

Acta Medica Okayama

Volume 42, Issue 1

1988

Article 4

FEBRUARY 1988

Ultrastructure of monoaminergic terminals in the intermediolateral nucleus of the cat spinal cord.

Kiminao Mizukawa*

I-Ming Tseng†

Ryoichi Tobita‡

Nagayasu Otsuka**

*Okayama University,

†Okayama University,

‡Okayama University,

**Okayama University,

Ultrastructure of monoaminergic terminals in the intermediolateral nucleus of the cat spinal cord.*

Kiminao Mizukawa, I-Ming Tseng, Ryoichi Tobita, and Nagayasu Otsuka

Abstract

Monoaminergic innervation of the intermediolateral nucleus of the cat spinal cord was investigated by fluorescence histochemistry and electron microscopy. Large numbers of monoaminergic terminals were labeled by prior administration of the false neurotransmitter 5-hydroxydopamine (5-OHDA). Ultrastructurally, 5-OHDA-labeled terminals fell into three types. Type I, which made up 55% of the labeled terminals, contained abundant, large and densely labeled vesicles and only a few small and unlabeled vesicles. This type was "bouton de passage". Type II, which made up 40% of the terminals, made asymmetrical synaptic contacts with typical postsynaptic structures. This type contained many small vesicles, some of which were labeled, and a few large dense-core vesicles. Type III, which made up 5% of the terminals, made close contact with presynaptic nerve endings containing abundant small unlabeled clear vesicles. The type III terminals contained many large and densely labeled vesicles and a few small flattened vesicles, most of which were unlabeled.

KEYWORDS: ultrastructure, monoaminergic terminals, 5-hydroxydopamine, cat spinal cord, intermediolateral nucleus

*PMID: 3364212 [PubMed - indexed for MEDLINE]

Ultrastructure of Monoaminergic Terminals in the Intermediolateral Nucleus of the Cat Spinal Cord

Kiminao Mizukawa*, I-Ming Tseng, Ryoichi Tobita and Nagayasu Otsuka

Department of Anatomy, Okayama University Medical School, Okayama 700, Japan

Monoaminergic innervation of the intermediolateral nucleus of the cat spinal cord was investigated by fluorescence histochemistry and electron microscopy. Large numbers of monoaminergic terminals were labeled by prior administration of the false neurotransmitter 5-hydroxydopamine (5-OHDA). Ultrastructurally, 5-OHDA-labeled terminals fell into three types. Type I, which made up 55% of the labeled terminals, contained abundant, large and densely labeled vesicles and only a few small and unlabeled vesicles. This type was "bouton de passage". Type II, which made up 40% of the terminals, made asymmetrical synaptic contacts with typical postsynaptic structures. This type contained many small vesicles, some of which were labeled, and a few large dense-core vesicles. Type III, which made up 5% of the terminals, made close contact with presynaptic nerve endings containing abundant small unlabeled clear vesicles. The type III terminals contained many large and densely labeled vesicles and a few small flattened vesicles, most of which were unlabeled.

Key words : ultrastructure, monoaminergic terminals, 5-hydroxydopamine, cat spinal cord, intermediolateral nucleus

Since the introduction of Falck-Hillarp fluorescence histochemistry (1), noradrenergic and serotonergic fluorescence varicosities have been demonstrated throughout the gray matter of the spinal cord. The densest plexus of monoaminergic fibers has been observed in the intermediolateral cell column of the spinal cord of various mammals (2-8). The intermediolateral cell column in the lateral horn of the spinal cord is an important nucleus for controlling autonomic function. These monoaminergic fibers from the supra-spinal central nervous system completely control the cardiovascular auto-

nomie functions (6, 9, 10).

To investigate the ultrastructure of monoaminergic terminals, potassium permanganate fixation coupled with labeling using false neurotransmitters such as 5-hydroxydopamine (5-OHDA) and 6-hydroxydopamine (6-OHDA) has been employed (11). Many investigations of the central and peripheral nervous systems have been performed by this method (12-15). However, there are few ultrastructural and semiquantitative investigations of these monoaminergic terminals in the intermediolateral nucleus. The present study aims to clarify the ultrastructure of the terminals using preadministered 5-OHDA-as-

*To whom correspondence should be addressed.

sisted electron microscopy.

Materials and Methods

Ten cats of both sexes (body weight 2-3 kg) were used in this study. Five were used for fluorescence histochemistry and the remaining five cats were treated with 5-OHDA and used for electron microscopy.

Fluorescence histochemical examination. Five animals were intraperitoneally injected with Nialamide (100 mg/kg of body weight), an inhibitor of monoamine oxidase (MAO). Five to 6 h after the injection, the cats were anesthetized with Nembutal and perfused with chilled 2% glyoxylic acid phosphate buffer solution (pH 7.0) through the left ventricles. Tissues of the thoracic spinal cord (T7-T8) of the cats were rapidly removed, placed in isopentane cooled with liquid nitrogen, and lyophilized. The resulting tissue blocks were then reacted with paraformaldehyde gas at 80°C for 1 h. The blocks were embedded in paraffin in vacuo and 10 μ m transverse and longitudinal serial sections were prepared. Each section was mounted in Entellan and examined under an Olympus fluorescence microscope (B. V., Y50).

Electron microscopic examination. Under Nembutal anesthesia, a laminectomy was performed on five animals at the T7-T8 level to expose the surface of the spinal cord. Then 5-50 mg of 5-OHDA dissolved in 0.5 ml saline was injected by microsyringe into the subarachnoid space of the thoracic cord. After 6-8 h, the animals were reanesthetized and perfused via the left ventricles with a mixture of 1% glutaraldehyde and 1% paraformaldehyde solution adjusted with 0.1 M phosphate buffer to pH 7.4. The thoracic cord was immediately removed and cut into small blocks which were fixed in the same fixative for 2 h. The blocks were then rinsed with phosphate buffer solution and postfixed in 1% osmium tetroxide for 2 h. The blocks were dehydrated through a graded acetone series and embedded in an EPON 812 mixture, and semithin and ultrathin sections were prepared. The semithin sections were stained with toluidine blue to permit the identification of the intermediolateral nucleus. The ultrathin sections, some of which were serial, were stained with uranyl acetate and lead acetate and observed with

a JEM 100B transmission electron microscope (JEOL).

Results

Fluorescence histochemical investigation. In the lateral horn, characteristic green noradrenergic fluorescence and yellowish serotonergic fluorescence intensively accumulated around neurons of the intermediolateral nucleus. Noradrenergic fluorescence existed in the ventral side of the intermediolateral cell column, while serotonergic fluorescence was found on the dorsal side (Fig. 1a). In horizontal sections, intensive fluorescence with a beaded arrangement was observed. Bundles of fluorescent fibers were also observed to pass in a transverse direction (Fig. 1b). Fluorescent fiber bundles crossed from side to side, providing bilateral innervation to the intermediolateral nuclei segmentally.

Electron microscopic investigation. Many 5-OHDA-labeled terminals of various types could be observed in the intermediolateral nucleus (Fig. 2). Following the administration of 5-OHDA, many cores in the large synaptic vesicles (80-100 nm in diameter) and the small synaptic vesicles (40-60 nm in diameter) characteristically increased in electron density. A characteristic feature was that most of these electron dense-cores in the small synaptic vesicles were eccentrically located. They were presumed to be monoaminergic terminals, and could be classified into three types on the basis of their synaptic structural specialization.

Type I. This type contained mainly large dense-core vesicles, together with a few small electron dense-core vesicles and many unlabeled vesicles. These terminals were 0.5-1.5 μ m in diameter and were usually surrounded by glial elements. There was a relatively wide space between cell structures (Figs. 3a, b). They were never observed to

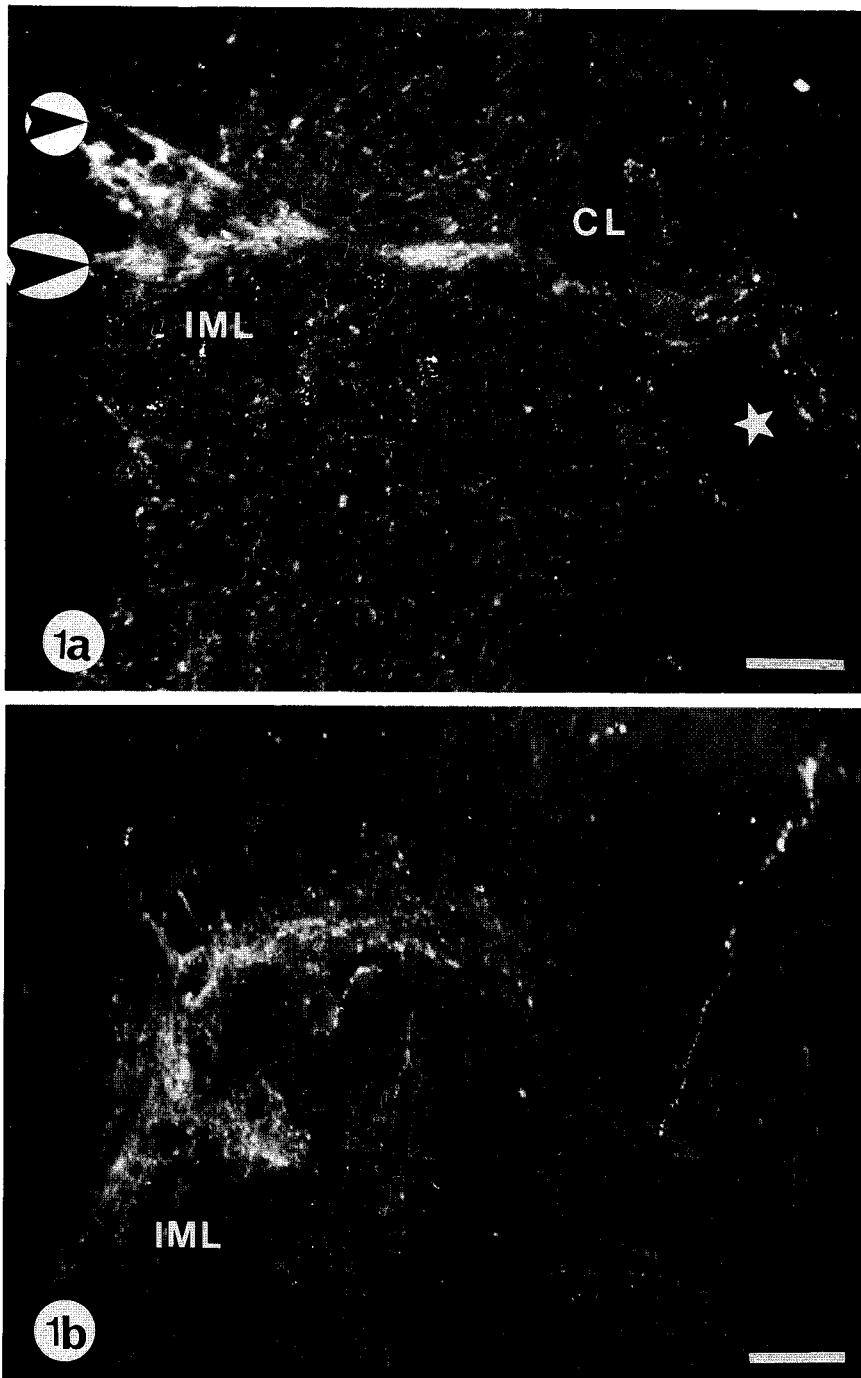


Fig. 1 Fluorescence micrographs of the cat thoracic cord.

1a. Transverse section. Dense monoaminergic fluorescence exists in the intermediolateral nucleus (IML). Dense fluorescent fiber bundle is observed in transverse direction to opposite side of IML, segmentally. Large arrow head indicates green noradrenaline fluorescence and small arrow head indicates yellowish serotonin fluorescence. ★, central canal; CL, column of Clarke. Bar = 0.1 mm.

1b. Longitudinal section. Dense accumulation of fluorescence is observed around the IML. Bar = 0.1 mm.

form typical synaptic contacts in any of many serial ultrathin sections. In some cases, these type I terminals were found in the neighborhood of the cell soma of intermediolateral nucleus neurons (Fig. 4a). Type I

boutons could also be observed in sagittal sections of axons confirming that this type presents a "bouton de passage" (Figs. 4a, b).

Type II. This type contained only a few

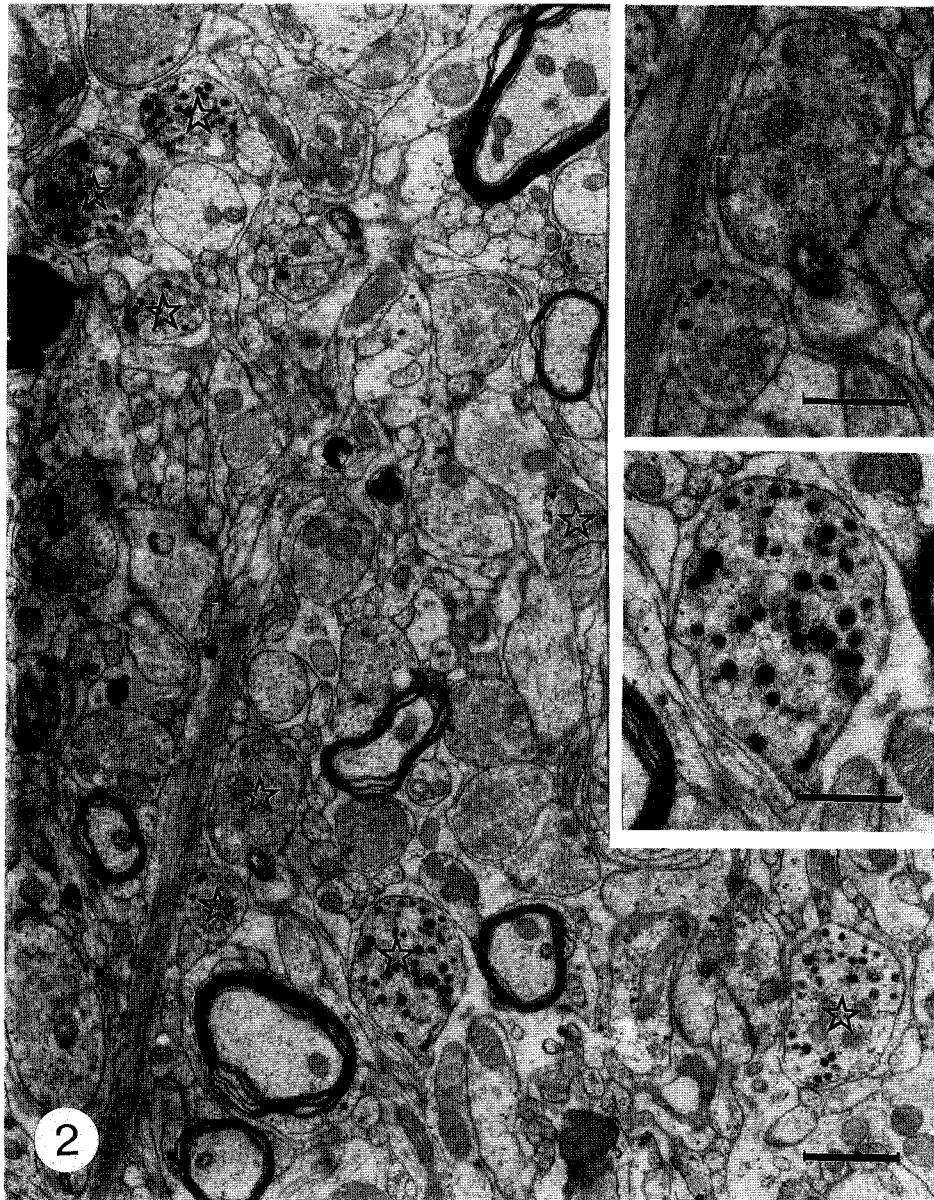


Fig. 2 Low and high magnification electron micrographs of the intermediolateral nucleus after administration of 5-hydroxydopamine (5-OHDA). There are many monoaminergic terminals (☆) labeled with 5-OHDA. Small 5-OHDA-labeled dense-core synaptic vesicles are prominent in the upper inset, and large 5-OHDA-labeled synaptic vesicles are prominent in the lower inset. Bars = 0.5 μ m.

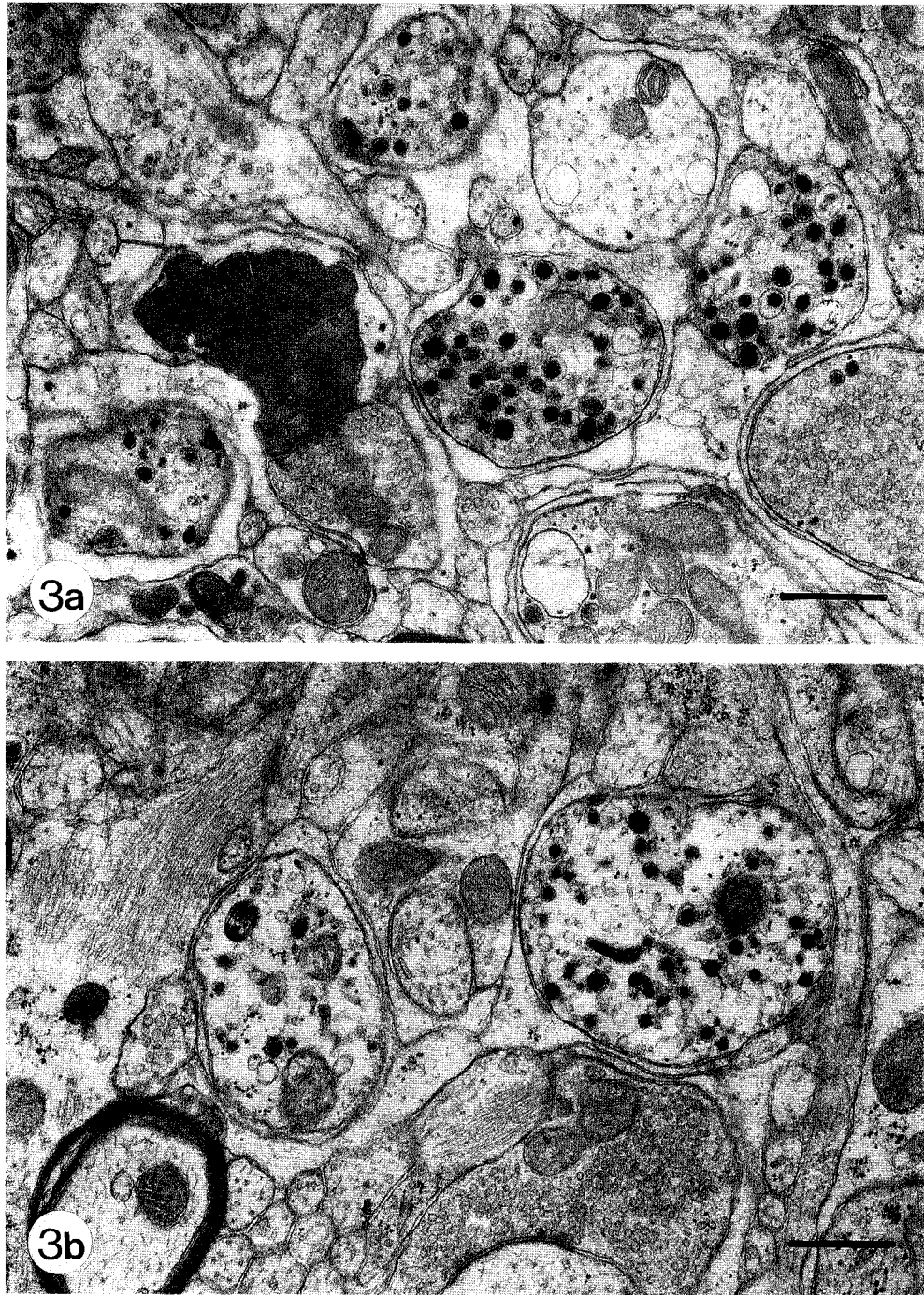


Fig. 3 High magnification electron micrographs of type I monoaminergic terminals labeled with 5-hydroxydopamine (5-OHDA). These terminals do not have typical synaptic structures, but do have many large 5-OHDA-labeled dense-core synaptic vesicles. Bars = 0.5 μ m.

large dense-core vesicles and many small dense-core vesicles. This type of terminal was 0.5–1.5 μm in diameter and had a typical asymmetric synaptic structure (Figs. 5a, b).

Type III. This type of axonal terminal contained both large and small electron densely labeled vesicles and occasionally a few flattened small vesicles. This type of terminal was 0.7–1.5 μm in diameter. The synaptic contact was to a presynaptic nerve

ending containing many non-labeled clear vesicles, and was of the axo-axonic type (Figs. 6a, b).

A semiquantitative analysis of the monoamine-labeled axonal terminals in the region of the intermediolateral nucleus showed that 495 labeled terminals occurred in this nucleus in five experimental animals, and that 55% of the labeled axonal terminals were type I, 40% were type II and 5% were type

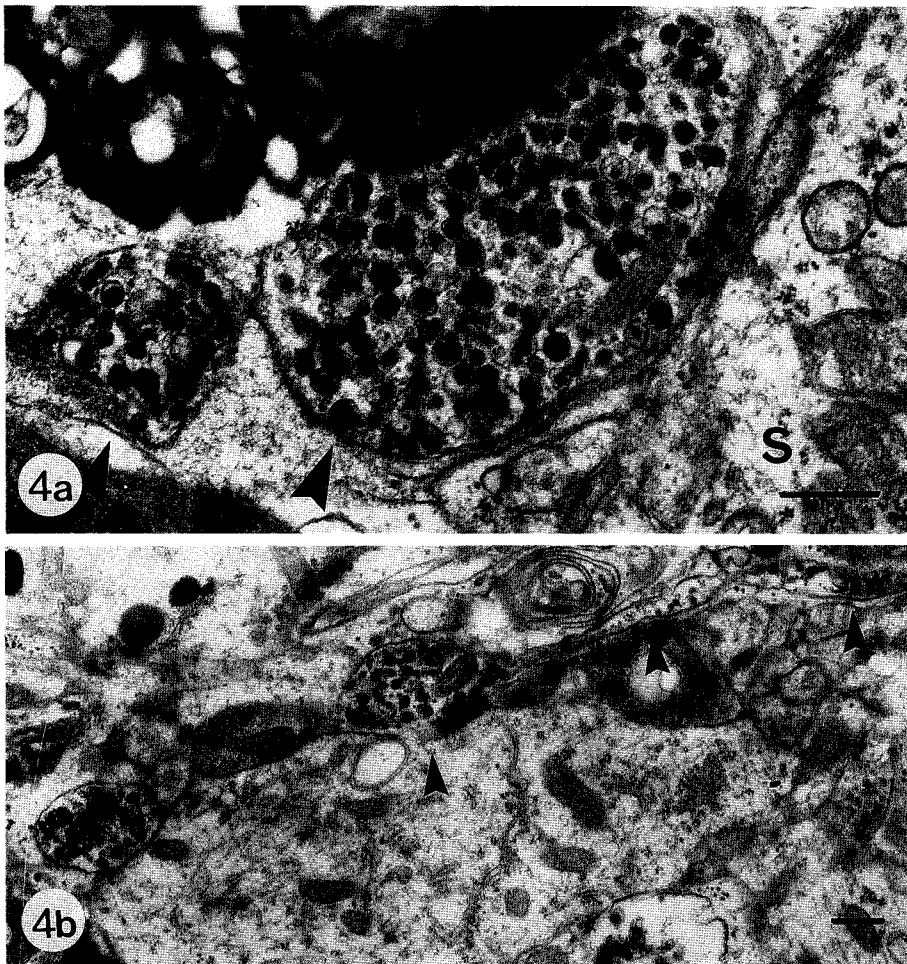


Fig. 4 High magnification electron micrographs of type I monoaminergic terminals labeled with 5-hydroxydopamine (5-OHDA).

4a. Two varicose terminals exist in the vicinity of neurons of the intermediolateral nucleus. S, Soma of a neuron of the intermediolateral nucleus.

4b. Sagittal section of long axon incorporating type I boutons. Arrow heads indicate the "bouton de passage" type of 5-OHDA-labeled varicose terminals. Bars = 0.5 μm .

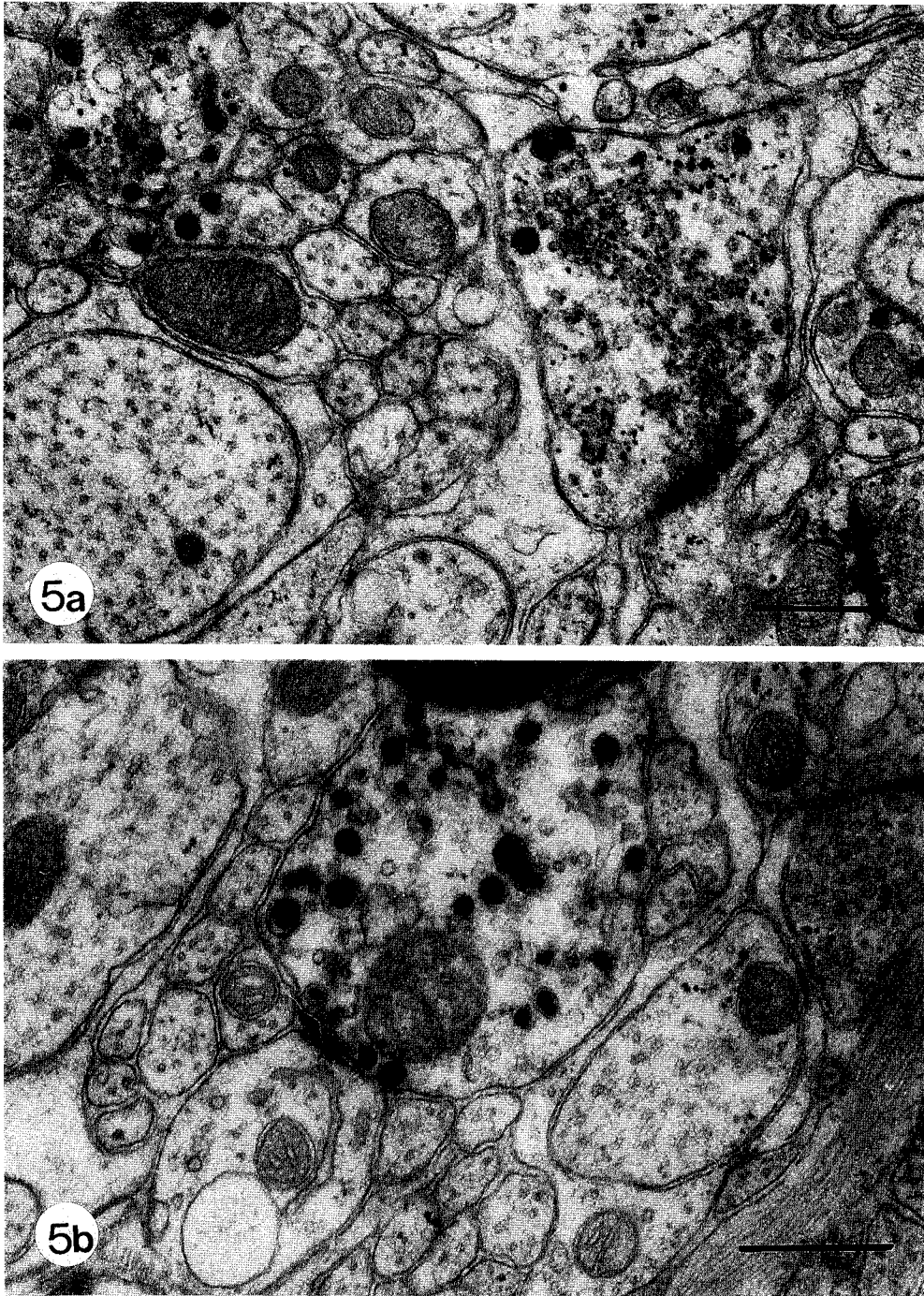


Fig. 5 High magnification electron micrographs of type II monoaminergic terminals labeled with 5-hydroxydopamine (5-OHDA). The terminals have a typical synaptic contact and contain many large and small 5-OHDA-labeled dense-core synaptic vesicles. Bars = 0.5 μ m.

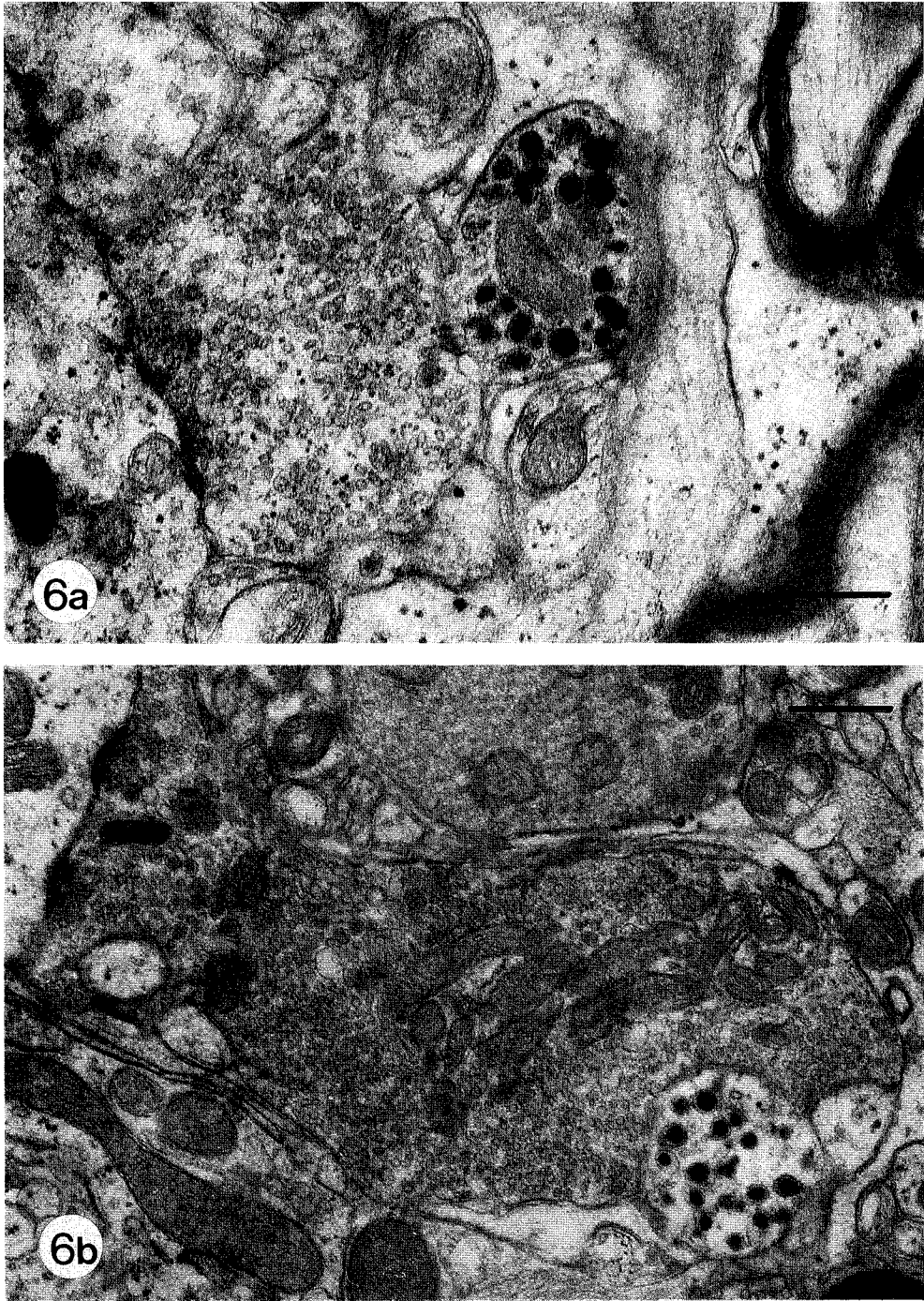


Fig. 6 High magnification of electron micrographs of type III monoaminergic terminals labeled with 5-hydroxydopamine (5-OHDA). The terminals have a synaptic contact with a presynaptic nerve ending containing many unlabeled vesicles. Bars = 0.5 μ m.

Table 1 Three types of monoaminergic terminals labeled with 5-hydroxydopamine in the intermediolateral nucleus of the cat thoracic cord

	Type		
	I	II	III
No ^a	274	199	22
(%)	(55)	(40)	(5)

a: A total of 495 labeled terminals were counted in samples from five cats.

III (Table 1.). The frequency was similar for each animal examined.

Discussion

Many fluorescence histochemical studies have shown that monoaminergic axonal terminals are richly distributed throughout the gray matter of the spinal cord and that the densest fluorescence accumulation is observed in the intermediolateral cell column (2-4, 6-8). According to recent immunohistochemical studies (16-18), many serotonin terminals exist in this nucleus. It has been confirmed that the intermediolateral nucleus receives many monoaminergic fibers including serotonin fibers bilaterally from brain stem nuclei (3-6, 10, 16, 18, 19). It is widely accepted that the monoaminergic plexus in the intermediolateral cell column plays a role in central cardiovascular control (9, 10). To investigate the ultrastructure of the monoaminergic terminals, Richardson (20) developed the potassium permanganate fixation method which, however, did not yield good preservation of the ultrastructure. Modified methods of glyoxylic acid perfusion combined with potassium permanganate fixation have been developed (14, 19, 21), but the structural synaptic membrane specialization is poorly preserved compared with the routine aldehyde and osmium tetroxide fixation method.

Recent advances in immunohistochemistry

have provided specific labels for terminals which contain noradrenaline and serotonin (16-18), but it is very difficult to study serotonergic and noradrenergic terminals of the spinal cord simultaneously by electron immunohistochemistry.

For these reasons, 5-OHDA, a false neurotransmitter, combined with routine aldehyde and osmium fixation, was employed in the present study. The disadvantage of this method is the inability to distinguish noradrenaline terminals from serotonin terminals because both terminals are able to incorporate 5-OHDA (13, 15). However, the ultrastructure of these terminals is well preserved, permitting three different types to be identified. Using this method, three different types of monoaminergic terminals were previously identified in the anterior horn region (15), but their numerical proportion differed from that found in the present study of the intermediolateral nucleus. Monoaminergic terminals often did not show typical synaptic contacts. This finding is in agreement with other observations (14, 19, 21-24). Descending noradrenaline fibers usually stimulate the intermediolateral nucleus (9, 10). Type III terminals are rarely observed and might be associated with an atypical function (25). Other investigators (14, 15, 23) have pointed out the existence of inhibitory synapses in this region. Type III terminals might coexist with some neuropeptides which have an inhibitory action.

In conclusion, the autonomic intermediolateral nucleus appears to be mainly influenced by monoaminergic type I and II boutons. It might also be influenced by type III terminals which seem on morphological grounds to have a different type of function.

Acknowledgments. The first author wishes to thank Prof. Y. Sano, Department of Anatomy, Kyoto Prefectural University of Medicine, for providing the opportunity to participate in this study and for giving valuable criticism of this manuscript, and Prof. P. L. McGeer, Kinsmen Lab-

orotory for Neurological Research, University of British Columbia, Canada, for his scientific and linguistic criticism.

References

1. Falck B, Hillarp N-Å, Thieme G and Torp A: Fluorescence of catecholamines and related compounds with formaldehyde. *J Histochem Cytochem* (1962) **10**, 348-354.
2. Carlsson A, Falck B, Fuxe K and Hillarp N-Å: Cellular localization of monoamines in the spinal cord. *Acta Physiol Scand* (1964) **60**, 112-119.
3. Dahlström A and Fuxe K: Evidence for the existence of monoamine neurons in the central nervous system. II. Experimentally induced changes in the intraneuronal amine levels of bulbospinal neuron systems. *Acta Physiol Scand* (1965) **64** (Suppl. 247), 1-36.
4. Fuxe K: Evidence for the existence of monoamine neurons in the central nervous system. IV. Distribution of monoamine nerve terminals in the central nervous system. *Acta Physiol Scand* (1965) **64** (Suppl. 247), 38-85.
5. Crutcher KA and Bingham WG Jr: Descending monoaminergic pathways in the primate spinal cord. *Am J Anat* (1978) **153**, 159-164.
6. Loewy AD, McKellar S and Saper CB: Direct projections from the A5 catecholamine cell group to the intermediolateral cell column. *Brain Res* (1979) **174**, 309-314.
7. Mizukawa K: The segmental detailed topographical distribution of monoaminergic terminals and their pathways in the spinal cord of the cat. *Anat Anz* (1980) **147**, 125-144.
8. McLachlan EM and Oldfield BJ: Some observations on the catecholaminergic innervation of the intermediate zone of the thoracolumbar spinal cord of the cat. *J Comp Neurol* (1981) **200**, 529-544.
9. Coote JH and MacLeod VH: The influence of bulbospinal monoaminergic pathways on sympathetic nerve activity. *J Physiol* (1974) **241**, 453-475.
10. Neumayr RJ, Hare BD and Franz DN: Evidence for bulbospinal control of sympathetic preganglionic neurons by monoaminergic pathways. *Life Sci* (1974) **14**, 793-806.
11. Tranzer J-P and Thoenen H: Electronmicroscopic localization of 5-hydroxydopamine (3,4,5-trihydroxyphenyl-ethylamine), a new 'false' sympathetic transmitter. *Experientia* (1967) **23**, 743-745.
12. Richards JG and Tranzer J-P: The ultrastructural localization of amine storage sites in the central nervous system with the aid of a specific marker, 5-hydroxydopamine. *Brain Res* (1970) **17**, 463-469.
13. Iyata Y, Nojyo Y, Matsuura T, Yoshikawa H and Sano Y: Electron microscopy of the arcuate nucleus of normal and 5-hydroxydopamine treated cats. *Cell Tissue Res* (1975) **160**, 139-153.
14. Katoh Y, Isomura G and Shimizu N: Fine structural organization of the lateral horn of the rat spinal cord as studied by glyoxylic acid-permanganate fixation. *Arch Histol Jpn* (1980) **43**, 445-458.
15. Mizukawa K and Takeuchi Y: Electron microscopic investigation of monoaminergic terminals to α -motoneurons in the anterior horn of the cat spinal cord. *Acta Med Okayama* (1982) **36**, 85-93.
16. Steinbusch HWM: Distribution of serotonin-immunoreactivity in the central nervous system of the rat, cell bodies and terminals. *Neuroscience* (1981) **6**, 557-618.
17. Kojima M, Takeuchi Y, Goto M and Sono Y: Immunohistochemical study on the distribution of serotonin fibers in the spinal cord of the dog. *Cell Tissue Res* (1982) **226**, 477-491.
18. Mizukawa K, Otsuka N and Hattori T: Serotonin-containing nerve fibers in the rat spinal cord, electron microscopic immunohistochemistry. *Acta Med Okayama* (1986) **40**, 1-10.
19. Satoh K, Kashiba A, Kimura H and Maeda T: Noradrenergic axon terminals in the substantia gelatinosa of the rat spinal cord, an electron-microscopic study using glyoxylic acid-potassium permanganate fixation. *Cell Tissue Res* (1982) **222**, 359-378.
20. Richardson KC: Electron microscopic identification of autonomic nerve endings. *Nature* (1966) **210**, 756.
21. Takahashi Y, Tohyama M, Satoh K, Sakumoto T, Kashiba A and Shimizu N: Fine structure of noradrenaline nerve terminals in the dorsomedial portion of the nucleus tractus solitarii as demonstrated by a modified potassium permanganate method. *J Comp Neurol* (1980) **189**, 525-535.
22. Hökfelt T: On the ultrastructural localization of noradrenaline in the central nervous system of the rat. *Z Zellforsch Mikrosk Anat* (1967) **79**, 110-117.
23. Réthelyi M: Cell and neuropil architecture of the intermediolateral (sympathetic) nucleus of cat spinal cord. *Brain Res* (1972) **46**, 203-213.
24. Swanson LW, Connelly MA and Hartman BK: Further studies on the fine structure of the adrenergic innervation of the hypothalamus. *Brain Res* (1978) **151**, 165-174.
25. Uchizono K: Morphological background of excitation and inhibition at synapses. *J Electron Microsc* (1968) **17**, 55-66.

Received July 30, 1987; accepted October 27, 1987