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Brief visual experience induces immediate early gene expression in the cat visual cortex

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ABSTRACT **Brief visual experience causes rapid physiological changes in the visual cortex during early postnatal development. A possible mediator of these effects is the immediate early genes whose protein products are involved in the rapid response of neurons to transsynaptic stimulation. Here we report evidence that the levels of immediate early gene mRNAs in the visual cortex can be altered by manipulating the visual environment. Specifically, we find that brief (1 h) visual experience in dark-reared cats causes dramatic transient inductions of *egr1*, c-*fos*, and *junB* mRNAs in the visual cortex but not in the frontal cortex. Levels of c-*jun* and c-*myc* mRNAs are unaffected. These results suggest that select combinatorial interactions of immediate early gene proteins are an important step in the cascade of events through which visually elicited activity controls visual cortical development.**

The neonatal visual cortex is a highly plastic structure whose development is guided by visual experience during early postnatal life. The clearest example of such environmental effects on development of the visual cortex is to rear animals with one eye sutured closed (monocular deprivation), a condition that leads to dramatic anatomical and physiological abnormalities (1, 2). In normal development, sensitivity to monocular deprivation is limited to a "critical period" that, in cats, begins several weeks after birth, peaks at 5–6 weeks, and then gradually disappears over the next several months (3, 4).

Studies of cats reared in total darkness (dark rearing) have shown that the critical period is not a simple age-dependent maturational process; rather, visual input plays a role in controlling the time course of visual cortical plasticity. Dark rearing from birth maintains many properties of the immature cortex and extends this critical period well beyond its normal age limit (5, 6). Brief exposure (6 h) of dark-reared animals to light results in the rapid development of adult-like response properties (7, 8) and activates mechanisms that lead to the elimination of plasticity (9). These results strongly indicate that the critical period is controlled by interactions between visual input and on-going gene transcription processes.

Understanding how patterns of gene expression are affected by visual experience would be a major advance in clarifying the mechanisms that control the critical period of the visual cortex. As a first step toward this goal, the present study focused on the capacity of visual experience to influence the expression of the cellular immediate early genes (IEGs). These genes are an appropriate starting point because there is increasing evidence that they play an active role in converting brief environmental input into lasting changes in cellular function. IEGs are rapidly activated in neurons in response to transsynaptic stimuli and their induction leads to alterations in the transcriptional activity of target genes that contain the response elements for the IEG proteins (10–12). It appears that in the nervous system (13, 14), as in other systems (10–12), the induction of different combinations of IEGs leads to different and specific changes in cellular responses to environmental input.

The results reported here provide evidence that the levels of visual cortical IEG mRNAs can be altered by manipulating the visual environment. A particularly intriguing finding is that brief (1 h) visual experience in dark-reared cats causes dramatic transient inductions in a select group of IEG mRNAs in the visual cortex but not in the frontal cortex. The regional specificity of this response and its restriction to a subset of IEG mRNAs suggests that select combinatorial interactions of IEG proteins are an important step in the cascade of events through which visually elicited activity controls visual cortical development.

MATERIALS AND METHODS

Animals. Control cats (normal reared) were reared in a colony with a 12-h light/12-h dark cycle until they reached 5 (N5, $n = 4$) or 20 (N20, $n = 4$) weeks of age. Dark-reared cats were placed with their mother in total darkness within 24 h after birth and were raised in the absence of light until either 5 (D5, $n = 3$) or 20 (D20, $n = 5$) weeks of age. Dark-reared cats that received subsequent exposure to light were raised in total darkness until 5 weeks of age, at which time they received, immediately prior to sacrifice, either 1 h of visual experience (DE5[1h], $n = 3$) or 6 h of visual experience per day on two successive days (DE5[2d], $n = 3$). Comparative data were obtained from normal adult (>1 year) cats ($n = 3$), normal adults placed in prolonged (8 weeks) darkness ($n = 3$), and normal adults placed in darkness for 2 weeks and then given 1 h of visual experience ($n = 2$). All animals were sacrificed by an overdose of sodium pentobarbital. Dissection of visual cortex included nearly all of area 17 and part of area 18. The comparison dissection included the frontal cortex, much of the motor cortex, and part of the somatosensory cortex and is hereafter referred to as the frontal cortex. Brain regions were dissected, immediately frozen in liquid nitrogen, and stored at −80°C until used for RNA isolation.

RNA Isolation. RNA was isolated from brain tissue by the procedure of Chomczynski and Sacchi (15). RNA concentrations and purity were estimated spectrophotometrically by measuring the absorbance at 260 nm, 280 nm, and 320 nm. Integrity of the RNA was assessed by the pattern of ethidium bromide fluorescence after electrophoresis on denaturing formaldehyde/agarose gels.

Probes. Probes used for hybridizations were as follows: c-*fos*, a 1.7-kilobase (kb) *Pst* I fragment of a rat cDNA clone

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Abbreviations: IEG, immediate early gene; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

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(pcfos3, kindly provided by M. Greenberg and J. Belasco, Harvard Medical School); c-*myc*, a 1.5-kb *Sst* I fragment derived from the human cDNA pHSR-1 (ATCC 41010); *egr1*, a 700-base-pair *Pst* I fragment derived from a rat *egr1* cDNA (kindly provided by V. Sukhatme, University of Chicago, Chicago); c-*jun*, a 2.6-kb mouse cDNA pJAC.1 (ATCC 63026); *junB*, a 1.9-kb mouse cDNA p465.20 (ATCC 63025); GAPDH, a 500-base-pair *Xba* I–*Hin*dIII fragment of a human glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA (phcGAP, ATCC 57090).

Northern Blot Analysis. Northern blots were performed as described by Rosen *et al.* (16). In brief, total cellular RNA (7.5 μg) was fractionated on 1.3% agarose/2.2 M formaldehyde surface-tension gels. After fractionation, the RNA was transferred to a charged nylon membrane (Nytran, Schleicher & Schuell). All filters were hybridized in a solution containing 50% (vol/vol) formamide, 5× SSC (1× SSC = 0.15 M sodium chloride/0.015 M sodium citrate), 1× Denhardt's solution (0.02% bovine serum albumin/0.02% polyvinylpyrrolidone/0.02% Ficoll), 0.1% SDS, sheared denatured salmon sperm DNA (100 μg/ml), and the appropriate probe at 50°C for 24 h. DNA probes were labeled to a specific activity of 1×10^9 dpm/μg by using a random oligonucleotide priming kit (Boehringer Mannheim) in the presence of [α-^{32}P]dCTP (3000 Ci/mmol; 1 Ci = 37 GBq; New England Nuclear). After washing in 0.1× SSC/0.1% SDS at 50°C, membranes were exposed to x-ray film (Kodak XAR-5) with an intensifying screen (DuPont Cronex Lightning Plus) at −80°C. The resulting Northern blots demonstrated that, under the high-stringency conditions used, each IEG probe under investigation detected an appropriately sized band in cat visual cortex (17).

Slot Blot Hybridization. Total RNA (5 μg) from each sample was denatured and applied to a charged nylon membrane using a slot blot apparatus. All filters were hybridized and washed as described for Northern blots. Visual and frontal cortical RNA samples were analyzed on separate filters; therefore, the absolute RNA levels cannot be compared between the two structures. Each filter was apposed to film for various times to ensure an exposure whereby all samples were detectable and within the usable range of the film. After hybridization with an IEG probe, filters were stripped and rehybridized with the GAPDH probe. GAPDH mRNA levels were found to remain essentially constant during the time points under study (see Fig. 1). Dark-rearing and exposure to light also did not alter the levels of this mRNA and, therefore, it was used as a control for loading errors in slot-blot hybridization experiments. All values were expressed as the ratio of absorbance of individual IEG mRNAs to that of GAPDH mRNA.

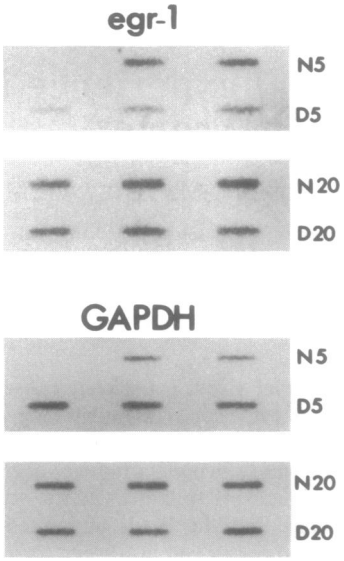

FIG. 1. Slot-blot analysis of visual cortical RNA probed with *egr1* and GAPDH probes. Normal (N) and dark-reared cats (D) were compared at 5 and 20 weeks of age. Data from several cats are not shown in this portion of the slot blot.

RESULTS

We first determined whether the absence of visual input affected the steady-state level of IEG mRNAs by comparing visual and frontal cortical RNA from individual normal and dark-reared cats at 5 and 20 weeks of age. We analyzed the expression of c-*fos*, *egr1*, c-*jun*, *junB*, and c-*myc* mRNAs. Levels of *egr1* mRNA were lowered slightly by dark rearing at both 5 (40%) and 20 (33%) weeks in the visual but not

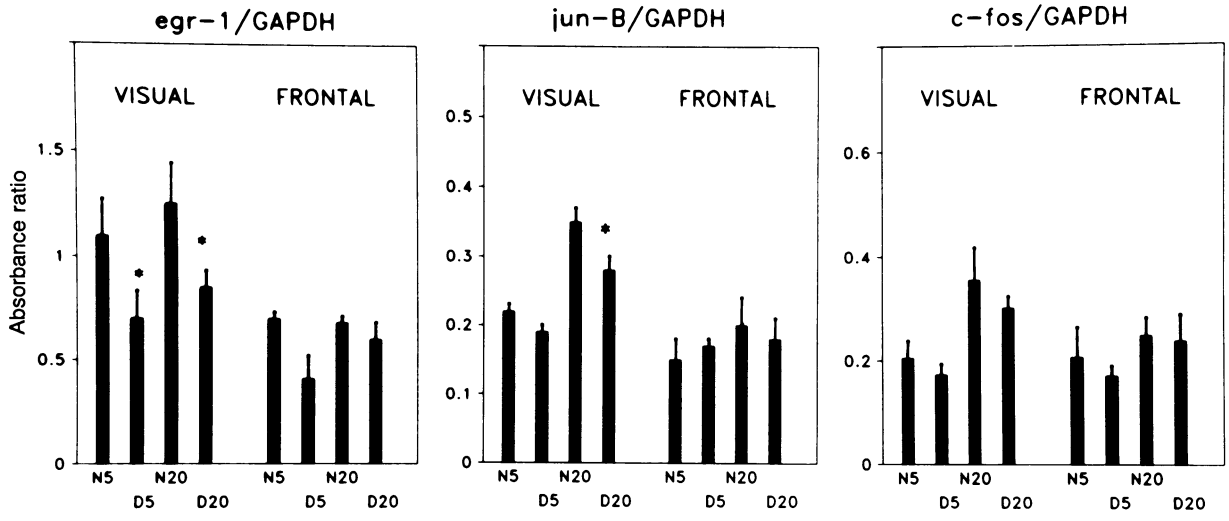

FIG. 2. Effect of dark rearing on IEG mRNA levels in the visual cortex and the frontal cortex. Results of densitometric analyses for *egr1*, *junB*, and c-*fos* mRNAs in various rearing conditions are shown. All values are expressed as the ratio of the absorbance of individual IEG mRNAs, as indicated, to that of GAPDH mRNA (mean ± SEM). At each age, Mann–Whitney *U* tests were used to compare normal and dark-reared animals. An * indicates statistically significant differences [*egr1*, N5 vs. D5 ($P = 0.028$) and N20 vs. D20 ($P = 0.018$); *junB*, N20 vs. D20 ($P = 0.036$)]. (Abbreviations are as in Fig. 1.) There were no significant differences for c-*fos*. Similarly, there were no significant effects of dark rearing on the levels of c-*jun* or c-*myc* mRNAs (data not shown).

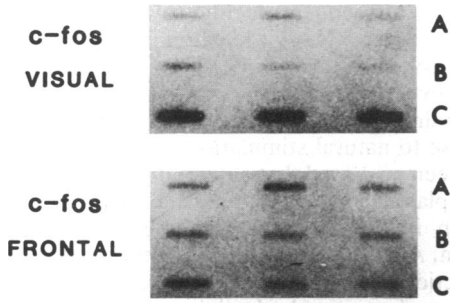

FIG. 3. Induction of c-*fos* mRNA in the visual cortex but not the frontal cortex of dark-reared animals as a result of brief visual experience. Total RNA (5 μg) from three animals in each of three rearing conditions were analyzed. Rows: A, Dark-reared animals at 5 weeks of age (D5); B, dark-reared animals at 5 weeks of age given 2 days of visual experience [DE5(2d)]; C, dark-reared animals at 5 weeks of age given 1 h of visual experience [DE5(1h)].

frontal cortex (Figs. 1 and 2). A less-marked reduction (22%) was found with *junB* mRNA, which was selectively decreased in the visual cortex only at 20 weeks of age in dark-reared animals. The levels of c-*jun*, c-*fos*, and c-*myc* mRNAs were not significantly different between rearing conditions in either structure. To ensure that the effects seen were not due to darkness *per se*, we compared normal adult cats ($n = 3$) with normal adult cats who were placed in total darkness for 8 weeks ($n = 3$) and found no difference in visual cortex for any of the IEGs tested (data not shown). This result suggests that the effect of dark rearing on IEG mRNA levels is limited to the early postnatal period. Clearly, the absence of visual input during postnatal development has a very selective effect on IEG mRNA expression. These results indicate that although most IEGs are expressed at a basal level irrespective of visual activity, a select population of IEGs (in this case *egr1* and *junB*) show a dependence upon visual input for the acquisition of their normal steady-state mRNA level in the visual cortex.

Next we investigated whether exposure to visual input would induce alterations in IEG mRNA levels in dark-reared cats. We examined RNA from both visual and frontal cortices of 5-week-old dark-reared cats that received, prior to sacrifice, either a single 1-h visual experience or a 6-h visual experience per day on two successive days. A 1-h visual experience led to dramatic increases (2- to 4-fold) in the levels of *egr1*, c-*fos*, and *junB* mRNAs in the visual cortex but not the frontal cortex (Figs. 3 and 4). The absolute activation of these genes is probably higher than that observed here, since the 1-h point is unlikely to correspond to the exact peak of expression of these IEGs. The absence of IEG activation in the frontal cortex shows that the response seen was not a generalized neural effect and rules out nonspecific events associated with either the anesthesia or the procedure used to isolate the tissue. The transient nature of this response is indicated by the disappearance of the induced IEG mRNAs in the visual cortex of the animals exposed to light on two successive days. The other members of the IEG family examined (c-*myc* and c-*jun*) showed no alterations under the conditions tested. These results indicate that specific IEGs are involved in the processes by which visual input activates the developing visual cortex.

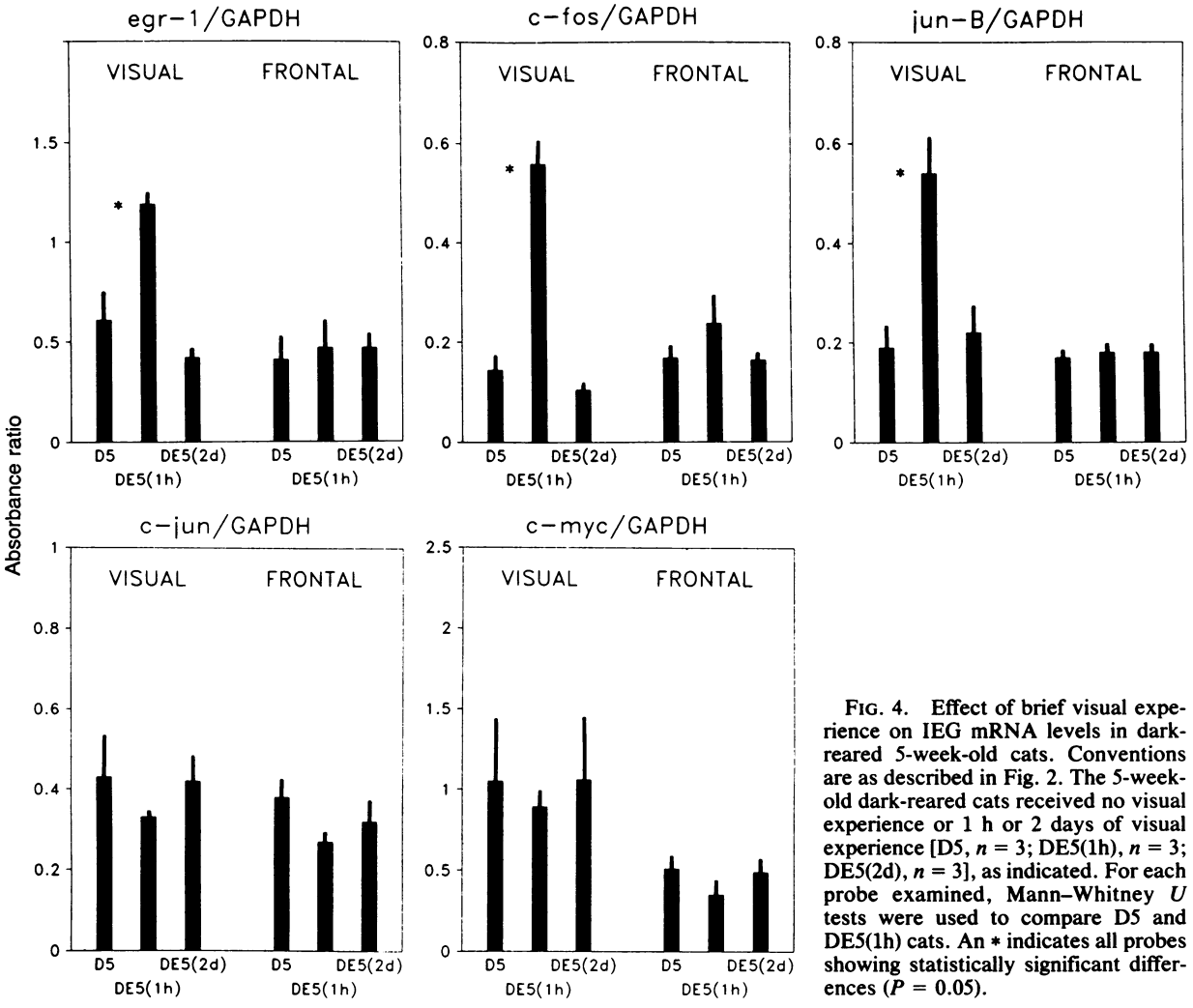

FIG. 4. Effect of brief visual experience on IEG mRNA levels in dark-reared 5-week-old cats. Conventions are as described in Fig. 2. The 5-week-old dark-reared cats received no visual experience or 1 h or 2 days of visual experience [D5, $n = 3$; DE5(1h), $n = 3$; DE5(2d), $n = 3$], as indicated. For each probe examined, Mann–Whitney U tests were used to compare D5 and DE5(1h) cats. An * indicates all probes showing statistically significant differences ($P = 0.05$).

The question arises as to whether the induction of these IEGs by brief visual experience plays a specific role during early postnatal life or whether it simply reflects the change in neural activity. To test these possibilities, we did a similar analysis on normal adult cats that were placed in darkness for 2 weeks and then exposed to visual input for 1 h prior to sacrifice [DEA(1h) cats; $n = 2$]. These cats were compared to normal adult cats and to normal adult cats that experienced prolonged darkness prior to sacrifice. As demonstrated for *junB* mRNA in Fig. 5, all three IEGs showed marked increases in their levels, specific to the visual cortex, after a 1-h visual experience in DEA(1h) cats. Quantitation, however, indicated that the magnitude of the induction in these cats was much smaller (c-*fos*, 1.7-fold; *egr1*, 1.4-fold; *junB*, 2.0-fold) than the magnitude in dark-reared 5-week-old kittens (c-*fos*, 3.9-fold; *egr1*, 2.3-fold; *junB*, 2.8-fold).

DISCUSSION

The results of this study demonstrate the induction of changes in the levels of IEG mRNAs in the cat visual cortex as a result of manipulating visual input, its natural stimulus. Rearing cats from birth without visual input causes a slight reduction of *egr1* and *junB* mRNA levels, specifically in the visual cortex, but has no effect on levels of c-*fos*, c-*myc*, or c-*jun* mRNAs. Acquisition of normal levels of both *egr1* and *junB* mRNAs, thus, relies on the presence of normal visual input. More importantly, our studies show that the introduction of visual experience induces the activation of *egr1*, *junB*, and c-*fos* mRNAs within 1 h in the visual cortex of dark-reared cats. A similar effect was recently reported for *egr1* mRNA in the rat visual cortex (18). The present findings that the inductions are specific to the visual cortex and that IEG mRNA levels have returned to their preexposure levels after 2 days of visual experience link the IEG response to the rapid physiological changes that brief experience causes in the visual cortex. Since it is well-established that development of the visual cortex is activity-dependent (19–22), the present findings that IEGs are activity-induced suggest that their induction may play a role in adaptive alterations in gene expression that define the long-term changes seen in neuronal behavior. Moreover, the induction of some, but not other, IEGs suggests that a combinatorial model of IEG activation may be involved in this process. It is interesting to note that the IEGs induced by brief visual experience show a common developmental pattern in visual cortex during postnatal life (17). *egr1*, c-*fos*, and *junB* mRNAs show a sharp rise from very low levels at birth to peak levels during the critical period and a subsequent decline to adult levels. Levels of c-*jun* and c-*myc* mRNAs, which did not show induction, are relatively constant throughout the critical period (17). Perhaps the developmental pattern of expression of IEGs within a given brain structure is related to the inducibility of IEGs in response to natural stimulation.

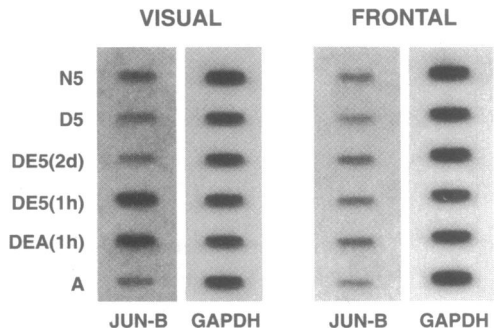

FIG. 5. Portion of a slot blot showing examples of the induction of *junB* mRNA in the visual cortex of a 5-week-old dark-reared cat exposed to visual input for 1 h [DE5(1h) cat], a 5-week-old dark-reared cat exposed to visual input on two successive days [DE5(2d)], and an adult cat placed in prolonged darkness and then given 1 h of visual experience [DEA(1h) cat]. The induction did not occur in the frontal cortex. Levels of GAPDH mRNA were not affected by visual manipulation in either structure.

It has been proposed that activation of IEGs is involved in neuronal plasticity (13, 14), and the characteristics of IEG expression in the visual cortex are consistent with this suggestion. An equally plausible hypothesis, however, is that IEG inductions simply reflect neural activity. Since both IEG induction and neuronal plasticity are activity-dependent, resolution of this issue is not simple. The comparison of inducibility in young vs. old cats in this study was aimed at this issue. Inductions occurred at both ages but were quantitatively greater in young animals. This result can be interpreted in several ways. One is that the induction of IEGs could occur frequently (e.g., every morning) and simply reflect a change in activity level. Studies of brief visual input during the dark phase of a 12-h light/12-h dark cycle suggest a less-simple interpretation. Such stimulation induces c-*fos* and *egr1* mRNA and proteins in the suprachiasmatic nuclei and related visual structures involved in controlling the circadian rhythm (23, 24). These stimulation conditions, however, do not induce changes in the expression of these IEGs in the lateral geniculate nucleus, and thus there may be differences between the geniculocortical pathway and other compartments of the visual system in induction of IEGs. The greater magnitude of the induction in young animals could indicate that induction of IEGs plays a special role during the critical period in visual cortex. The induction of IEGs could play a special role during early life if, for example, (*i*) the effects of IEGs on the target genes exceeded a specific threshold or were magnified during the critical period; (*ii*) the array of target genes was different at different ages; or (*iii*) different cell types were effected in young vs. old animals.

Recent studies have begun to uncover the mechanisms by which IEGs translate extracellular events to exert effects on their target genes. In the nervous system (13, 14), as in other systems (10–12), different combinatorial inductions of IEGs lead to different and specific changes in cellular responses to environmental input. Notably, different groups of IEGs respond to different types of stimulation in the nervous system. For example, *egr1* (also known as *zif-268*, NGFI-A, and Krox-24) is elevated in hippocampus by electrical stimulation that causes either seizures or long-term potentiation, whereas c-*fos* is induced only by seizures (25–28). c-*jun* is induced by trophic factors but not by depolarization, whereas c-*fos* is induced by both (29). It appears that the selective activation of specific subsets of IEGs provides a mechanism by which the expression of specific target genes is regulated.

Considered in this context, the activation of the c-*fos* and *junB* genes by visual exposure and the absence of an effect on c-*jun* expression are particularly interesting. The proteins produced by members of the *fos* and *jun* gene families can form heterodimeric complexes collectively termed AP-1 (30–33). The AP-1 complex is capable of binding to target genes and altering their transcriptional state. Genes involved in a wide array of cellular functions have been identified that contain AP-1 binding sites in their regulatory elements (10–12, 30–33). Recent studies have suggested that specificity of effects upon transcription can be generated by the selective combinatorial interaction of different members of the *fos* and *jun* families. Heterodimers of c-Fos and c-Jun proteins appear to be capable of activating many genes including c-*jun* itself. However, c-Fos and JunB protein heterodimers appear to be capable of activating certain target genes and repressing other genes (e.g., the c-*jun* gene) (34–36). Given the capacity of members of the *fos* and *jun* families, as well as *egr1*, to regulate transcriptional processes, it is tempting to speculate

that the particular IEG activation pattern seen in the visual cortex upon exposure to light may signal not only the activation of genes important to acquiring mature cortical patterns but also the repression of genes whose continued expression is necessary for the maintenance of visual cortical plasticity. Identification of the target genes of different IEG combinations will be of major importance in evaluating the possible role of IEGs in neuronal plasticity, and several possible candidate genes have been suggested (e.g., refs. 37–40).

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1. Hubel, D. H., Wiesel, T. N. & LeVay, S. (1977) *Philos. Trans. R. Soc. London Ser. B* **278**, 377–409.
2. Shatz, C. J. & Stryker, M. P. (1978) *J. Physiol. (London)* **281**, 267–283.
3. Hubel, D. H. & Wiesel, T. N. (1970) *J. Physiol. (London)* **206**, 419–436.
4. Olsen, C. R. & Freeman, R. D. (1980) *Exp. Brain Res.* **39**, 17–21.
5. Cynader, M. & Mitchell, D. E. (1980) *J. Neurophysiol.* **43**, 1026–1040.
6. Mower, G. D., Berry, D., Burchfiel, J. L. & Duffy, F. H. (1981) *Brain Res.* **220**, 255–267.
7. Buisseret, P., Gary-Bobo, E. & Imbert, M. (1978) *Nature (London)* **272**, 816–817.
8. Buisseret, P., Gary-Bobo, E. & Imbert, M. (1982) *Dev. Brain Res.* **4**, 417–426.
9. Mower, G. D., Christen, W. G. & Caplan, C. J. (1983) *Science* **221**, 178–180.
10. Verma, I. M. & Graham, R. W. (1987) *Adv. Cancer Res.* **49**, 29–52.
11. Johnson, P. F. & McKnight, S. L. (1989) *Annu. Rev. Biochem.* **58**, 799–839.
12. Mitchell, P. J. & Tjian, R. (1989) *Science* **245**, 371–378.
13. Sheng, M. & Greenberg, M. E. (1990) *Neuron* **4**, 477–485.
14. Morgan, J. I. & Curran, T. (1991) *Annu. Rev. Neurosci.* **14**, 421–451.
15. Chomczynski, P. & Sacchi, N. (1987) *Anal. Biochem.* **162**, 156–159.
16. Rosen, K. M., Lamperti, E. D. & Villa-Komaroff, L. (1990) *Biotechniques* **8**, 398–403.
17. McCormack, M. A., Rosen, K. M., Villa-Komaroff, L. & Mower, G. D. (1992) *Mol. Brain Res.* **12**, 215–223.
18. Worley, P. F., Christy, B. A., Nakabeppu, Y., Bhat, R. V., Cole, A. J. & Baraban, J. M. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 5106–5110.
19. Mower, G. D., Caplan, C., Christen, W. G. & Duffy, F. H. (1985) *J. Comp. Neurol.* **235**, 448–466.
20. Stryker, M. P. & Harris, W. A. (1986) *J. Neurosci.* **6**, 2117–2131.
21. Swindale, N. V. (1988) *J. Comp. Neurol.* **267**, 472–478.
22. Constantine-Paton, M., Cline, H. T. & Debski, E. (1990) *Annu. Rev. Neurosci.* **13**, 129–154.
23. Rusak, B., Robertson, H. A., Wisden, W. & Hunt, S. P. (1990) *Science* **248**, 1237–1240.
24. Aronin, N., Sagar, S. M., Sharp, F. R. & Schwartz, W. J. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 5959–5962.
25. Morgan, J. I., Cohen, D. R., Hempstead, J. L. & Curran, T. (1987) *Science* **237**, 192–197.
26. Douglas, R. M., Dragunow, M. & Robertson, H. A. (1988) *Mol. Brain Res.* **4**, 259–262.
27. Saffen, D. W., Cole, A. J., Worley, P. F., Christy, B. A., Ryder, K. & Baraban, J. M. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 7795–7799.
28. Cole, A. J., Saffen, D. W., Baraban, J. M. & Worley, P. F. (1989) *Nature (London)* **340**, 474–476.
29. Bartel, D. P., Sheng, M., Lau, L. F. & Greenberg, M. E. (1989) *Genes Dev.* **3**, 304–313.
30. Angel, P., Imagawa, M., Chiu, R., Stein, B., Imbra, R. J., Rahmsdorf, H. J., Jonat, C., Herrlich, P. & Karin, M. (1987) *Cell* **49**, 729–739.
31. Lee, W., Mitchell, P. & Tjian, R. (1987) *Cell* **49**, 741–752.
32. Bohmann, D., Bos, T. J., Admon, A., Nishimura, T., Su, H., Vogt, P. K. & Tjian, R. (1987) *Science* **238**, 1386–1392.
33. Rauscher, F. J., III, Sanbucetti, L. C., Curran, T., Distel, R. J. & Spiegelman, B. M. (1988) *Cell* **52**, 471–480.
34. Chiu, R., Angel, P. & Karin, M. (1989) *Cell* **59**, 979–986.
35. Schutte, J., Viallet, J., Nau, M., Segal, S., Fedorko, J. & Minna, J. (1989) *Cell* **59**, 987–997.
36. Lucibello, F. C., Lowag, C., Newberg, M. & Muller, R. (1989) *Cell* **59**, 999–1007.
37. Benowitz, L. I. & Routtenberg, A. (1987) *Trends Neurosci.* **10**, 527–532.
38. Guimaraes, A., Zaremba, S. & Hockfield, S. (1990) *J. Neurosci.* **10**, 3014–3024.
39. Aoki, C. & Siekevitz, P. (1985) *J. Neurosci.* **5**, 2465–2483.
40. Hendry, S. H., Jones, E. G. & Burstein, N. (1990) *J. Neurosci.* **8**, 2438–2450.