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c-Fos expression in the visual system of the tree shrew (*Tupaia belangeri*)

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c-Fos is a nuclear phosphoprotein coded by the proto-oncogen *c-fos* which can be detected immunohistochemically after both physiological and pathological stimuli. This property is of great importance, because it offers a valuable tool for morphofunctional identification of activated neurons. We have studied the neuronal activity in the visual pathway of *Tupaia belangeri* within the following anatomical structures: retina, superior colliculus (SC), dorsal lateral geniculate nucleus (dLGN), pulvinar (Pu), parabigeminal (PBG) nucleus and primary visual cortex (V1) analyzing the c-Fos expression after exposing the tree shrews to different light stimuli (white light –control positive group–, green light, blue light and darkness conditions –control negative group–). Our findings suggest that in the retina, the ganglion cells and the cells of the inner nuclear layer respond better to blue and green light stimuli, when comparing the c-Fos expression between white, green, blue lights and darkness conditions. However, in the SC, dLGN, Pu, PBG nucleus and V1 another pattern of c-Fos expression is observed: a maximum expression for the control positive group, a minimum expression for the control negative group and intermediate expressions within the blue and green light groups. *Conclusion*: the expression levels of c-Fos protein are able to show significant differences between distinct light stimuli in all anatomical structures studied (retina, SC, dLGN, Pu, PBG and V1) of *T. belangeri*.

1. Introduction

The survival instinct of animals always involves detection and evaluation of the information coming from the outer and inner environments. Considering human beings, perception is mostly oriented towards vision, for this reason, it is relevant to examine deeply the principles which manage the organization, and moreover, to analyze the structure of centers involved in perception, processing and transmission of the visual information of higher vertebrates.

Tree shrews (*Tupaia belangeri*) are small, diurnal squirrel-like animals. They have a particularly elaborated and distinct parcellation of anatomical and physiological features in their visual system, occupying a strategic position in the phylogeny. Their taxonomic classification has been controversial and discussions were mostly about whether or not tree shrews should be considered primates. Various authors described morphological traits that supposedly linked *Tupaias* with primates, with insectivores, or with rodents (Carlsson, 1922; Le Gros Clark, 1924; Le Gros Clark, 1925; Simpson, 1945; Jane et al., 1965; Van Valen, 1965; Tigges and Shantha, 1969; Butler, 1972; Thenius, 1979; Martin, 1990). Moreover, chromosomal homologies between human and *T. belangeri* were established with confidence by direct comparison with a third, already characterized species, *Eulemur macaco macaco* (Müller et al., 1997) using a multi-directional chromosome painting with probes derived by bivariate fluorescence-activated flow sorting of chromosomes from human, black lemur (*Eulemur macaco macaco*) and tree shrew (*T. belangeri*) to better define the karyological relationship of tree shrews and primates (Müller et al., 1999). Tree shrews are nowadays positioned as a separate order *Scandentia* with the extant species integrated into the families *Tupaiidae* and *Ptilocercidae* (Butler, 1972; Olson et al., 2005).

The tree shrew retina is almost a pure cone retina (96% of cones and 4% of rods, Foelix et al., 1987), and many of the interesting features of primate and cat visual system organization can be found within the tree shrew brain, which make them a valuable species for studying the functional organization of the visual system. There is a good agreement on the nature of the cone pigments of the *T. belangeri* retina. Measurements made with a microspectrophotometer indicated that one pigment has a peak at a wavelength of approximately 555 nm (long-wavelength sensitive cones [LWS], 97.05%) and the other one at a wavelength of approximately 427.8 nm (short-wavelength sensitive cones [SWS], 2.95%). There are no middle-wavelength sensitive cones [MWS] present (Petry and Hárosi, 1990).

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In the last 20 years, several genes activated in response to a trans-synaptic stimulus or electrical activity of the neuronal membrane have been identified (Greenberg et al., 1985; Barzilai et al., 1989). These genes can be classified in two general groups, (a) immediate early genes (IEGs), which transcription is transiently activated in a few minutes after the stimulus (Dragunow and Robertson, 1988; Morgan and Curran, 1991); and (b) late response genes, which expression is induced much more slowly, in the lapse of hours (Goldman et al., 1988; Barzilai et al., 1989). In particular, the *c-fos* proto-oncogene (which belongs to the IEGs group) codes for the nuclear phosphoprotein c-Fos, which can be detected immunohistochemically in the nucleus of the neurons after both physiological and pathological stimuli. This property is of great importance, because it offers a valuable tool for morphofunctional identification of activated neurons (Kaczmarek, 2002).

c-Fos induction by light has been mostly studied in rats, mice, rabbits and hamsters, in the suprachiasmatic nucleus in relation to changes of general illumination and circadian rhythms (Rea, 1989; Aronin et al., 1990; Rusak et al., 1990; Chambille et al., 1993; Earnest and Olschowka, 1993; Vuillez et al., 1994); in response to global flashes of light in the retina and central visual pathways (Sagar and Sharp, 1990; Craner et al., 1992; Koistinaho et al., 1993) and after pattern visual stimulation (Yoshida et al., 1993; Montero and Jian, 1995; Harada et al., 1996; Bussolino et al., 1998; Correa-Lacarcel et al., 2000; Lima et al., 2003).

Sagar et al. (1988), in order to determine whether the c-Fos protein and Fos-related proteins can be induced in response to polysynaptic activation, stimulated electrically the rat hindlimb motor/sensory cortex and examined the Fos expression immunohistochemically. Three hours after the onset of stimulation, focal nuclear Fos staining was seen in motor and sensory thalamus, pontine nuclei, globus pallidus and cerebellum. Moreover, 24-h water deprivation resulted in Fos expression in paraventricular and supraoptic nuclei. Later on, Sagar and Sharp (1990) analyzed the synaptic induction of Fos in the rabbit retina. Dark-adapted retinas had virtually no Fos immunostaining. Retinas of dark-adapted rabbits that were exposed to 3 Hz diffuse flashing white light for 1 h and sacrificed 2 h later displayed nuclear Fos immunostaining in a minority of neurons. These included presumptive amacrine cells of the inner nuclear layer and either displaced amacrine cells or ganglion cells of the ganglion cell layer.

Yoshida et al. (1993) analyzed the distribution and levels of c-Fos expression in response to changes in the light/dark cycle in the rat retina by in situ hybridization histochemistry. Under a 12 h light:12 h dark cycle (LD 12:12), *c-fos* mRNA levels in the inner nuclear layer and the ganglion cell layer increased transiently for 30 min immediately following the onset of the light period, whereas in the outer nuclear layer, *c-fos* mRNA expression was observed throughout the dark period. During a period of extended darkness following the dark period of the LD 12:12, *c-fos* mRNA levels in the outer nuclear layer remained cyclic – low during the day and high during the night – suggesting an endogenously generated rhythm. Furthermore, since a 30 min pulse of light during the dark period decreased c-Fos expression in the outer nuclear layer, whereas a similar pulse of darkness during the light period resulted in an increase, environmental light/dark cues are also suggested to influence *c-fos* gene expression.

Montero and Jian (1995) used localized patterned stimulation in rats to investigate the feasibility of stimulus-dependent induction of the gene *c-fos* in neurons of cortical and subcortical visual centers of this mammal. Moving and stationary visual patterns, consisting of gratings and arrays of dark dots, induced Fos-like immunoreactivity in populations of neurons in retinotopically corresponding stimulated regions of the dorsal and ventral lateral geniculate nucleus, stratum griseum superficiale of the superior colliculus, nucleus of the optic tract, and primary (striate) visual cortex. Stimulus-induced Fos-like immunoreactivity neurons in the striate cortex were predominantly distributed in layers IV and VI, while few labeled neurons were present in layers II–III, and almost none in layer V.

Harada et al. (1996) examined whether light-controlled *c-fos* gene expression was mediated by a cone specific pathway in the rat retina, placing them under continuous lighting for 21 days. The luminance in the cages ranged from 1200 to 2000 lux. The presence of cones was determined by peanut agglutinin and rods by the monoclonal antibody 1-E7. After long-term exposure to continuous light, the 1-E7 immunoreactivity was not detected in the outer nuclear layer (ONL). The cone photoreceptors and cells in the ganglion cell layer (GCL) and inner nuclear layer (INL) survived. In normal retinas, there was strong hybridization for c-Fos expression in the GCL and the INL 30 min after the onset of the light cycle.

Bussolino et al. (1998) studied the retina photoreceptor and ganglion cells isolated from chicks that *in vivo* were exposed to light, observing a different phospholipid labeling capacity than those from chicks in the dark. In the light exposed animals, the phospholipid labeling in the ganglion cells was higher than in those maintained in the dark, whereas in the photoreceptor cells, the opposite occurred, that is, the phospholipid labeling was higher in the dark than in the light conditions. The light–dark differences for phospholipid labeling correlated with the expression of c-Fos: when c-Fos expression increased (both in mRNA and in c-Fos protein content), phospholipid labeling increased concomitantly. That is, in ganglion cells, c-Fos expression and the phospholipid synthesis was higher in light with respect to dark, whereas in photoreceptor cells, c-Fos expression and phospholipid synthesis was higher in dark with respect to light.

Correa-Lacarcel et al. (2000) characterized the c-Fos expression patterns in various centers of the visual pathway of adult rats monocularly stimulated either by continuous or flickering light at different frequencies. Results showed different immunocytochemical patterns in all centers studied, the lateral geniculate complex; superior colliculus; and primary visual cortex, depending on the physical characteristics of the stimulus (blinking frequency or light wavelength). After stimulation of the left eye, the ipsilateral pathway presented a substantial density of immunoresponsive cells, which was greater than expected with respect to the number of fibers that project ipsilaterally from the retina to the lateral geniculate complex and the superficial layers of the superior colliculus. A surprisingly high positive immunoresponsiveness was obtained in all cases with coherent light stimulation in the red spectrum (634 nm).

Lima et al. (2003) studied the c-fos gene expression to characterize the residual vision of retinal degeneration mice after applying static and dynamic photic stimuli. They found diffuse light induced Fos-positive nuclei in the INL and GCL of the mice retina.

The aim of this research is to study the neuronal activity in the visual pathway of *T. belangeri* within the following anatomical structures: retina, superior colliculus (SC), dorsal lateral geniculate nucleus (dLGN), pulvinar (Pu), parabigeminal (PBG) nucleus and primary visual cortex (V1), using the c-Fos expression, after exposing the tree shrews to different light stimuli. Up to date there is neither any published study about the c-Fos expression in tree shrews in general, nor in their visual system in particular. Therefore, it will be analyzed whether the levels of c-Fos expression are able to show significant differences between the distinct types of stimuli and whether those differences take place at each studied level of the visual pathway of *Tupaia belangeri*.

2. Materials and methods

Adult tree shrews (*T. belangeri*) of both sexes and weighing 156–237 g ($n = 8$) were used. They were kept in our breeding colony at the Anatomy Unit, Department of Medicine, University of Fribourg (Switzerland).

2.1. Conditions and experimental design

2.1.1. Design of the cages

For the experiments, animals were kept in a wire mesh cage, size 50 cm × 50 cm × 50 cm, which had an attached nest box. This cage was covered on its inferior two thirds with aluminum foil to help to improve the reflection of the experimental light, which was mounted above the cage (see Section 2.1.3). During the week preceding each experiment, the selected tree shrew was kept for 1–2 days inside the experimental cage under LD 12:12 conditions (lights on at 6:30 a.m.) to allow the animal to get used to the new environment and avoid biases of c-Fos expression. Subjects had *ad libitum* access to food and water throughout the experiments.

All the experiments were performed according to the published guidelines of the Swiss Academy of Medical Sciences and the Swiss Academy of Natural Sciences, regarding care and use of animals for experimental procedures.

2.1.2. Description of the experimental groups

We designed four experimental groups for our study, including two animals in each one ($n = 8$): (1) control positive group, (2) control negative group, (3) blue light group and (4) green light group.

Tree shrews were kept under LD 12:12 conditions with white light during the 1–2 days period of adaptation in the experimental cage. After the last light period, before sacrifice, animals were kept in a 15 h-dark-period after which they were stimulated with 1 h of light (either white light –control positive group–, blue light –blue light group–, or green light –green light group–) or anesthetized and perfused without undergoing any further stimulation –control negative group–. To handle the animals for anesthesia we used either the regular white light of the experimental room (for the control positive group) or a dim blue, green or red light pocket-lamp (for the respective blue light group, green light group and control negative group). Once they were anaesthetized, we covered the eyes of the tree shrews from the blue light group, green light group and control negative group with a non-transparent tape to proceed with the perfusion.

Considering the typical c-Fos expression pattern observed in the literature read and in our previous experience in rats and mice, it was expected to obtain the maximum c-Fos expression pattern in the control positive group, the minimum c-Fos expression pattern in the control negative group and intermediate c-Fos expression patterns within the range of the controls for the blue light group and the green light group and, perhaps, a different distribution of activated cells (short- and long-wavelengths pathways).

2.1.3. Design of the different monochromatic light environments

The different monochromatic light environments were achieved using Kodak color filters. The Kodak Wratten filters number 47B ($\lambda_{max} = 430$ nm; $T_{max} = 53\%$; half width$_{\lambda_{max}} = 55$ nm) and number 99 ($\lambda_{max} = 548$ nm; $T_{max} = 22.9\%$; half width$_{\lambda_{max}} = 34$ nm) were used, corresponding to human perception of blue and green, respectively. These filters were mounted onto a LED-lamp (18 LED of 2 mm each) with 2470 cd/m^2 intensity. This lamp was placed 156 cm high from the base of the experimental cage. We have measured the light intensities of the LED-lamp with and without the color filters using a digital photometer (J16, Tektronix, probe J6503 for cd/m^2 and probe J6502 for mW/m^2, Table 1).

2.2. Procedures

Tree shrews were anaesthetized, first, with 2 μl/g ketamin i.m. (Ketalar®, Parke-Davis, 50 mg/ml) and 10 min later with 0.5 μl/g sodium pentobarbital i.p. (Vetanarcol®, Veterinaria AG, 162 mg/ml), then transcardially perfused with 0.9% NaCl + 0.1 g procaine, followed by the fixative (2.5% para-formaldehyde + 1.5% picric acid in 0.1 M phosphate buffer (pH 7.4)). Then, we placed the head in a stereotactic apparatus (David Kopf Instruments, Tujunga, CA, USA), orientated according to the Frankfurter horizontal plane and cut the part of the brain to be investigated with a scalpel blade, performing one anterior cut at A9.0 and one posterior cut at P3.3, both parallel to the AP0.0 plane. In addition, the brain and eyes were post-fixed in the latter solution for 3 h and subsequently cryoprotected for 3 h in a 10% sucrose solution and overnight in a 20% sucrose solution.

Sixty micrometer-thick frozen brain sections were cut in a coronal plane with a freezing microtome and kept in a compartmentalized box for free-floating incubation of antibodies. The eyes were frozen with liquid nitrogen and 20 μm-thick frozen sections were cut in a transversal plane with a cryostat and mounted directly onto gelatin-subbed slides for performing the immunohistochemistry. The sections underwent an incubation in the primary rabbit anti-fos antibody for 48 h at 4 °C (anti-c-Fos [Ab-5], human [rabbit], #PC38T-100, VWR International, dilution 1:10000 in 0.01 M PBS pH 7.4, containing 1% normal goat serum and 0.1% BSA). After that, incubation in secondary antibody was performed (biotinylated anti-rabbit IgG, made in goat, #BA-1000, Vector Laboratories, Inc., dilution 1:300 in 0.1% BSA). The reaction was amplified by the ABC complex (Vectastain Elite ABC Kit [Standard], #PK-6100, Vector Laboratories, Inc.) and visualized by 0.05% 3,3′-diaminobenzidine +0.015% H_2O_2. The presence of c-Fos was seen as a black–brown reaction. In order to better identify the activated structures of the visual pathway, every second section was Nissl stained. To localize the anatomical structures, the Tigges and Shantha (1969) atlas was used as a reference. When performing pre-experimental controls for the specificity of the c-Fos antibody, we diluted out the primary antiserum to the point where it just stained the sites of interest in the tissue, going from dense staining to light or imperceptible staining with a dilution of about tenfold. Furthermore, no labeled cells were observed when the same procedure was used as described above but omitting the incubation in the first antibody.

Table 1
Schematic representation of the light intensities (I_v) measured under different monochromatic light environments.

		LED (mW/m^2)	LED + blue filter (mW/m^2)	LED + green filter (mW/m^2)
I_v	Direct	263.00	19.60	15.90
	Aluminium foil	3.88	0.15	0.17
	Base	16.07	0.82	0.78

"Direct" means pointing the photometer towards the light source; "Aluminum foil" means pointing the photometer to the aluminum foil covering the walls of the cage; "Base" means pointing the photometer towards the base of the experimental cage. LED, light emitting diode.

2.3. Quantification and statistics

To quantify the number of labeled cells at the desired levels of the visual pathway of *T. belangeri* 10 sections of each visual structure were chosen, except for the pulvinar and parabigeminal nucleus, where we chose 5 sections only of each due to size limitations. For the retina 10 transversal sections were selected at the level of the optic nerve; for the SC 10 coronal sections from P1.5 to A1.5 were chosen; for the dLGN 10 coronal sections from A2.5 to A4.0; for the PBG 5 coronal sections from P0.5 to A1.0 were selected; for the Pu 5 coronal sections from A1.0 to A3.5 were chosen and for the V1 10 coronal sections from P1.0 to A2.0. The 10 or 5 sections selected from each visual structure always spanned its whole rostral to caudal limits. Photomicrographs of the desired anatomical structures were taken with Leitz DMRBE and Leica MZ16FA photomicroscopes and a digital Leica camera DFC 320.

Six different regions of interest (ROI) were selected to quantify the number of c-Fos positive cells, one for each anatomical structure studied. For the retina, a ROI of 280 μm × 375 μm, starting at the pigment epithelium and located close to the optic nerve was selected; for the SC, we used three times a ROI of 570 μm × 720 μm, the first one starting at the stratum zonale and slightly overlapped for encompassing only the superficial layers; for the dLGN, a ROI of 1.1 mm × 1.5 mm, spanning between the optic tract and layer 6 of the dLGN; for the Pu, a ROI of 520 μm × 710 μm containing the most of the central nucleus; for the PBG, a ROI of 530 μm × 700 μm, including the whole nucleus was selected and, finally, for the V1, we used three times a ROI of 0.53 mm × 1 mm, starting the first one at the top of layer I and slightly overlapped for encompassing all the layers were used.

The pictures were analyzed with the software Image J (Rasband, W.S., Image J, U.S. National Institutes of Health, Bethesda, Maryland, USA, http://rsb.info.nih.gov/ij/, 1997–2006) and the measurements were exported to SPSS 11.0 and Microsoft Excel for statistical analysis and graphical representation of the results.

Table 2
Descriptive analysis of the immunoreactive c-Fos cell counting.

A	C	N	Mean	S.D.	Variance	Min.	Max.
Retina GCL	W	1	128.0	20.7	426.5	100.0	145.0
		2	134.4	19.3	371.3	110.0	155.0
	D	1	91.6	21.1	446.3	61.0	112.0
		2	100.8	11.7	136.7	84.0	114.0
	B	1	326.2	34.6	1197.2	283.0	379.0
		2	336.8	28.3	800.7	297.0	368.0
	G	1	230.2	41.9	1755.2	196.0	301.0
		2	258.6	34.4	1184.3	210.0	293.0
Retina INL	W	1	598.0	58.9	3469.5	508.0	672.0
		2	641.2	42.7	1826.7	575.0	691.0
	D	1	494.0	82.0	6722.5	415.0	612.0
		2	467.2	73.2	5362.7	400.0	558.0
	B	1	821.0	16.8	281.5	798.0	839.0
		2	810.4	13.6	183.8	797.0	829.0
	G	1	867.0	105.8	11187.5	781.0	1039.0
		2	844.6	43.7	1911.3	790.0	901.0
Retina ONL	W	1	198.8	68.0	4628.2	99.0	283.0
		2	180.4	38.6	1489.3	141.0	242.0
	D	1	218.0	108.8	11844.0	85.0	337.0
		2	138.2	28.8	831.7	103.0	175.0
	B	1	237.0	44.7	1993.5	198.0	303.0
		2	213.4	13.5	182.3	200.0	235.0
	G	1	198.8	36.7	1347.7	165.0	259.0
		2	207.8	22.7	514.7	179.0	234.0

Table 2 (*Continued*)

A	C	*N*	Mean	S.D.	Variance	Min.	Max.
SC	W	1	1825.2	66.0	4355.7	1735.0	1921.0
		2	1824.0	96.7	9343.5	1697.0	1964.0
	D	1	1442.0	52.9	2801.5	1362.0	1502.0
		2	1439.0	44.4	1968.5	1388.0	1486.0
	B	1	1725.6	47.1	2215.8	1675.0	1803.0
		2	1737.8	40.1	1609.2	1688.0	1782.0
	G	1	1620.8	144.4	20841.2	1438.0	1822.0
		2	1662.4	50.4	2544.3	1598.0	1739.0
dLGN	W	1	2249.0	53.9	2905.5	2184.0	2304.0
		2	2297.4	36.1	1299.8	2256.0	2346.0
	D	1	2046.6	36.3	1316.3	2001.0	2100.0
		2	2120.4	11.0	121.3	2000.0	2027.0
	B	1	2171.8	60.2	3627.7	2078.0	2241.0
		2	2192.0	78.9	6228.5	2093.0	2302.0
	G	1	2181.8	52.1	2708.7	2136.0	2240.0
		2	2150.0	84.6	7151.5	2018.0	2239.0
Pu	W	1	3058.0	59.9	3592.5	2988.0	3121.0
		2	3066.4	90.8	8241.8	3001.0	3219.0
	D	1	2485.4	118.3	14003.3	2348.0	2675.0
		2	2444.0	150.6	22673.5	2212.0	2596.0
	B	1	2798.0	99.1	9814.5	2633.0	2890.0
		2	2845.8	109.6	12019.7	2699.0	2995.0
	G	1	2780.0	85.9	7377.5	2653.0	2890.0
		2	2688.6	165.5	27389.3	2490.0	2871.0
PBG	W	1	183.8	36.0	1293.7	148.0	235.0
		2	200.0	32.0	1022.5	159.0	232.0
	D	1	80.2	20.1	404.7	61.0	113.0
		2	85.0	11.6	135.0	72.0	100.0
	B	1	128.6	33.6	1131.3	77.0	169.0
		2	112.6	26.3	692.3	81.0	146.0
	G	1	127.2	28.2	794.7	90.0	166.0
		2	118.4	13.5	183.3	100.0	131.0
Cx	W	1	3525.2	53.4	2853.7	3458.0	3571.0
		2	3512.4	18.3	334.3	3488.0	3534.0
	D	1	2623.4	194.0	37625.3	2428.0	2893.0
		2	2724.8	104.2	10850.7	2576.0	2836.0
	B	1	2889.2	296.8	88071.2	2659.0	3240.0
		2	2923.2	181.9	33099.7	2699.0	3105.0
	G	1	2912.4	109.8	12047.3	2782.0	3044.0
		2	2854.4	128.3	16471.8	2679.0	3027.0

A, areas analyzed in the visual system; C, experimental conditions (different monochromatic light environments/dark adaptation); *N*, number of experimental animal within each experimental group; mean, arithmetical average of c-Fos positive neurons per experimental animal within each experimental group; S.D., standard deviation; Min., minimum cell count per experimental animal; Max, maximum cell count per experimental animal; GCL, ganglion cell layer; INL, inner nuclear layer; ONL, outer nuclear layer; SC, superior colliculus; dLGN, dorsal lateral geniculate nucleus; Pu, pulvinar nucleus; PBG, parabigeminal nucleus; Cx, primary visual cortex; W, white light (control positive group); D, dark, no light (control negative group); B, blue light group; G, green light group.

Counting of c-Fos positive nuclei in a set of selected sections was manually performed by three collaborators who were unaware of the experimental groups by means of a graphic tablet (WACOM). Differences between the individual measurements were below 1%. The criteria applied for counting labeled nuclei was to mark each labeled cell which limits were clearly identified. The values shown correspond to the mean of the three measurements. A descriptive analysis (Table 2) of the different experimental groups was performed.

3. Results

3.1. Retina

The retina of *T. belangeri* contains the following distribution of cellular layers (Foelix et al., 1987): (1) a pigment epithelium layer; (2) a layer of photoreceptors cells, the outer nuclear layer (ONL); (3) an outer plexiform layer (OPL); (4) an inner nuclear layer (INL); (5) an inner plexiform layer (IPL); (6) a ganglion cell layer (GCL); and (7) a fiber layer.

Our results in the retina showed c-Fos expression during white, blue, green light stimulations and dark adaptation in the GCL, INL and ONL (Fig. 1). In the GCL, INL and ONL we obtained a higher expression of c-Fos for the blue and green light groups compared with the control positive and control negative groups (Fig. 7).

3.1.1. Ganglion cell layer and inner nuclear layer

In the GCL, the highest c-Fos expression was obtained in the blue light group (152%, 244.6% and 35.6% more labeled cells than in the control positive group, control negative group and green light group, respectively) and the lowest one in the control negative group (26.7%, 71% and 60.6% less labeled cells than in the control positive group, blue light group and green light group). The green light group showed 86.3%, 154.1% more c-Fos positive cells than the control positive group and the control negative group, respectively, and 26.3% less labeled cells compared with the blue light group. The control positive group was higher than the control negative group with 36.4% more labeled cells.

In the INL, both blue light group and green light group had the highest c-Fos positive cell counts, with no difference between them (less than 5%). They showed 34.9% and 74% more labeled cells than the control positive group and the control negative group, respectively. The control positive group was higher than the control negative group with 28.9% more c-Fos positive cells.

3.1.2. Outer nuclear layer

Considering now the ONL we obtained the highest c-Fos positive cell count for the blue light group (18.8%, 26.4% and 10.8% more labeled cells than in the control positive group, control negative group and green light group, respectively) and the lowest for the control negative group (6.1%, 21% and 12.4% less labeled cells compared with the control positive group, blue light group and green light group, respectively). The green light group showed 7.2% and 14.1% more labeled cells than the control positive group and the control negative group, respectively, and 9.7% less labeled cells compared with the blue light group. The control positive group was higher than the control negative group with 6.5% more labeled cells.

3.2. Superior colliculus, dorsal lateral geniculate nucleus, pulvinar, parabigeminal nucleus and primary visual cortex

In the other anatomical structures studied of the visual pathway of *T. belangeri* (Figs. 2–6), we obtained a similar c-Fos expression pattern in each structure: the highest c-Fos expression for the control positive group, the lowest c-Fos expression for the control negative group and intermediate c-Fos expressions within the range of the controls for the blue and green light groups (Fig. 7).

3.2.1. Superior colliculus

The SC of *T. belangeri* is a laminar structure which, following the nomenclature internationally accepted from Huber and Crosby (1943), could be divided in eight cellular layers: (1) *Stratum zonale*; (2) *Stratum griseum superficiale*; (3) *Stratum opticum*; (4) *Stratum griseum mediale*; (5) *Stratum album mediale*; (6) *Stratum griseum profundum*; (7) *Stratum album profundum*; and (8) *Stratum griseum centrale*.

In the SC, although we analyzed the superficial layers together (*stratum zonale*, *stratum griseum superficiale*, *stratum opticum*, *stratum griseum mediale*) without differentiating between them, we observed that the immunoreactivity is more dense at the rostral level rather than at the caudal one. We obtained the highest c-Fos expression in the control positive group (26.7%, 5.4% and 11.1% more labeled cells than in the control negative group, blue light group and green light group, respectively) and the lowest c-Fos expression in the control negative group (21.1%, 16.8% and

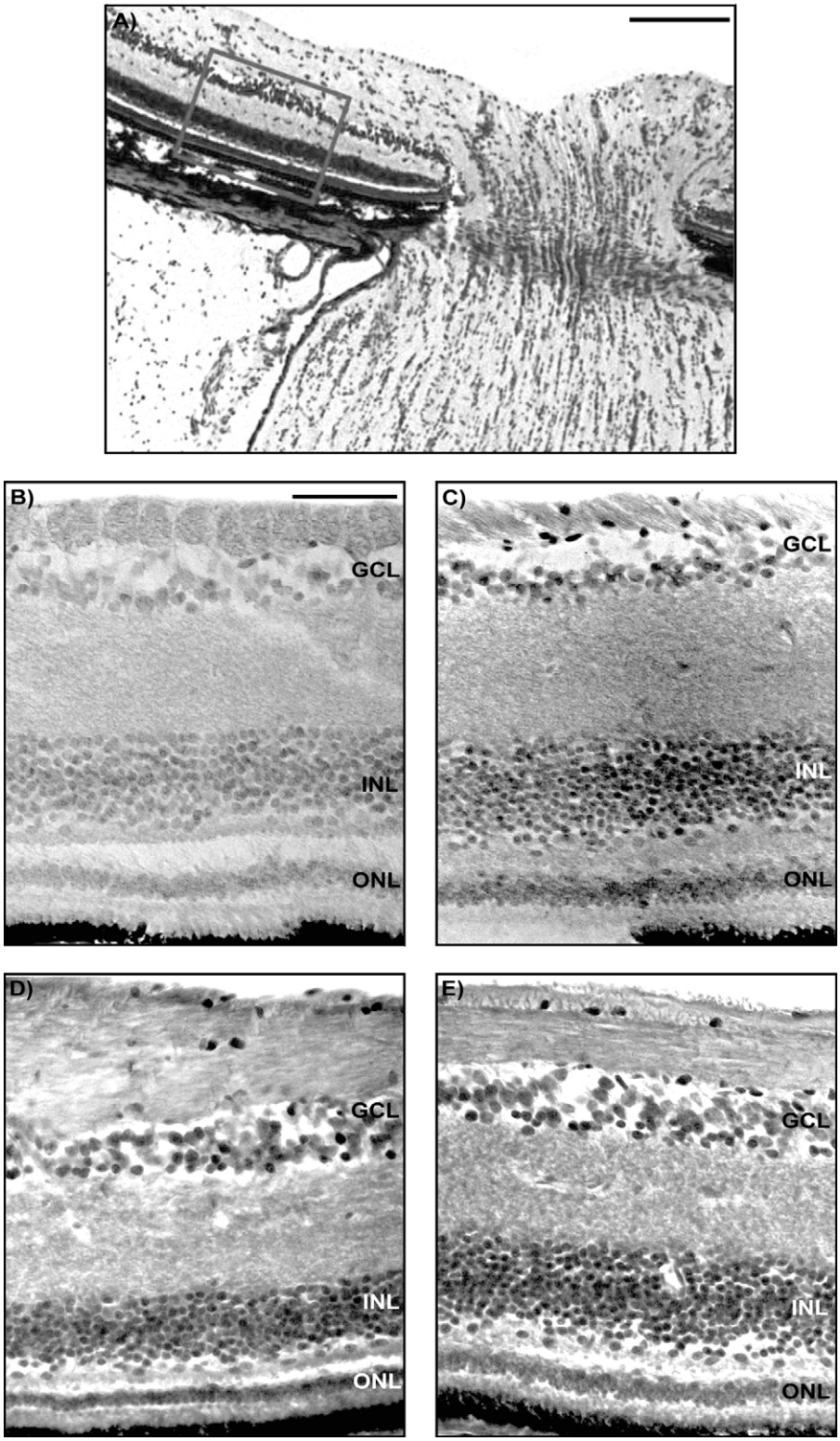

Fig. 1. Photomicrographs of 20-μm-thick transversal sections of the retina of *Tupaia belangeri* (A) stained with Cresyl violet and showing the localization and size of the ROI employed (280 μm × 375 μm) at the level of the retina for cell counting, and (B–E) displaying c-Fos positive cells after different environmental light/dark conditions. (B) Control negative group; (C) control positive group; (D) blue light group; (E) green light group. GCL, ganglion cell layer; INL, inner nuclear layer; ONL, outer nuclear layer. Scale bars: (A) 250 μm, (B–E) 50 μm.

12.3% less labeled cells than in the control positive group, blue light group and green light group, respectively). The blue light group and green light group showed intermediate c-Fos expressions between the controls, with 5.9% more labeled cells for the blue light group compared with the green light group (Figs. 2 and 7).

3.2.2. Dorsal lateral geniculate nucleus

The dLGN of tree shrews is a lens-shaped structure, consisting of six well-defined cellular layers, separated by interlaminar layers of sparse cell density. We number the layers from medial to lateral, 1–6.

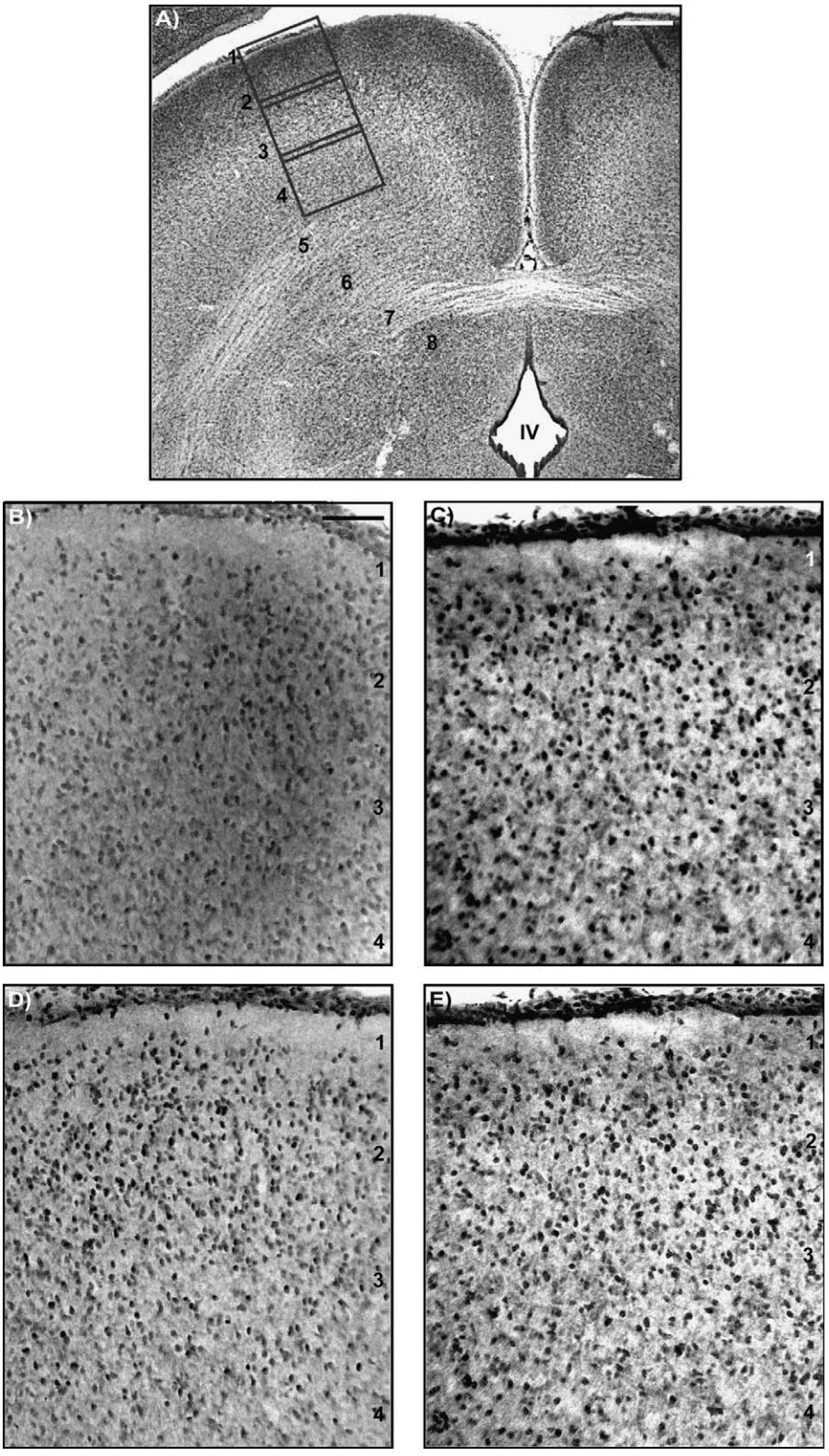

Fig. 2. Photomicrographs of 60-μm-thick coronal sections of the superior colliculus (SC) of *Tupaia belangeri* (A) stained with Cresyl violet and showing the localization and size of the ROI employed (three times an ROI of 570 μm × 720 μm) at the level of the SC for cell counting, and (B–E) displaying c-Fos positive cells after different environmental light/dark conditions. (B) Control negative group; (C) control positive group; (D) blue light group; (E) green light group. The numbers 1–8 in (A) and 1–4 in (B–E) indicate the layers of the SC. IV, fourth ventricle. Scale bars: (A) 500 μm, (B–E) 100 μm.

In the dLGN (Figs. 3 and 7), we also analyzed the whole structure without separating the different layers and obtained a higher c-Fos expression for the control positive group than for the control negative group and the green light group (9.1% and 5% more labeled cells, respectively). The control positive group had no significant difference in the number of c-Fos positive cells compared with the blue light group (less than 5%). The control negative group presented no significant difference compared with

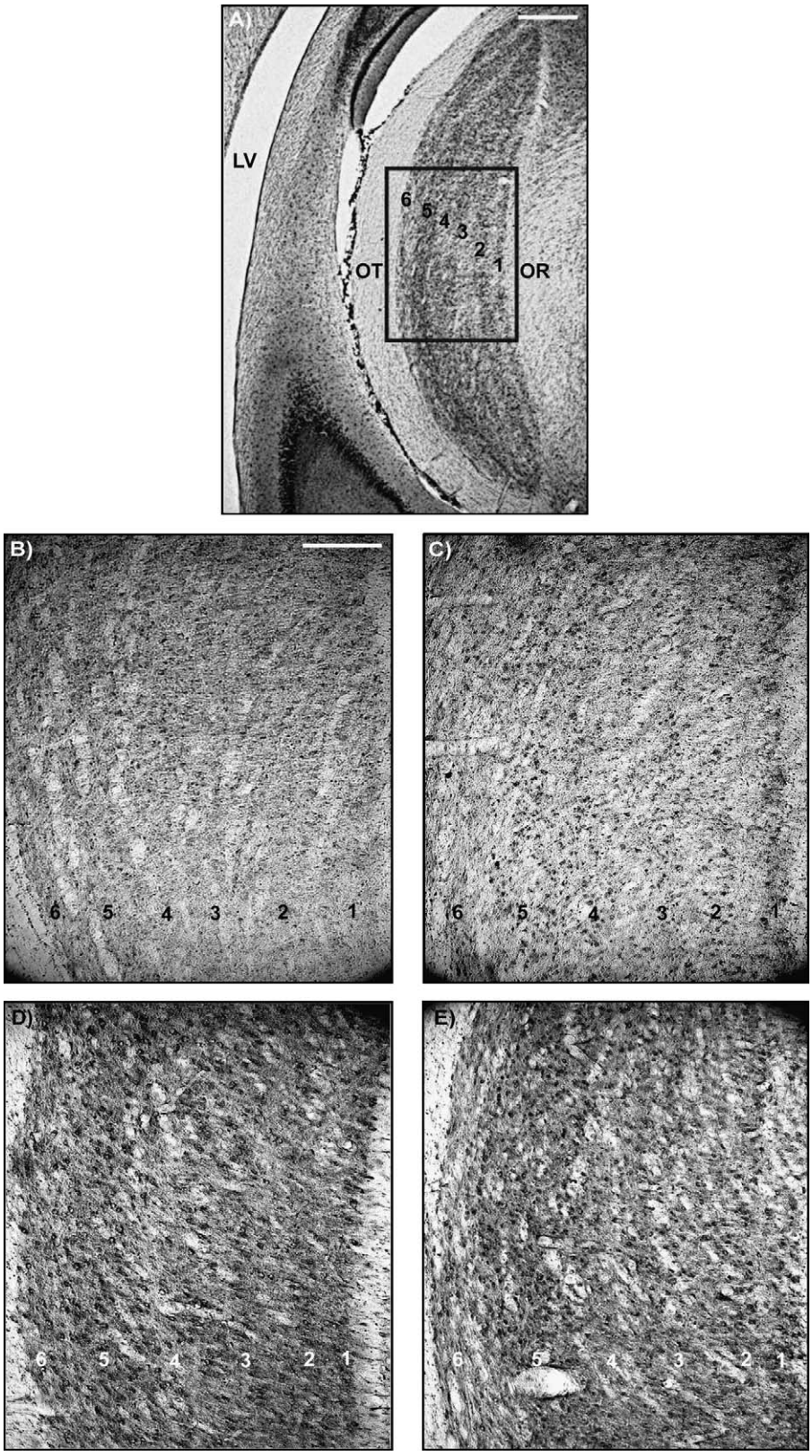

Fig. 3. Photomicrographs of 60-μm-thick coronal sections of the dorsal lateral geniculate nucleus (dLGN) of *Tupaia belangeri* (A) stained with Cresyl violet and showing the localization and size of the ROI employed (1.1 mm × 1.5 mm) at the level of the dLGN for cell counting, and (B–E) displaying c-Fos positive cells after different environmental light/dark conditions. (B) Control negative group; (C) control positive group; (D) blue light group; (E) green light group. The numbers 1–6 indicate the six layers of the dLGN. LV, lateral ventricle; OT, optic tract; OR, optic radiation. Scale bars: (A) 500 μm, (B–E) 250 μm.

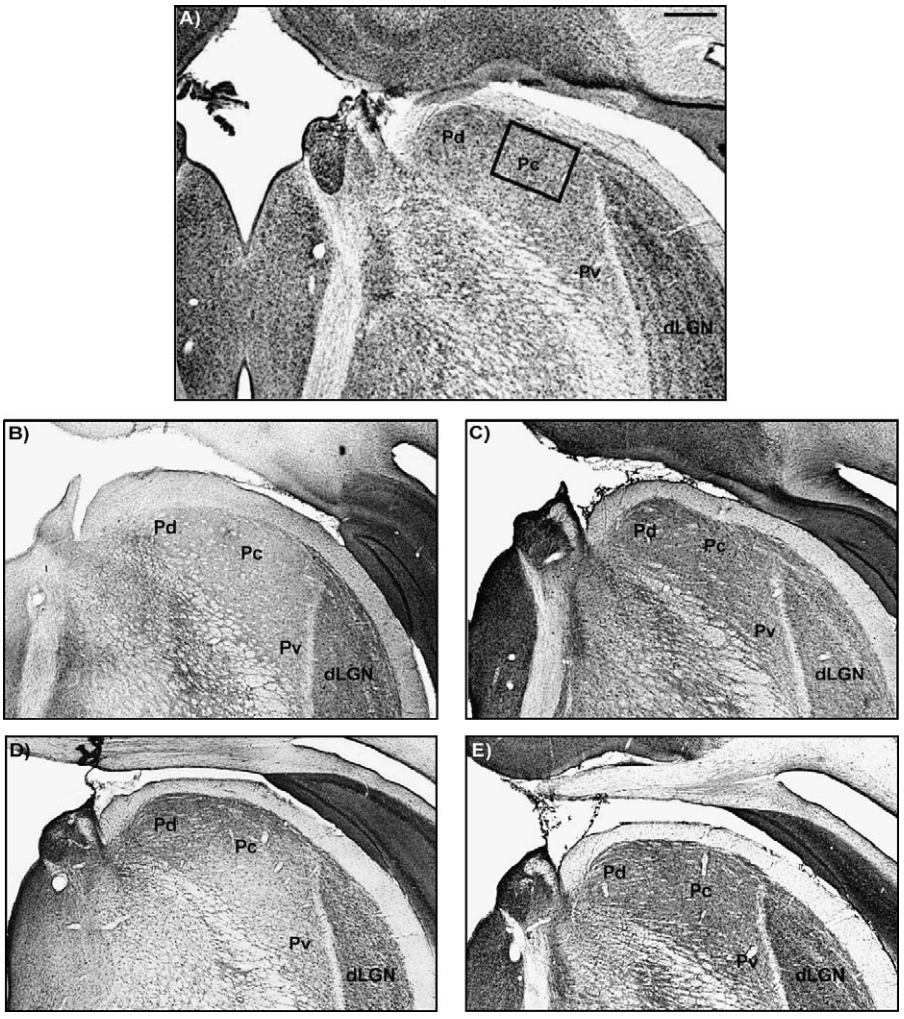

Fig. 4. Photomicrographs of 60-μm-thick coronal sections of the pulvinar (Pu) of *Tupaia belangeri* (A) stained with Cresyl violet and showing the localization and size of the ROI employed (520 μm × 710 μm) at the level of the Pu for cell counting, and (B–E) displaying c-Fos positive cells after different environmental light/dark conditions. (B) Control negative group; (C) control positive group; (D) blue light group; (E) green light group. Pd, pulvinar dorsal; Pc, pulvinar central; Pv, pulvinar ventral; dLGN, dorsal lateral geniculate nucleus. Scale bars: (A– E) 500 μm.

the blue and the green light groups and the blue and green light groups had no difference between them in c-Fos positive cells (less than 5%).

3.2.3. Pulvinar

The pulvinar nucleus of *T. belangeri* consists of 4 different subdivisions (Lyon et al., 2003): (1) dorsal nucleus, Pd; (2) central nucleus, Pc; (3) ventral nucleus, Pv; (4) posterior nucleus, Pp.

In the pulvinar nucleus, we obtained the highest c-Fos expression pattern in the control positive group (24.2%, 8.5% and 12% more labeled cells compared with the control negative group, the blue light group and the green light group, respectively) and the lowest in the control negative group (19.5%, 12.7% and 9.9% less labeled cells compared with the control positive group, blue light group and green light group, respectively). The blue and green light groups showed no significant difference between (less than 5%) them and an intermediate c-Fos expression pattern between the controls (Figs. 4 and 7).

3.2.4. Parabigeminal nucleus

The PBG is a cellular group at mesencephalic level, adjacent to the *brachium colliculi superior* and considered the homologue of the isthmus nucleus of the inferior vertebrates (Dacey and Ulinski, 1986).

In the PBG (Figs. 5 and 7), we got the highest c-Fos labeled cells for the control positive group (with 132%, 59.1% and 56.3% more c-Fos positive cells than the control negative group, the blue light group and the green light group, respectively) and the lowest one for the control negative group (57%, 31.5% and 32.7% less c-Fos positive cells if compared with the control positive group, the blue light group and the green light group). The blue and the green light groups showed no significant difference between them and an intermediate c-Fos expression pattern between the controls.

3.2.5. Primary visual cortex

Considering the primary visual cortex, we can describe it as a laminar structure, which prominent feature in a Nissl stained section, is the densely packed layer IV. This layer is not homogeneous in structure; in the center of this layer there is a cell-sparse cleft, which is surrounded by a thin compact row of cells on either side. Previous studies have used this cell-sparse cleft as a border for two subdivisions of layer IV (IVa and IVb) related to projections from different dLGN layers. Immediately superior to layer IV there is a zone of loosely packed cells that has been called IIIc. The cell packing density gradually increases above IIIc and this zone of increased packing density is referred to as IIIb. A reproducible delineation between layers IIIb, IIIa and II is not possible, as there is poor demarcation of cytology or packing

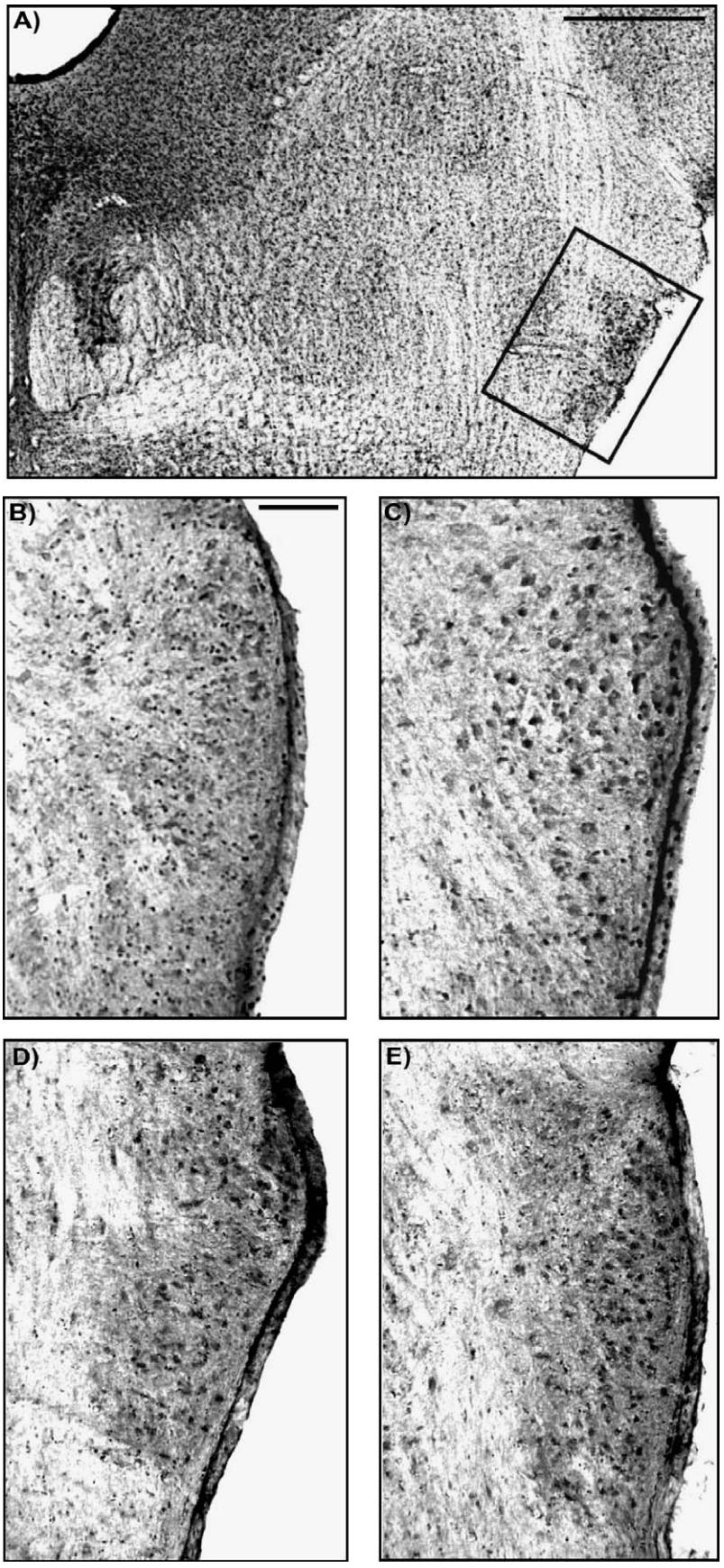

Fig. 5. Photomicrographs of 60-μm-thick coronal sections of the parabigeminal nucleus (PBG) of *Tupaia belangeri* (A) stained with Cresyl violet and showing the localization and size of the ROI employed (530 μm × 700 μm) at the level of the PBG for cell counting, and (B– E) displaying c-Fos positive cells after different environmental light/dark conditions. (B) Control negative group; (C) control positive group; (D) blue light group; (E) green light group. Scale bars: (A) 500 μm, (B–E) 100 μm.

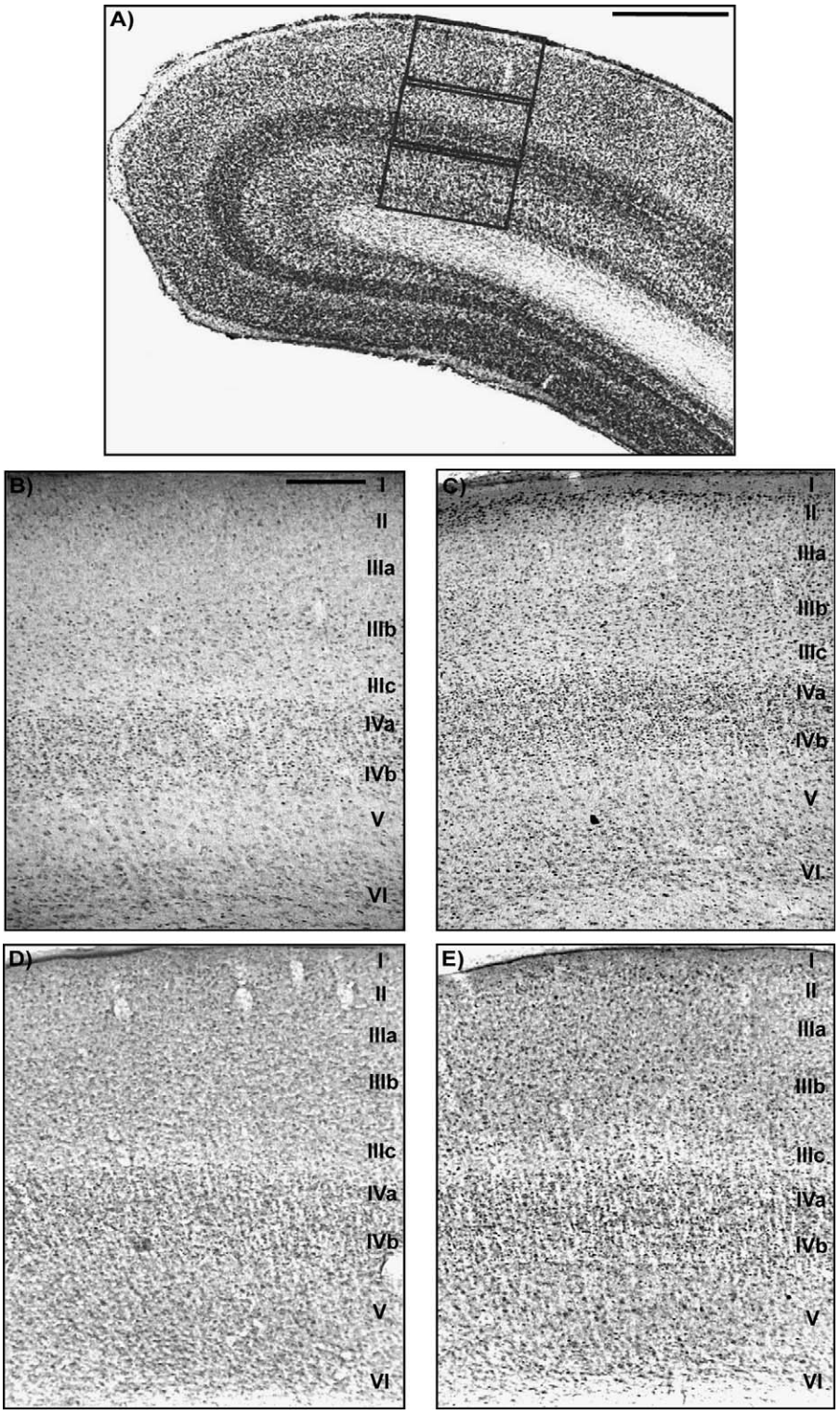

Fig. 6. Photomicrographs of 60-μm-thick coronal sections of the primary visual cortex (Cx) of *Tupaia belangeri* (A) stained with Cresyl violet and showing the localization and size of the ROI employed (three times a ROI of 0.53 mm × 1 mm) at the level of the Cx for cell counting, and (B–E) displaying c-Fos positive cells after different environmental light/dark conditions. (B) Control negative group; (C) control positive group; (D) blue light group; (E) green light group. Scale bars: (A) 1 mm, (B–E) 250 μm. Numbers I–VI refer to the cortical layers.

density of the nerve cells. The layer I contains very few neurons, whereas more glial than nerve cells are found in layer I, the packing density of the cellular elements is the lowest of all laminas. Inferior to layer IV is found layer V with large and small pyramidal cells dominating this lamina and the layer VI (the most inferior one) (Zilles, 1978; Lund et al., 1985).

In the primary visual cortex (Figs. 6 and 7), we analyzed the whole structure without separating the different layers and counted the highest number of c-Fos positive cells in the control positive group (31.6%, 21.1% and 22% more labeled cells than for the control negative group, the blue light group and the green light group, respectively) and the lowest number for the control

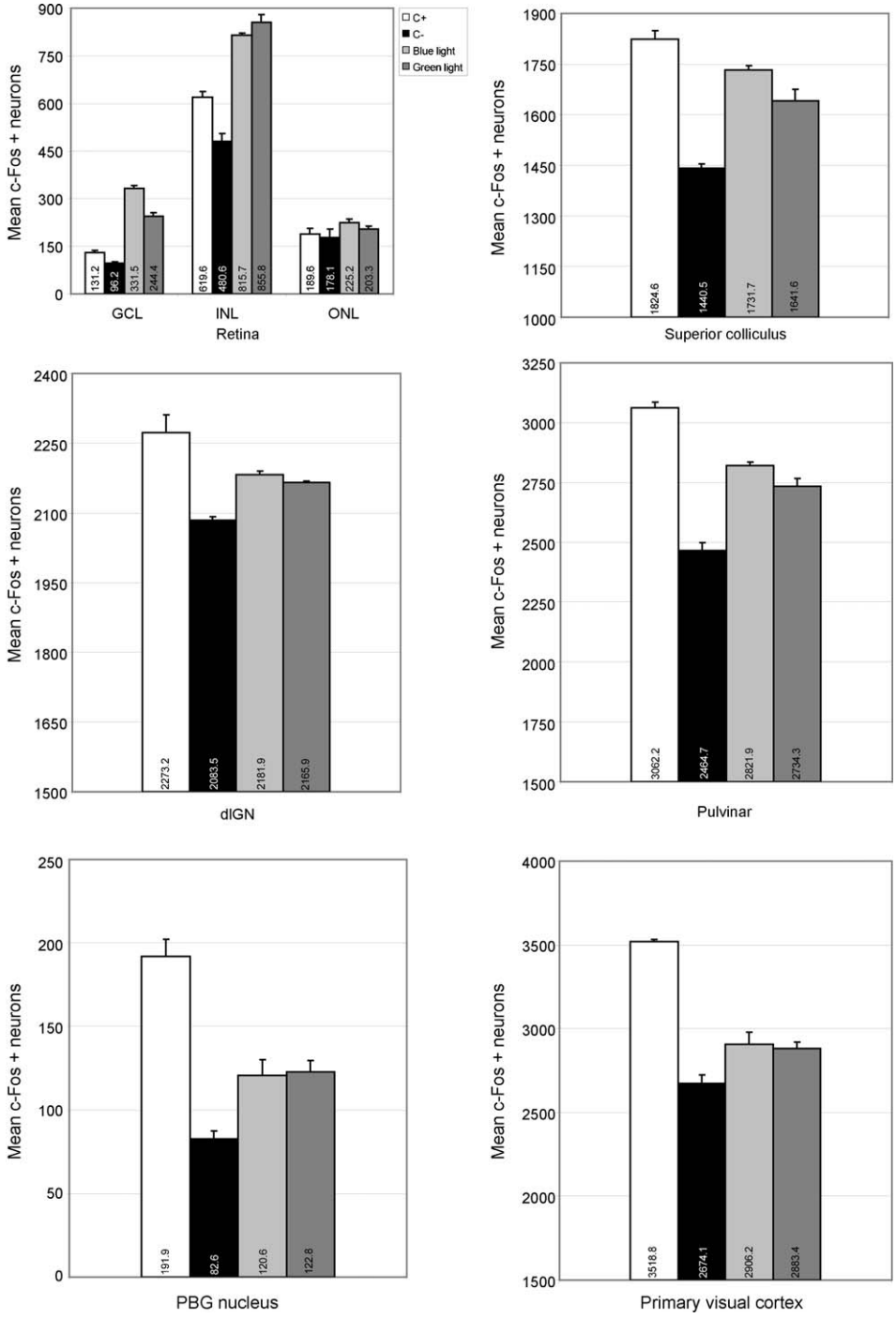

Fig. 7. Histograms of the c-Fos positive cell counts for the different anatomical structures of the visual pathway in *Tupaia belangeri* (values = mean ± error mean).

negative group (with 24%, 8% and 7.3% less labeled cells compared with the control positive group, the blue light group and the green light group, respectively). The blue and the green light groups showed no significant difference between them (less than 5%) and an intermediate c-Fos expression pattern between the controls. Although we analyzed all layers together, we observed more immunoreactivity in the layers II, IIIb, IV and VI in the control positive group, the blue light group and the green light group. This c-Fos expression pattern is modified in the control negative group, where we can only observe this intense immunoreactivity in layer IV.

4. Discussion

The main findings of the present study are: (1) the expression levels of c-Fos protein are able to show differences between distinct light stimuli in all anatomical structures studied of the visual pathway of *T. belangeri*; (2) in the retina, the ganglion cells and the cells of the inner nuclear layer respond better to blue and green light stimuli rather than white light or dark adaptation; (3) the superior colliculus, dorsal lateral geniculate nucleus, pulvinar, parabigeminal nucleus and the primary visual cortex show an identical pattern of c-Fos expression: the highest c-Fos expression

for the control positive group, the lowest c-Fos expression for the control negative group and intermediate c-Fos expressions within the range of the controls for the blue and the green light groups; (4) the immunohistochemical detection of c-Fos protein levels is a powerful tool as a neuronal activity marker for the morphofunctional study of the central nervous system (CNS). Although it is suggested that combining other methods with this technique, such as neuronal tracers, immunohistochemical detection of neurotransmitter systems, electrophysiology or all together, could help in getting a more accurate information of the c-Fos positive neurons.

As there are no studies in which the c-Fos protein expression in tree shrews was studied in general, and in their visual system in particular, this discussion will be focused on the comparison of the present results with those obtained in other experimental animals, mainly in rats.

4.1. Retina

The environmental cycle of light and darkness is involved in the regulation of transcription or protein synthesis in retinal cells. Therefore, the pattern of *c-fos* gene expression varies within the subpopulations of retinal neurons (Yoshida et al., 1993).

Our results in the retina show c-Fos expression during different light stimulations and dark adaptation in the GCL, INL and ONL.

4.1.1. Ganglion cell layer and inner nuclear layer

Our results in the GCL and in the INL show that after light stimulation there is expression of c-Fos in these two layers.

Sagar and Sharp (1990) found c-Fos expression in retinas of dark-adapted rabbits that were exposed to 3 Hz diffuse flashing white light for 1 h. Yoshida et al. (1993) reported a strong c-Fos expression in the INL as well as in the GCL of rats 30 min after the onset of light by a fluorescent lamp (60 lux). Koistinaho and Sagar (1995) obtained also c-Fos positive nuclei in the GCL and the INL of dark-adapted rabbits exposed to flashing light (3 Hz, 60 ms, 300 lux) for 2 h. Bussolino et al. (1998) found that light differences for phospholipid labeling correlate with the expression of c-Fos: that is, in the GCL the c-Fos expression and phospholipid synthesis is higher in light with respect to dark. Lima et al. (2003) obtained also c-Fos positive nuclei in the GCL and the INL of dark-adapted retinal degeneration mice exposed to 1 h of diffuse light (900 lux) or optokinetic stimuli (180 lux).

In the GCL and INL from the analyzed *T. belangeri* retinas of our study, the lowest c-Fos expression pattern was obtained in the control negative group. Considering that many cells in the INL and the GCL depolarize with illumination (Ehinger and Dowling, 1987), depolarization may be involved in c-Fos expression in the INL and GCL by light exposure (Yoshida et al., 1993), therefore it seems obvious that in absence of illumination, there is lower c-Fos expression. It has long been recognized that there are neurons in the retina of *T. belangeri* which respond to the onset of light (ON-center cells) and the offset of light (OFF-center cells) (Norton et al., 1985). DeBruyn and Casagrande (1983) found that, on the one hand, the ganglion cells projecting to the dLGN layers containing ON-center cells have dendrites which appear to ramify close to the cell body in the upper part of the inner plexiform layer (IPL); and on the other hand, ganglion cells which project to the dLGN layers containing OFF-center cells have dendrites which appear to extend further from the cell body into the lower part of the IPL. Thus, in the tree shrew, as in other species (Famiglietti and Kolb, 1976; Nelson et al., 1978) the spatial segregation of ON- and OFF-center cells appears to be organized initially in the retina. Therefore, we explain the fact that during the dark period exist c-Fos expression in the GCL and the INL because there are phasic and tonic OFF-center cells active, which after a dark period (in this study 15 h), the phasic cells are inhibited, but not the tonic ones. For that reason we consider that the c-Fos expression for the control negative group corresponds to the tonic OFF-center cells.

In the GCL and INL we obtained a higher expression of c-Fos for the blue and the green light groups compared with the control positive and control negative groups, probably because of spectrally inhibitory mechanisms at the level of horizontal and/or amacrine cells.

Inhibition shapes visually evoked retinal signals. GABA (*c*-aminobutyric acid) is a transmitter found in both synaptic layers of the retina mediating visually evoked inhibition (Lukasiewicz et al., 2004). Two distinct types of ionotropic GABA receptor, GABAA and GABAC, are abundant in the IPL, where they influence signaling from bipolar cells to amacrine and ganglion cells. Inhibitory signaling pathways modulate the visual signal as it passes from photoreceptors to bipolar cells and again as it passes from bipolar cells to ganglion cells. The initial inhibitory synaptic interactions that occur at the outer plexiform layer (OPL) are mediated by horizontal cells (Lukasiewicz et al., 2004). A second stage of inhibition occurs in the IPL, and is mediated by amacrine cells. Lateral inhibitory interactions in the OPL contribute to the surround portion of the receptive fields of bipolar and ganglion cells (Werblin and Dowling, 1969; Mangel, 1991; McMahon et al., 2004). However, several studies suggest that the OPL generated surround signal is not mediated by GABA (Verweij et al., 1996; Kamermans et al., 2001; Verweij et al., 2003; McMahon et al., 2004). In contrast, lateral inhibition in the IPL, which contributes to the surround of ganglion cells, is mediated by GABA (Cook and McReynolds, 1998; Flores-Herr et al., 2001). In addition, GABAergic inhibition in the IPL also may shape the temporal responses of ganglion cells (Dong and Werblin, 1998) and is a key player in the signaling that determines their motion and direction sensitivity (Caldwell et al., 1978).

The effects of the inhibitory feedback pathways from horizontal cells to cones can be revealed when the properties of the light stimuli are adjusted to favor the contributions of these pathways to the cones relative to light absorption in the cones themselves. Thus, when large spots or annuli are used to stimulate the retina with long-wavelength light, the photoresponses of medium and short-wavelength cones are expected to be of a more depolarizing pattern (Twig et al., 2003).

Any light stimulus excites to a certain extent all types of cone and therefore, the responses of chromaticity-type (C-type) horizontal cells reflect the summation of hyperpolarizing and depolarizing inputs. Since the receptive field size of C-type cells depends upon the wavelength and the intensity of the stimulating light, a complex relationship between amplitude and polarity of the photoresponse and the spatial and spectral properties of the light stimulus is expected (Twig et al., 2003).

4.1.2. Outer nuclear layer

Considering the ONL, we obtained the highest c-Fos positive cell count for the blue light group and the lowest for the control negative group. The control positive group was higher than the control negative group. The green light group showed more labeled cells than the control positive group and the control negative group, respectively, and less labeled cells compared with the blue light group.

The authors who have studied the ONL report a higher c-Fos expression in the dark period compared with light conditions (Yoshida et al., 1993; Bussolino et al., 1998). Yoshida et al. (1993) report that during the entire light phase, *c-fos* mRNA in the ONL remained at a low level at all the time points examined. Thus, the *c-fos* mRNA level in the ONL showed cyclic changes, with high levels during the dark period and low levels during the light period. Bussolino et al. (1998) found that in photoreceptor cells the c-Fos

expression and phospholipid synthesis is higher in dark with respect to light. In our study, although the experimental design was different than the studies of Yoshida et al. (1993) and Bussolino et al. (1998), we do observe lower c-Fos expression in the ONL with light/dark conditions if comparing with the GCL and the INL, however those values are still higher than the control negative group, which is the lowest c-Fos expression of the ONL.

4.2. Superior colliculus, dorsal lateral geniculate nucleus, pulvinar, parabigeminal nucleus and primary visual cortex

Concerning the rest of the structures studied (SC, dLGN, Pu, PBG and V1) our results show an identical c-Fos expression pattern in each structure: the highest c-Fos expression for the control positive group, the lowest c-Fos expression for the control negative group and intermediate c-Fos expressions for the blue and the green light groups within the controls.

Why is the pattern in these structures different from the one seen in the retina? It could be that in the retina the visual information remains segregated, so that we obtained c-Fos expression for the white, blue and green lights and for the condition of darkness in a segregated pathway. However, as soon as the visual information is processed at later stages we obtained the highest c-Fos expression for the white light stimulation, a smaller c-Fos expression for the blue and green light stimulations, and the smallest c-Fos expression under darkness conditions. We obtained a c-Fos expression corresponding to the information which has already started converging, what would increase the c-Fos expression for the white light, due to the fact of converging the blue and the green light pathways.

4.2.1. Superior colliculus

In the superior colliculus, we performed a quantitative analysis considering all the superficial cell layers together.

A few published reports (Montero and Jian, 1995; Correa-Lacarcel et al., 2000; Lima et al., 2003) exist which analyze the immunoreactivity for the c-Fos protein at the level of subcortical structures. Montero and Jian (1995) found Fos immunoreactive populations of neurons in retinotopically corresponding regions of the *stratum griseum superficiale* and in the *stratum opticum* after exposing dark-adapted rats to moving and stationary visual stimuli. Correa-Lacarcel et al. (2000) found that the frequency of light stimulus determinates differently the level of c-Fos expression in each center of dark-adapted rats studied. Thus, the SC displayed high c-Fos expression when rats were stimulated with medium and, mostly, low frequency light. On the contrary, continuous and high frequency light elicited low c-Fos expression levels in these centers. Lima et al. (2003) only stated to have found Fos staining after diffuse light stimulation in several retinorecipients structures of the mouse brain, such as the superior colliculus.

Although we have not quantified the differences between the labeled cells within the rostral and caudal parts of the superior colliculus, we have observed that the immunoreactivity is more dense at the rostral level rather than at the caudal one. This fact is consistent with the observations of Laemle (1968), who reported that the retino-collicular afferent fibers penetrate into the lateral portion of the superior colliculus. Most of these fibers go through the optic layer and they are distributed, afterwards, to the rest of the layers. However, some fibers enter directly into the *stratum griseum superficiale*. Therefore, we find more c-Fos immunoreactivity within the superficial cell layers (retinorecipient) than within the deeper ones.

Our results support previous anatomical and electrophysiological studies which propose that the superior colliculus is an important part of the CNS, because it is involved in both localization of sensory stimuli (which, in the case of the visual stimuli, start converging at the level of the superficial layers) and in the initiation of behavioral responses, which allow space orientation (Harting et al., 1973; Dean et al., 1989; Lee and Hall, 1995).

4.2.2. Dorsal lateral geniculate nucleus

In the tree shrew, the dLGN contains six distinct layers, as mentioned before: layers 6, 4, 3 and 2 (with 6 nearest the optic tract) receive input from the contralateral eye, and layers 5 and 1 receive input from the ipsilateral eye (Norton et al., 1985). Conway and Schiller (1983) found that the medial two dLGN layers (1 and 2) contain almost entirely ON-center cells, while layers 3, 4 and 5 contain almost entirely OFF-center cells.

Our results for the dLGN agree with those obtained by other authors as Montero and Jian (1995) and Correa-Lacarcel et al. (2000). Montero and Jian (1995) found Fos immunoreactive populations of neurons in retinotopically corresponding columns of the dLGN after exposing dark-adapted rats to moving and stationary visual stimuli. Correa-Lacarcel et al. (2000) found that the frequency of light stimulus determinates differently the level of c-Fos expression in each center studied. Thus, the dLGN displayed high c-Fos expression when rats were stimulated with medium and, mostly, low frequency light. On the contrary, continuous and high frequency light elicited low c-Fos expression levels in these centers.

The dLGN serves, as other sensory centers of the dorsal thalamus, as a mandatory relay for the transmission of the information from the sensory organ (the retina) to the visual cortex. Therefore, the presence of c-Fos expression in this structure is consistent with the assumption that the retinal stimulation should provoke the stimulation of the transmission neurons at the dLGN, which should be manifested in an increase of the c-Fos immunoreactivity. The nervous stimulation provokes a rapid and transient increment of the levels of c-Fos protein in the CNS (Morgan and Curran, 1991).

4.2.3. Pulvinar and parabigeminal nucleus

Up to date, there exists no published study in which the c-Fos expression either at the level of the pulvinar or at the parabigeminal nucleus of any experimental animal was studied, neither as a main aim of the study nor as a secondary one. Therefore, we will just indicate our results concerning the c-Fos expression in the pulvinar and parabigeminal nucleus of *T. belangeri*.

In the pulvinar and parabigeminal nucleus we obtained the highest c-Fos expression pattern in the control positive group and the lowest in the control negative group. The blue and green light groups have shown no significant difference between them and an intermediate c-Fos expression pattern between the controls.

As it was already mentioned in Section 4.2, as soon as the visual information is processed at later stages of the retina, we obtained the highest c-Fos expression for the white light stimulation, a smaller c-Fos expression for the blue and the green light stimulations, and the smallest c-Fos expression under darkness conditions. We obtained a c-Fos expression corresponding to the information which has already started converging, what would increase the c-Fos expression for white light, due to the fact of converging the blue and the green light pathways. Therefore, we suggest that there are segregated and converged visual information together at the level of both pulvinar and parabigeminal nucleus of *T. belangeri*.

4.2.4. Primary visual cortex

The medial dLGN layers 1 and 2 project to layer IVa, while layers 4 and 5 project to layer IVb. Therefore, there may be a population of cortical neurons in layer IVa which receive input from the ON-center dLGN cells and respond primarily to the onset of light, and a separate population of cells in layer IVb which receive input from

OFF-center dLGN cells and respond primarily to the offset of light. This was consistent with anatomical data showing that dLGN layer 1 (ON, ipsilateral) and 2 (ON, contralateral) project to layer IVa while layer 4 (OFF, contralateral) and 5 (OFF, ipsilateral) project to IVb (Norton et al., 1985).

Opposite to the rest of the analyzed structures of the visual system, where there are only a few studies about c-Fos expression, we can find numerous studies about the expression of immediate early genes in the visual cortex, in which it was not only studied the immunoreactivity for the c-Fos protein, but also it was analyzed the *c-fos* mRNA levels and other immediate early genes as *zif-268* or *c-jun* (Rosen et al., 1992; Zhang et al., 1994; Koistinaho and Sagar, 1995; Herrera and Robertson, 1996; Kaplan et al., 1996; Kaczmarek and Chaudhuri, 1997).

Our results at the level of the visual cortex are limited exclusively to the primary visual cortex (V1), without studying the extrastriate cortex. We analyzed the whole structure without separating the different layers and counted the highest number of c-Fos positive cells in the control positive group and the lowest number for the control negative group. The blue and the green light groups showed no significant difference between them and an intermediate c-Fos expression pattern between the controls.

Although we analyzed all layers together, we found moreover, qualitative or distribution differences observing more immunoreactivity in the layers II, IIIb, IV and VI in the control positive group, blue light group and green light group. This c-Fos expression pattern is modified in the control negative group, where we can only observe this intense immunoreactivity in layer IV. Amir and Robinson (1996) exposed dark-adapted rats to ultraviolet light for 30 min and observed intense c-Fos expression in the primary visual cortex. The largest concentration of Fos-labeled cells was in layer IV; layers II/III and VI were moderately stained whereas layer V showed only sparse Fos labeling. Layer I was unstained. Kaplan et al. (1996) reported intensely c-Fos stained cells almost exclusively above and below layer IV after 1 h of visual experience to dark-adapted cats. Montero and Jian (1995) and Correa-Lacarcel et al. (2000), also found a higher c-Fos expression in the layers II/III, IV and VI after exposing dark-adapted rats to moving and stationary visual stimuli and to continuous/pulsed coherent light, respectively.

The information processing in the cerebral cortex of mammals is considered to be based on interactions of neuronal signals through both vertical and horizontal pathways, i.e. laminar and columnar synaptical connections. The analysis of our results seems to suggest that not only a columnar pattern, but also a horizontal transmission which plays a crucial role integrating the visual information was observed. Moreover, the presence of differences in the c-Fos expression within the different groups is consistent with the coexistence of diverse pathways, or at least different interactions for the different types of stimuli, and also depended on the magnitude of it, suggesting as reported by Norton et al. (1985) that the segregation of ON- and OFF-center cells which is established in the retina, is preserved in the tree shrew through the first synapse in the striate cortex.

The quantitative analysis of our results seem, therefore, to confirm the validity of the immunohistochemical method to detect the c-Fos protein at visual pathway levels, appointing that the method is able to discriminate between different types of stimuli and, therefore, is suited as a valuable tool for the morphofunctional study in the CNS.

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