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Distribution of NADPH-diaphorase in the superior colliculus of *Cebus* monkeys, and co-localization with calcium-binding proteins

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Abstract

We examined the distribution of the enzyme dihydronicotinamide adenine dinucleotide phosphate-diaphorase (NADPH-d) in the superior colliculus (SC) of the New World monkey *Cebus apella*, and the co-localization of this enzyme with the calcium-binding proteins (CaBPs) calbindin-D28K, parvalbumin and calretinin. Despite the intensely labeled neuropil, rare NADPH-d-positive cells were observed in the stratum griseum superficiale (SGS). Most of the labeled cells in the SC were found in the intermediate layers, with a great number also in the deeper layers. This pattern is very similar to that described in the opossum (*Didelphis marsupialis*) and in the cat, and different from the pattern found in the rat, which shows labeled cells mainly in the SGS. Cells doubly stained for NADPH-d and CaBPs were observed throughout the SC, although in a small number. Of the NADPH-d-positive cells, 20.3% were doubly labeled for NADPH-d and parvalbumin, 10.2% revealed co-localization with calretinin, and 5.6% with calbindin. The low number of double-stained cells for NADPH-d and the CaBPs indicates that these molecules must participate in different functional circuits within the SC.

Keywords: Superior colliculus; NADPH-diaphorase; NO synthase; Calcium-binding proteins; Calbindin; Calretinin; Parvalbumin; Primates

1. Introduction

NADPH-diaphorase (NADPH-d) histochemistry stains selective cell populations, by the NADPH-dependent reduction of tetrazolium salts to visible formazans, producing a Golgi-like image of cells (Thomas and Pearse, 1961). Various studies have shown the localization of diaphorase within neurons, which also contain various neuroactive substances, including somatostatin, neuropeptide Y, substance P and acetylcholine, among others. This co-localization is highly selective to specific population of cells in different locations in the central nervous system (CNS) (Vincent et al., 1983a,b; Sandell, 1985). Other studies have demonstrated that in the nervous system NADPH-d is a nitric oxide (NO) synthase responsible for the formation of NO from arginine. NO is a neuronal messenger molecule produced in response to an increase in intracellular calcium ions that interact with guanylate cyclase and increase levels of cyclic guanosine monophosphate (cGMP) in target cells (Knowles et al., 1989; Hope et al., 1991; Dawson et al., 1991).

Previous works have mapped this neural messenger system in different regions of the brain of vertebrate species (Sandell, 1986; Volchan and Franca, 1994; Satoh et al., 1995; Costa et al., 1996; Franca et al., 1997; Vargas et al., 1998; Soares et al., 2001a). Some studies have demonstrated that the distribution pattern of NADPH-d-positive neurons in the superior colliculus (SC) varies among species. For example, in rats NADPH-d-positive neurons were observed mainly in the superficial layers, while in the deep layers, a few multipolar NADPH-d-positive cells occurred in groups continuous with dense aggregations of cells in the lateral periaqueductal gray matter (Leigh et al., 1990; Vincent and Kimura, 1992; Tenório et al., 1995). In cats few labeled cells were found within the stratum zonale (SZ). Cellular labeling was sparse within the stratum griseum

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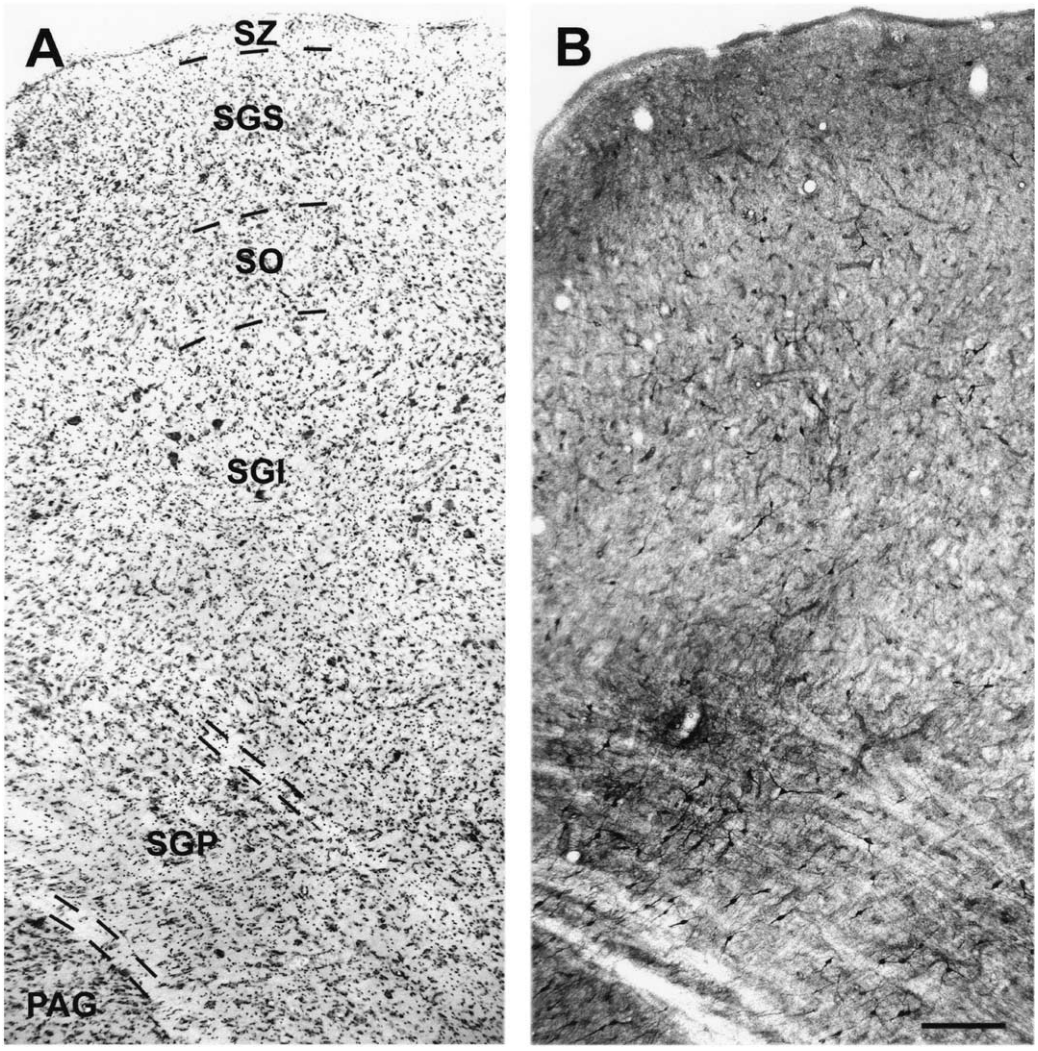

Fig. 1. Photomicrographs of coronal sections of the *Cebus* SC stained by Nissl method (A), and for NADPH-diaphorase (B). The dashed lines indicated the approximate limits of the SC layers. PAG, periaqueductal gray; SGI, stratum griseum intermediale; SGP, stratum griseum profundum; SGS, stratum griseum superficiale; SO, stratum opticum; SZ, stratum zonale. Scale bar = 200 μm.

superficiale (SGS) and the stratum opticum (SO). By contrast, fiber and cell labeling was more intense in the stratum griseum intermediale (SGI) and in the deep layers (Scheiner et al., 2000). Similarly, in opossums the number of NADPH-d stained cells was low in the SGS and SO, but high in the intermediate and deep layers (Giraldi-Guimarães et al., 1999). Finally, in humans, the SC showed a high density of small NOS-positive cells in the SGS, and medium-sized, lightly staining neurons in the SGI and stratum griseum profundum (SGP) (Egberongbe et al., 1994). As in the rat and cat, a lattice of NADPH-d-positive fibers was described in the intermediate gray layers of the SC of the monkey (Wallace, 1988). However, at this moment there is not a detailed description of the distribution of NADPH-d-positive neurons throughout the layers of the monkey SC. Here, we examine the distribution of the enzyme NADPH-diaphorase in the SC of the New World monkey *Cebus apella*.

In a previous study we described the distribution of the calcium binding proteins (CaBPs) calbindin-D28K, parvalbumin and calretinin in the SC of the *Cebus* monkey (Soares et al., 2001b). These CaBPs are implicated in the buffering and transport of calcium in the CNS, where they are present in distinct subpopulations of neurons (Jones and Hendry, 1989; Celio, 1990). Inasmuch as the activation of the NO synthase is related to calcium influx (Garthwaite et al., 1988; Bredt and Snyder, 1989), another goal of this study is to compare the distribution of the NADPH-d with that of the CaBPs using histochemical-immunocytochemical double-staining technique.

2. Materials and methods

Eight adult *C. apella* monkeys of both sexes weighing between 1.2 and 2.6 kg were used in this study. All experimental protocols were conducted following the NIH guidelines for animal research and they were approved by the committee for animal care and use of the Instituto de Biofísica Carlos Chagas Filho, UFRJ. The animals were deeply anesthetized with sodium pentobarbital (30 mg/kg) and perfused with normal saline followed by 2% paraformaldehyde in phosphate-buffered saline (PBS); 2% paraformaldehyde in PBS + 2.5% glycerol; PBS + 5% glycerol; and PBS + 10% glycerol. Serial 40 μm thick sections were obtained using a cryostat. Adjacent series were stained for cell bodies with cresyl violet, NADPH-diaphorase histochemistry, and for immunocytochemistry for calbindin, parvalbumin and calretinin. In four animals double staining of NADPH-d with each one of those three CaBPs were made in adjacent sections.

For NADPH-d histochemistry, free-floating sections were washed in PBS and reacted at 37 °C in a solution containing 1 mM β-NADPH, reduced form, 0.4 mM nitrobluetetrazolium (NBT), 10% dimethyl-sufoxide (DMSO) and 0.3% triton X-100 in PBS, pH 7.4, for 1–2 h. For double labeling, sections reacted for NADPH-d were washed in PBS and then immunoreacted as described below.

For immunocytochemical reactions, sections were incubated overnight with calbindin-D28K (1:2500), parvalbumin (1:3000) monoclonal antibody or calretinin (1:2000) polyclonal antibody (Swant–Swin Antibodies), in a solution containing 0.05% of bovine albumin and 0.3% of triton X-100 in 0.01 M PBS, pH 7.4. They were then incubated for an additional hour in biotinylated anti-mouse or anti-rabbit secondary antibody, and then processed by the avidin–biotin method with ABC kits (Vector) and 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.

Sections were examined under brightfield microscopy and photomicrographs were obtained using a Zeiss Axiocam attached to the microscope and processed using Adobe Photoshop version 5.0. The percentage of double-stained cells was calculated relative to the number of NADPH-d-positive cells counted in one coronal section at the middle level of the SC, in two animals where the blue-colored cells of the reaction for NADPH-d and the brown-colored cells of immunocytochemical reaction for CABPs were clearly distinguishable. At least 100 NADPH-d-positive cells were randomly selected throughout all layers of the SC in each section and analyzed for co-localization.

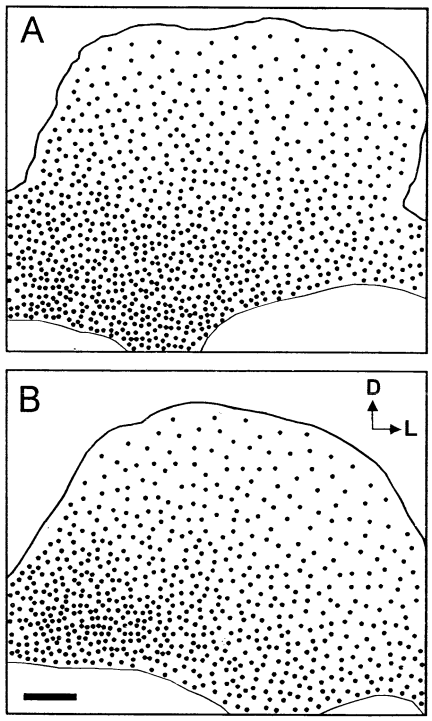

Fig. 2. Schematic representation of the distribution of labeled cells (dots) for NADPH-diaphorase in two coronal sections, in caudal (A) and rostral (B) portions of the SC. Scale bar = 500 μm.

3. Results

Fig. 1A shows a coronal section of the *Cebus* SC stained by the Nissl method, where approximate limits of the layers were delineated. The pattern revealed by the NADPH-d histochemistry in an adjacent section of the SC is shown in the Fig. 1B. Fig. 2 shows a schematic representation of the distribution of labeled cells throughout the layers of the SC at caudal (A) and rostral (B) levels. We observe a greater density of labeled cells in caudal than in rostral sections. In addition, there is a greater concentration of cells in the medial portions of the deeper layers throughout the rostro-caudal extent. Rare multipolar and bipolar NADPH-d-positive cells were found in the SGS, which, however, depicts a heavy stained neuropil (Fig. 3A). These cells were mainly small (10–20 μm) in size with large dendrites pointing toward the SZ (Fig. 4A and B). In the SO we also observe a small number of small and medium-sized (20–30 μm) multipolar, horizontal and vertical bipolar cells (Fig. 3B, Fig. 4C and D).

Numerous small to medium-sized neurons, of all morphologies, were found in the SGI (Fig. 3C, Fig. 4E and F). Finally, the SGP is the region that presents the greatest density of labeled neurons, mostly medium multipolar and vertical bipolar cells (Fig. 3D, Fig. 4G and H). A net of high activity NADPH-d fibers was found within the intermediate and mainly in the deeper layers of the SC (Fig. 5). These fibers were denser at

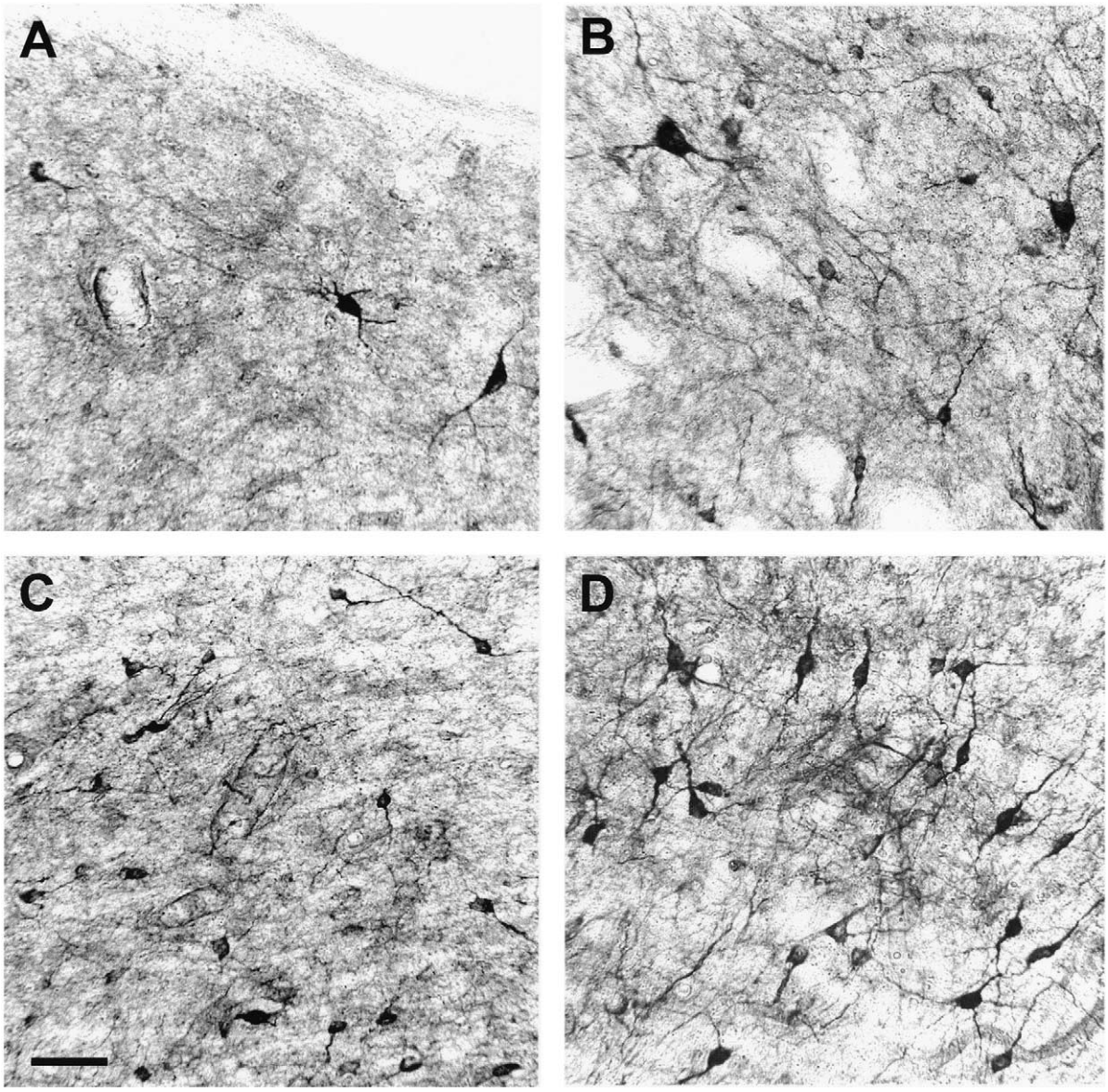

Fig. 3. Photomicrographs of a coronal section through the SC stained for NADPH-diaphorase, showing the pattern of distribution and morphology of labeled cells in the different SC layers. (A) SZ and SGS; (B) SO; (C) SGI and (D) SGP. Scale bar = 50 μm.

caudal than rostral levels; however, they were not organized in patches as described in cat and mouse (Wallace, 1986, 1988).

A small number of cells doubly stained for NADPH-d histochemistry and CaBPs immunocytochemistry was found in the SC of the *Cebus*. In sections that were doubly stained, NADPH-d-positive cells appear in blue and CaBPs positive cells appear in brown. Double-labeled cells are identified by the presence of both, the brown product of the immunocytochemistry, that in general appears in the center of the cell body, surrounded by the blue product of the NADPH-d histochemistry which can also be observed in the beginning of the processes (Fig. 6).

Calbindin-D28K-positive cells were more abundant in the superficial layers, where NADPH-d-positive cells appear in small numbers. We observed a very small percentage (5.6%; 13/231) of neurons doubly stained for NADPH-d and calbindin-D28K. These neurons were usually small and medium-sized cells located in the SO and SGP (Fig. 6A and B). Parvalbumin-positive cells were evenly distributed throughout all layers. They were observed beside NADPH-d-positive cells, mainly in the deeper layers. The highest percentage of double-stained cells (20.3%; 48/236) was for NADPH-d and parvalbumin. These cells were mainly medium-sized multi- or bipolar neurons located in the SGI (Fig. 6C and D). Finally, double staining for NADPH-d and calretinin

was detected in a small percentage of neurons (10.2%; 24/235), mainly bipolar or multipolar in shape, and located in intermediary and deep layers (Fig. 6E and F).

4. Discussion

The present study reveals a small number of NADPH-d-positive cells in the SGS, which shows,

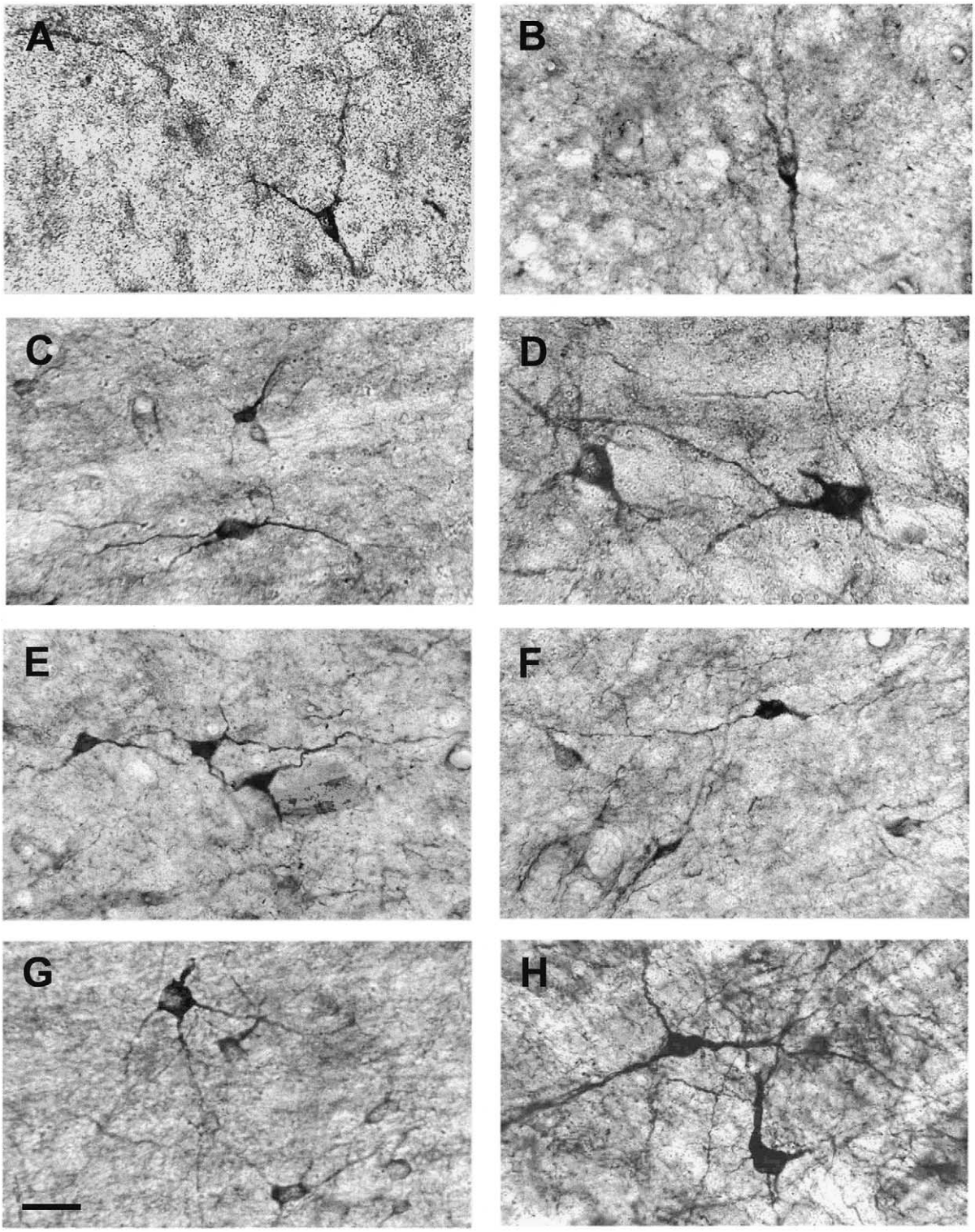

Fig. 4. Higher magnification photomicrographs depicting NADPH-d-positive cell types in various layers of the SC. (A, B) SGS; (C, D) SO; (E, F) SGI and (G, H) SGP. Scale bar = 30 μm.

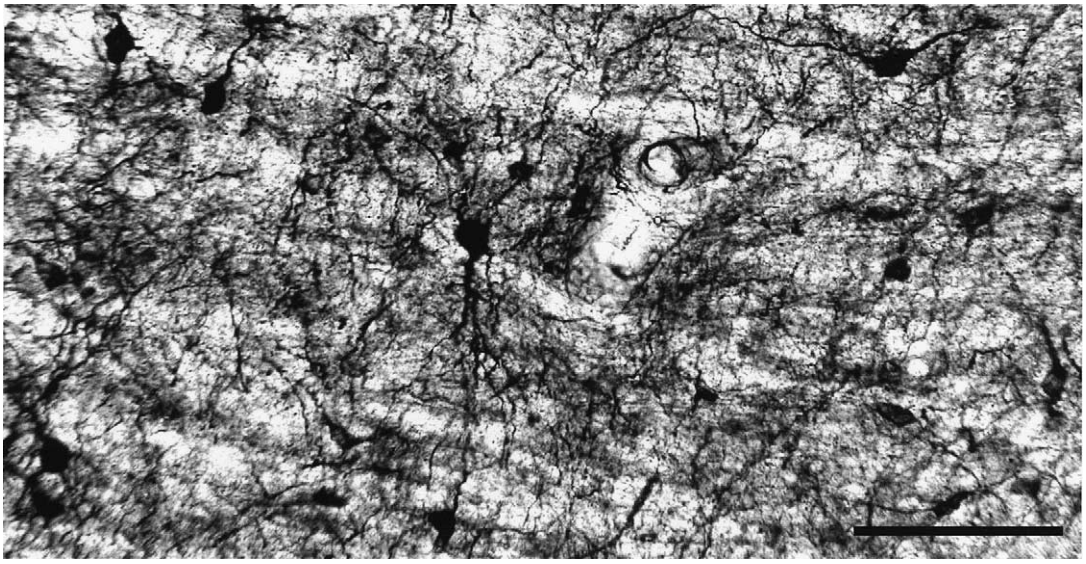

Fig. 5. Photomicrograph of the deep layers of the SC showing a net of labeled fibers for NADPH-d. Scale bar = 100 μm.

however, an intensely labeled neuropil. Most of the labeled cells were found in the intermediate and deep layers, with a greater density in the medial portion of the SC. This pattern is very similar to that described for both the opossum *Didelphis marsupialis* (Giraldi-Guimarães et al., 1999) and the cat (Scheiner et al., 2000), however, differs from the results of the rat: labeled cells are located mainly in the superficial layers (Gonzáles-Hernández et al., 1992; Tenório et al., 1995, 1996). These results suggest inter-species differences concerning the connections and functions of cells of the various layers of the SC.

Wallace (1986, 1988) studied the occurrence of patches of high NADPH-d activity forming a lattice within the SGI in various species. Those lattices were prominent in the mouse and cat, occurred more faintly in the monkey (*Macaca fascicularis*) and were not found in human. In *Cebus* monkeys we observed a net of fibers with high enzyme activity in the SGI and mainly in the SGP, but they did not form patches as the ones described in the mouse and cat.

The mammalian SC has a high concentration of NO synthase (Wallace, 1986, 1988; Vincent and Kimura, 1992), but very little is known about the function of NO in this structure. The study of Wallace (1986) in the mouse and rat showed that the interruption of the afferent pathways from the brainstem abolishes the lattices of high diaphorase activity in the intermediate layers of the SC, without altering the enzyme activity in the superficial layers. The presence of NADPH-d amorphous reaction product in the superficial layers of the SC also is not abolished by eye enucleation (Tenório et al., 1998), and even increases after optic nerve transection (Yan et al., 1995). A possible source for this staining could be projections from cells intrinsic to the deeper portions of the colliculus that contain high diaphorase activity.

Various works have demonstrated the chemical diversity of NO-synthesizing neurons (Vincent et al., 1983a,b; Sandell, 1985; Wong-Riley et al., 1998). Co-localization of NADPH-d and the CaBPs has been studied in various regions of the brain. The coexistence of NADPH-d with calbindin-D28K was studied in the magnocellular secretory nuclei of the rat hypothalamus, where a selective pattern of distribution and only a partial coexistence was found, suggesting that they are involved in specific physiological functions shared by restricted neuronal cell populations (Alonso et al., 1992).

In the monkey cerebral cortex, co-localization was found between NADPH-d and calbindin-D28K in all nonpyramidal small cells (type II) and in 4% of nonpyramidal large (type I) NADPH-d neurons, but not between NADPH-d and parvalbumin (Yan et al., 1996). The rat striatal NOS-positive neurons lack parvalbumin and calretinin although some of these cells contain calbindin-D28K (Kita et al., 1990; Bennett and Bolam, 1993a,b). In the SC of rats, NOS-positive neurons do not contain calbindin-D28K, and receive glutamatergic inputs; moreover, some of these neurons express GABA (20%) or parvalbumin (15%) (Soares-Mota et al., 2001). However, Gonzales-Soriano et al. (2002) demonstrated a lack of colocalization of parvalbumin and NADPH-d, and the presence of double-labeled neurons for calbindin-D28K and NADPH-d (10%) in the SC of rabbits. This co-localization of NADPH-d and calbindin-D28K or parvalbumin in some cells could imply that these CaBPs may participate in the regulation of the synthesis of the NO by changing the concentration of calcium in the cytoplasm of the cell (Garthwaite et al., 1988; Bredt and Snyder, 1989).

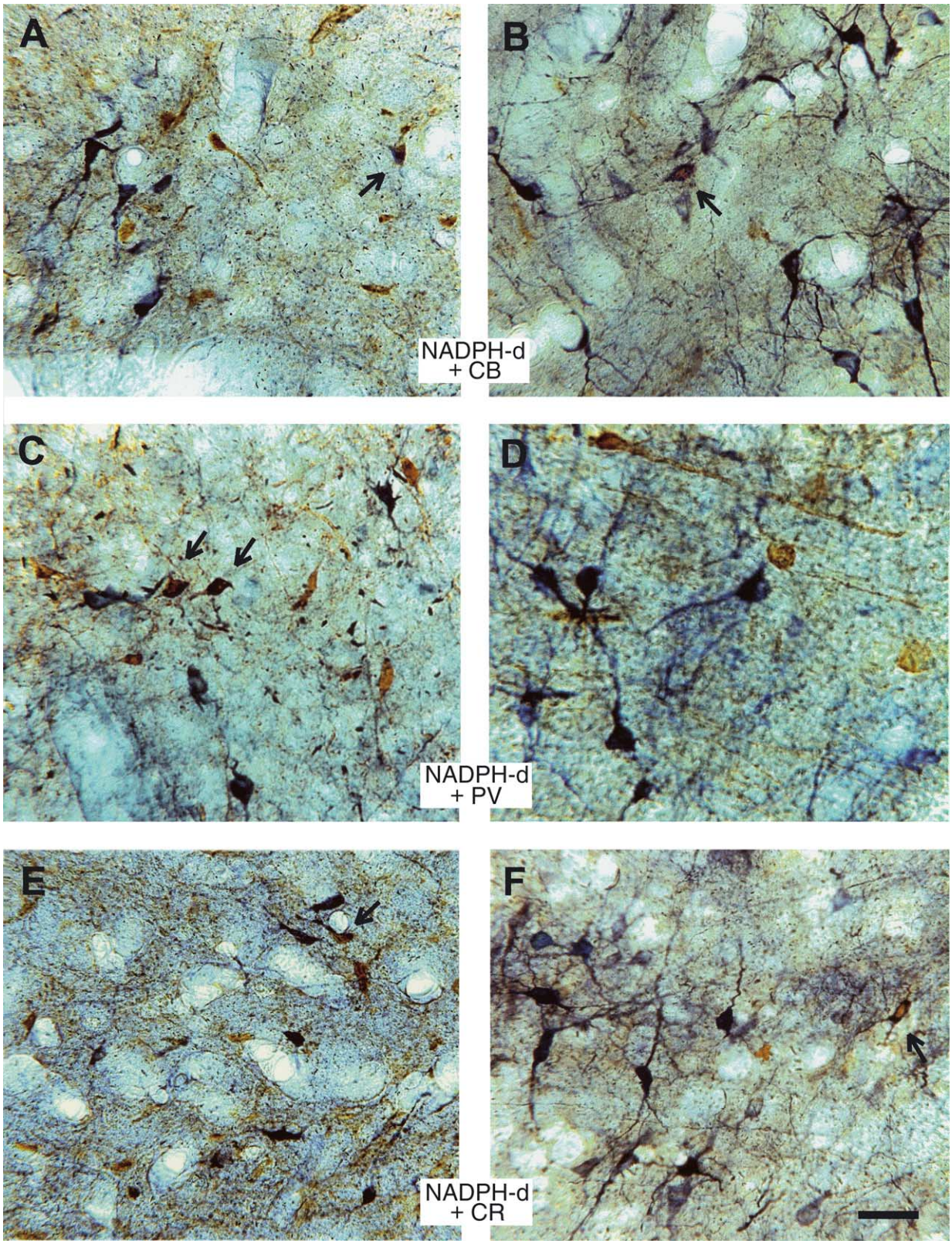

Fig. 6. Photomicrographs of double-stained sections for NADPH-d and CaBPs: calbindin-D28K (A, B), parvalbumin (C, D), calretinin (E, F). NADPH-d-positive cells appear in blue and cells labeled by immunocytochemistry in brown in SO (A), SGI (C, E) and SGP (B, D, F) layers. Arrows point to double-labeled cells. Scale bar = 30 μm.

However, most of the NADPH-d-positive neurons may use other CaBPs, such as calmodulin, or may have a different mechanism for the control of the calcium concentration.

In this study we have shown that only a small percentage of NADPH-d-positive neurons in the SC of *Cebus* monkeys also express parvalbumin, calbindin-D28K or calretinin. This small degree of co-localization of NADPH-d and CaBPs in the SC of *Cebus* monkeys suggests that these proteins may be active as independent elements in the primate SC.

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References

Alonso, J.R., Sánchez, F., Arévalo, R., Carretero, J., Aijón, J., Vásquez, R., 1992. CABP D-28k and NADPH-diaphorase coexistence in the magnocellular neurosecretory nuclei. NeuroReport 3, 249–252.

Bennett, B.D., Bolam, J.P., 1993a. Characterization of calretinin-immunoreactive structures in the striatum of the rat. Brain Res. 609, 137–148.

Bennett, B.D., Bolam, J.P., 1993b. Two populations of calbindin D28k-immunoreactive neurons in the striatum of the rat. Brain Res. 610, 305–310.

Bredt, D.S., Snyder, S.H., 1989. Nitric oxide mediates glutamate-linked enhancement of cGMP levels in the cerebellum. Proc. Natl. Acad. Sci. USA 86, 9030–9033.

Celio, M.R., 1990. Calbindin D-28k and parvalbumin in the rat nervous system. Neuroscience 35, 375–475.

Costa, E.T., Nascimento, J.L.M., Picanço-Diniz, C.W., Quaresma, J.A.S., Silva-Filho, M., 1996. Histochemical characterization of NADPH-diaphorase activity in area 17 of diurnal and nocturnal primates and rodents. Braz. J. Med. Res. 29, 1355–1362.

Dawson, T.M., Bredt, D.S., Fotuhi, M., Hwang, P.M., Snyder, S.H., 1991. Nitric oxide synthase and neuronal NADPH diaphorase are identical in brain and peripheral tissues. Proc. Natl. Acad. Sci. USA 88, 7797–7801.

Egberongbe, Y.I., Gentleman, S.M., Falkai, P., Bogerts, B., Polak, J.M., Roberts, G.W., 1994. The distribution of nitric oxide synthase immunoreactivity in the human brain. Neuroscience 59, 561–578.

Franca, J.G., do-Nascimento, J.L., Picanço-Diniz, C.W., Quaresma, J.A., Silva, A.L., 1997. NADPH-diaphorase activity in area 17 of the squirrel monkey visual cortex: neuropil pattern, cell morphology and laminar distribution. Braz. J. Med. Biol. Res. 30, 1093–1105.

Garthwaite, J., Charles, S.L., Chess-Williams, R., 1988. Endothelium-derived relaxing factor release on activation of NMDA receptors suggests role as intercellular messenger in the brain. Nature 336, 385–388.

Giraldi-Guimarães, A., Tenório, F., Bruning, G., Mayer, B., Mendez-Otero, R., Cavalcante, L.A., 1999. Nitric oxide synthase expression in the opossum superior colliculus: a histochemical, immunohistochemical and biochemical study. Brain Behav. Evol. 54, 303–313.

Gonzáles-Hernández, T., Conde-Séndin, M., Meyer, G., 1992. Laminar distribution and morphology of NADPH-diaphorase containing neurons in the superior colliculus and underlying periaqueductal gray of the rat. Anat. Embryol. 186, 245–250 (Berlin).

Gonzales-Soriano, J., Contreras-Rodrigues, J., Martinez-Sainz, P., Martin-Palacios, S., Marin-Garcia, P., Rodriguez-Veiga, E., 2002. NADPH- diaphorase distribution in the rabbit superior colliculus and co-localization with calcium-binding proteins. J. Anat. 200, 297–308.

Hope, B.T., Michael, G.J., Knigge, K.M., Vincent, S.R., 1991. Neuronal NADPH diaphorase is a nitric oxide synthase. Proc. Natl. Acad. Sci. USA 88, 2811–2814.

Jones, E.G., Hendry, S.H.C., 1989. Differential calcium binding protein immunoreactivity distinguishes classes of relay neurons in monkey thalamic nuclei. Eur. J. Neurosci. 1, 222–246.

Kita, H., Kosaka, T., Heizmann, C.W., 1990. Parvalbumin-immunoreactive neurons in the rat neostriatum: a light and electron microscopic study. Brain Res. 536, 1–15.

Knowles, R.G., Palacios, M., Palmer, R.M.J., Moncada, S., 1989. Formation of nitric oxide from L-arginine in the central nervous system: a transduction mechanism for stimulation of the soluble guanylate cyclase. Proc. Natl. Acad. Sci. USA 86, 5159–5162.

Leigh, P.N., Connick, J.H., Stone, T.W., 1990. Distribution of NADPH diaphorase positive cells in the rat brain. Comp. Biochem. Physiol. 97C, 259–264.

Sandell, J.H., 1985. NADPH diaphorase cells in the mammalian inner retina. J. Comp. Neurol. 238, 466–472.

Sandell, J.H., 1986. NADPH diaphorase histochemistry in the macaque striate cortex. J. Comp. Neurol. 251, 388–397.

Satoh, K., Aral, R., Ikemoto, K., Narita, M., Nagai, T., Ohshima, H., Kitahama, K., 1995. Distribution of nitric oxide synthase in the central nervous system of *Macaca fuscata*: subcortical regions. Neuroscience 66, 685–696.

Scheiner, C., Arceneaux, R., Guido, W., Kratz, K., Mize, R., 2000. Nitric oxide synthase distribution in cat superior colliculus and co-localization with choline acetyltransferase. J. Chem. Neuroanat. 18, 147–159.

Soares, J.G.M., Gattass, R., Souza, A.P.B., Rosa, M.G.P., Fiorani, M., Jr, Brandão, B.L., 2001a. Connectional and neurochemical subdivisions of the pulvinar in *Cebus* monkeys. Visual Neurosci. 18, 25–41.

Soares, J.G.M., Botelho, E.P., Gattass, R., 2001b. Distribution of calbindin, parvalbumin and calretinin in the superior colliculus and lateral geniculate nucleus of *Cebus apella* monkeys. J. Chem. Neuroanat. 22, 139–146.

Soares-Mota, M., Henze, I., Mendez-Otero, R., 2001. Nitric oxide synthase-positive neurons in the rat superior colliculus: colocalization of NOS with NMDAR1 glutamate receptor, GABA, and parvalbumin. J. Neurosci. Res. 64, 501–507.

Tenório, F., Giraldi-Guimarães, A., Mendez-Otero, R., 1995. Developmental changes of nitric oxide synthase in the rat superior colliculus. J. Neurosci. Res. 42, 633–637.

Tenório, F., Giraldi-Guimarães, A., Mendez-Otero, R., 1996. Morphology of NADPH-diaphorase-positive cells in the retinoceptive layers of the developing rat superior colliculus. Int. J. Dev. Neurosci. 14, 1–10.

Tenório, F., Giraldi-Guimarães, A., Santos, H.R., Cintra, W.M., Mendez-Otero, R., 1998. Eye enucleation alters intracellular distribution of NO synthase in the superior colliculus. NeuroReport 9, 145–148.

Thomas, E., Pearse, A.G.E., 1961. The fine localization of dehydrogenases in the nervous system. Histochemical 2, 266–282.

Vargas, C.D., Sousa, A.O., Bittencourt, F.L., Santos, C.M., Pereira, A., Jr, Bernardes, R.F., Rocha-Miranda, C., Volchan, E., 1998. Cytochrome oxidase and NADPH-diaphorase on the afferent relay branch of the optokinetic reflex in the opossum. J. Comp. Neurol. 398, 206–224.

Vincent, S.R., Kimura, H., 1992. Histochemical mapping of nitric oxide synthase in the rat brain. Neuroscience 46, 755–784.

Vincent, S.R., Johanson, O., Hokfelt, T., Skirboll, L., Elde, R.P., Terenius, L., Kimmel, J., Goldstein, M., 1983a. NADPH-diaphorase: a selective histochemical marker for striatal neurons containing both somatostatin- and avian pancreatic polypeptide (APP)-like immunoreactivities. J. Comp. Neurol. 217, 252–263.

Vincent, S.R., Satoh, K., Armstrong, D.M., Fibiger, H.C., 1983b. NADPH-diaphorase: a selective histochemical marker for the cholinergic neurons of the pontine reticular formation. Neurosci. Lett. 43, 31–36.

Volchan, E., Franca, J.G., 1994. Distribution of NADPH-diaphorase-positive neurons in the opossum neocortex. Braz. J. Med. Biol. Res. 27, 2431–2435.

Wallace, M.N., 1986. Spatial relationship of NADPH-diaphorase and acetylcholinesterase lattices in the rat and mouse superior colliculus. Neuroscience 19, 381–391.

Wallace, M.N., 1988. Lattices of high histochemical activity occurred in the human, monkey and cat superior colliculus. Neuroscience 25, 569–583.

Wong-Riley, M.T.T., Hung, Z., Liebl, W., Nie, F., Xu, H., Zhang, C., 1998. Neurochemical organization of the macaque retina: effect of TTX on levels and gene expression of cytochrome oxidase and nitric oxide synthase and on the immunoreactivity of Na+ K-ATPase and NMDA receptor subunit I. Vis. Res. 38, 1455–1477.

Yan, X.X., Garey, L.J., Liang, Y., Von Bussmann, K.A., Jen, L.S., 1995. Increased expression of NADPH-diaphorase in visual centres after unilateral optic nerve transection in the rat. J. Brain Res. 36, 485–488.

Yan, X.X., Jen, L.S., Garey, L.J., 1996. NADPH-diaphorase-positive neurons in primate cerebral cortex colocalize with GABA and calcium-binding proteins. Cerebral Cortex 6, 524–529.