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Differential Expression of Zif268 and c-Fos in the Primary Visual Cortex and Lateral Geniculate Nucleus of Normal *Cebus* Monkeys and after Monocular Lesions

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ABSTRACT

The transcription factors c-Fos and Zif268 have been used as markers of neuronal activity, and they also have been implicated in neuronal plasticity. In this study, we investigated the expression of c-Fos and Zif268 proteins in the lateral geniculate nucleus (LGN) and in the cortical primary visual area (V1) of normal adult *Cebus apella* monkeys and in animals with monocular lesions. In the LGN, the reaction for c-Fos showed immunopositive cells in both magnocellular (M) and parvocellular (P) layers; however, the label was heavier in P layers. In animals that suffered monocular lesions, the immunocytochemistry for c-Fos showed more labeling in layers related to the normal eye compared with those of the lesioned eye. No specific label was observed after the reaction for Zif268 in the LGN. In V1, the reaction for both Zif268 and c-Fos showed a pattern of lamination in which heavier labeling was found in layers 2/3, 4A, 4C, and 6. After monocular lesions, we observed a clear pattern of ocular dominance columns in V1 for both c-Fos and Zif268, in which the columns related to the normal eye are more heavily labeled than those related to the lesioned eye. This pattern is more evident in layer 4C after c-Fos reaction, whereas, after Zif268, it is more clearly observed in layers 2/3. These results suggest that, in addition to be regulated by functional activity, these transcription factors are involved in different processes during cortical reorganization. J. Comp. Neurol. 482:166–175, 2005. © 2004 Wiley-Liss, Inc.

Indexing terms: immediate early genes; primate; V1; plasticity; ocular dominance column

The transcription factors Zif268 and c-Fos are rapidly and transiently induced by synaptic stimulation, so they have been used as markers of neuronal activity in the brain of several species. Various studies have shown that the expression of these proteins in the visual cortex increases with light stimulation after a period of dark adaptation (Worley et al., 1991; Chaudhuri and Cynader, 1993; Montero and Jian, 1995; Kaplan et al., 1996). The expression of immediate early genes (IEGs) has also been involved in neuronal plasticity (Kaczmarek, 1993; Wallace et al., 1995). The difference in the laminar distribution pattern of Zif268 and c-Fos proteins in the visual cortex of young and adult cats suggests a relation between IEGs expression and the state of visual cortical plasticity (Kaplan et al., 1996). Also in cat, cortical reorganization

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TABLE 1. Summary of the Animals with Retinal Lesions

Animal	Sex	Extent and location of retinal lesion	Survival time after lesion (days)
Monkey 1 (MS1)	Female	75°, Temporal	28 Days
Monkey 2 (MS2)	Female	80°, Temporal	1 Day
		60°, Superior Pole	7 Days
Monkey 3 (MS5)	Female	60°, Superior Temporal	14 Days
		60°, Temporal	28 Days
Monkey 4 (MS8)	Male	45°, Superior Temporal	14 Days

after partial sensory deafferentation of the lateral geniculate nucleus (LGN) and visual cortex occurs concomitantly with changes in IEGs expression (Arckens et al., 2000).

Zif268 is expressed throughout the visual cortex of monkeys at high basal levels, with the highest levels of the protein appearing in layers 2/3, 4Cβ, and 6, although there is little variation among the species studied (Chaudhuri and Cynader, 1993; Silveira et al., 1996; Okuno et al., 1997; Markstahler et al., 1998). In contrast, c-Fos is expressed at low basal levels, although it is also more abundant in layers 2/3, 4C, and 6 (Kaczmareck et al., 1999). Experiments with monocular stimulation in monkeys showed that 3 hours of privation followed by light stimulation was sufficient for subsequent induction of Zif268 or c-Fos expression, revealing ocular dominance columns (Chaudhuri and Cynader, 1993; Chaudhuri et al., 1995; Silveira et al., 1996; Okuno et al., 1997; Markstahler et al., 1998; Kaczmareck et al., 1999).

The expression of these IEGs was also studied in the dorsal LGN of rats and cats. Moving and stationary visual patterns induced c-Fos expression in populations of neurons in retinotopically corresponding stimulated regions of the LGN of rats (Montero and Jian, 1995). The zif268 mRNA and protein were not observed in the LGN of cats, where c-fos is expressed homogeneously, although in low amounts, over the entire LGN under normal visual conditions (Zhang et al., 1994; Arckens et al., 2000).

In the present work, we studied the immunocytochemical pattern of c-Fos and Zif268 expression in LGN and V1 in the diurnal New World monkey *Cebus apella*. Here we describe, for the first time, the expression of c-Fos in LGN of primates and show that this expression is activity dependent. We also studied the pattern of ocular dominance columns, evidenced by the expression of both Zif268 and c-Fos, at different times after retinal lesions, to investigate the relation of these transcription factors to cortical plasticity after visual deafferentation in *Cebus* monkeys.

MATERIALS AND METHODS

Seven *C. apella* monkeys of both sexes, weighing between 2.8 and 4.0 kg, were used. All experimental protocols were conducted following the NIH guidelines for animal research and were approved by the committee for animal care and use of the Instituto de Biofísica Carlos Chagas Filho, UFRJ. Two normal animals and one monkey with a severe cataract in the right eye, diagnosed 4 months before the perfusion, were exposed to light for 2 hours following an overnight dark adaptation. These animals were then deeply anesthetized with sodium pentobarbital (30 mg/kg) and perfused transcardially with normal saline, followed by 4% paraformaldehyde in phosphate-buffered saline (PBS), 4% paraformaldehyde in PBS + 2.5% glycerol, PBS + 5% glycerol, and PBS + 10% glycerol.

Four animals were submitted to retinal lesions in the right eye. Each animal suffered two or three lesions in different portions of the optic disk border. These lesions were performed 1, 7, 14, or 28 days before the perfusion (Table 1). In the beginning of each session of retinal lesion, the animals were anesthetized with intramuscular administration of 0.5 ml/kg of a 4:1 mixture of 6% ketamine hydrochloride (Ketalar; ParkeDavis) and 2% dihydrotiazine hydrochloride (Rompun; Bayer). Atropine sulfate (0.15 mg/kg) and benzodiazepine (0.8 mg/kg) were injected to prevent tracheobronchic secretion and stress, respectively. The pupil was dilated with 1% tropicamide and 10% phenylephrine hydrochloride, and the topical anesthetic tetracaine hydrochloride was applied to the cornea. A lens OMRA-WF Mainster Wide Field (Ocular Instruments, Inc.) with objective of ×5 soaked in a solution of 2% methylcelulose was positioned on the cornea of the animal to allow the visualization of the ocular fundus. Lesions were made by photocoagulation with a neodymium laser (Ophthalas 532 nm) mounted in a rift ophthalmoscope (Top-Con AIT-20; Carl Zeiss 30 SL-M), using various numbers of spots (5–10) with a spot size of 50 μm, duration of 0.1 seconds, and energy level of 0.9 W.

After intracardiac perfusion, the eyes with retinal lesions and the brain were removed for future histological processing. The retinas were blocked and cut at 30 μm on a cryostat. Horizontal sections were stained with neutral red and examined in the light microscope to verify the efficiency of the lesions. Serial 40- or 50-μm-thick coronal or sagittal sections of the brain were obtained with a cryostat. Adjacent series were stained for cell bodies with cresyl violet and reacted for cytochrome oxidase (CO) histochemistry (Silverman and Tootell, 1987) and for immunocytochemistry for Zif268 and c-Fos. For immunocytochemical reactions, sections were incubated for 16–48 hours with Zif268 (1:1,500–2,000) rabbit polyclonal antibody [Egr-1 (C-19); Santa Cruz Biotechnology, Santa Cruz, CA] or c-Fos (1:15,000–20,000) polyclonal antibody (Ab-5; Oncogene, Bethesda, MD), in a solution containing 0.05% of bovine albumin and 0.3% of Triton X-100 in 0.001 M PBS, pH 7.4. They were then incubated for an additional 1 hour in biotinylated anti-rabbit secondary antibody and then processed by the avidin-biotin method with ABC kits (Vector, Burlingame, CA) and nickel-enhanced diaminobenzidine. The sections were rinsed in PBS, mounted on gelatin-coated slides, dehydrated, and coverslipped. Control sections were prepared by omitting the primary antibody in the incubation solution. These sections showed no specific staining. For each animal, all sections were immunoreacted in a same battery, simultaneously, to minimize variability in the reaction product among different immunoruns.

Sections were examined under brightfield microscopy and photomicrographed with a Zeiss Axiocam attached to the microscope. All photomicrographs used in the density analysis were made by using the same microscope imaging settings and brightfield conditions. The determination of the cortical region corresponding to the retinotopic representation of the different lesions was made based on the visuotopic maps of V1 in *Cebus* (Gattass et al., 1987).

To quantify the intensity of the reactions in the ocular dominance columns labeled by IEGs immunocytochemistry, we used the Image program (Scion) to measure the mean density values within a 200- × 200-μm box centered in each ocular dominance column, in layers 2/3, 4C, and 6. For each animal, we measured five dark and five light

columns located in the middle of the region of representation of each lesion, in three different sections. Student's paired *t*-test was used to determine the statistical significance of differences between the mean density values of the ocular dominance columns. To quantify the contrast between the dark and the pale columns, we used a contrast index, CI = (max – min)/(max + min), where max is the maximal and min is the minimal mean density value. In addition, in one case, animal MS2, mean density and contrast index curves across the cortical layers were obtained by means of Matlab routines. For this analysis we used 200-μm-wide cortical strips including all layers: five from regions corresponding to columns receiving inputs from the lesioned eye, five from columns receiving inputs from the normal eye inside the "scotoma," and five from regions of normal cortex outside the "scotoma." Each sample was digitized with a 4-μm/pixel resolution and with 256 gray tones. Each point in the final density curve is the average of 250 pixels.

RESULTS

LGN

In the normal *Cebus*, the LGN is formed by two magnocellular (M) and four parvocellular (P) layers, as illustrated in Figure 1A in a coronal section stained by the Nissl method. In this species, the P layers are not totally individualized and join each other in some portions of the nucleus. In the LGN of normal animals, the reaction for c-Fos showed immunopositive cells in both M and P layers; however, the label was more intense in the P layers, which showed large numbers of labeled cells (Fig. 1B). No specific label was observed after the reaction for Zif268 in the LGN.

In the animal with cataract in the right eye, the reaction for cytochrome oxidase revealed alternate poor (M1, P2, and P4) and rich (M2, P1, and P3) CO layers, which correspond to the deprived and normal eye, respectively (Fig. 1C). Likewise, the immunocytochemistry for c-Fos revealed intense labeling in the layers corresponding to the normal eye and poor labeling in the layers of the lesioned eye (Fig. 1D). Similarly, for the LGN of animals with retinal lesions in one of the eyes, we also observed in the regions corresponding to the lesions rich and poor in labeling for c-Fos and CO in alternate layers.

V1

The distribution patterns of the proteins Zif268 and C-Fos were first studied in V1 of normal *Cebus* monkeys that were perfused after approximately 2 hours of exposure to normal day light early in the morning. Figure 2 shows adjacent coronal sections of V1 stained by the Nissl method, where approximate limits of the layers were delineated (Fig. 2A), and c-Fos (Fig. 2B) and Zif268 (Fig. 2C) immunocytochemistry reactions. The reactions for both Zif268 and c-Fos revealed a pattern of lamination in which a larger number of labeled nuclei were found in layers 2/3, 4A, 4C, and 6. However, the reaction for Zif268 showed a greater density of labeled nuclei than the reaction for c-Fos, mainly in layers 2/3. The number of c-Fos- or of Zif268-immunopositive neurons was small in layers 4B and 5.

In the animals that suffered monocular lesions, the laser lesions at the border of the optic disk caused local destruction of all retinal layers (Fig 3A), cutting the fibers of passage in the ganglion fiber layer. In V1, these lesions caused cortical "scotomas" in the retinotopically corresponding regions evidenced by the alternating dark and light columns of CO. The immunocytochemistry reactions for both c-Fos and Zif268 also reveled dark and light ocular dominance columns in the same cortical regions, where the dark columns labeled by these proteins correspond to the dark columns of CO, which are related to the normal eye.

Figure 3 illustrates the data from one animal (MS2) that underwent a retinal lesion on the peripheral representation of the visual field 7 days before perfusion and another lesion on the central representation 1 day before perfusion. The central lesion caused the occurrence of ocular dominance columns in a region of the same retinotopy in the operculum, whereas the peripheral lesion revealed columns in the calcarine region. The CO columns were very conspicuous in layer 4C both in the operculum (Fig. 3C) and in the calcarine (Fig. 3D). The columns labeled by c-Fos immunocytochemistry showed a higher contrast in the layer 4Cβ, but were also well visible in layers 2/3, 4A, 4Cα, and 6 (Fig. 3E,F). Unlike the case for c-Fos, the reaction for Zif268 showed a higher contrast between columns in layers 2/3 and a lower contrast in layers 4A, 4C, 5, and 6 (Fig. 3G,H). Figure 4 illustrates the mean density curves across all cortical layers from regions corresponding to columns receiving inputs from the lesioned eye (LEC), from the normal eye inside the "scotoma" (NEC), and from regions of normal cortex outside the "scotoma" (control). The contrast index curves show the difference between the labeling in columns related to the normal eye and the labeling in columns related to lesioned eye inside the "scotoma." The difference observed is due to a decrease in the labeling of the columns related to lesioned eye and not to an increase in the columns of the normal eye, as can be observed by comparing the mean density curves of these regions with the curve obtained in a normal region outside the "scotoma."

c-Fos columns can also be clearly observed in Figure 5, which illustrates in the same section ocular dominance columns in the operculum after a central lesion and in the calcarine caused by a peripheral lesion in animal MS5. We observed a significant difference in the reaction intensity between the normal and the lesioned eye columns in both the operculum and the calcarine regions.

Figure 6 shows sections of V1 of different animals reacted for c-Fos and for Zif268 in regions corresponding to lesions of 1 day (Fig. 6A,B), 7 days (Fig. 6C,D), 14 days (Fig. 6E,F), and 28 days (Fig. 6G,H). Although the quality of the reaction has varied in the different animals, we can observe a clear contrast between ocular dominance columns for both c-Fos and Zif268 immunoreactions, in all animals and with the different lesion times. This is better illustrated in Figure 7, in which we plotted the mean density of the ocular dominance columns in layers 2/3, 4C, and 6 for the different lesion times. This figure shows that the difference in labeling between the dark and the pale columns was significant in all cases ($P < 0.01$). When we analyze the contrast index between dark and pale columns (Fig. 8) in the different layers, we see that the reaction for c-Fos showed the highest contrast in layers 4C for all lesion times, except for the 28-day lesion. On the other hand, the Zif268 reaction showed the highest contrast in layer 2/3, except for the 7-day lesion.

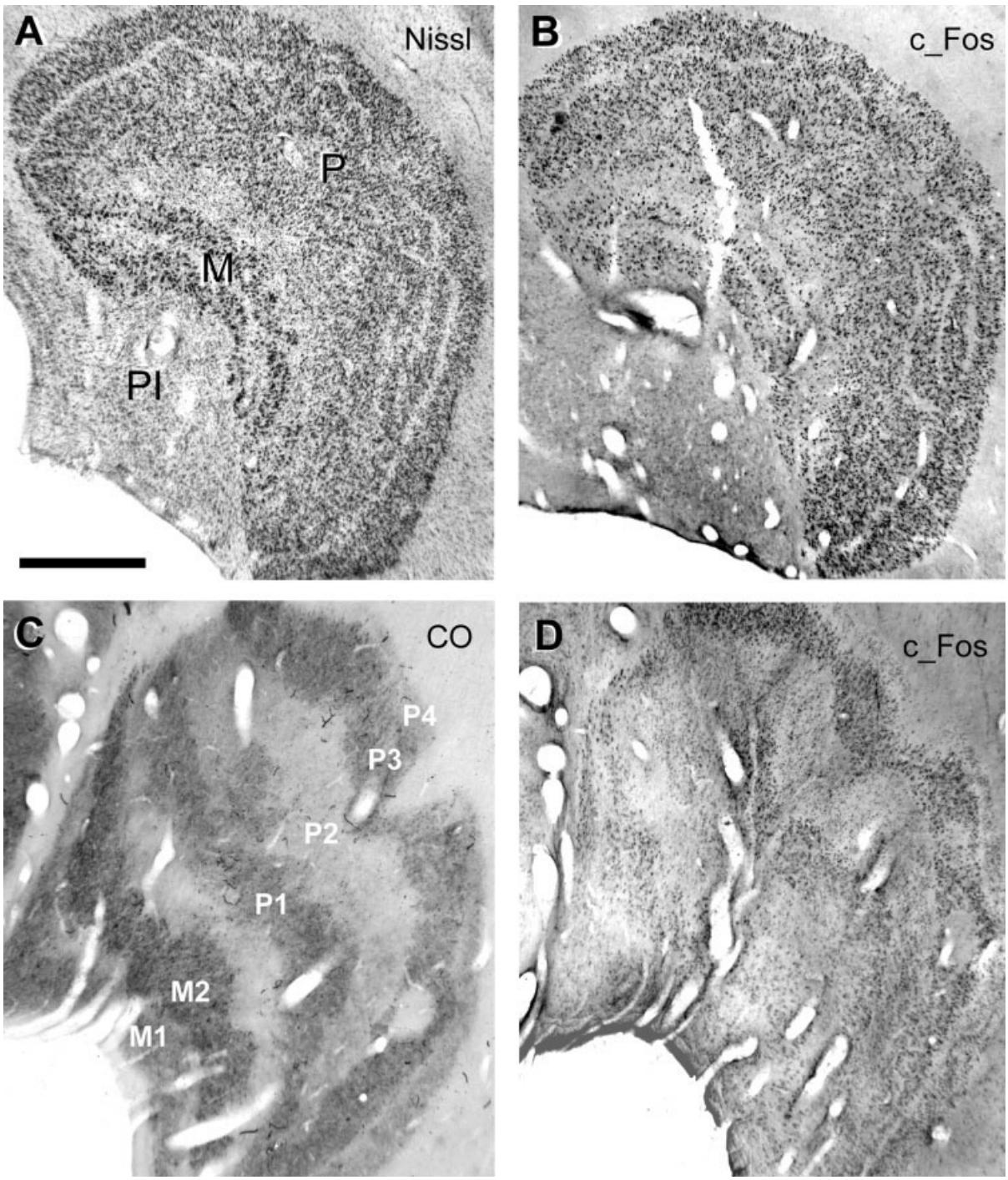

Fig. 1. Photomicrographs of coronal sections of the lateral geniculate nucleus of *Cebus*. **A:** Section of the LGN of a normal animal stained by Nissl method showing the magnocellular (M) and parvocellular (P) layers. **B:** Section adjacent to that illustrated in A showing c-Fos-immunopositive cells. **C:** Section of the LGN of the animal with cataract in the right eye, reacted for cytochrome oxidase, showing the contralateral layers (M1, P2, and P4) poorly labeled and the ipsilateral layers (M2, P1, and P3) intensely labeled. **D:** Adjacent section reacted for c-Fos showing more intense label in ipsilateral layers, as in C. PI, pulvinar inferior. Scale bar = 1 mm.

DISCUSSION

Here we describe for the first time the pattern of c-Fos immunocytochemistry labeling in the LGN of primates. Analysis of the results shows that c-Fos expression is activity dependent, inasmuch as there is a clear reduction of c-Fos labeling in the layers corresponding to the lesioned eye. No c-Fos labeling was observed in the LGN of *Macaca mulatta* (Brooks et al., 2004). This discrepancy could be due to interspecies differences or to the different antibodies used in these studies. In cat, under normal

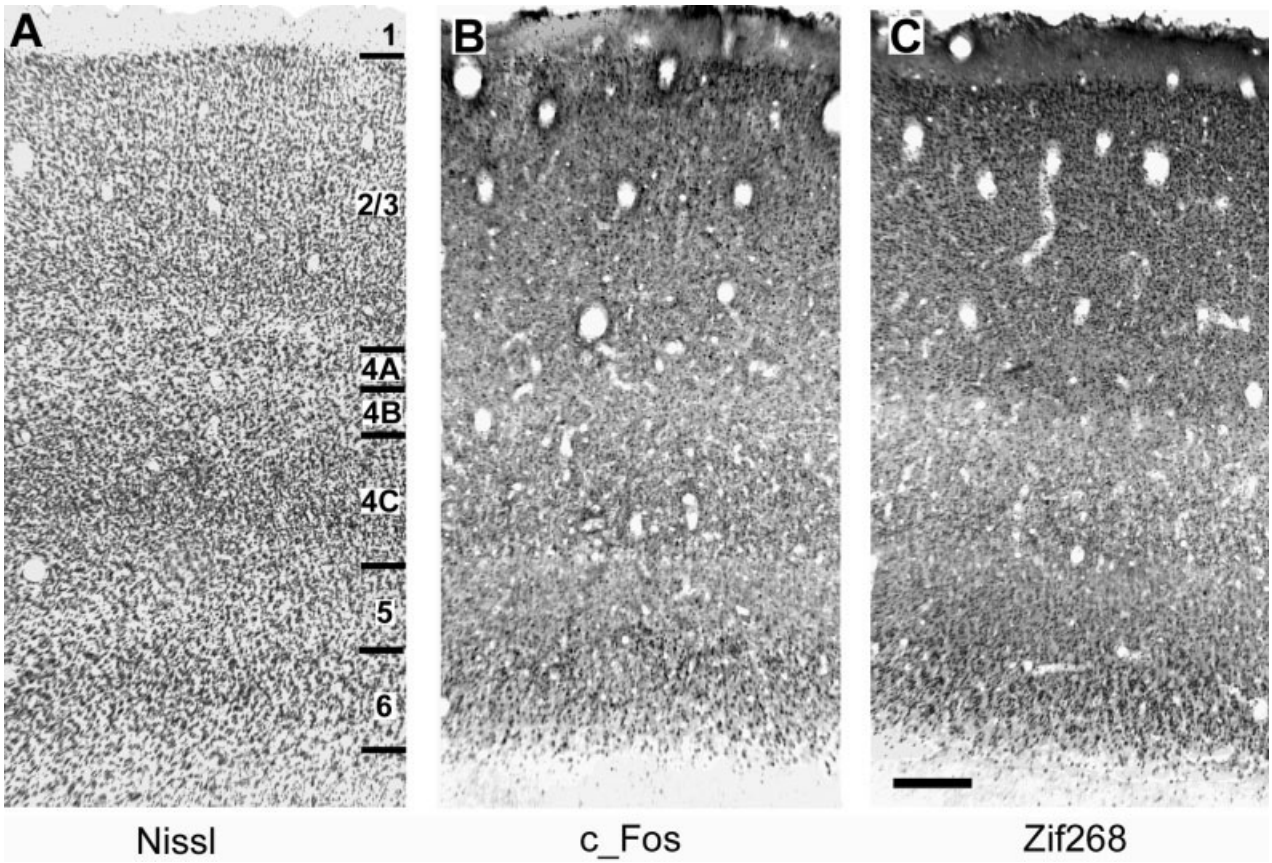

Fig. 2. Photomicrographs of adjacent coronal sections of V1 of a normal *Cebus* monkey. **A:** Section stained by the Nissl method in which the approximate limits of the layers are delineated. **B,C:** Sections stained by immunocytochemistry for proteins c-Fos (B) and Zif268 (C) showing the normal lamination pattern for these immediate genes in V1. Scale bar = 200 μm.

visual conditions, c-fos is expressed homogeneously throughout the LGN (Zhang et al., 1994), whereas, after retinal lesions, the LGN showed decreased amounts of c-fos mRNA in the expected region based on the location and extent of the retinal lesions (Arckens et al., 2000). As already described in previous work with primates (Chaudhuri and Cynader, 1993) and cat (Arckens et al., 2000), no specific label for Zif268 in the LGN in *Cebus* was found in this study.

Similar to V1 of vervet monkeys (Kaczmareck et al., 1999), in V1 of *Cebus* we also found high levels of c-Fos in layers 2/3, 4C, and 6. However, unlike the results in vervet monkeys that showed reduced contrast of c-Fos immunostaining when ocular dominance columns were analyzed, in this study c-Fos immunostaining clearly distinguished the ocular dominance columns mainly in layers 4Cβ, where the contrast between columns was highest. The conspicuous result for c-Fos protein labeling obtained in this study could be due to the different anti-c-Fos antibody used: c-fos–Ab-5 Oncogene (catalog No. PC38; Calbiochem-Novabiochem, La Jolla, CA).

Previous works report small differences in the distribution of Zif268 in V1 of various species studied. The pattern of Zif268 labeling found in *Cebus* monkey in the present study is more similar to that described by Okuno et al. (1997) for macaque, in which the number of Zif268-immunopositive neurons was large in layers 2/3, 4A, 4Cβ, and 6 and small in layers 4B, 4Cα, and 5. In vervet monkeys, Zif268 is found at high basal levels in layers 2/3 and 6 and at moderate levels in layers 4Cβ and 5 (Chaudhuri and Cynader, 1993; Chaudhuri et al., 1995). In marmoset, the reaction for Zif268 showed intense staining in layers 2/3, 4A, 5, and 6. Layer 4Cβ showed moderate staining, whereas layers 4Cα and 4B exhibited low levels of reactivity (Markstahler et al., 1998).

After retinal lesions, we found a clear ocular dominance pattern in V1 of *Cebus* monkeys with Zif268 expression, which was more conspicuous in layers 2/3 and 6, similar to the results described by Silveira et al. (1996) for *Cebus* monocularly deprived by eyelid suture for 48 hours. These results are also similar to those described for *Macaca* after eye enucleation, in which the contrast between adjacent columns was highest in the upper layers and lowest in layer 4C (Chaudhuri et al., 1995). This pattern of Zif268 expression is different from that observed for c-Fos protein, in which c-Fos columns were more evident in layer 4C.

The columns observed after c-Fos immunoreaction are similar to the ocular dominance columns evidenced by CO histochemistry, which shows the highest contrast in the highly monocular layer IVCβ (Rosa et al., 1992; Horton and Hocking, 1998). Melzer and Steiner (1997), in a study of stimulus-dependent expression of IEGs in rat somato-

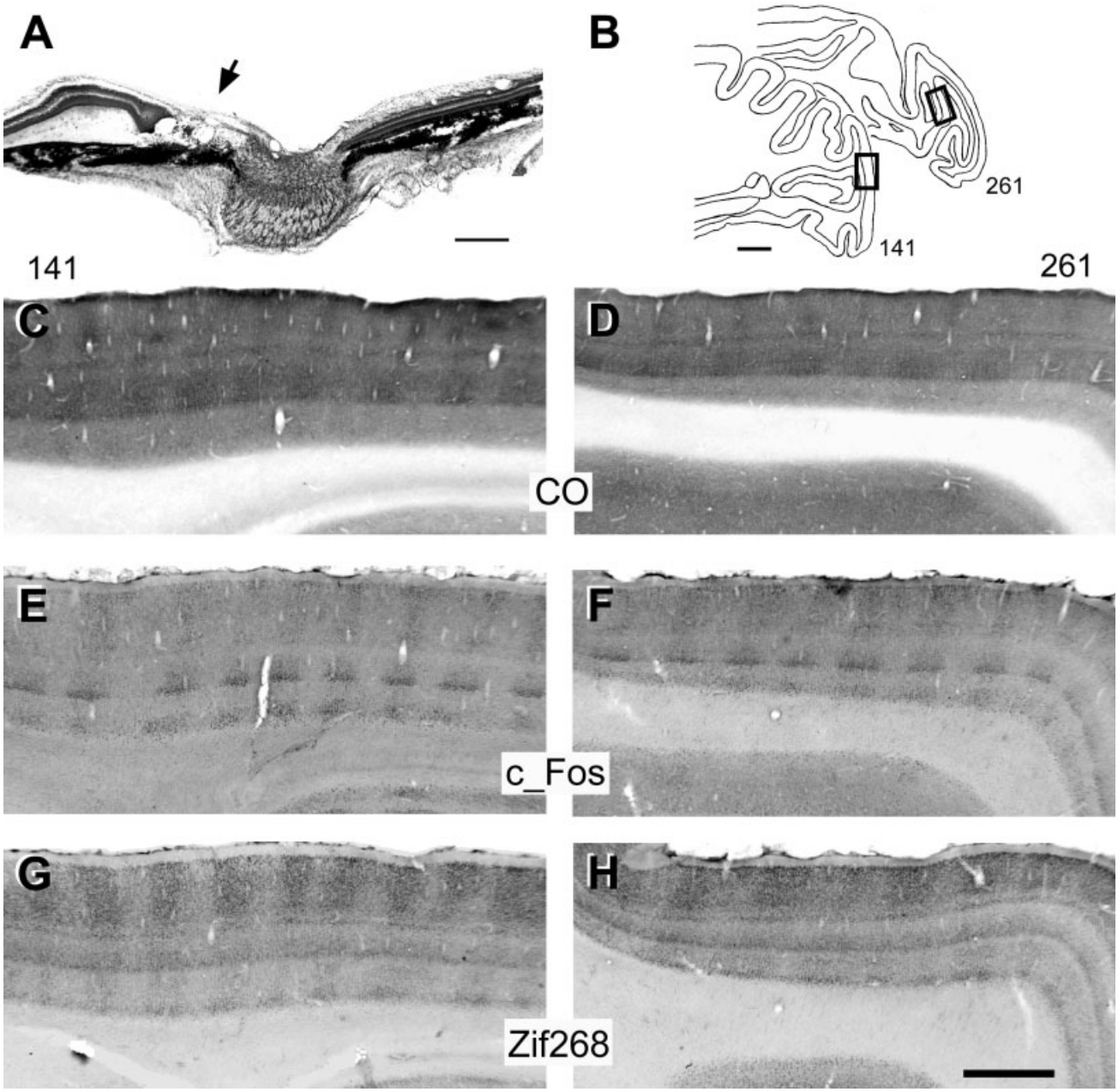

Fig. 3. Ocular dominance columns in V1 after retinal lesions with 1 and 7 days of survival (animal MS2). **A:** Retinal section passing through the optic disk stained by neutral red. The arrow points to the site of destruction of the retinal layers at the central lesion. **B:** Schematic drawing of sagittal sections of the right hemisphere with the regions of V1 corresponding to the cortical "scotomas" (rectangles) relative to lesion of 1 day in operculum (section 141), illustrated in C, E, and G, and relative to lesion of 7 days in the calcarine (section 261), illustrated in D, F, and H. **C,D:** Photomicrographs of the sections drawn in B reacted for cytochrome oxidase showing, in the layer 4 of V1, the dark columns corresponding to the intact eye and the light columns corresponding to the lesioned eye. **E,F:** Adjacent sections reacted for c-Fos. **G,H:** Sections reacted for Zif268. Scale bars = 500 μm in A; 5 mm in B; 500 μm in H (applieas to C–H).

sensory cortex, found the highest proportion of c-fos-labeled perikarya in layer IV of the activated barrel column. These results suggest that c-fos, similarly to CO, might be related to cell activity. However, some studies have proposed the involvement of IEGs with cortical plasticity. For cat, Arckens et al. (2000) showed that the cortical reorganization after partial sensorial deafferentation of the LGN and visual cortex occurs concomitantly with changes in zif268 and c-fos expressions. Long postlesion survival times resulted in a time-dependent restoration of IEGs expression from the border to the center of the cortical lesion.

In our study, we observed well-contrasted columns for the different survival times used; however, the longest time used was 28 days. Chaudhuri et al. (1995) found a blurring of the ocular dominance columns in the upper

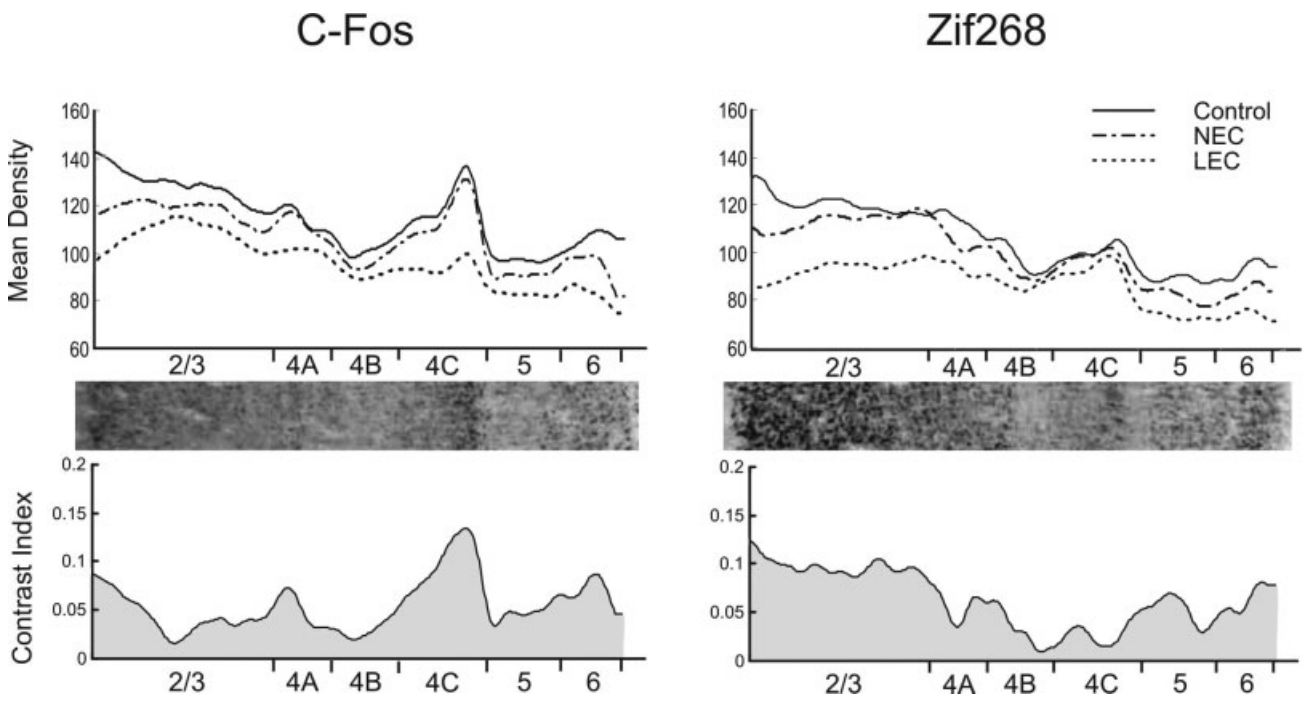

Fig. 4. Mean density and contrast index curves across all cortical layers of V1 after reaction for c-Fos and Zif268 in animal MS2. **Top:** Mean density curves obtained from columns corresponding to the lesioned eye (LEC), to the normal eye inside the "scotoma" (NEC), and to regions of normal cortex outside the "scotoma" (control). **Bottom:** Contrast index curves illustrating the difference between LEC and NEC regions inside the "scotoma." **Middle:** Photomicrographs of a cortical band in the region of normal cortex reacted for c-Fos (left) and Zif268 (right).

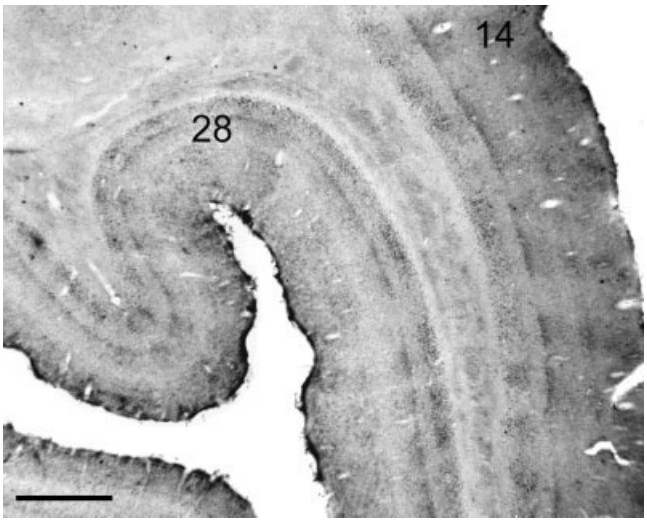

Fig. 5. Photomicrograph of a sagittal section of V1 reacted for c-Fos. This animal (MS5) had a retinal lesion (14 days) at the central representation of the visual field and another lesion in the peripheral representation (28 days). Note the ocular dominance columns in the cortical "scotomas" in the operculum, corresponding to the central retinal lesion (at right), and in the region of the calcarine, corresponding to lesion of 28 days. Scale bar = 1 mm.

layers in animals that had a 3-month survival period following enucleation. They suggested that this could reflect encroachment of the cortical territory dominated by the intact eye and, thus, indicate an ongoing process of cortical reorganization and redistribution of inputs.

Studies on cultured neurons and in animals treated with N-methyl-D-aspartate (NMDA) have shown that glutamate binding on NMDA receptors is capable of inducing both c-fos and zif268 expression. However, c-fos expression may also be induced by the opening of voltage-sensitive calcium channels after activation of AMPA-kainate receptors by glutamate (Murphy et al., 1991; Ghosh et al., 1994; Bading et al., 1993; Lerea et al., 1993; Thompson et al., 1995). Zif268 expression is highest in regions where NMDA-dependent plasticity, such as hippocampal long-term potentiation (LTP), occurs. Whereas both c-fos and zif268 expression are increased in the hippocampus by seizure activity, electrical stimulation conditions that result in LTP induce zif268 but not c-fos expression (Morgan et al., 1987; Douglas et al., 1988; Cole et al., 1989; Jeffery et al., 1990; Wisden et al., 1990; Worley et al., 1991).

Various studies have implicated Zif268 in the regulation of the protein expression required for plasticity, such as synapsin I (Thiel et al., 1994; Petersohn et al., 1995), and of the induction of transforming growth factor-β1 by nerve growth factor (NGF; Kim et al., 1994). Wallace et al. (1995) showed that Zif268 induction is highest in regions, such as the visual cortex, that exhibit dendritic and synaptic changes in response to environmental complexity compared with regions in which no morphological responses have been detected, such as frontal area 2. In addition, c-fos was reported to regulate genes related to neuronal growth, like NGF and growth-associated protein-43 (Hengerer et al., 1990; Nedive et al., 1992).

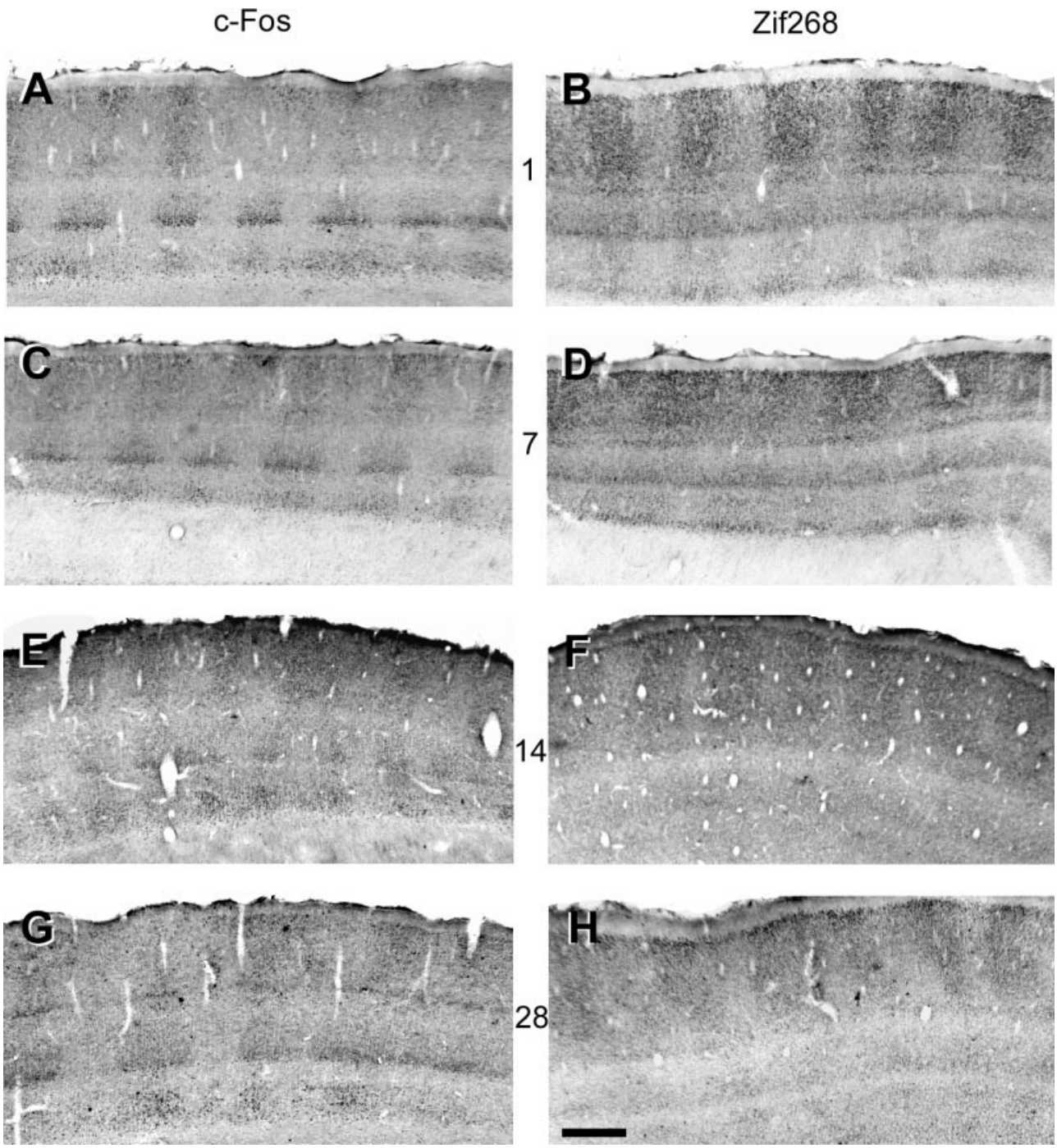

Fig. 6. Photomicrographs of sections of V1 to illustrate c-Fos (left column) and Zif268 (right column) ocular dominance columns at different survival times: 1 day (**A,B**), 7 days (**C,D**), 14 days (**E,F**), and 28 days (**G,H**). Scale bar = 500 μm.

The fact that zif268 columns are less evident in layer 4C could be due to the thalamocortical connections being non-NMDA receptor driven, similar to findings described for others species (Kaczmarek et al., 1997), given that NMDA receptors apparently are the major mediators for Zif268 expression. It is likely that Zif268 expression in layer 4C is driven by intracortical circuits. In addition, other reports have suggested that layer 4 is less liable to plastic changes than the extragranular layers. The critical period for plasticity in layer 4 ends earlier than in other layers, and in older animals residual plasticity occurs only outside layer 4 (LeVay et al., 1980; Singer et al., 1982; Draw et al., 1992; Chaudhuri and Cynader, 1995; Kaplan et al., 1996).

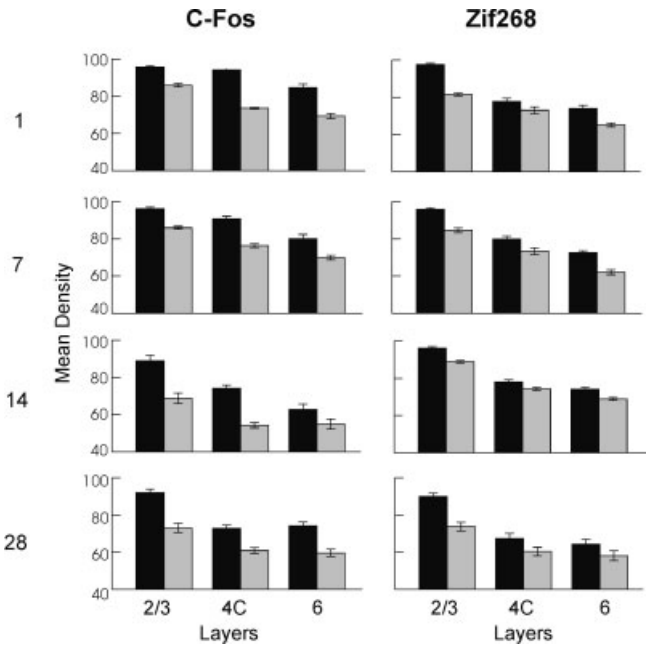

Fig. 7. Mean density of the ocular dominance columns labeled by c-Fos (left) and Zif268 (right) reactions. Black and gray bars represent the mean density of normal and lesioned eye columns, respectively, measured in layers 2/3, 4C, and 6 in the regions of V1 corresponding to lesions of 1, 7, 14 and 28 days. Error bars = SEM. All differences were statistically significant ($P < 0.01$).

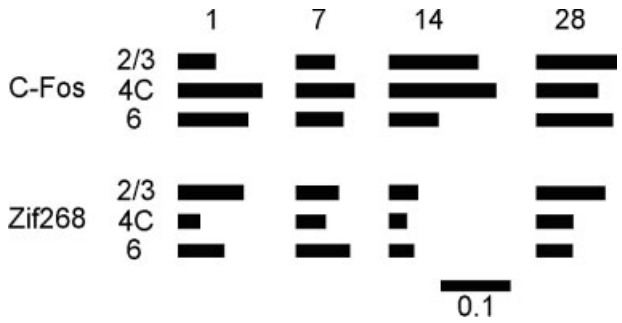

Fig. 8. Representation of contrast indexes between dark and pale columns labeled for c-Fos and Zif268 measured in layers 2/3, 4C, and 6, in the regions of V1 corresponding to lesions of 1, 7, 14, and 28 days. Contrast strength is represented by the horizontal length of each bar. Scale bar shows contrast index of 0.1.

Obata et al. (1999) suggest that the laminar pattern of brain-derived neurotrophic factor (BDNF) elevation within the area of the cortical scotoma mirrors the laminar distribution of functional recovery, as well as that of the horizontal connections, which tend to target cells in the superficial and deep layers and are relatively absent in layer 4. Previous works have shown changes in cortical receptive field properties immediately after a retinal lesion, and, after a few months of recovery, portions of the cortex silenced after the lesion became responsive to stimuli in a region immediately outside the scotoma. However, the region of the LGN silenced by the retinal lesion remained unresponsive, and there were no new geniculocortical connections. These facts indicate that cortical reorganization could be due to intrinsic cortical alterations, such as the increase in the strength of the horizontal connections, which can involve sprouting of axons of long-range laterally projecting neurons, as well as synaptogenesis (Gilbert and Wiesel, 1992; Darian-Smith and Gilbert, 1994, 1995; Chino, 1995; Rosa et al., 1995).

A low level of c-Fos and Zif-268 expression in columns related to the lesioned eye suggests that the expression may be regulated by functional activity similar to what has been described in previous work (Worley et al., 1991; Chaudhuri and Cynader, 1993; Silveira et al., 1996; Kaczmareck et al., 1999). However, the difference between c-Fos and Zif268 expressions in different layers of lesioned regions of V1 in *Cebus* monkey may indicate that, although both c-Fos and Zif-268 are found mainly in layers 2/3, 4C, and 6 and the same extracellular signal can induce both, these transcription factors are involved in different processes of the cortical reorganization.

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