Figs 2.1 and 2.2).

While visual deprivation slightly decreased *c-fos* expression in the rodent visual cortex (Yamada et al., 1999), visual input has been shown to significantly drive the expression of this IEG. For example, Correa-Lacarcel and colleagues (2000) have shown that white light stimulation, as well as various intensities of laser flickering, induces c-Fos in the rat V1. Interestingly, the distribution of c-Fos immunoreactive neurons was changed as a function of the stimulation paradigm: while continuous or high frequency flickering stimuli significantly increased the number of c-Fos immunolabeled neurons, stimulation of animals with middle and low frequency flickering laser light did not significantly alter c-Fos expression in V1 neurons of the rat. In all experimental groups, c-Fos immunopositive neurons were detected in cortical layers II/III, IV and VI, and to a lesser extent in layer V (Correa-Lacarcel et al., 2000). Montero (1995) has shown that exposure of animals to patterned visual stimuli (gratings and dots) triggered a significant increase in the number of c-Fos immunolabeled neurons, primarily in layers IV and VI, and to a lesser extent in the supragranular layers (Montero and Jian, 1995).

To the best of our knowledge, no systematic anatomical characterization of the distribution of *c-jun*-, *junB*- and *junD*- and *arc*-expressing cells, in dark-reared and light stimulated rodents has been conducted in V1. However, Kaminska and colleagues (1996) showed that dark-adaptation followed by light stimulation markedly increased the DNA-binding activity of both AP-1 and NGFI-A. It has also been reported that although FosB and JunD are the two primary components of the AP-1 in dark-reared rats, light stimulation for 2 hours recruits JunB, c-Jun and c-Fos to the formation of this transcription factor (Kaminska et al., 1996). Interestingly, longer stimulation periods (6-24 hours) led to a marked decrease in

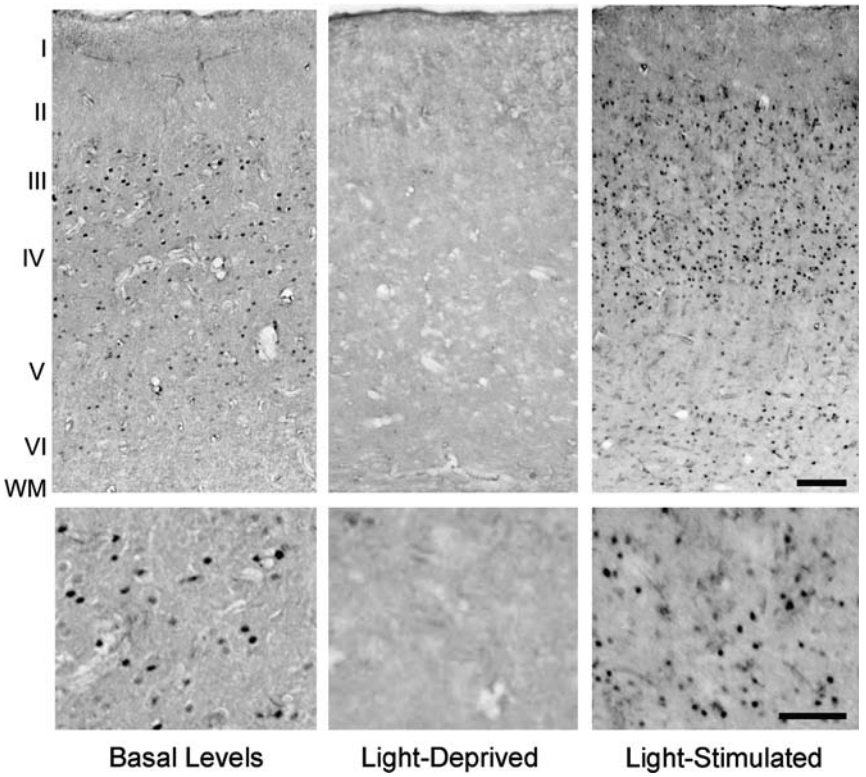

Figure 2.1. Immediate early gene regulation by light experience in the mammalian primary visual cortex (V1). The opossum (*Didelphis aurita*) embodies an ancestral pattern of organization of the central nervous system and, therefore, has been extensively used as a model to study the general principles of the visual system anatomical and functional organization. In the opossum, NGFI-A/Egr-1 is expressed at high basal levels (left column). Light-deprivation (24 hours; middle column) markedly decreases the number of NGFI-A/Egr-1 positive cells, while a 2 hour exposure of ambient light following light-deprivation (right column) elicits a marked upregulation of the protein encoded by this IEG in V1. Very similar results have been obtained with these protocols of light deprivation and stimulation in a number of other mammalian species, including rodents, cats and monkeys, indicating that the regulation of this IEG by light is evolutionarily conserved in the visual cortex of the mammalian lineage. I–VI—cortical layers 1 through 6; WM—white matter. Scale bars (in μm) = 100 (top) and 50 (bottom). Reprinted from Brain Res. Bull., 61, Pinaud et al., Light-induced Egr-1 expression in the striate cortex of the opossum, pp. 139-146, Copyright (2003), with permission from Elsevier.

c-Fos and c-Jun levels, while a sustained expression of JunD, JunB and FosB was detected, suggesting that AP-1 composition, and likely its activity and the identity of its target genes, is regulated by visual activity (Kaminska et al., 1996).

3.2. Cat

As observed in rodents, NGFI-A is expressed at high basal levels in the cat visual cortex, with highest expression detected in cortical layers II/III and VI

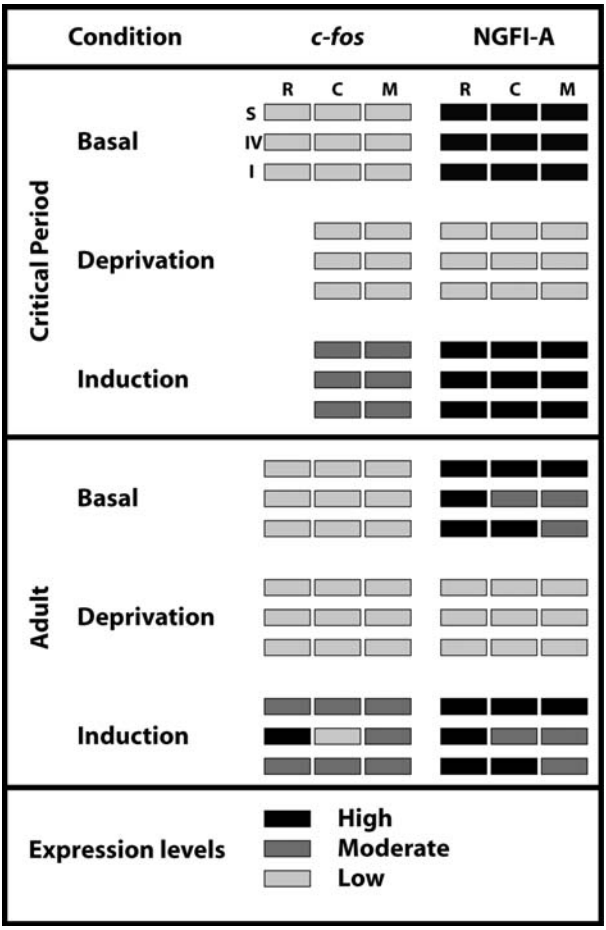

Figure 2.2. Table comparing c-fos and NGFI-A/zif268 expression under different sensory conditions in the rat, cat and monkey visual cortex. The information presented here was compiled from laminar expression profiles of the mRNA and protein products of both genes. In-situ hybridization or immunohistochemical staining levels were assigned one of three levels (low, moderate or high) based on the original histological data and descriptions, as cited in the text and detailed in Kaczmarek and Chaudhuri (1997). These three qualitative levels of staining are represented accordingly by the darkness of the pattern. Each cell in this figure depicts the staining intensity separately for the thalamo-recipient (IV), supragranular (S), and infragranular (I) layers of the rat (R), cat (C), and vervet monkey (M). In most instances of the original table (Kaczmarek and Chaudhuri, 1997), authors 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. Reproduced with the kind permission of Prof. L. Kaczmarek and reprinted from Brain Res. Rev., 23, L. Kaczmarek and A. Chaudhuri, Sensory regulation of immediate-early gene expression in mammalian visual cortex: implications for functional mapping and neural plasticity, pp. 237-256, Copyright (1997), with permission from Elsevier.

(Zhang et al., 1994; Kaplan et al., 1996). Likewise, protocols of visual deprivation also affected the expression of this IEG in V1 of the cat (Rosen et al., 1992; Zhang et al., 1995) (Fig. 2.2). For example, a marked downregulation of NGFI-A (zif268) mRNA levels in both supragranular and infragranular layers of V1 was described after unilateral sectioning of the optic tract (Zhang et al., 1995). Subsequent work showed that 1-week of dark-rearing significantly downregulated NGFI-A protein levels throughout all cortical layers of the cat V1 (Kaplan et al., 1996). These results differ from those obtained in the rodent V1, where deprivation of visual input has a greater impact on NGFI-A levels in cortical layer IV (Worley et al., 1991), likely reflecting the different cortical architecture of V1 between these two species, as well as dissimilar processing/computational strategies achieved by these two networks.

Kaplan and colleagues (1996) demonstrated that a brief exposure to light after dark-rearing triggers a robust induction of NGFI-A in an age-dependent, cortical layer-specific fashion. Adult cats underwent a substantial increase in NGFI-A protein levels in both supragranular and infragranular layers, with a small number of immunopositive neurons detected in the thalamorecipient layer IV. Conversely, young animals (5-weeks) underwent a marked induction of NGFI-A in all cortical layers (Kaplan et al., 1996) (Fig. 2.2).

c-fos mRNA basal levels were shown to be low and predominantly expressed in the cortical layer VI of the cat striate cortex (Zhang et al., 1994). It has also been reported that c-Fos expression is affected by light experience. Even though basal levels of this IEG are markedly low, dark-rearing has been shown to slightly reduce protein levels in both young and adult animals (Rosen et al., 1992; Kaplan et al., 1996). Similarly, unilateral optic tract sectioning decreased c-fos mRNA levels in layers II/III and V/VI in the cat V1 (Zhang et al., 1995) (Fig. 2.2). c-jun and junD basal expression levels have been shown to be primarily distributed through the supragranular layers and cortical layer VI in area 17 (Zhang et al., 1994). Interestingly, unlike what has been observed for c-fos and NGFI-A, optic tract lesioning was shown to increase c-jun levels, as detected by *in-situ* hybridization, in the infragranular layers of the striate cortex (Zhang et al., 1995). In addition, dark-rearing for 20-weeks, but not 5-weeks, affected the expression levels of *junB*, but not *c-jun*, in the cat V1 (Rosen et al., 1992). Dark-adaptation followed by light stimulation (1 hour) triggered a robust increase in both c-fos and junB (components of the AP-1) mRNA levels in the feline visual cortex (Rosen et al., 1992). This increase in c-fos and junB mRNA levels following light stimulation was shown to be transient, given that exposure of animals to light for two successive days led to a significant decrease in mRNA levels of both genes (but not *c-jun*) (Rosen et al., 1992).

Subsequent detailed anatomical characterization of the distribution of light-induced c-Fos expression revealed that two hours after stimulus onset, the highest density of c-Fos positive neurons was detected in the supragranular layers (II/III) as well as in cortical layer VI (Fig. 2.2). In addition, persistent visual stimulation (6 hours) revealed that c-Fos immunoreactivity levels returned to baseline (control) levels (Beaver et al., 1993). The transient nature of *c-fos* expression following

sustained sensory drive has also been described for the IEG *junB* (Rosen et al., 1992); recall that the protein products of both IEGs are major components of the AP-1 transcription factor.

Finally, a systematic comparison between the levels of *c-fos* and NGFI-A during adulthood versus the critical period revealed that light-induced expression of both IEGs occurred at all cortical layers of young animals (5-weeks old), while the highest expression levels of both genes in adult animals were detected in supragranular and infragranular layers, with the lowest expression detected for cortical layer IV (Kaplan et al., 1996) (Fig. 2.2). These data suggest that the discrepant expression profiles of these IEGs during development, as compared to adulthood, may reveal differential activation patterns in V1, perhaps related to various plasticity states, across separate cortical layers (discussed below).

3.3. Monkey

High basal expression of the IEG NGFI-A has also been described for the V1 of both New- and Old-World primates (Chaudhuri and Cynader, 1993; Silveira et al., 1996; Okuno et al., 1997; Soares et al., 2005). Similar to data obtained from cats, the greatest density of NGFI-A positive neurons was detected in cortical layers II/III and VI, with a moderate number of immunolabeled cells in the thalamo-recipient layer IV (IVCb) and cortical layer V (Chaudhuri et al., 1995; Okuno et al., 1997) (Fig. 2.2).

Monocular deprivation, either by means of intraocular TTX infusion, enucleation or eyelid suture, was shown to decrease significantly NGFI-A basal levels in the ocular dominance columns (ODCs) associated with the deprived eye (Chaudhuri and Cynader, 1993; Silveira et al., 1996). Interestingly, the IEG downregulation that occurred as a function of visual deprivation was detected in a very small time window, as early as 1-5 hours after the interference with sensory input (Chaudhuri and Cynader, 1993; Chaudhuri et al., 1995; Silveira et al., 1996).

As discussed above, monocular deprivation in the adult markedly downregulated NGFI-A expression in the ODCs associated with the deprived eye. Interestingly, Silveira and colleagues (1996) have shown that this form of sensory deprivation did not reveal ODCs in 3-month old Cebus monkeys, while 6-month old animals exhibited scantily developed ODCs (Silveira et al., 1996). Conversely, Kaczmarek and colleagues (1999) were able to detect ODCs in the Vervet monkey as early as post-natal day (PD) 6, and robustly at PD 40 and 90 (Kaczmarek et al., 1999) (Fig. 2.2). Although a clear explanation for these seemingly discrepant findings has yet to be systematically revealed—some possibilities are discussed by Kaczmarek and colleagues (1999)—these findings suggest that ODCs, as revealed by NGFI-A expression, are functionally shaped late in the process of development of the visual cortex.

In the vervet monkey, light stimulation followed by dark-rearing triggered a significant increase in NGFI-A expression levels in ODCs associated with the stimulated eye, while minimal levels have been described for the ODCs associated with the deprived eye (Chaudhuri et al., 1995). In this work, NGFI-A

immunolabeling appeared to be detected evenly across cortical layers (Chaudhuri et al., 1995) (Fig. 2.2).

The expression of both c-fos mRNA and protein levels has also been investigated in the Vervet monkey V1. It was found that the laminar profile of c-fos mRNA expression undergoes virtually no change with age: animals as young as 6-days of age exhibited high expression in supragranular layers (II/III), layer IVC and VI; this profile was also observed for adult monkeys (Kaczmarek et al., 1999). Immunocytochemistry directed at c-Fos protein yielded a remarkably similar laminar distribution, as compared to *in-situ* hybridization approaches directed at c-fos mRNA (Kaczmarek et al., 1999). Finally, similar findings were recently obtained in the New World monkey *Cebus apella* (Soares et al., 2005).

JunD expression has been assessed in the macaque monkey. Highest expression was detected in the superficial part of layer II and in layer VI, while moderate immunostaining was detected in the remainder of layer II and cortical layers III and IVA. Low JunD expression was detected in layers IVB, IVC and V (Okuno et al., 1997). To the best of our knowledge, *c-jun*, *junB* and *arc* expression have not been assessed in the primate V1.

4. Plasticity in the Visual Cortex and IEG Expression

Little doubt remains that IEG expression depends on sensory input in the mammalian visual cortex. It is also clear that neuronal activity is necessary, but not sufficient, to drive IEG expression in the CNS (Sharp et al., 1993; Arckens et al., 2000; Pinaud, 2004). For example, visual drive reliably triggers activity of LGN neurons, yet, in a number of experimental models, no NGFI-A expression has been detected after visual stimulation in these thalamic neurons in a number of mammalian species (Arckens et al., 2000; Pinaud et al., 2003b; Soares et al., 2005). Given that IEG expression likely relies on specific activity patterns and qualities, substantial interest in the field has been shifting from their use as activity-markers towards studies of their specific roles in cellular physiology. As discussed in a number of chapters in this volume, the proteins encoded by IEGs play a wide variety of roles in the physiology of neurons and neural networks, including memory formation, cell death and trophic regulation, as a few examples. In addition, a group of IEGs, including some discussed in this chapter, appear to play key roles in the induction and/or maintenance of plasticity in neurons. Given that the expression of these IEGs is strongly correlated with neuronal activity patterns that have been associated with plasticity in the CNS, recent studies have focused on these "candidate-plasticity genes" as tools to map circuits undergoing experience-dependent reorganization, as well as the specific roles of these proteins in these processes.

One of the most commonly employed experimental paradigms that reliably triggers plasticity-associated modifications in cortical circuitry is the exposure of animals to an enriched environment (EE). This experimental protocol fundamentally involves exposing freely-behaving animals to a complex sensory setting (related to visual processing: higher contrast, stimuli frequency, depth,

colors) and comparing gene expression patterns obtained from these animals with those obtained from control groups, which are often composed of freely-ranging animals that have been exposed to impoverished visual environments (Rosenzweig et al., 1972; van Praag et al., 2000; Pinaud, 2004; Pinaud and Tremere, 2005).

Exposure of animals to an EE triggers dramatic changes in CNS architecture and physiology (reviewed in Rosenzweig et al., 1972; van Praag et al., 2000; Pinaud, 2004; Pinaud and Tremere, 2005). For example, in the visual cortex exposure of animals to an EE significantly increases the dendritic arborization of primary visual cortical neurons and overall neuronal density, as well as mean synaptic disc diameter and synapse-to-neuron ratio, both of which are direct correlates of enhanced synaptic transmission (Volkmar and Greenough, 1972; Bhide and Bedi, 1984, 1985; Beaulieu and Colonnier, 1987, 1988; van Praag et al., 2000). These morphological correlates of visual experience have been shown to translate into enhanced physiological responses in V1 neurons. For example, EE animals exhibit a higher percentage of orientation-selective neurons when compared to animals raised in impoverished visual conditions. Moreover, sharper orientation tuning and increased responsivity to light were reported for EE animals (Beaulieu and Cynader, 1990a, b). Finally, contrast sensitivity and acuity were reported to be enhanced in enriched animals (Beaulieu and Cynader, 1990a, b; Prusky et al., 2000). These findings clearly indicate that exposure of animals to an EE triggers significant anatomical and functional plastic changes in visual cortical circuitry.

In an effort to separate the effects of activity-driven gene expression from those genetic mechanisms associated with the induction of plasticity in the visual cortex, we and others have investigated candidate-plasticity gene expression patterns that result from ambient light-stimulation paradigms, and compared them with those obtained by exposing animals to complex visual environments (Pinaud et al., 2001; Pinaud et al., 2002a). Our working hypothesis was that if IEG expression is associated with the induction and/or regulation of plastic changes in V1, then EE animals will exhibit a different gene expression profile as compared with those animals that experienced simple patterns of visual stimulation (and presumably did not undergo experience-dependent changes in cortical circuitry).

Our questions regarding the relationship of IEG expression to a putative plasticity-inducing set of stimuli were addressed in the following experiment. A population of young adult rats was divided into three experimental groups that were housed in typical home cages of two to three animals per cage. The first experimental group was named the enriched environment group (EE) because each day, at the same hour, these animals were placed into the enriched environment complex. Following 1 hr in the EE complex, animals were returned to their standard housing. The full duration of the experiment was 21 days, so each animal had 21 exposures to the EE. As one control group, we maintained a group of animals in housing conditions that were identical to the home cages used for the EE animals. These animals were simply maintained in parallel with

the experimental group with no interference from an investigator; this group was named the undisturbed (UD) control group. We anticipated that the daily handling of EE animals to and from the EE could influence gene expression and cause an overestimation of the EE effect on IEG expression. To capture what proportion of IEG expression could be attributed to this movement of animals and any stress associated with going into and out from the experimental setting we created a second control group called the handling only (HO) group. These subjects were housed in conditions that were identical to both the EE and UD groups. The HO animals were handled at the same time that the EE group underwent relocation to the enriched setting. Unlike EE animals, HO animals were immediately placed back into standard home cages (Pinaud et al., 2001, 2002a; Pinaud, 2004).

In these experiments, we focused our analyses on the expression of the IEGs NGFI-A and *arc*. As briefly described above, both of these IEGs are well positioned to mediate activity-dependent plastic changes in neurons, including those that result from EE exposure. For example, target genes that are regulated by the transcription factor NGFI-A include part of the neurotransmitter release machinery, such as synapsin I, synapsin II and synaptobrevin, as well as structural genes such as neurofilament (Pospelov et al., 1994; Thiel et al., 1994; Petersohn et al., 1995; Petersohn and Thiel, 1996). The protein encoded by the IEG *arc*, on the other hand, is translated in an activity-dependent manner in polyribossomal complexes located in the post-synaptic membrane, often at the base of dendritic spines (Lyford et al., 1995; Steward et al., 1998; Steward and Worley, 2001b). This attribute of *arc* expression potentially places this protein in a good position to orchestrate dendritic reconfiguration as a function of activity (Pinaud et al., 2001; Pinaud, 2004). Finally, the expression of both genes depend on calcium influx associated with NMDA receptor activation, a subtype of glutamatergic receptors that has been repeatedly implicated in paradigms of plasticity and enhanced synaptic efficacy (reviewed in Collingridge and Lester, 1989; Hollmann and Heinemann, 1994; Mayer and Armstrong, 2004).

Both NGFI-A mRNA and protein levels were assessed in V1 of EE, HO and UD animals. As reported previously by other groups, NGFI-A mRNA was detected at high basal levels in UD animals (Wallace et al., 1995; Kaczmarek and Chaudhuri, 1997; Pinaud et al., 2002a). Even though high mRNA levels were detected in all cortical layers except for layer I, the highest signal was observed in cortical layers III and V (discussed below) (Pinaud et al., 2002a). Optical density analysis revealed that the mRNA levels detected in HO controls were not significantly different than those levels observed in UD controls. Conversely, EE animals underwent a significant upregulation of NGFI-A mRNA levels in all cortical layers of V1, when compared to HO and UD controls (Fig. 2.3) (Pinaud et al., 2002a).

Immunocytochemistry directed at NGFI-A protein also showed that exposure to a complex visual environment triggered a marked upregulation of this IEG in all cortical layers of V1, with the exception of layer I, as compared to the protein levels observed in HO and UD animals (Pinaud et al., 2002a) (Fig. 2.4). Similar to our findings obtained with NGFI-A mRNA distribution, the highest increase in

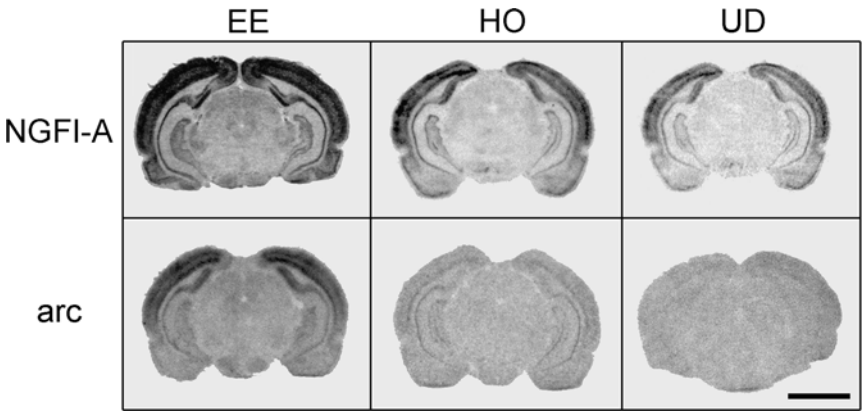

Figure 2.3. Experience-dependent expression of immediate early genes NGFI-A and *arc* in the rodent visual cortex. In-situ hybridization autoradiograms depicting NGFI-A (top row) and arc (bottom row) expression in animals that were exposed to a complex visual environment (EE) for 1 hour/day for a total of 21 days, as compared to the expression levels of animals that were manipulated (HO) or left unidisturbed in their home cages (UD). Both NGFI-A and arc are markedly upregulated by exposure to the EE condition, as compared to both control groups. Expression levels for both IEGs are not different across both control groups. Highest induction in response to the EE for both IEGs was detected in cortical layers III and V. Scale bar = 500 μm. NGFI-A, nerve growth factor-induced gene A; arc, activity-regulated cytoskeletal gene; EE, enriched environment group; HO, handled-only group; UD, undisturbed group.

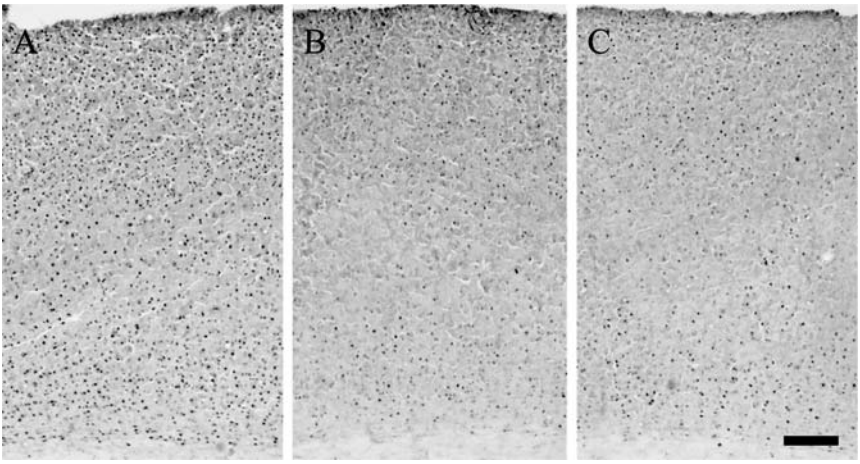

Figure 2.4. NGFI-A protein levels are increased in all cortical layers of the primary visual cortex in rats exposed to an enriched visual environment. Compared to undisturbed (UD) and handled-only (HO) animals, animals exposed to an enriched environment (EE) exhibited an increased number of immunopositive nuclei in cortical layers II/III and VI, while a more modest increase was found in layer IV (A). Both HO (B) and UD (C) controls displayed basal levels of NGFI-A immunoreactivity in all cortical layers, layer IV being the cortical layer with fewest NGFI-A positive nuclei. No NGFI-A positive cells were detected in layer I for all experimental groups. Scale bar = 200 μm.

the number of NGFI-A immunopositive neurons was detected in cortical layers III and V, while the smallest increase in NGFI-A expression was detected in cortical layer IV (discussed below).

Together these findings suggest that it was the complexity of the visual environment—and likely the neuronal activity patterns associated with this experience—rather than simply the light stimulation, that triggered a differential regulation of the IEG NGFI-A in the rodent V1. The underlying notion behind this interpretation considers that both UD and HO groups were fully awake, displayed similar activity levels and were maintained in identical luminance environments, as compared to EE animals and, therefore, were also visually stimulated. Nevertheless, in both HO and UD groups, NGFI-A mRNA and protein levels were substantially different from those obtained in the V1 of EE animals.

In contrast to the findings obtained with NGFI-A, arc mRNA levels were found at moderately low basal levels in the rodent V1 (Pinaud et al., 2001) (Fig. 2.3). Quantitative analysis of arc mRNA levels in HO and UD controls revealed that these groups were not significantly different from each other. Conversely, arc levels in EE animals were markedly increased when compared to both control groups (Pinaud et al., 2001). This upregulation was detected in all cortical layers (Fig. 2.3). Interestingly, and similar to the findings obtained with NGFI-A, the highest arc mRNA levels were detected in cortical layers III and V, with the smallest increases observed in the thalamo-recipient layer IV (discussed below). Together, these findings provided direct evidence for a reliable and significant upregulation of both IEGs following EE exposure. In addition these data suggested that NGFI-A and arc may be part of the machinery involved in the early inducible genomic response associated with experience-dependent plastic changes in the rodent visual cortex, rather than simply echoing neuronal activity.

The significant increase in IEG expression in the V1 of EE animals may therefore be associated with increased levels, or specific patterns, of neuronal activity that result from visual experiences in a complex environment. One interesting possibility for the recruitment of plasticity-associated gene expression programs in V1 is that mechanisms for detecting sub-optimal architecture and processing capabilities are in place, and were activated, in this cortical region following EE exposure. During experience in the EE setting, detection of sensory or information overload may trigger these programs to initiate a potential optimization of cortical architecture and synaptic weights, in order to appropriately process information contained in this new, rich sensory environment. Experimental testing of this hypothesis may shed light on the genetic mechanisms associated with the regulation of plasticity in the CNS.

Alternatively, novelty may play a role in the differential regulation of IEG expression following EE exposure. In fact, other IEGs such as *c-fos* and *c-jun* have been previously demonstrated to be positively regulated by novelty in a number of forebrain areas, including the somatosensory and visual cortices (Papa et al., 1993; Zhu et al., 1995). Interestingly, noradrenergic input, which has been

proposed to gate attentional processing as it relates to arousal and behavioral responsivity to novelty, has been shown to be required for the maintenance of basal expression of NGFI-A in the rodent forebrain (Cirelli et al., 1996), as well as for light-induced expression of this IEG in the rat V1 (Pinaud et al., 2000). However, in the EE experiments conducted by our group, novelty is not a probable player in the regulation of IEG expression given that expression levels were assessed in animals that were familiarized with the EE for three weeks (Pinaud et al., 2001, 2002a).

One of the most noticeable effects in our preparations was that the highest expression of both NGFI-A and *arc* was detected in cortical layers III and V, and the smallest increases in gene expression were consistently detected in the thalamo-recipient layer IV of EE animals (Pinaud et al., 2001, 2002a). These findings are intriguing and may be related to electrophysiological results suggesting that cortical layers II/III and V are potentially more plastic, as indicated by the ease of triggering experience-dependent alterations in their functional properties and synaptic strength, as has been observed in long-term potentiation (LTP) and long-term depression (LTD) (Daw et al., 1992; Darian-Smith and Gilbert, 1994; Glazewski and Fox, 1996; Petersen and Sakmann, 2001). On the contrary, these activity-driven changes in synaptic strength were more difficult to trigger in layer IV neurons, suggesting that the thalamo-recipient layer is less plastic than other cortical strata. The potentially low plasticity observed in layer IV may be associated with the stability in cortical map representation, as well as the preservation of fidelity of information transfer at this level (Pinaud, 2004, 2005). Although systematic studies are required to support or refute this hypothesis, should it be supported, the low candidate-plasticity gene expression detected in layer IV following EE exposure may provide a read-out of a decrease in activity for the machinery involved in triggering or regulating experience-dependent network reorganization in V1. Similarly, enhanced expression levels in layers III and V following EE exposure may provide a direct visualization of neurons in V1 that are participating in a neural plastic response triggered by exposure to an enriched visual environment.

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