

Neurochemistry study of spinal cord in non-human primate (*Sapajus* spp.)

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Abstract

The spinal cord is involved in local, ascending and descending neural pathways. Few studies analyzed the distribution of neuromediators in the laminae of non-human primates along all segments. The present study described the classic neuromediators in the spinal cord of the non-human primate *Sapajus* spp. through histochemical and immunohistochemical methods. Nicotinamide adenine dinucleotide hydrogen phosphate-diaphorase (NADPH-d) method showed neuronal somata in the intermediolateral column (IML), central cervical nucleus (CCN), laminae I, II, III, IV, V, VI, VII, VIII and X, besides dense presence of nerve fibers in laminae II and IX. Acetylcholinesterase (AChE) activity was evident in the neuronal somata in laminae V, VI, VII, VIII, IX, CCN, IML and in the Clarke's column (CC). Immunohistochemistry data revealed neuronal nitric oxide synthase (nNOS) immunoreactivity in neuronal somata and in fibers of laminae I, II, III, VII, VIII, X and IML; choline acetyltransferase (ChAT) in neuronal somata and in fibers of laminae VII, VIII and IX; calcitonin gene-related peptide (CGRP) was noticed in neuronal somata of lamina IX and in nerve fibers of laminae I, II, III, IV, V, VI and VII; substance P (SP) in nerve fibers of laminae I, II, III, IV, V, VI, VII, VIII, IX, X, CCN, CC and IML; serotonin (5-HT) and vesicular glutamate transporter-1 (VGLUT1) was noticed in nerve fibers of all laminae; somatostatin (SOM) in neuronal somata of laminae III, IV, V, VI, VII, VIII and IX and nerve fibers in laminae I, II, V, VI, VII, X and IML; calbindin (Cb) in neuronal somata of laminae I, II, VI, VII, IX and X; parvalbumin (PV) was found in neuronal somata and in nerve fibers of laminae III, IV, V, VI, VII, VIII, IX and CC; finally, gamma-amino

butyric acid (GABA) was present in neuronal somata of laminae V, VI, VII, VIII, IX and X. This study revealed interesting results concerning the chemoarchitecture of the *Sapajus* spp. spinal cord with a distribution pattern mostly similar to other mammals. The data corroborate the result described in literature, except for some differences in CGRP, SP, Cb, PV and GABA immunoreactivities present in neuronal somata and in nerve fibers. This could suggest certain specificity for the neurochemistry distribution in this non-human primate species, besides adding relevant data to support further studies related to processes involving spinal cord components.

Introduction

The motor and sensory information processing is mainly dependent on neurotransmitters and neuropeptides released in the spinal cord.¹ The control of nociceptive information involves neuropeptides such as somatostatin (SOM), substance P (SP) and calcitonin gene-related peptide (CGRP) mainly localized in neurons present in the dorsal horn of the spinal cord.^{2,3} Neurons containing these neuropeptides modulate glutamate and gamma-amino butyric acid (GABA) levels in synaptic terminals at the dorsal horn or may project to other areas of the central nervous system (CNS) associated with pain modulation, as observed in cats and monkeys.⁴

Serotonin (5-HT) is a neuromodulator mainly distributed in autonomic areas of the spinal cord, such as intermediolateral column (IML) and lamina X.⁵ Acetylcholine is a classical neurotransmitter present in motor neurons projecting efferents to the somatic musculature and in IML neurons responsible for preganglionic innervation of autonomic ganglia.^{6,7} The distribution of acetylcholine has been indirectly and directly assessed by acetylcholinesterase enzyme (AChE) and choline acetyltransferase (ChAT), respectively. These enzymes enable a thorough neuroanatomic mapping, showing intense staining in motoneurons of the ventral horn and preganglionic neurons in the IML in rats and dogs.^{8,9}

The calcium-binding proteins, such as calbindin (Cb) and parvalbumin (PV), are important neuronal markers widely distributed in the CNS and peripheral nervous system, acting in the storage and/or transport of intracellular Ca²⁺ participating in synaptic modulation, especially in nociception.^{10,11} Cb spinal cord distribution is evident in the superficial dorsal horn, participating in nociceptive pathways, while PV is concentrated in the ventral horn, including motoneurons, associated with motor functions.¹² Studies show that the actions of

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calcium-binding proteins are related to the coexistence of neurotransmitters or neuromodulators, as previously described through the colocalization with nitric oxide enzyme (NO), which collaborates with synaptic plasticity and spinal nociceptive regulation.¹²

The presence of nicotinamide adenine dinucleotide hydrogen phosphate-diaphorase (NADPH-d) enzyme corresponds to the nitric oxide synthase (NOS), which produces nitric oxide (NO). Thus, the distribution of NO is indirectly revealed through NADPH-d histochemistry.¹² In rats, NADPH-d is colocalized with GABA in laminae I and II, similar to other mammalian species.³ However, NADPH-d mapping in the thoracic segment showed some differences between rodents and birds with no staining in autonomic preganglionic neurons in pheasants. This data suggests differences in the phylogenetic development, which is of

great importance in neuroanatomical studies across species.^{12,13} Thus, the use of neurochemical markers has helped morphological and functional characterization of neuronal populations in the spinal cord.¹⁴

Previous neuropeptide mapping studies in the spinal cord did not thoroughly describe their distribution along all laminae and were mainly limited to some segments, as observed in rats, cats, dogs, birds and monkeys, mainly in Old World monkeys.^{2,12,13,15-19} The *Sapajus* spp., previously described as *Cebus apella*, belongs to the group of New World monkeys and is extremely agile and active, presenting an important use of forelimbs to perform activities, such as digging in the soil, searching and breaking open objects.²⁰ Their brain morphology is comparable to other Old World monkeys and they are an interesting experimental model used in cyto- and chemoarchitecture studies,²¹⁻²³ although their spinal cord neuroanatomy has not yet been described. Therefore, the purpose of this study was to analyze the classical distribution of neuromediators in the *Sapajus* spp. spinal cord by using immunohistochemical and histochemical methods.

Materials and Methods

Three young male tufted capuchin monkeys (*Sapajus* spp.), housed in the Tufted Capuchin Monkey Procreation Center (Araçatuba School of Dentistry, São Paulo State University - UNESP) were used in the present study. The experimental procedures followed the guidelines of the Brazilian Institute for the Protection of the Environment (IBAMA) and of the Institutional Ethical Committee for Animal Research (protocol number 02257-2012).

Tissue preparation

The monkeys were anesthetized, perfused and the spinal cords were collected and cryoprotected, as described in previous studies.^{24,25} Histological sections were obtained using a freezing microtome (coronal plane at 40 μ m), stored in antifreeze solution at -30°C until histochemistry and immunohistochemistry methods were performed.

Histochemistry and immunohistochemistry

The enzymatic activity of NADPH-d and AChE was assessed by the histochemistry method described in previous studies.^{21,26} Adjacent series were selected for immunohistochemistry. Free-floating sections were washed in phosphate buffer (0.1 M PBS, pH 7.4), pretreated in 0.3% hydrogen peroxide solution diluted in PBS and then incubated

with avidin and biotin blocking solution (Avidin/Biotin Blocking Kit, SP-2001, Vector Laboratories Inc., Burlingame, CA, USA), for 30 min each. After PBS rinses, the sections were incubated overnight in a non-specific labeling blocking solution containing PBS, normal donkey serum and 1.5% bovine serum albumin. After that, sections were incubated in

primary antiserum (Table 1) and diluted in blocking solution (PBS + 0.03% triton X-100 + normal donkey serum), for 48 h at 4°C. Next, the sections were incubated in biotinylated antibody and then in avidin-biotin complex (Vectastain, Kit Standard, PK-4000, Vector) for 1 h each at room temperature and the peroxidase activity was developed using diaminoben-

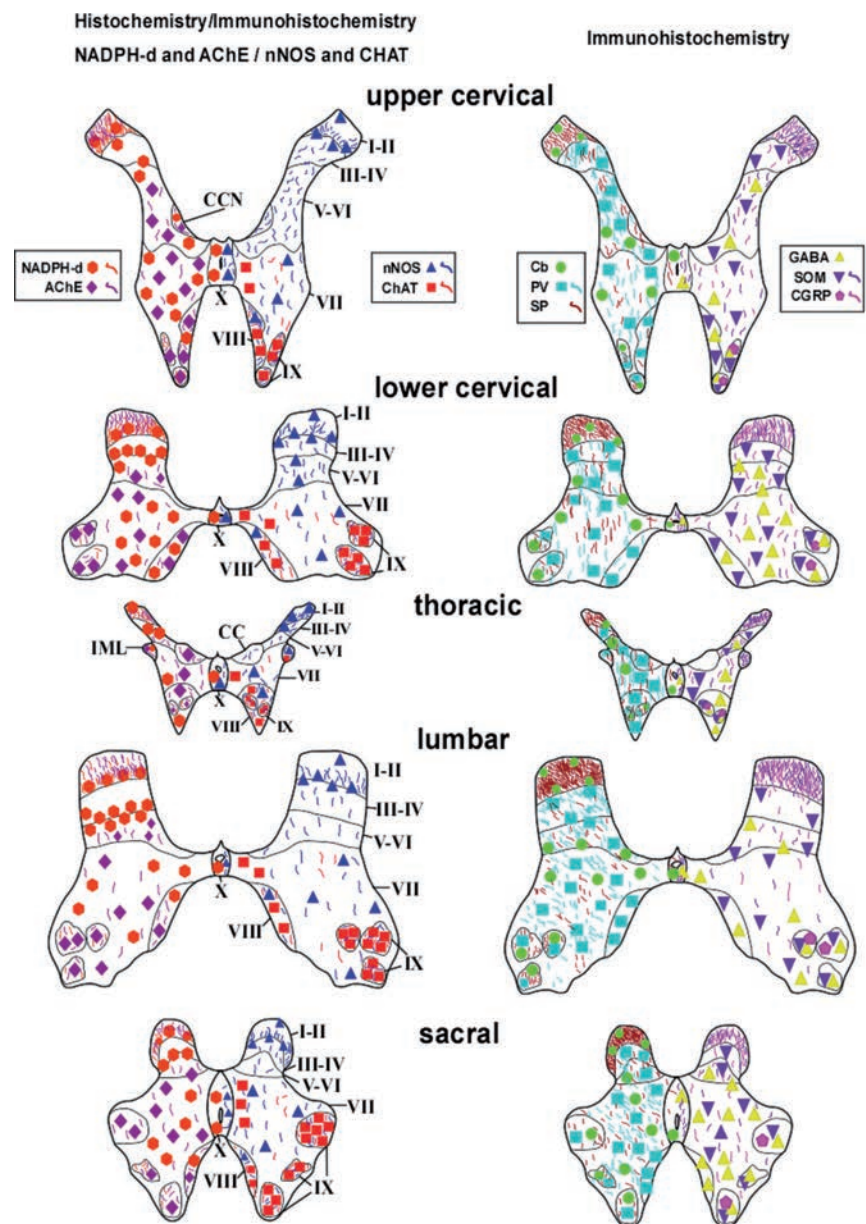


Figure 1. Schematic view of neurochemical distribution in cervical, thoracic, lumbar and sacral spinal cord segments of *Sapajus* spp. Left column: left antimere representing histochemical distribution of NADPH-d and AChE, whereas right antimere represents immunoreactivity distribution to nNOS and ChAT. Right column: left antimere represents immunoreactivity to Cb, PV and SP, whereas right antimere represents neuromediators GABA, SOM, and CGRP. Histochemistry symbols for neuronal somata: orange hexagon, NADPH-d; purple rhombus, AChE. NADPH-d and AChE nerve fibers shown by dashes using same colors of neuronal somata. Immunohistochemistry symbols for neuronal somata: blue triangle, nNOS; red square, ChAT; green circle, Cb; blue square, PV; yellow triangle, GABA; purple inverted triangle, SOM; lilac pentagon, CGRP. Nerve fibers shown by dashes using same colors of respective neuronal somata.

zidine-chromogen reagent. Finally, the sections were mounted on gelatin coated slides, air dried, dehydrated in increasing ethanol solutions, delipified in xylene, mounted on DPX and cover slips. For control experiments all antibodies were omitted and no specific staining was observed. The sections were analyzed by light microscopy (Scope A1/Axioplan2, Carl Zeiss, Jena, Germany) and images were captured using a digital camera (Nikon Corporation, Tokyo, Japan) coupled to a Leica DMR microscope and NIS-Elements BR 3.0 software (Nikon Corporation).

Results

The histochemical methods used were adequate to reveal the laminar divisions and cytoarchitecture of the spinal cord of *Sapajus* spp. monkeys. The organization of laminae along the cord segments is shown in the schematic drawings (Figure 1). In general, all segments demonstrated the basic cytoarchitectonic organization observed in the spinal cord of mammals, such as distribution of neurons in all laminae and the presence of some neuronal groups. The latter were represented by:

i) central cervical nucleus (CCN), localized in the medial part of laminae V and VII, at the upper cervical levels; ii) Clarke's column (CC), situated in the medial portion of laminae V and VI of the thoracic segment; iii) intermediolateral column (IML), situated in the lateral part of lamina VII of thoracic and upper lumbar segments. These variations were peculiar to spinal segments (Figure 1).

Histochemistry

NADPH-d. Neuronal somata were observed in the CCN and fibers of passage and possible terminals with varicosities were mostly evi-

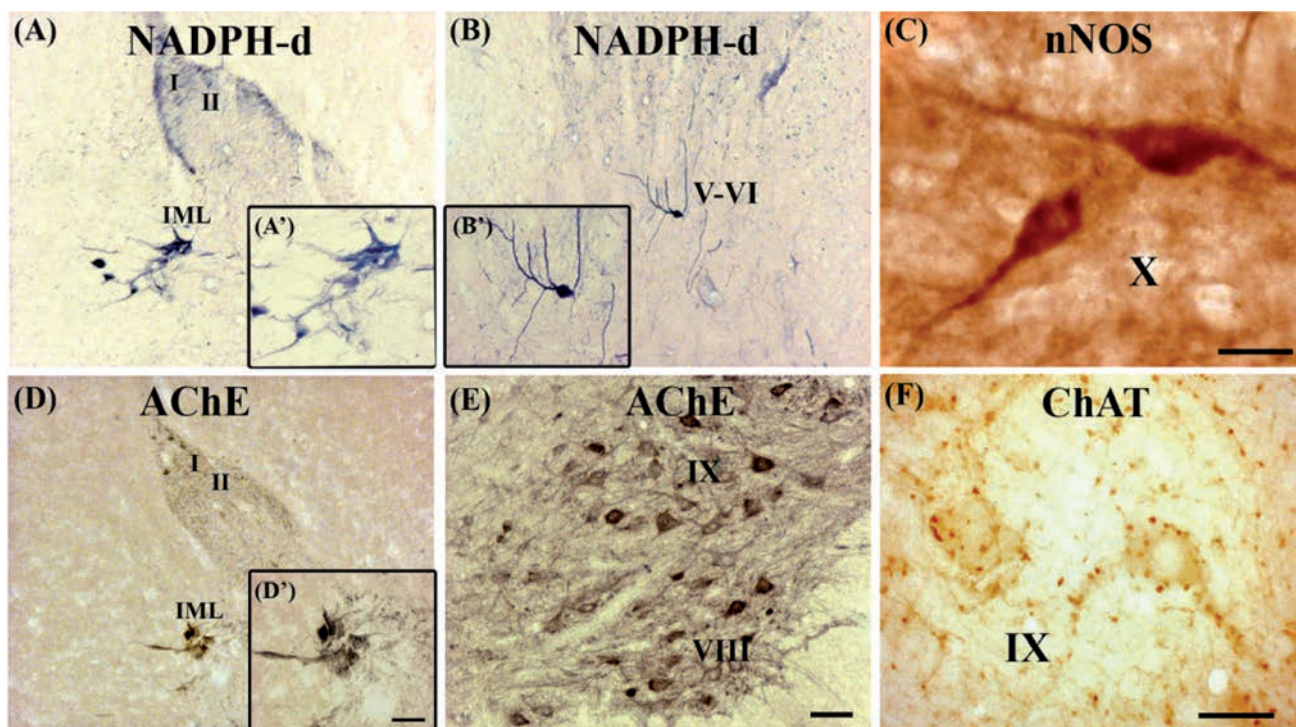


Figure 2. Light microscope photomicrographs showing chemoarchitecture of *Sapajus* spp. spinal cord. NADPH-d and AChE histochemistry is shown in A-B and D-E, respectively. A, D) Neuronal somata in IML from thoracic segment. B) NADPH-d neuronal somata within laminae V-VI from sacral segment; whereas E shows AChE motor neurons within laminae VIII and IX from lower cervical segment. nNOS-ir neuronal somata in lamina IV from lower cervical segment can be visualized in C and ChAT-ir terminals can be visualized within lamina IX from lumbar segment in F. Scale bars: A, B, D, E) 1000 μ m; A', B', D', F) 500 μ m; C) 200 μ m.

Table 1. Description of commercial primary and secondary biotinylated antibodies used for mapping the spinal cord of *Sapajus* spp.

Antibody	Source	Species	Dilution
Serotonin (5-HT)	Immunostar® #20080	Rabbit	1/20000
GABA	Sigma® A2052	Mouse	1/1000
Anti-vesicular glutamate transporter (VGLUT1)	Millipore® MAB 5502	Mouse	1/2000
Calbindin (Cb)	Swant® #CB38a	Mouse	1/5000
Parvalbumin (PV)	Sigma® P 3088	Mouse	1/5000
Somatostatin (SOM)	Peninsula® T-4103	Rabbit	1/2000
Calcitonin gene-related peptide (α -CGRP)	Peninsula® T-4031.0400	Rabbit	1/3000
Substance P (SP)	Chemicon® AB 1566	Rabbit	1/2000
Anti-mouse biotinylated	Vector® #BA-1000	Goat	1/800
Anti-rabbit biotinylated	Vector® #BA-9200	Goat	1/800

dent in laminae I and II (Figures 1 and 2A). Positively stained neuronal somata were also found in the IML and in laminae I, II, III, IV, V, VI, VII, VIII and X (Figures 1 and 2B).

AChE. Fibers were evident in laminae I and II (Figures 1 and 2D). Neuronal somata and fibers were observed in laminae V, VI and VII including CCN, IML and CC (Figures 1 and 2D). The ventral horn showed nerve fibers and neuronal somata evidently stained for AChE in lamina VIII and IX (Figures 1 and 2E).

Immunohistochemistry

nNOS. Neuronal nitric oxide synthase-immunoreactive (nNOS-ir) fibers were observed in all laminae along the spinal cord. nNOS-ir neuronal somata were noted in laminae I, II, III, VII, VIII, X and in IML (Figures 1 and 2C). Neuronal somata were also observed in lamina V, only at the lower cervical portion.

ChAT. Choline acetyltransferase-immunoreactive (ChAT-ir) neuronal somata and fibers were noticed in laminae VII, VIII and IX, with

ChAT-ir terminals surrounding motoneurons in lamina IX (Figures 1 and 2F).

CGRP. Calcitonin gene-related peptide-immunoreactive (CGRP-ir) nerve fibers and terminals were distributed in laminae I and II (Figures 1 and 3A). Few terminals were seen in laminae III, IV, V, VI and VII. Diffuse CGRP-ir neuronal somata were observed in lamina IX (Figures 1 and 3A').

SP. Substance P-immunoreactive (SP-ir) nerve fibers and terminals were noted in laminae I, II, III, IV, V, VI, VII, VIII, X, CC and IML

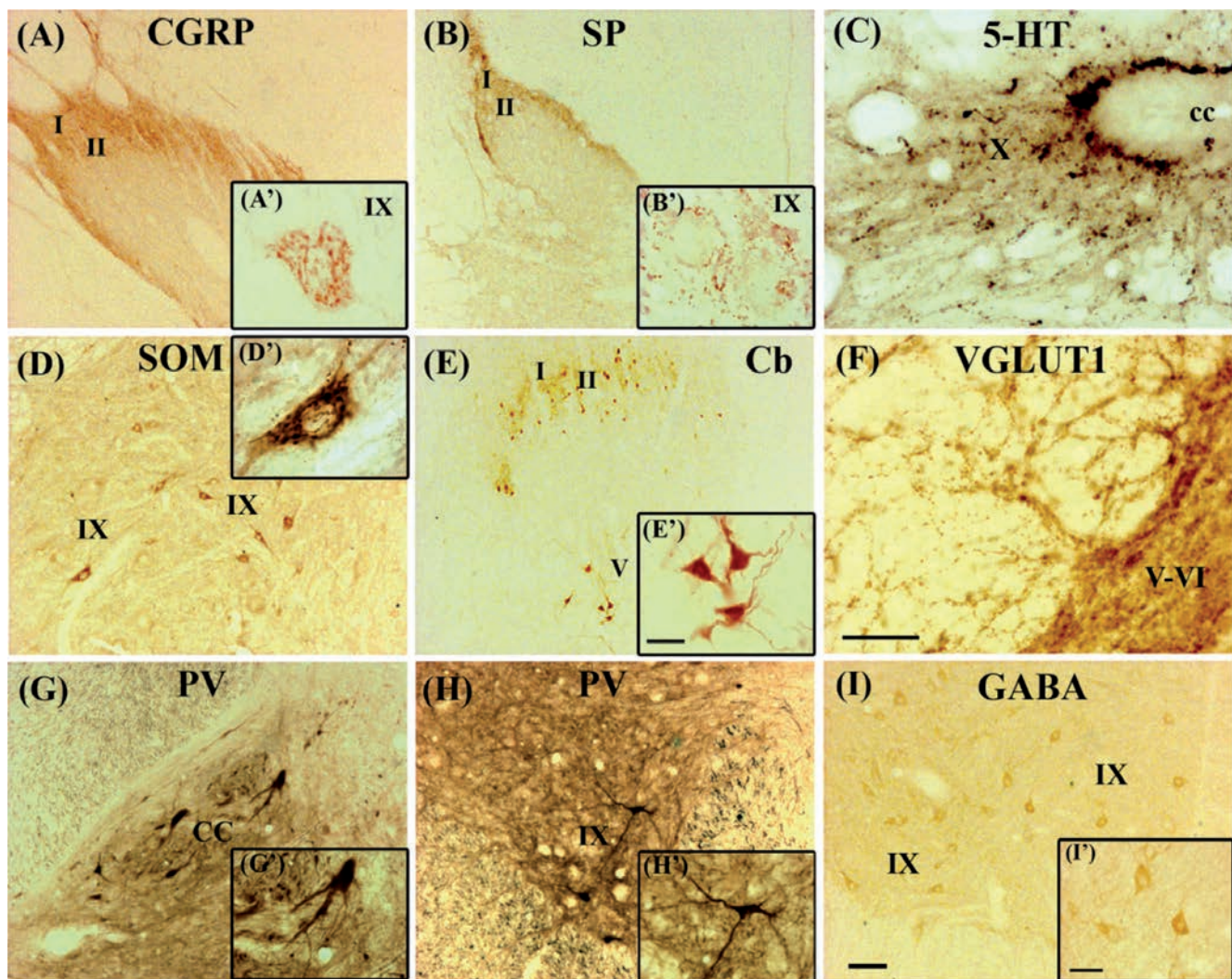


Figure 3. Light microscope photomicrographs showing neurochemistry of *Sapajus* spp. spinal cord. A) CGRP immunoreactivity in terminals within laminae I and II from upper cervical segment and a motor neuron from lamina IX (high magnification, A'). B) SP immunoreactivity in terminals within laminae I and II from thoracic segment. B') SP-ir motor neurons within lamina IX, surrounded by several terminals at high magnification. C) 5-HT immunoreactivity observed in terminals within lamina X from thoracic segment. D) SOM immunoreactivity observed in neurons; whereas a neuronal somata is shown in lamina IX from lumbar segment at high magnification (D'). E) Cb immunoreactivity in terminals and neuronal somata within lamina I and II from lumbar segment; whereas neuronal somata are shown in lamina V at high magnification (E'). F) VGLUT1 immunoreactivity in terminals within laminae V-VI, in transition region between white and gray matter from lower cervical segment. G,H) PV immunoreactivity in terminals and neuronal somata (high magnification in G' and H') at Clarke's column and lamina IX, respectively, from thoracic segment. I) GABA immunoreactivity in neuronal somata distributed within lamina IX (high magnification in I'). Scale bars: A,B,D,E,G,H,I) 1000 µm; C,E,G',H',I') 500 µm; A', B', D', E') 200 µm.

(Figures 1 and 3B). SP-ir terminals encircling the neuronal somata of motoneurons in lamina IX of all spinal segments (Figure 3B').

5-HT. Serotonin-immunoreactive (5-HT-ir) nerve fibers and terminals were homogeneously distributed along the spinal cord in all laminae; however, they were more evident in laminae I, X and IML (Figure 3C) and less evident in laminae II and VII.

SOM. Somatostatin-immunoreactive (SOM-ir) nerve fibers were noted in laminae I, II, V, VI, VII, X and IML. A small number of SOM-ir neuronal somata exhibiting immunoreactive cytosolic granules were evident in laminae III, IV, V, VI, VII, VIII and IX (Figures 1, 3D and 3D').

Cb. Calbindin-immunoreactive (Cb-ir) neuronal somata were distributed within laminae I, II and in the lateral portion of lamina V with neuronal somata showing long axons and dendrites (Figures 3E and 3E'). Few and diffuse Cb-ir neuronal somata were observed in laminae VI, VII, IX and X (Figure 1).

VGLUT1. Vesicular glutamate transporter-1-immunoreactive (VGLUT1-ir) nerve fibers and terminals were present throughout the segments with more evident staining in CCN, IML, lamina I and CC. Interestingly, fibers and terminals were noticed at the margin of the gray matter inside the lateral and ventral funiculus (Figure 3F).

PV. Parvalbumin-immunoreactive (PV-ir) neuronal somata and nerve fibers were localized in most laminae, excluding I, II and X (Figure 1). PV-ir fibers and neuronal somata with profuse axons and dendrites were distributed in the gray matter in CC (Figures 3G and 3G') and in laminae III, IV, V, VI, VII, VIII and IX (Figures 1, 3H and 3H').

GABA. Gamma-aminobutyric acid-immunoreactive (GABA-ir) neuronal somata were most evident in lower cervical and sacral segments in laminae V, VI, VII, VIII, IX and X (Figures 1 and 3I), while no immunostaining was observed in superficial laminae.

Discussion

The distribution of classical neuromediators and cytoarchitectonic arrangements in the spinal cord of *Sapajus* spp. monkey has not yet been described. This non-human primate exhibits spinal cord laminar cytoarchitectural organization similar to other mammals, including the Rexed's laminae study in cats, and some studies in Old World monkeys and humans.^{2,12,14,15,17,27-30} The spinal circuitry of mammals is characterized by its complexity compatible with the fine motor control of limbs and their feedback systems. The maintenance of a basic cytoarchitectonic and neurochemical

structure of the spinal cord indicates a phylogenetically conserved evolutionary advantage. In parallel, rats and primates have comparable motor systems, which enable similar skilled movements of the limbs.³¹ In fact, the complexity of motor control probably exceeds the actions needed along the animal's lifetime.³² Hence, few differences among mammalian species would be expected in the spinal cord cytoarchitecture, although primates have an evident evolutionary difference that permitted the use of hands with an opposable set of digits. It is also important to mention that when primates are not moving, they use the forelimbs to perform other activities, such as digging in the soil and in tree holes, besides breaking objects open.²⁰

The histochemistry method for NADPH-d along the spinal cord of *Sapajus* spp. monkey showed nerve fibers and possible nerve endings in lamina II and neuronal somata in laminae II-VIII, X and IML. NADPH-d activity indirectly indicates the possible localization of neuronal somata that produce nitric oxide (NO), which is an important neurotransmitter for modulation of the locomotion circuitry and autonomic nervous system control.^{33,34} For this reason, nitrenergic neuronal somata distribution was also analyzed by immunohistochemistry using nNOS antibody. As expected, NADPH-d distribution was very similar to nNOS immunostaining. We observed that the distribution of nNOS-ir neuronal somata was not as extensive as NADPH-d, corroborating previous studies which indicated that some neuronal territories may not present colocalization.^{35,36}

It has been demonstrated that cholinergic and non-cholinergic neurons have acetylcholinesterase enzyme (AChE)⁷ and the immunohistochemical technique against choline acetyltransferase (ChAT) enzyme was able to confirm the cholinergic neurons.³⁷ Our histochemistry results showed AChE in neuronal somata and fibers of all laminae along the spinal cord, as evidenced in the rat spinal cord.³⁷ On the other hand, immunohistochemistry for ChAT showed immunostaining in neuronal somata of laminae VII, VIII and IX, which is in agreement with the literature demonstrating comparable data in rats, cats and monkeys.^{7,38} In addition, the presence of cholinergic terminals in motor neurons was evident in our immunohistochemistry results for ChAT, corroborating other studies.¹⁸ The present study also demonstrated AChE- and ChAT-stained neuronal somata in the IML, corresponding to the neurochemistry of preganglionic neurons.^{7,8,39} The data from the present study showed agreement with previous works describing the AChE distribution and ChAT mRNA in the spinal cord of small mammals,³⁹ non-human primates and humans.^{7,18}

CGRP-ir fibers were observed within lami-

nae I and II of *Sapajus* spp., analogous to other species.^{2,40,41} CGRP has been localized in several areas of the central and peripheral nervous system. The superficial laminae of the spinal cord receives CGRP primary afferent projections from dorsal root ganglion neurons, participating in sensory systems of the spinal cord, including pain processing.^{40,42,43} Despite this evident distribution of CGRP-ir fibers in the spinal cord of *Sapajus* spp., it was verified that some neuronal somata within lamina IX presented granular immunoreactivity in the cytosol, corroborating another study indicating a possible CGRP role in motor systems as a trophic factor.² A previous study described CGRP in neurons close to lamina IX, such as in laminae VII and VIII of dogs;² however, we only observed neuronal somata CGRP-ir in lamina IX of *Sapajus* spp.

The glutamate amino acid is a widely excitatory neurotransmitter in the mammalian CNS.²⁷ It is synthesized in the cytoplasm and transported along the axon in synaptic vesicles to the terminals. The glutamate distribution is very broad and its three carriers, VGLUT1, VGLUT2 and VGLUT3, can be visualized by immunohistochemistry.^{44,45} The present study mapped VGLUT1, since it was already characterized in the rat spinal cord.^{27,44,45} Several VGLUT1-ir terminals were observed in all monkey medullary segments and laminae, including lamina I. This finding partly corroborates data from the rat spinal cord, since the literature emphasizes the absence of VGLUT1 terminals in the rat lamina I.^{27,44} The VGLUT immunoreactive terminals in the superficial laminae establish synapse with SP nerve terminals from the dorsal root ganglion, possibly modulating sensory information.⁴⁴ Furthermore, the literature emphasizes that VGLUT1 terminals are associated with proprioception pathways, as they are present in mechanoreceptor afferents and in many cutaneous afferents.⁴⁵ Many VGLUT1 terminals in the spinal cord are probably projections from pyramidal cells of the neocortex that express VGLUT1 mRNA.⁴⁵

Substance P is a neuropeptide involved in pain sensibility.⁴⁶ Its immunoreactivity is present in neuronal somata in laminae I-III, V and IML, besides in terminals and fibers of superficial laminae in rats⁴⁶ and cats.⁴⁷ SP-ir neuronal somata were not found in the *Sapajus* spinal cord. In contrast, some terminals were observed within all laminae, with special abundance in the dorsal horn, IML and establishing possible contact with motoneurons of lamina IX, corroborating previous studies in man and monkey.⁴⁸ Previous studies showed SP immunoreactivity in neuronal somata from the rat spinal cord, following colchicine treatment, which allows better visualization of neuropeptides in nervous tissue.^{46,49}

Former studies indicated that Cb is mainly distributed in neurons within laminae I and II of dogs,¹¹ rats² and *Macaca fascicularis*,¹⁶ in agreement with our results. Further studies in *Macaca fascicularis* showed Cb-ir neurons in lamina VII and nerve fibers in laminae I, II, VIII and IX,¹⁶ whereas our results demonstrated neuronal somata in laminae I, II, V, VI, VII, IX and X. Another calcium-binding molecule, the parvalbumin, was observed in nerve fibers and neuronal somata of all laminae, excluding laminae I, II and X in contrast to studies in rats that describe the presence of PV in all laminae.⁵⁰ PV staining delimited the funiculus in the white matter as cuneiform funiculus and lateral corticospinal tract in *Sapajus* spp. PV neuronal somata were seen in the gray matter including the CC, an area that projects afferents related to unconscious proprioception to the cerebellum,^{51,52} as previously verified in dogs.²

GABA is an inhibitory neurotransmitter present all over the CNS.⁵³ GABA is present in spinal cord superficial laminae in rats, acting as a pre-synaptic neurotransmitter in contact with nerve terminals, mostly glutamatergic.^{54,55} GABAergic mRNA expression was observed within neurons of lamina X in rats, in dorsal and lateral regions of the central canal, also known as a central autonomic area.⁵⁶ Besides this, GABA plays an important role in learning and motor coordination during the developmental stages of the CNS.⁵⁷ Our results showed GABA-ir neuronal somata in laminae V-X of all segments. Most of these results indicate that GABA localization in the spinal cord of *Sapajus* spp. is consistent with what has already been described in literature, possibly participating in autonomic⁵⁶ and motor control.⁵⁷ Although no immunoreactivity was observed in superficial laminae of the *Sapajus* spinal cord, the employed antibody showed specificity in rats^{58,59} and other studies described GABA in the dorsal horn of rhesus monkey.⁴ It is possible that the absence of immunostaining was due to the histological fixation step, impairing antigen recognition by the antibody.

Serotonin (5-HT) is a neuromediator of sensory processing⁶⁰ and motor activity,⁶¹ contributing to wide functions in the spinal cord. 5-HT is present in diffuse terminals and in some neurons in laminae VII and X of rats.⁵ In monkeys, 5-HT terminals are present in all laminae in the dorsal, ventral and lateral horns of the spinal cord^{62,63} and in some neurons in lamina X.^{64,65} The present results disclosed a diffuse and strong immunolabeling of 5-HT terminals in all segment levels and laminae, particularly in laminae I, X and IML, corresponding to varicose nerve fibers described in other monkeys.^{64,65} Some studies suggest that these terminals are originated from the raphe nucleus and ventrolateral medulla in primates.⁶⁶

Somatostatin is a neuropeptide that is widely expressed in the CNS of mammals.^{28,67} Previous studies demonstrated that SOM is restricted to neuronal circuits in the dorsal horn.^{68,69} Furthermore, SOM was found in the dorsal root ganglia, suggesting an important modulatory role upon pain input, mainly the acute type.^{67,68} SOM-ir fibers were clearly found in lamina X corroborating with other studies in monkeys^{64,70} and humans.²⁸ Moreover, terminals in the IML were also described in the human spinal cord.⁷¹ SOM-ir neuronal somata in the dorsal (lamina III) and ventral horns (laminae VIII and IX) were also verified in other mammals.⁷⁰ These data indicate that SOM probably participates not only in the modulation of sensory information of superficial laminae, but also in motor and in autonomic modulations in *Sapajus* spp.

It can be concluded that the neurochemical features found in the spinal cord of *Sapajus* spp. have similarities with other mammal species. Nevertheless, some results were different from the literature, such as CGRP, which was not observed in neuronal somata in laminae VII and VIII. In addition, SP was only found in nerve fibers and terminals; Cb was present in neuronal somata in laminae V, VI, VII and X; PV and GABA was absence in dorsal horn. These results showed some particularities in the distribution of classical neuromediators in this species, supporting further functional studies of the spinal cord in *Sapajus* spp.

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