Eur J Anat, 7 (3): 121-125 (2003)

# Differential density of blood vessels in the mesencephalon of Macaque brain stem

**C. Barcia and M.T. Herrero**

*Experimental Neurology and Neurosurgery, Department of Human Anatomy and Psychobiology, School of Medicine, Campus Espinardo, 30071 Murcia, Spain*

## Abstract

The blood vessels (BV) of the brain stem show different patterns of development, and nutrient requeriments, metabolism and the activity of the different nuclei of the mesencephalon differ from one nucleus to another. The density of BV indirectly explains the blood flow intensity and physiological activity of the nuclei, and may explain the varying degrees of vulnerability of neurons to pathologies. To ascertain the different vascular densities of the mesencephalic nuclei, in the present work we used stereological methods to measure BV density in the Ventral Tegmental Area (VTA), the Substantia Nigra *pars compacta* (SNpc), the A8 Catecholaminergic Cell group (A8), the Periaqueductal Grey Matter (PAG) and the Locus Coeruleus (LC) of two intact macaques. The results pointed to a high density of BV in the LC; a low density in the SNpc, and an intermediate density in the VTA, A8 and PAG. These results confirm the high blood metabolism of the LC and suggest that the vulnerability of SNpc neurons may be related to the observed low density of BV.

**Key Words**: Blood vessels – Mesencephalon – Acetyl cholinesterase – Stereology – Macaque

## Introduction

BV development and formation in the brain may occur by means of a number of different processes (Risau, 1997). For instance, monocytes, macrophages, platelets, mast cells and other leukocytes are involved in mechanisms that release several angiogenic factors that may stimulate neovascularization (Pinedo et al., 1998; Seljelid et al., 1999). There is a close relationship between neurons and BV, while differences in the density of vascularization may explain neuronal activity and metabolism. In this work, we measured the BV density of several nuclei of the brain stem, analysing the Periaqueductal Grey Matter (PAG), the Catecholaminergic Cell group of the Periretrorubral area (A8), the Substantia Nigra *pars compacta* (SNpc), the Ventral Tegmental Area (VTA) and the Locus Coeruleus (LC).

## Materials and Methods

*Animals, tissue preparation and staining*

Two adult male cynomolgus monkeys were used for this study. The monkeys were sacrificed by pentobarbital injection after ketamine anaesthesia. The brains were then removed and divided along the midline into two blocks of tissue. One block from each animal was fixed for 3 days in 4% paraformaldehyde dissolved in 0.1 M phosphate buffer. The mesencephalon was cut into 40-µm-thick serial sections (Microm®, HM400). Series of sections in both blocks regularly spaced at intervals of 1440 µm were stained with thionine in order to analyse the cellular morphology; they were analysed by acetyl cholinesterase histochemistry, according to Graybiel and Ragsdale (1978) to visualise the outlines of the anatomical regions (Figure 1A),

Submitted: September 24, 2003
Accepted: November 14, 2003

Correspondence to:
Dr. Carlos Barcia. Experimental Neurology and Neurosurgery, Department of Human Anatomy and Psychobiology, School of Medicine, Campus Espinardo, 30071 Murcia, Spain
E-mail: barcia@um.es; mtherrer@um.es

and by tyrosine hydroxylase immunohistochemistry (Incstar) to determine the dopaminergic regions (Figure 1B). Adjacent sections were stained by reticulin impregnation in order to visualise BV (Naoumenko and Feigin, 1974). This histochemical reaction, which impregnates reticulin fibres, allows the microcirculatory network to be visualised (Figure 2). Sections from two animals were stained simultaneously and under the same experimental conditions.

*Quantification method*

The Substantia Nigra *pars compacta* (SNpc), the Ventral Tegmental Area (VTA), the Periaqueductal Grey Matter (PAG), the Catecholaminergic cell group A8 (A8) and the Locus Coeruleus (LC) were delineated in all sections using acetyl cholinesterase histochemistry (Graybiel and Ragsdale, 1978) and tyrosine hydroxylase immunohistochemistry on two sets of adjacent sections (Figure 1). BV were then quantified using stereological methods and a computer-assisted image analysis system (MIP 4 ADVANCED; Consulting de Imagen Digital S.L. Madrid, Spain) with a Leitz laborlux D microscope connected to a DXC-101P camera (SONY) through a Zeiss zoom set at 12.5x and a 0.1x adapter. A 20x objective was used to observe microvessels.

The number of blood vessels was measured in 260-μm squares, 200 μm (x) and 200 μm (y) apart, using systematic fields covering the whole surface area of the analysed regions. Several stereological estimation techniques were used: i) BV were counted only when they cut the upper and left limits of the square and were classified within four categories according to the number of ramifications visible in the entire field of view (Cat1: BV without ramifications; Cat2: BV with 1 ramification; Cat3: BV with 2 ramifications; and Cat4: BV with 3 or more ramifications). Ramifications were counted only when they appeared in an area of the disector. The number of BV in each category (Cat1, Cat2, Cat3, Cat4) were summed in each field and the mean number of BV branches was estimated using the following formula [(Cat1)+(Cat2)x2+(Cat3)x3+(Cat4)x4]. All parameters were expressed as a density of BV per $mm^3$ for each anatomical region and each animal.

The volume occupied by the blood vessels was measured in the same squares using the computerised image analysis system. First, segmentation of blood vessels was performed on the basis of a grey level threshold that was identical for all sections, and the surface area occupied by the BV was determined for each field.

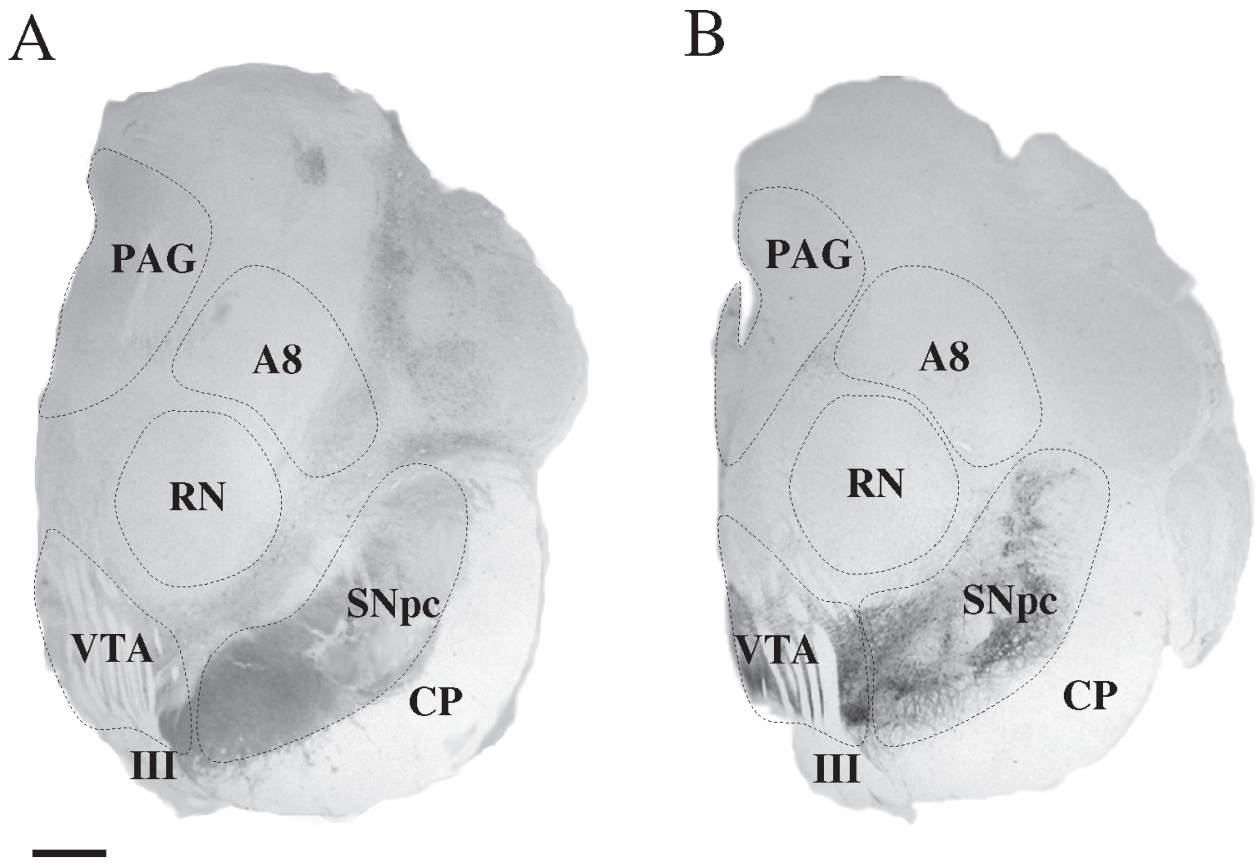


**Fig. 1.-** Sections of hemimesencephalon of cynomolgus monkey (*Macaca fascicularis*) at the level of the III cranial nerve stained by Acetylcholinesterase (**A**) and TH (**B**). Note the outlines of the mesencephalic regions. PAG: Periacueductal Grey Matter; A8: Periretrorubral catecholaminergic cell group; RN: Red nucleus; VTA: Ventral Tegmental Area; SNpc: Substantia Nigra *pars compacta*; III: 3rd Cranial nerve; CP: Cerebral peduncle. Scale bar: 1mm.

Material also stained by reticulin that did not belong to BV was excluded on the basis of size (Figure 3). Then, the volume of the blood vessels was estimated according to their diameter, considering them to be cylindrical in shape. The results were expressed as the mean volume occupied by the BV per $mm^3$ in each region for each animal.

*Statistical analysis*

Data are represented as the mean ± SEM. Statistical analysis was performed using a one-way ANOVA test with the anatomical regions and animal treatment as parameters (SigmaStat. Statistical Software, Copyright© 1992-1997). The null hypothesis was rejected for an a risk equal to 5%.

## Results

BV were clearly labelled in the mesencephalic nucleus and LC. Pigmented neurons of the SNpc and LC were labelled and were observed to be closely related to blood vessels, in accordance with a previous study [Felten and Crutcher, 1979]. The nuclei of glial cells were also labelled and were distributed throughout the mesencephalon (Figures 2 and 4).

Quantification of the BV revealed that the SNpc shows low levels of BV density, in accordance with previous results (Majewska-Michalska, 1997). The highest degree of vascularization was found in the LC (Figure 4). Significant differences were found in the LC and SNpc with respect to the other nuclei measured, and BV density in the PAG was significantly higher than in A8 (Figures 4 and 5). Similar findings were obtained with two different methods (high degree of BV in LC and low in SNpc). PAG, A8 and VTA showed similar results (Figures 4 and 5).

## Discussion

The results show that the LC has a high degree of vascularization, as previously described (Felten and Crutcher, 1979; Raichle et al., 1975). This feature suggests that the metabolism of this nora-

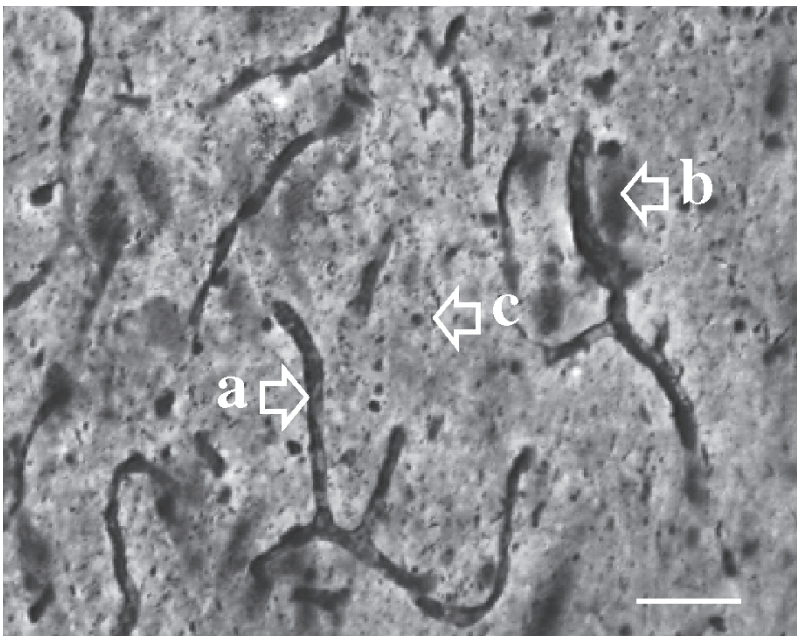


**Fig. 2.-** Reticulin staining performed in the mesencephalon of cynomolgus monkey (*Macaca fascicularis*). Note the BV (arrow "a"), neurons (arrow "b") and glial cell nuclei (arrow "c"). Scale bar: 40 µm.

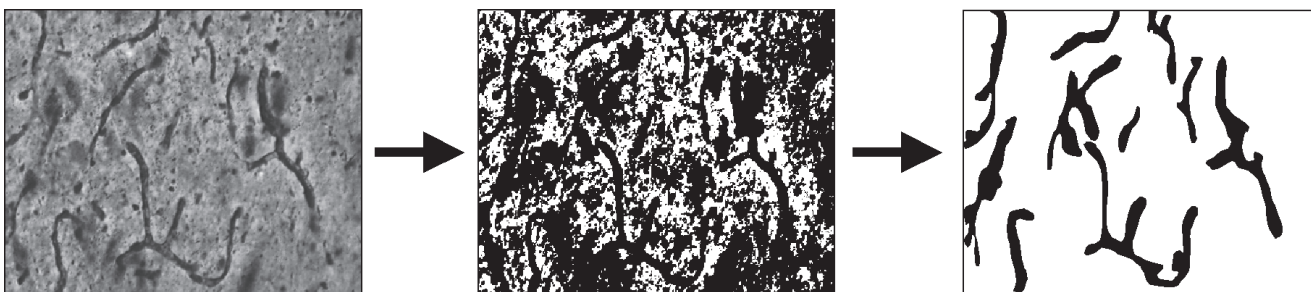

**Fig. 3.-** Example of segmentation performed in the photomicrographs in order to estimate the area occupied by BV. The computer separates objects on the basis of a grey level threshold, and the surface area occupied by the BV was determined for each field. Material also stained by reticulin that did not belong to BV was excluded.

drenergic nucleus requires high concentrations of oxygen and/or nutrients.

The low density of blood vessels in the SNpc (in contrast with the VTA) suggests that the vulnerability of dopaminergic neurons to degeneration could be related to this. In fact, this region is the most vulnerable to cell death in Parkinson's disease and in MPTP-treated monkeys. Along these lines, changes in BV density have been described in parkinsonian patients (Faucheux et al., 1999) and in MPTP-treated monkeys (Barcia et al., 2002). Both these findings suggest that BV may play a role in neuroprotection or in the rescue of surviving neurons. However, increased vascularization is a hallmark of the inflammatory response and it has been proposed that inflammation may induce injury in neurons (Barcia et al., 2004).

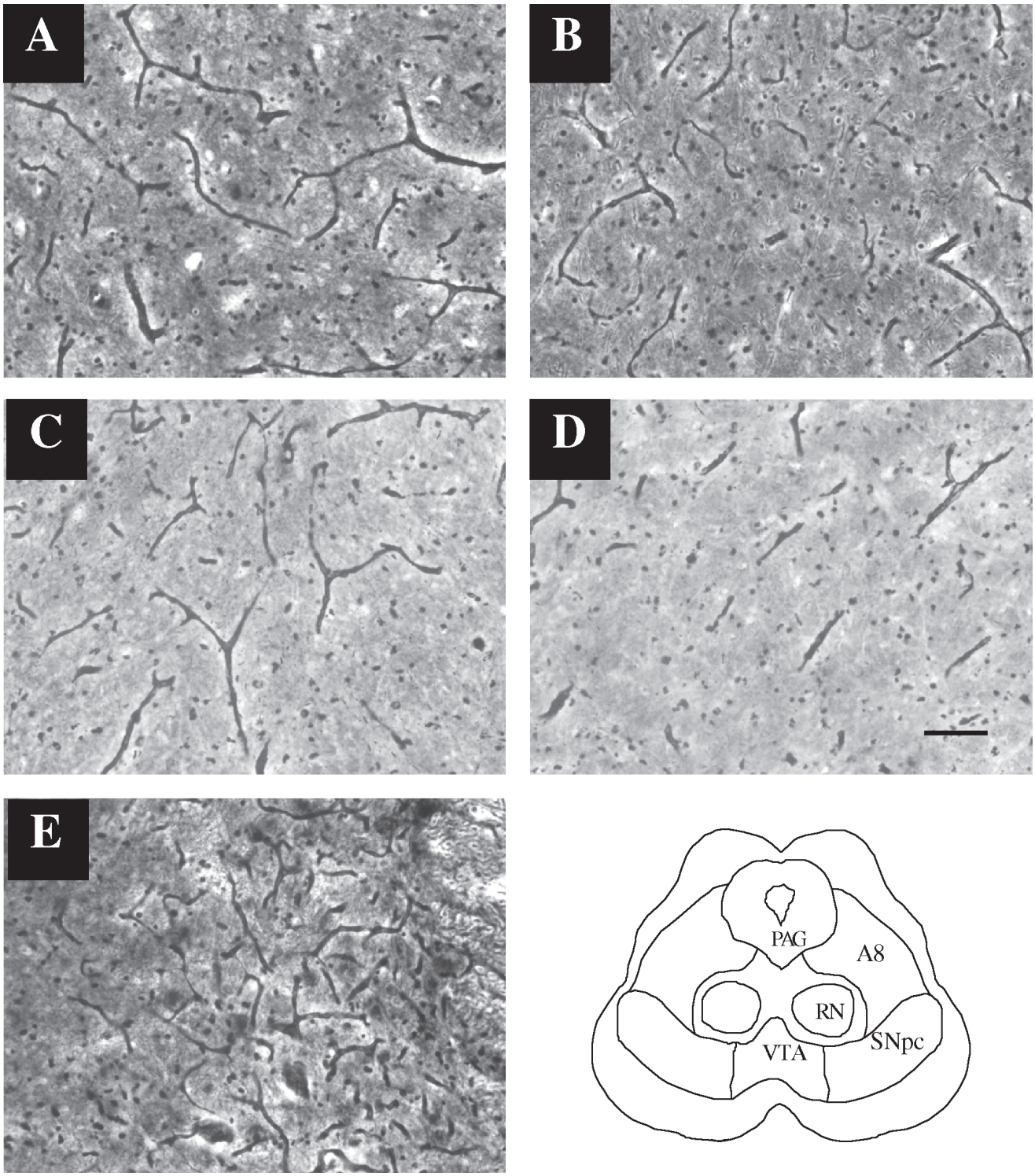


**Fig. 4.-** Photomicrographs of BV in the mesencephalon of cynomolgus monkey (*Macaca fascicularis*). Note the differential BV density. The SNpc (D) shows a low density of BV, while the LC displays a high degree of vascularization (E). **A**: PAG; **B**: A8; **C**: VTA; **D**: SNpc and **E**: LC. Scale bar: 50 μm.

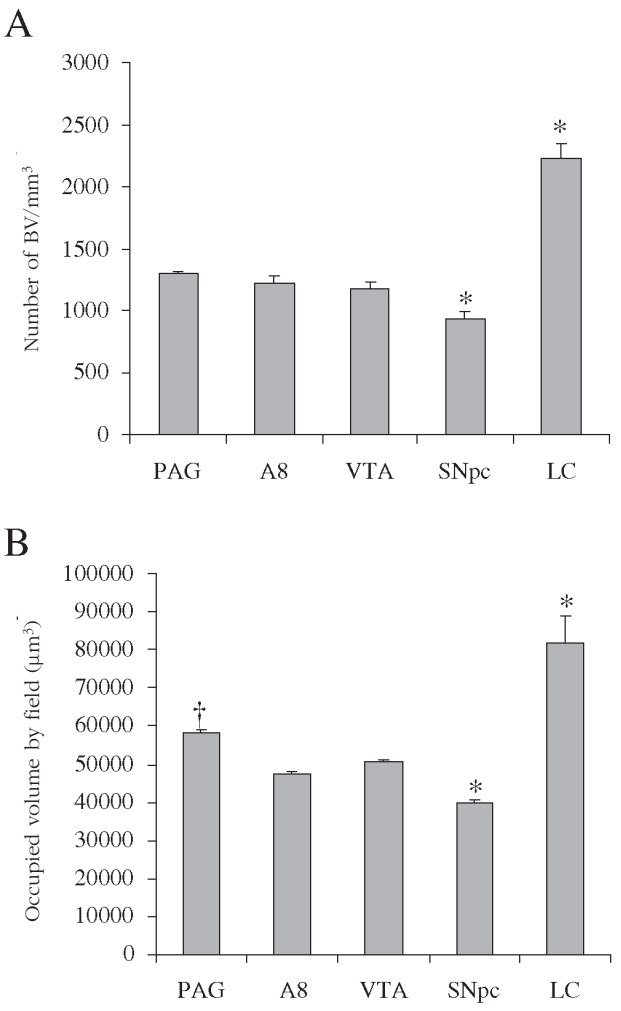


**Fig. 5.-** Estimation of number (**A**) and the volume (**B**) occupied by BV in mesencephalic regions. Note the low levels of vascularization of the SNpc and the high levels of the LC. * $p<0.05$ with respect to the other nuclei. † $p<0.05$ with respect to A8.

## ACKNOWLEDGEMENTS

This study was supported by Spanish grants from SAF 2001-0479 and FIS PI02/1335.

## REFERENCES

BARCIA C, SÁNCHEZ-BAHILLO A, BAUTISTA-HERNÁNDEZ V, NAVARRO-RUIZ J, FERNÁNDEZ-VILLALBA E, FAUCHEUX B, HIRSCH EC and HERRERO MT (2002). Blood vessels and neurodegeneration in Parkinson's disease. Study in chronic MPTP-treated monkeys. In: Nicholson & Faull (eds). *The Basal Ganglia VII, Kluwer Academic/Plenum Publishers, New York,* 52: 341-347.

BARCIA C, EMBORG ME, HIRSCH EC and HERRERO MT (2004). Blood vessels and parkinsonism. *Frontiers in Bioscience*, 9: 277-282.

FAUCHEUX BA, BONNET AM, AGID Y and HIRSCH EC (1999). Blood vessels change in the mesencephalon of patients with Parkinson's disease. *Lancet,* 353: 981-982.

FELTEN DL and CRUTCHER KA (1979). Neuronal-vascular relationships in the raphe nuclei, locus coeruleus, and Substantia Nigra in primates. *Am J Anat*, 155: 467-481.

GRAYBIEL AM and RAGSDALE CW Jr (1978). Histochemically distinct compartments in the striatum of human, monkeys, and cat demonstrated by acetylthiocholinesterase staining. *Proc Natl Acad Sci USA,* 75: 5723-5726.

MAJEWSKA-MICHALSKA E (1997). Vascularization of the brain in guinea pig. IV. Angioarchitectonics of the tectum, tegmentum, and crura mescencephali. *Folia Morphol (Warsz)*, 56: 47-53.

NAOUMENKO J and FEIGIN I (1974). A simple silver solution for staining reticulin. *Stain Technol,* 49: 153-155.

PINEDO HM, VERHEUL HM, D'AMATO RJ and FOLKMAN J (1998). Involvement of platelets in tumour angiogenesis? *Lancet*, 352: 1775-1777.

RAICHLE ME, HARTMAN BK, EICHLING JO and SHARPE LG (1975). Central noradrenergic regulation of cerebral blood flow and vascular permeability. *Proc Natl Acad Sci USA,* 72: 3726-3730.

RISAU W (1997). Mechanisms of angiogenesis. *Nature,* 386: 671-674.

SELJELID R, JOZEFOWSKI S and SVEINBJORNSSON B (1999). Tumor stroma. *Anticancer Res*, 19: 4809-4822.

STERIO DC (1984). The unbiased estimation of number and sizes of arbitrary particles using the disector. *J Microsc,* 134: 127-136.