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POSITION AND SIZE OF THE AXON HILLOCK IN VARIOUS GROUPS OF NEURONS*

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The origin of the axon was studied in Golgi–Kopsch impregnated specimens prepared from the spinal cord and brain of adult rats. Five types of neurons were sampled: large ventral horn neurons, neurons in the intermediate zone and ventral horn of the spinal cord, antenna-type neurons in the spinal dorsal horn, neurons in the thalamus, and neurons in the hypothalamus. The axon originated from the perikaryon in 76% of the large ventral horn neurons and in 64% of the neurons in the thalamus. In contrast, the axon emerged from one of the dendrites in 75% of the neurons in the intermediate zone and the ventral horn of the spinal cord and in 68% of the neurons in the hypothalamus. In the case of the antenna-type neurons in the spinal dorsal horn, the axon often originated from one of the dendrites, but never from a dorsally oriented dendrite. The mean distance of the axon hillock of dendritic origin was the longest in the neurons in the intermediate zone and the ventral horn of the spinal cord. The size of the axon hillock was proportional to the size of the perikaryon. The impregnated portion of the axon was longest in the large ventral horn neurons.

Keywords: Axon hillock – spinal neurons – thalamic neurons – hypothalamic neurons – rat

INTRODUCTION

The origin of the axon (the axon hillock) is a unique area of multipolar neurons. Its primary role is the initiation of the action potential, but the axon hillock is the portion of the neuron from where the axoplasmic transport starts. The axon hillock and the ensuing initial segment are easily recognizable on Golgi specimens. Their ultrastructure has been described [10]. Even a superficial view of Golgi specimens, however, reveals numerous exceptions.

It is generally assumed that the axon emerges from the perikaryon, i.e. from the region where the dendrites converge. Schematic drawings and neuronal models unquestionably pin the axon to the perikaryon. In spite of this general assumption, Golgi specimens and intracellular stained neurons often reveal axons originating from one of the dendrites.

*Dedicated to Professor József Hámori on the occasion of his 70th birthday.

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The aim of this study was to detect the regularities in the position of axon emergence. Neurons with various morphological characters were compared in order to learn whether the size of the perikaryon, the shape of the dendritic tree or the myelinization of the axon facilitate a perikaryal vs. a dendritic origin of the axon.

MATERIALS AND METHODS

The Golgi–Kopsch impregnation procedure was used to study the structures of the neurons in the brain and spinal cord of young adult albino rats (body weight 120–140 g). Pieces of the lumbar and cervical spinal cord were cut in the transverse and sagittal planes. The brains of the same animals were cut in the coronal plane.

Neurons with a visible axon hillock were studied with a LUCIA image analyzing system (Laboratory Imaging, Ltd.) connected to a conventional microscope. The neurons were characterized by the dendritic arborization and the position of the axon hillock was determined. The dendritic arborization was either dense or sparse. The axon hillock was found partly on the perikaryon and partly along one of the dendrites. The axon hillock was often attached directly to the initial portion of one of the main dendrites. In this latter case, the origin was classified as perikaryal. In each neuron the surface area of the perikaryon was measured and the length of the diameter was calculated. Additionally, the diameter of the axon hillock and the length of the impregnated portion of the axon were measured. When the axon hillock was found along one of the dendrites, the distance between the edge of the perikaryon and the midpoint of the axon hillock was also measured.

A detailed quantitative analysis was made on the large neurons in the spinal ventral horn (presumably motoneurons; n = 55), on the medium-sized and small neurons in the spinal intermediate zone and ventral horn (n = 94), on the "tufted" thalamic neurons (n = 64) and on the small neurons in the medial-basal hypothalamus (n = 77). The large "antenna neurons" in the spinal dorsal horn were also analyzed, but no measurements were made because of the small sample size (n = 22).

RESULTS

I. Morphology of the neurons (size of the perikaryon, dendritic arbors, position of the axon hillock)

The *large neurons in the ventral horn* had several thick, straight dendrites. The axon emerged mainly from the perikaryon, independently from the dendrites (Figs 1 and 2). The axon hillock was prominent in size, and tapered gradually. Long portions of the straight initial axon segment were impregnated. Occasionally, the initial axon portion was immediately followed by an impregnated structure with an irregular contour line (Fig. 2). This ragged structure was interpreted as an irregular silver deposit between the lamellae of the first portion of the myelin sheath.

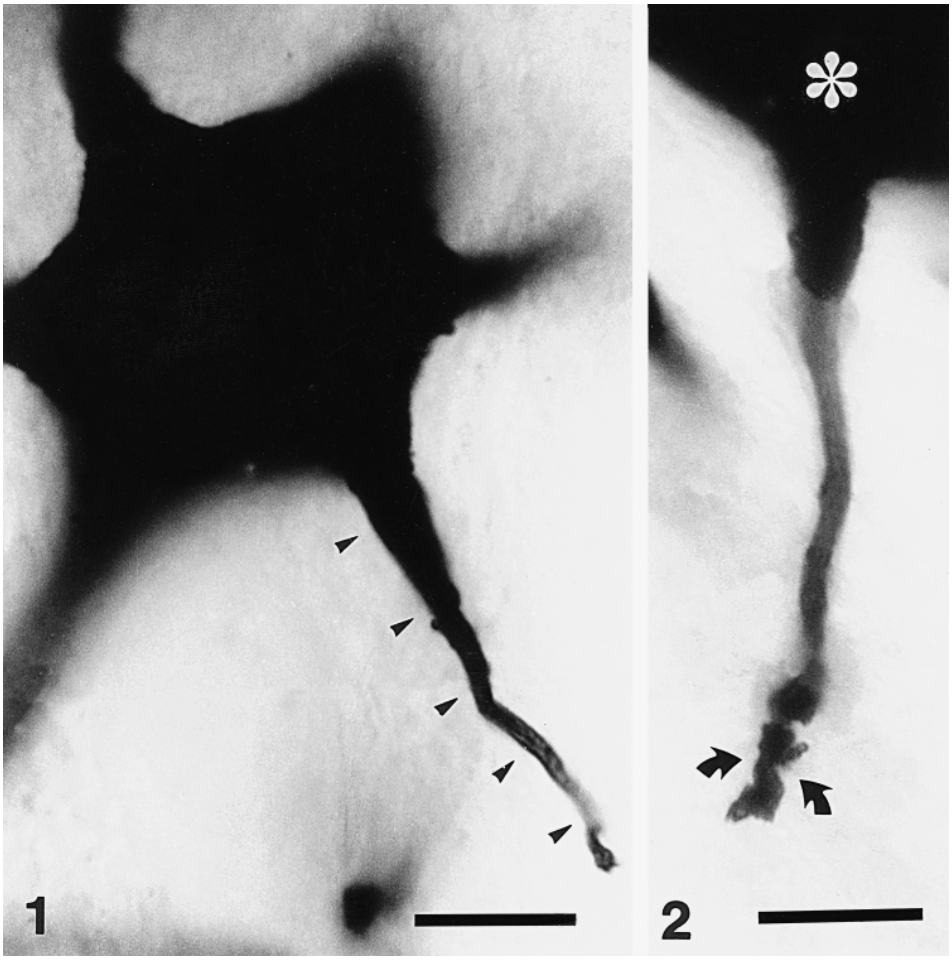

Fig. 1. Photomontage showing a large ventral horn neuron (transverse plane) with axon hillock and initial axon segment (arrowheads). The axon originates with a prominent, funnel-shaped hillock directly from the perikaryon and takes a relatively straight course with minor inflections. The impregnated axon portion ends abruptly. Several dendrites with various diameters originate from the perikaryon. Scale: 10 µm. *Fig. 2.* Photomontage showing a small detail of the perikaryon of a large ventral horn neuron (asterisk). The axon originates from the perikaryon with a giant-size axon hillock and courses with minor inflections for a long distance. The irregular silver precipitation at the end of the impregnated axon portion appears to belong to the myelin sheath. Scale: 10 µm

Small and medium-sized neurons are typical *in the spinal intermediate zone and ventral horn.* They had a few long and straight dendrites oriented to the transverse plane of the spinal cord. The dendrites divided only occasionally (Fig. 3). The thin axon of the neurons often originated from one of the main dendritic trunks or from one of the dendrites far away from the perikaryon. Occasionally, an axon of

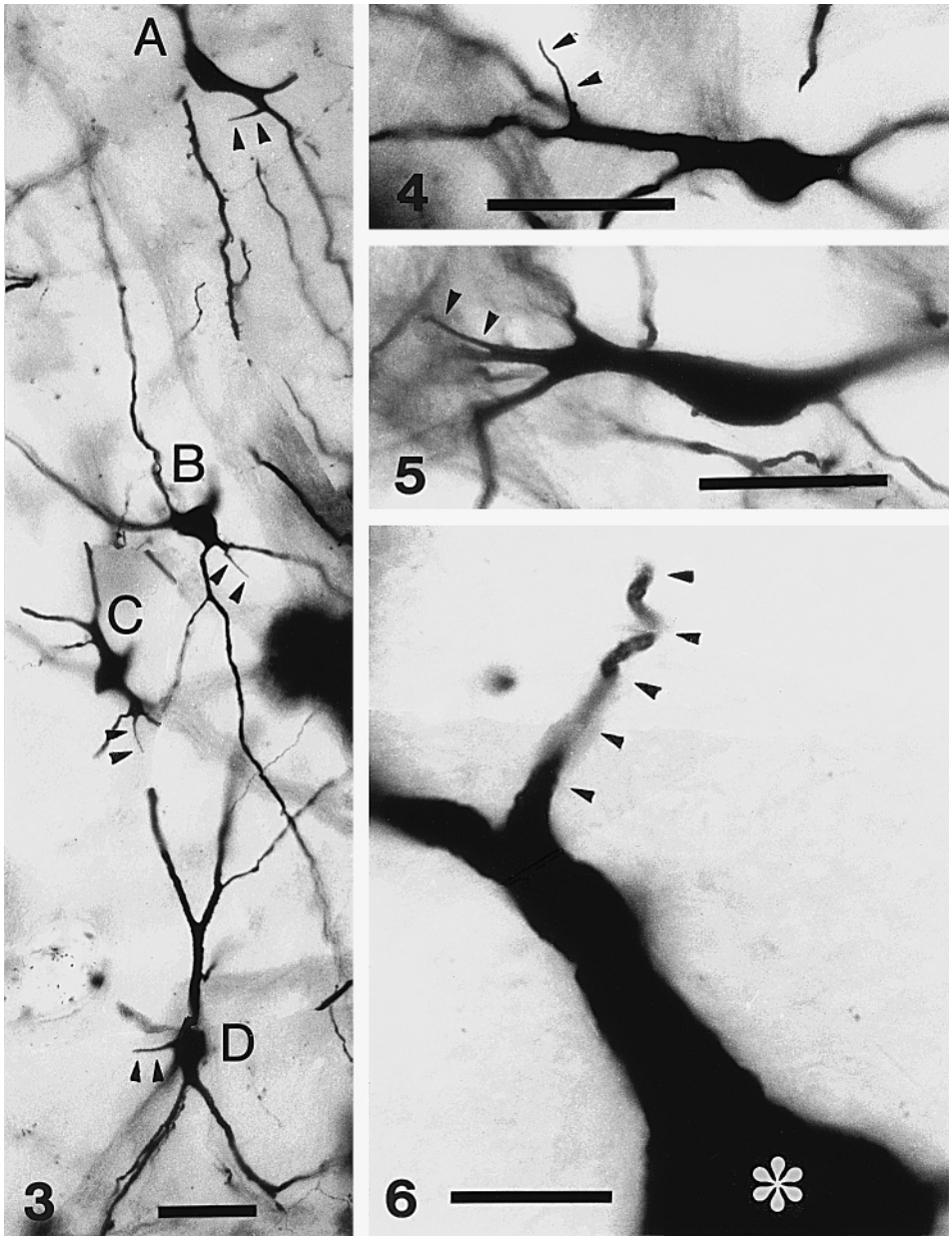

Fig. 3. Photomontage showing medium-sized and small neurons in the intermediate zone of the spinal cord (transverse plane). The perikarya of the impregnated neurons (labeled A, B, C and D) are round, triangular or spindle-shaped. The dendrites have a smooth surface, bifurcate occasionally and course straight for long distances. In neurons A, B and C the axon originates from one of the dendrites (arrowheads), while in neuron D the axon originates from the perikaryon (arrowheads). The impregnated portion of the axon is usually short, and the axon ends abruptly. Scale: 50 µm. *Figs 4, 5.* Photomicrographs

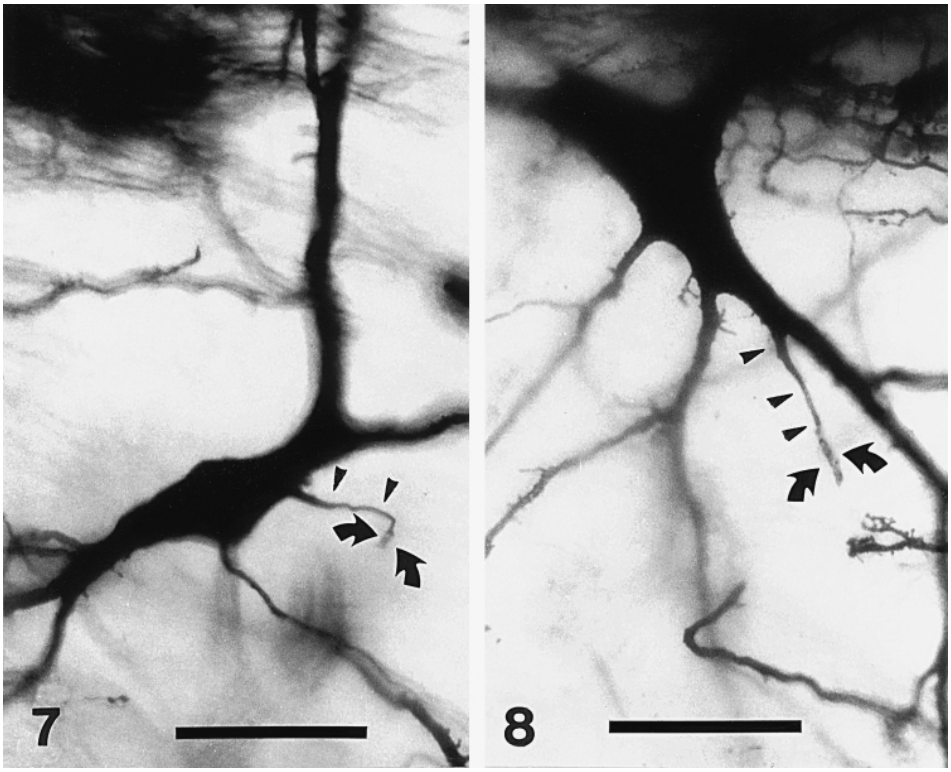

Fig. 7. Photomicrograph showing a spindle-shaped perikaryon in lamina III of the dorsal horn (sagittal plane) with dendrites coursing dorsally (right side) and ventrally (left side). The axon (arrowheads) originates from the perikaryon. The final portion of the impregnated process (between curved arrows) may correspond to the first portion of the myelin sheath. Scale: 50 μm. *Fig. 8.* Photomicrograph showing a large neuron at the dorsal border of lamina III (sagittal plane) with extensive dendritic arborization (the ventrally directed dendritic arbor is visible; the dorsal dendrites are in a different focal plane). The axon (arrowheads) originates from a ventrally directed secondary dendrite. The terminal portion of the impregnated axon is labeled with an irregular silver precipitate (between curved arrows) similar to that seen in Fig. 2. Scale: 50 μm

perikaryal origin could also be found. This group of neurons provided the most extreme examples as concerns the dendritic origin of the axon. Figure 4 shows a neuron with a round perikaryon and two main dendrites. The axon emerges from the second bifurcation of one of the main dendrites. Figure 5 depicts a neuron with a spin-

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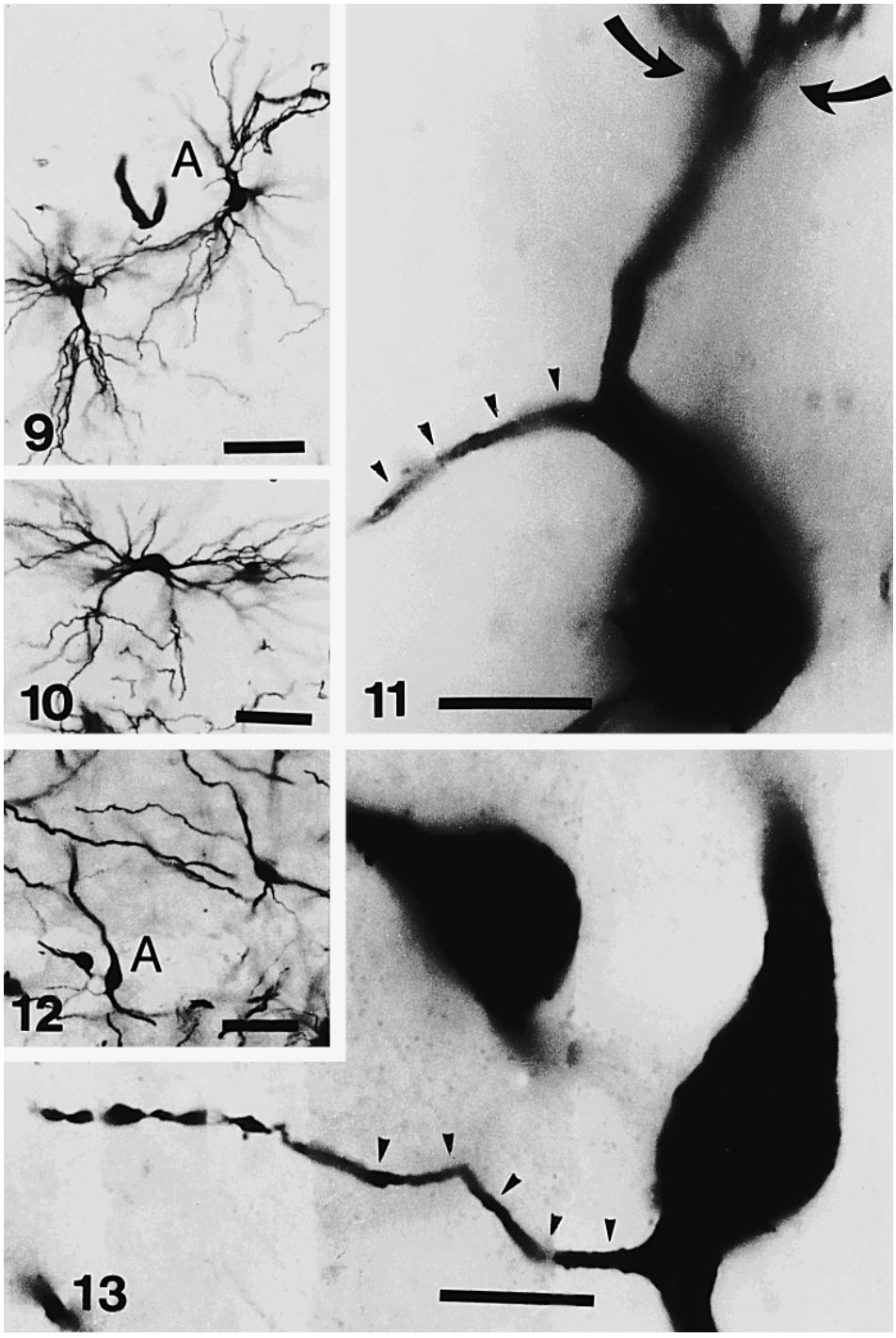
showing round and spindle-shaped neurons, the axons of which (arrowheads) originate from secondary or tertiary dendrites far from the perikaryon. Scale: 50 μm. *Fig. 6.* Photomicrograph showing the origin and initial course of the axon of a neuron in the intermediate zone of the spinal cord. The axon (arrowheads) originates from one of the main dendrites. (A small detail of the perikaryon is labeled with an asterisk.) The distal portion of the impregnated axon portion shows the spiraling course. Scale: 10 μm


A
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10
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Figs 9, 10. Photomicrographs showing medium-sized neurons in the dorsal portion of the thalamus (transverse plane). The perikaryon is at the center of the dense dendritic arborization (tufted neurons). Neuron labeled A in Fig. 9 can be seen at higher magnification in Fig. 11. Scale: 50 μm. *Fig. 11.* Photomicrograph showing details of neuron A in Fig. 9 at higher magnification. The axon (arrowheads) originates from the dorsally directed main dendrite not far from the perikaryon. This dendrite splits into a dense dendritic tree (between curved arrows) close to the upper margin of the picture (hence the name: tufted neurons). The impregnation of the axon ends abruptly. Scale: 10 μm. *Fig. 12.* Photomicrograph showing small neurons in the ventrobasal hypothalamus (transverse plane). The perikarya are spindle-shaped; the dendrites start at the poles of the perikaryon. The neuron labeled A can be seen at higher magnification in Fig. 13. Scale: 50 μm. *Fig. 13.* Photomontage showing the spindle-shaped neuron labeled A in Fig. 12. The axon (arrowheads) originates jointly with one of the dendrites. The thick initial segment takes a few turns and continues into a thin axon decorated with frequent varicosities. Scale: 10 μm

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dle-shaped perikaryon. The axon originates from one of the main dendrites distal to the second dendritic branch point (i.e. from a tertiary dendrite). The impregnated portion of the axons was relatively short; the terminal part of the initial segment frequently displayed a spiraling course (Fig. 6).

The *large "antenna type" neurons in the spinal dorsal horn* had dorsally and ventrally directed dendritic arborizations (Figs 7 and 8). The dorsal dendrites coursed vertically up to the most superficial regions of the dorsal horn (laminae I and II; Fig. 7), while the ventral dendrites arborized in laminae III and IV (Fig. 8). The axon originated either from the perikaryon (Fig. 7), or more frequently from one of the ventral dendrites (Fig. 8). None of the neurons observed had its axon originating from a dorsally oriented dendrite. In both neurons (Figs 7 and 8), the irregular, loose silver deposit at the end of the tapering initial axon segment seems to correspond to the beginning of the impregnated myelin sheath.

The *neurons in the thalamus* possessed frequently branching, dense dendritic trees coursing radially around the medium-sized perikarya (Figs 9 and 10). Figure 11 shows the characteristic branching pattern of the thalamic neurons (the same neuron as neuron A in Fig. 9): after a 20 to 25 μm straight course, the main dendrite splits abruptly into several secondary branches (therefore the name: tufted neurons). The axon emerges with a moderate axon hillock and the initial portion of the axon follows a short straight or slightly bending course (Fig. 11).

The typical *neurons in the medial basal hypothalamus* had small perikarya and a few (two, or rarely three), straight or slightly wavy dendrites (Fig. 12). The axon of the hypothalamic neuron in Fig. 13 originated from a dendritic trunk with a moderate axon hillock (the same neuron as neuron A in Fig. 12). The initial portion of the axon continued in a thin axon with multiple irregularly spaced *en passant* enlargements.

Table 1 summarizes for each group the percentage of neurons with axons originating from the perikaryon. Two-thirds or more of the large ventral horn spinal neurons and the thalamic neurons correspond to the classical image, i.e. the axon of the neuron emerges from the perikaryon. In contrast, axons in the same proportion of the neurons in the spinal intermediate zone and ventral horn as for the hypothalamic neu-

Table 1
Percentage of neurons with axons originating from the perikaryon

Type of neuron	No. of neurons studied	No. of neurons with axon originating from the perikaryon	Percentage (%)
Large ventral horn neurons	55	42	76
Neurons in the intermediate zone and ventral horn	94	24	25
Antenna-type neurons	22	9	41
Neurons in the thalamus	64	42	66
Neurons in the hypothalamus	77	25	32

rons emerged from dendrites. Antenna-type neurons in the dorsal horn take up an intermediate position, although the small number of neurons observed does not allow a generalization. It is important to emphasize, however, that the axons of this latter type of neurons were never found to originate from the dorsally oriented dendrites.

Table 2
Mean numerical data (μm) showing the size of the perikaryon, the diameter of the axon hillock, the distance of the axon hillock from the perikaryon and the length of the impregnated initial axon segment

Type of neuron	Diameter of the perikaryon (mean ± S.D.)	Diameter of the axon hillock (mean ± S.D.)	Distance of the axon hillock from perikaryon (mean ± S.D.)	Length of the impregnated axon (mean ± S.D.)
Large, ventral horn neurons (n = 55)	43.52 ± 3.67	7.16 ± 1.07	14.88 ± 5.27 (n = 13)	35.44 ± 5.24
Neurons in the intermediate zone and ventral horn (n = 94)	21.80 ± 3.18	3.54 ± 0.79	17.64 ± 7.58 (n = 70)	24.38 ± 4.05
Neurons in the thalamus (n = 64)	19.94 ± 1.57	3.40 ± 0.64	10.23 ± 4.66 (n = 22)	20.48 ± 6.83
Neurons in the hypothalamus (n = 77)	15.57 ± 1.19	2.64 ± 0.51	11.68 ± 5.70 (n = 52)	–

II. Quantitative data: size of the perikaryon, thickness of the axon hillock, distance of the axon hillock from the perikaryon, length of the impregnated axon (Table 2)

Diameter of the perikaryon. The large ventral horn neurons were the largest. The neurons in the intermediate zone and ventral horn were somewhat larger than the thalamic neurons. The neurons in the hypothalamus had the shortest average diameter.

The *diameter of the axon hillock* was proportional to the size of the perikaryon, both within a group of similar neurons and between the groups of different neurons. The large ventral horn neurons had the largest axon hillock, while the hypothalamic neurons had the smallest axon hillock. No systematic difference was found in the size of the axon hillock with respect to its perikaryal or dendritic position in any of the groups studied. A constant ratio of 0.16–0.17 was found between the average diameter of the perikaryon and the average diameter of the axon hillock in all four groups of neurons.

The *distance of the axon hillock from the edge of the perikaryon* had the largest average value in the group of neurons in the spinal intermediate zone and ventral horn. The diagram in Fig. 14 shows that in 6 neurons the beginning of the axon was more than 30 μm away from the edge of the perikaryon. The distance was somewhat shorter in the group of large ventral horn neurons and even shorter in the group of hypothalamic and thalamic neurons.

The *impregnated portion of the initial axon segment* was longest in the group of large ventral horn neurons (Fig. 15). It was much shorter in the group of neurons in the intermediate zone and ventral horn and even shorter in the thalamic neurons. The impregnated portion of the initial axon segment of the hypothalamic neurons continued in a much thinner axon portion with repeated *en passant* enlargements.

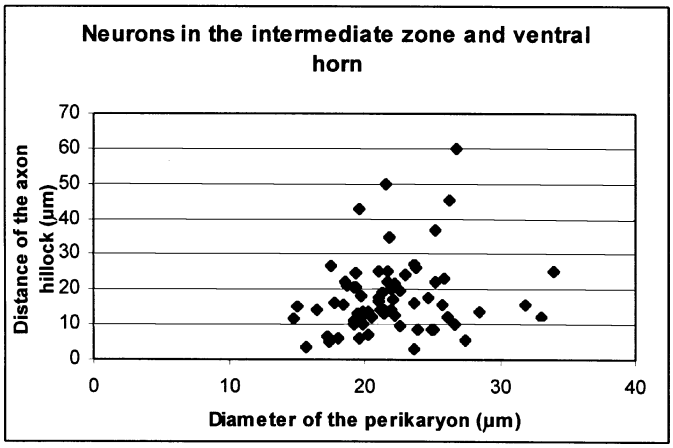

Fig. 14. Diagram showing the distance of the axon hillock from the edge of the perikaryon along a dendrite with respect to the size of the perikaryon. Each diamond represents a neuron in the intermediate zone and ventral horn

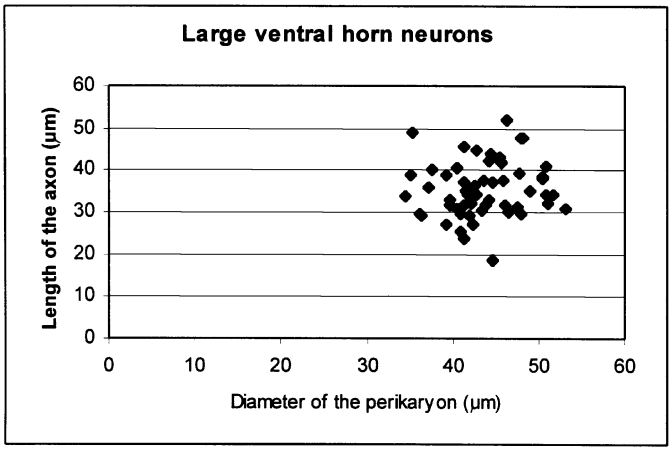

Fig. 15. Diagram showing the length of the impregnated portion of the axon with respect to the size of the perikaryon. Each diamond represents a large neuron in the ventral horn

DISCUSSION

Methodological considerations. It has to be emphasized that the axon hillock of the neurons is clearly recognizable in well-impregnated Golgi specimens. The tapering character, the straight or slightly bending course and even the contour line differentiate the axon hillock from the dendrites. Electron microscopic observations of the Golgi specimens showed that the silver deposit is confined to the perikaryon and processes. This indicates that the data obtained from the measurements are exact values.

The selected types of neurons are widely known. Large ventral horn neurons (motoneurons) are the prototype of multipolar neurons. Even the ultrastructure of the axon hillock of this type of neurons has been studied in the cat [4]. The medium-sized and small neurons in the spinal intermediate zone and ventral horn form functionally diverse groups. The orientation of their dendrites into the transverse plane of the spinal cord is a general morphological feature for the majority of these cells in the cat spinal cord [12]. Dorsal horn neurons with dendrites directed towards the superficial laminae in the cat were named antenna-type neurons by Szentágothai [15]. The tufted neurons in the thalamic nuclei are the thalamocortical relay neurons [6]. The dendritic and axonal arborizations of the neurons in the medial-basal hypothalamus were also described earlier [1, 8, 16].

Dendritic origin of the axon: cause and consequences. It is widely known that the axons of neurons may originate either from the perikaryon or from one of the dendrites. The present results indicate that the location of the axon hillock is a characteristic morphological feature of neurons. Neurons in which the axon takes its origin with higher probability from the perikaryon are the large ventral horn neurons in the spinal cord and the medium-sized, tufted thalamic neurons, most of them projecting

to the cerebral cortex [11]. Both of these types of neurons have extensive dendritic arborizations. Neurons in which the axon frequently originates from one of the dendrites are the small and medium-sized neurons in the spinal intermediate zone and ventral horn, and also the small hypothalamic neurons. The common morphological feature of these two groups of neurons is the sparse dendrites.

Axons originating from one of the dendrites were described by Ramon y Cajal. As an extreme example, he mentioned the crook-pattern neuron in the optic lobe of birds [11], in which the axon takes its origin from a straight dendrite far away from the perikaryon. This is the dendrite of the neuron that receives the fibers arriving from the retina. This peculiar arrangement serves the laws of economy of space, time and conductive matter, but the author did not offer any explanation for the phenomenon. On this basis, Ramon y Cajal formulated the dynamic polarization of neurons as axipetal dendritic and somatic polarization (towards the axon, wherever the axon is located) and dendrifugal or somatofugal axonal polarization. Much later, Morest [9] concluded that the outgrowth of the axon during neuronal development often precedes the final settlement of the perikaryon, and the site of the axon origin and the location of the nucleus of a developing neuron do not necessarily coincide. Although the perikaryon (nucleus) migrates intensively, in the case of the shepherd's crook cells in the chick embryo optic tectum it never reaches the position of the axon hillock [5].

On similar grounds, it is provisionally suggested that in neurons with few and sparsely branching dendrites the position of the migrating perikaryon (the location of the nucleus) is accidental along the continuum of the straight dendrites in the final phase of maturation. The perikaryon frequently migrates away from the fixed position of the axon hillock, and in these cases the axon originates from a dendrite. It would be unrealistic to speculate that the nucleus of a neuron is permanently "migrating" in the cytoplasm in mature neurons too, and the picture one sees in a Golgi specimen is the position of the nucleus at the moment of tissue fixation. The maximum distance of dislocation of the the axon was 59.92 μm for a neuron in the spinal intermediate zone (diameter of the perikaryon: 26.84 μm). It is interesting to compare this figure with that of Spruston et al. [14], who found the axon origin of a byocitin-filled substantia nigra dopamine neuron 215 μm away from the perikaryon.

Migration of the nucleus appears to be much less probable in neurons with extensive dendritic arborization. One may speculate that the spherically balanced dendritic tree keeps the nucleus in the centrum of the neuron, which is the site of emergence of the axon.

In many neurons the diameter of the dendrites at their origin from the cell body is proportional to the diameter of the cell body [2, 17]. This observation could be supplemented with the present finding that the mean diameter of the axon hillock within and between the various groups of neurons is proportional to the mean size of the perikarya. A larger sample and variety of neurons and the application of diverse staining techniques would be necessary to verify the validity of the ratio (0.16–0.17) found in this study.

It is widely accepted that only the unmyelinated portions of the axons can be impregnated with the Golgi technique. The continuous impregnation of the fine axons of the hypothalamic neurons in the present study supports this assumption in the positive sense. Therefore, the abrupt termination of the impregnation in the case of the other neurons could be seen as the beginning of the myelinization of the axons. The irregular silver deposit at the end of the impregnated axon in Figs 2, 7 and 8 could be interpreted as silver particles among the first myelin lamellae. Colbert and Johnston [3] filled the initial axon portion of hippocampal pyramidal neurons with an extracellular injection of byocitin, and measured the length of the axon up to the first myelin as 19.9 ± 0.75 μm ($n = 34$). This is somewhat smaller, but still within the range of similar figures measured in the groups of thalamic neurons. The theoretical neuronal model of Segev and London [13] puts the initiation of the action potential in the 1 μm thick axon about 200 μm away from the soma (soma size: 20 μm). This is several times larger than the longest length of the initial segment as found in the large ventral horn neurons in the present study.

The systematic and frequent dendritic origin of the axon in certain types of neurons raises questions in connection with the axoplasmic transport and dendritic integration. What kind of processes signal the origin of the axon to the perikaryon if this latter is not directly connected to the former? Does the dendrite from which the axon emerges have a unique position in the initiation of the action potential with respect to the other dendrites? This is irrelevant if all dendrites of a neuron receive synapses from the same kinds of presynaptic fibers. However, in the case of the antenna-type dorsal horn neurons, dorsal and ventral dendritic arbors receive different inputs due to the layered termination of primary afferent and other fibers [7]. Since the axon was never found to emerge from one of the dorsal dendrites, presynaptic fibers impinging on the ventral dendrites seem to have a much better opportunity to activate the antenna neurons.

In the background of sophisticated neurohistological and neurophysiological methods, Golgi specimens are of limited use for revealing really important morphological features of neurons. The frequent and systematic occurrence of axons originating from dendrites and the numerical data reported in the present study could be used in single neuron experiments and should be taken into consideration in neuronal modeling.

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