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Types of neurons of the subthalamic nucleus and zona incerta in the guinea pig — Nissl and Golgi study

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The studies were carried out on the subthalamus of adult guinea pigs. Golgi impregnation, Nissl and Klüver-Barrera methods were used for the study. In Nissl stained sections the subthalamic neuronal population consists of multipolar, fusiform, oval and pear-shaped perikarya. In two studied areas: nucleus subthalamicus (STN) and zona incerta (ZI) three types of neurons were distinguished. Type I, multipolar neurons with quadrangular, triangular or oval perikarya. They have 3–6 primary dendrites wich run slightly wavy and spread out in all directions. Type II, bipolar neurons with fusiform or semilunar perikarya, they have two primary dendrites. Type III, pear-shaped neurons with 1–2 dendritic trunks arising from one pole of the neuron. In all types of neurons axon emerges from the perikaryon or initial segment of a dendritic trunk and can be followed at a maximum distance of about 50 μm.

key words: subthalamic nucleus, zona incerta, types of neurons, Golgi and Nissl studies

INTRODUCTION

The aim of the present study is to set up some data of types of neurons in the subthalamic region of guinea pig. In available literature the neuronal morphology of the subthalamic nuclei was described only in a few mammals: in cat [12], galago [18] and maccaca [15,19], although the cytoarchitecture was investigated in many animals, mainly in laboratory and domestic mammals for example: in rat [13] and pig [26].

Experimental studies indicate that subthalamic nuclei give rise to a widespread system of descending and ascending projections that reach the reticular structures of the brain stem. They receive afferent fibres from the globus pallidus [2,6,20,22], cortex [3,14,21], nucleus parafascicularis thalami [9], tegmental pedunculopontin nucleus [20,23]. On the other hand the efferent fibres from the subthalamic nuclei reach mainly the globus pallidus and substantia nigra [5,6,11,17,24], the spinal cord [16,20] and the colliculus superior [10,20]. Some of the described connections may be of a reciprocal nature [4,5,11].

MATERIAL AND METHODS

The studies were carried out on the subthalamus of 6 adult guinea pigs. Preparations were made by means of the Bagiński and Golgi-Kopsch techniques, Nissl and Klüver-Barrera methods. The brains were cut into frontal and sagittal planes. The scraps, 60-μm-thick and 10-μm-thick, were prepared for the Golgi and Nissl methods, respectively. The microscopic images of the chosen impregnated cells were digitally recorded by means of a camera that was coupled with a microscope and an image processing system (VIST-Wikom, Warsow). From 50 to 100 such digital microphotographs were taken at different focus layers of the section for each neuron. The computerized reconstructions of microscopic images were made on the basis of these series. The neuropil was removed to clarify the picture.

RESULTS

The neurons were categorized by size and shape of soma, distribution of tigroidal substance, number and arborization of dendrites and location of axon.

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On the basis of these criteria in the nucleus subthalamicus and zona incerta the following types of neurons were distinguished:

Type I (Fig. 1, 2) multipolar neurons — they constitute about 55% of the total populations of STN and ZI neurons. In this type as regards the shape of perikaryon and dendritic trunks two subclasses can be distinguished: subclass 1a (Fig. 1) and subclass 1b (Fig. 2). Most of type I neurons consist of the 1a subclass, its neurons have 4–6 (rarely 3) rather thin primary dendrites which spread out in all directions and perikarya from quadrangular to oval in shape which measure from 20 to 35 μm. Their dendrites run slightly wavy, sometimes change their directions, and some of them may be followed at a distance of about 300 μm within the same frontal-section, more rarely within the sagittal-section. These dendrites divide once, twice or not at all but sometimes give off collaterals. Usually two or three dendritic trunks bifurcate after 10–40 μm from the soma into quite long branches, whereas the remaining dendrites can bifurcate at various distances. Tertiary branches are not numerous. Within the 1a subclass there are neurons with smooth dendrites but also dendrites with various numbers of processes. These processes have either delicate filiform, or stalked appendices, which are usually seen on secondary dendrites and their branches but occasionally they are seen on the dendritic trunks. The secondary and tertiary dendrites can be varicose or beaded along their whole extent. Axon emerges from the perikaryon often close to one of the dendritic trunks, orientates usually medially and is impregnated only in its initial segment.

The 1b subclass (Fig. 2) — neurons have three thick primary dendrites that conically arise from the mostly triangular soma. In general, two primary dendrites divide dichotomically after 10–30 μm and quite often once again at the distance of about 15–40 μm from the first bifurcation. The third primary dendrite may be even thicker than the remaining ones and may not divide but only gives off collaterals. The dendrites of these neurons are devoid of appendages. Axon hillock is prominent and gives off thin axon that usually directs antero-laterally and can be followed at a maximum distance of about 50 μm. No collaterals have been seen arising from the axon.

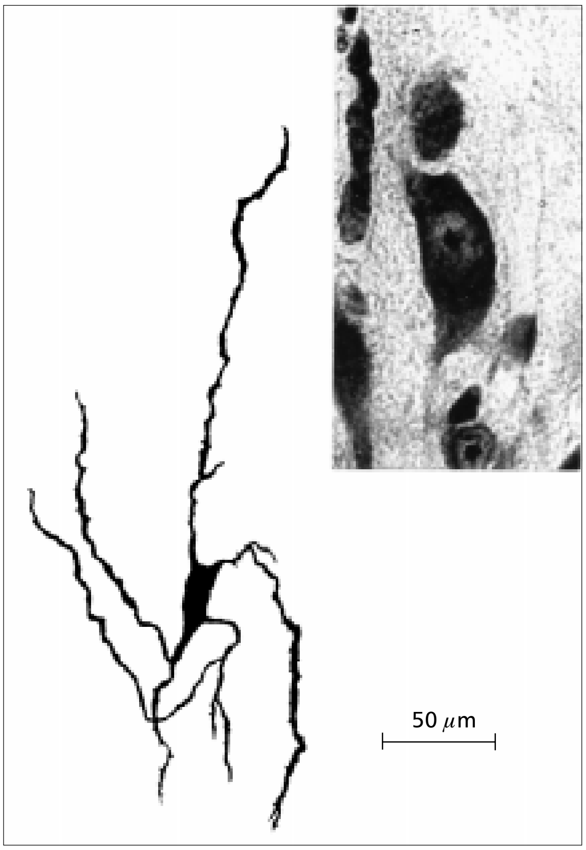

Figure 1. Computerized reconstruction of Golgi impregnated neuron of type I (1a subclass). The Nissl stained soma (insert).

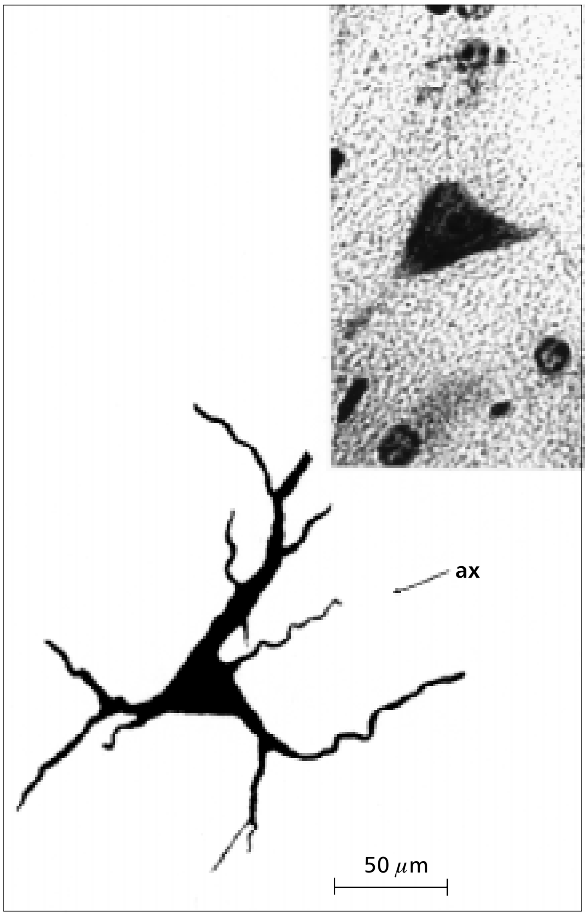

Figure 2. Computerized reconstruction of Golgi impregnated neurons of type I (1b subclass), ax — axon. The Nissl stained soma (insert).

Within STN it can be observed that in its medial region there is a preponderance of oval perikarya (1a subclass) whereas in the lateral part of STN in the majority are perikarya with quadrangular and triangular somata (1a and 1b subclasses) are thus in equal. In the zona incerta all the shapes of perikarya are uniformly placed throughout its whole extent. The neurons of type I account for about 34% of ZI neurons and about 75% of STN neurons. Their dendrites run in all directions causing the dendritic field to range in shape from oval to round.

Type II (Fig. 3) bipolar neurons — they account for about 40% of the total number of cells in both studied areas. The fusiform perikarya (rarely semilunar) measure from 20–40 μm along the long axis. Two primary dendrites emanate in opposite directions from the poles of the cell body whereas the dendrites of semilunar perikarya direct slightly ventrally. The primary dendrites bifurcate into secondary dendrites after 20–35 μm of their route, sometimes one of the primary dendrites divides dichotomically very close to the cell body or gives off collaterals. Some of the secondary dendrites may divide once again at a distance of about 30–50 μm from the first bifurcation. The dendrites can be covered either with relatively many or only a few delicate filiform spines or bead-like protuberances whereas others are devoid of them and they are smooth. The dendritic appendages are concentrated on the secondary and tertiary dendrites and their terminal segments; sporadically they are met on the dendritic trunks. The bipolar neurons (type II) have fewer appendages than the multipolar types (type I). The axon arises either from the cell body or from the proximal part of the dendritic trunk and runs antero-laterally and can be seen at the distance of about 30–40 μm. The dendrites of fusiform neurons run in rostral and caudal directions so the dendritic field is of stream-like shape. The neurons of the type II on the cross-sections in zona incerta are distributed uniformly throughout its rostro-caudal extent and they constitute about 65% of the total number of ZI cells. In STN the neurons of type II account for about 20% of all cells and they are mainly seen on the periphery of the nucleus.

Type III (Fig. 4) contains pear-shaped neurons — the least numerous in our material, about 5% of the total number of cells in STN and ZI. Their perikarya measure from 15 to 20 μm. They possess 1 or 2 thick dendritic trunks arising from one pole of the neuron. The dendritic trunks divide dichotomically into

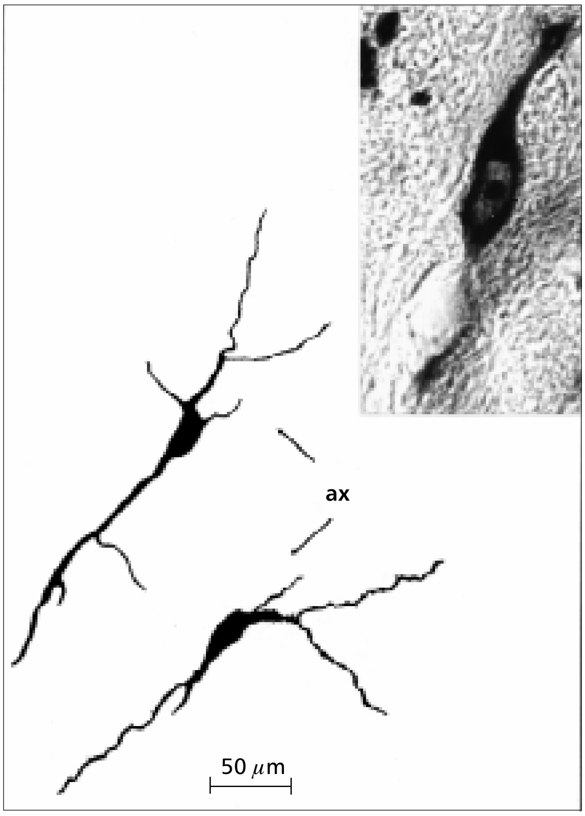

Figure 3. Computerized reconstruction of Golgi impregnated neurons of type II (ax — axon). The Nissl stained soma (insert).

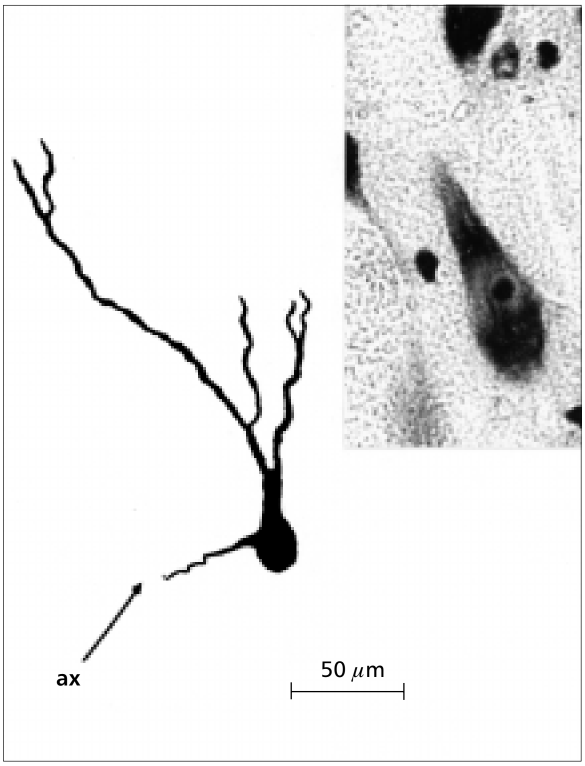

Figure 4. Computerized reconstruction of Golgi impregnated neuron of type III (ax — axon). The Nissl stained soma (insert).

secondary and tertiary dendrites after the course of about 20–60 μm and sporadically once again at a long distance from the perikaryon. Appendages are absent or are only occasionally seen. A thin axon emerges from the perikaryon at some distance from the dendritic trunk and directs usually medially (sometimes ventrally) and can be seen at the maximum distance of about 50 μm. The dendrites run in dorsal directions making the dendritic field fan-shaped. The neurons are observed mostly in STN in its medial region whereas in ZI they are only occasionally met.

Nissl staining

The lens-shaped nucleus subthalamicus is the best-developed nucleus of the subthalamic region. The posterior pole appears slightly backwards of the posterior pole of the zona incerta and extends forwards for about 600 μm. The nucleus is bordered dorsomedially by lenticular fasciculus, ventrolaterally by cerebral peduncule. On the dorsolateral side of STN lies zona incerta, on the ventromedial side mamillary peduncle (in back) and area hypothalamica lateralis (in front). In the nucleus subthalamicus there are visible multipolar and fusiform perikarya from 20–35 μm, and less numerous triangular, oval or pear-shaped perikarya from 15–25 μm. It is evident that there are differences in cell sizes and cell concentration between the medial and lateral parts. Pear-shaped and oval perikarya constitute the medial part whereas the lateral region consists mostly of quadrangular and triangular cells. Cells of the medial part are slightly smaller (from 15–30 μm) and more densely packed than in the lateral region of the STN. The perikarya of STN are intensively stained and contain relatively large, also deeply stained nucleus. In the neuroplasma there are a lot of thick and medium-size densely distributed granules of tigroid matter, which in fusiform, multipolar and pear-shaped cells deeply penetrates into the initial portion of the dendritic trunks.

Zona incerta, on the cross-sections, extends as a band of cells between the mamillary bodies and the nucleus reticularis thalami. Medially the ZI is surrounded by nucleus thalamicus fascicularis, ventrally by nucleus subthalamicus and ventrolaterally by capsula interna. Its length is about 900 μm. The zona incerta is mainly made of fusiform and multipolar cells from 25–40 μm but triangular and pear-shaped cells from 20–25 μm are sporadically met. All the perikarya are met through its whole extent. The perikarya of zona incerta are intensively stained and they have thick and medium-size granules of tigroid matter that enters deeply into the initial portion of primary dendrites, especially in fusiform cells.

DISCUSSION

In guinea pig in the Nissl pictures the subthalamic nuclei (STN and ZI) consist of multipolar, fusiform, triangular and pear-shaped cells. In the zona incerta the commonest are the fusiform and multipolar cells and they are uniformly distributed throughout its whole extent, similarly to the pig [26], whereas in rat [13] all shapes of perikarya appear only in three parts of ZI. Kawana and Watanabe [13], basing on their results, thought that axons of ZI neurons are topographically arranged in triple centrifugal fashion. The STN in guinea pig is divided into the medial and lateral region on the basis of cell sizes and their concentration. The medial region consists of all types of cells (quite often oval and pear-shaped) whereas in the lateral region mostly the multipolar and fusiform cells are seen. This is also compatible with the distribution of neurons of STN in the Golgi scraps. The mediolateral division of STN is supported by several authors [4,19,24,26]. It was determinated that the medial part of STN was related to the ventral pallidum [2,11] and substantia nigra [24], whereas the lateral part to the globus pallidus [2,11,25].

In guinea pig there are no clear borders between ZI and STN cells, thus this common incerto-subthalamic population was segregated into 3 types of neurons. Similarly to guinea pig also in other studied mammals (cat, prosimians, monkey), generally 3 categories of neurons were distinguished, but the criteria and nomenclature used by authors [12,18, 19] are different.

The multipolar nerve cells (type I) in guinea pig correspond most probably to the radiate or polygonal cells in primates [4,15,18,19] and to type II in the cat [12]. These cells show a similar shape of soma and dendritic field but vary in the differentiation of dendritic spine-like protuberances and appendages. The presence of a mixed population of neurons with different spines is confirmed by Rafols [19]. Electron microscopic studies [21,22,23,25] demonstrated that most terminals derived from pallidum, nucleus tegmental pedunculopontinus and cerebral cortex make synaptic contacts with dendritic spines and proximal dendritic trunks and also with somata and somatic spines. According to Chang and co-workers [7] two kinds of terminals can be observed in the

STN in rat. The terminals of the first class form synapses with thin dendrites in the rat; this can correspond to the neurons of type I (1a subclass) and to type II in guinea pig. The second class of terminals probably contains synapses interacting with somata and large dendrites; such thick dendrites possess cells of type III and I (1b subclass). Immunocytochemical techniques [1] suggest that neurons of the nucleus subthalamicus are glutamatergic and they are inhibited by pallidal stimulation [2], however there are different opinions as regards GABAergic neurons in STN [25].

Bipolar neurons (present study) correspond most probably to the elongate-fusiform cells in primates [4,15,18,19]. In our study the number of bipolar neurons in zona incerta are in the majority (about 65%) whereas in nucleus subthalamus they are in the minority (about 20%), like in the primate [19]. In addition the bipolar neurons have fewer appendages than the multipolar ones, which is also observed in galago [18]. In guinea pig many dendrites could be traced to their terminal segments within the same section, rather in the frontal than the sagittal sections. This suggests that most dendrites in the studied nuclei are orientated mediolaterally and only a few rostrocaudally. A similar orientation of dendrites was observed in the subthalamic nuclei in primates [15,19]. In guinea pig in both studied nuclei axon is seen only in its initial segment, which may mean that in this point axons are covered with myelin sheet. In type I and III, in general, axons usually are directed medially, but in type II axons emanate rostrally and laterally. In our material no collaterals have been seen arising from the axon, which was also reported by Pearson [18] and Rafols [19] in contrast to Chang [8] and Ivahori [12], who described many axon-collaterals.

The pear-shaped neurons, which are the least numerous in our material, have not been observed in previous Golgi studies. All types of neurons in the subthalamic nuclei of guinea pig have similar morphology and any cells which could correspond to the local interneurons described in the primate [15,18,19] were not seen in our material, just as in cat [12] and rat [7]. According to van der Kooy and Hattori [24] most if not all of rat STH neurons are projection cells. This can suggest that all cells of STN and ZI in guinea pig are tentatively considered to be projection cells. It must be kept in mind that Golgi methods selectively impregnate neurons, thus it is difficult finally to solve this problem.

REFERENCES

1. Albin RL, Aldridge JW, Young AB, Gilman S (1989) Feline subthalamic nucleus neurons contain glutamate-like but not GABA-like or glicine-like immunoreactivity. Brain Res, 491:185–188.
2. Bell K, Churchil L, Kalivas PW (1995) GABAergic projection from the neutral pallidum and globus pallidus to the subthalamic nucleus. Synapse, 20: 10–18.
3. Canteras NS, Shammah-Lagnado SJ, Silva BA, Ricardo JA (1988) Somatosensory inputs to the subthalamic nucleus: a combined retrograde and anterograde horseradish peroxidase study in the rat. Brain Res, 458: 53–64.
4. Carpenter MB (1982) Anatomy and physiology of the basal ganglia. Schaltenbrand G, Walker AE, Hassler R, Narabayashi H, Riechert T. Stereotaxy of the human brain. Anatomical, physiological and clinical applications. George Thieme Verlag. Stuttgart, pp. 233–262.
5. Carpenter MB, Strominger NL (1967) Efferent fibres of the subthalamic nucleus in the monkey. A comparison of the efferent projections of the subthalamic nucleus, substantia nigra and globus pallidus. Am J Anat, 121: 41–72.
6. Carpenter MB, Batton RR, Carleton SC, Keller JT (1981) Interconnections and organization of pallidal and subthalamic nucleus neurons in the monkey. J Comp Neurol, 197: 579–603.
7. Chang HT, Kita H, Kitai ST (1983) The fine structure of the rat subthalamic nucleus: an electron microscopic study. J Comp Neurol, 221: 113–123.
8. Chang HT, Kita H, Kitai ST (1984) The ultrastructural morphology of the subthalamic-nigral axon terminals intracellularly labeled with horseradish peroxidase. Brain Res, 299: 182–185.
9. Feger J, Bevan M, Crossman AR (1994) The projections from the parafascicular thalamic nucleus to the subthalamic nucleus and the striatum arise from seperate neuronal populations: A comparision with the corticostriatal and corticosubthalamic efferents in a retrograde fluorescent double-labelling study. Neuroscience, 60: 125–132.
10. Ficalora AS, Mize RR (1989) The neurons of the substantia nigra and zona incerta which project to the cat superior colliculus are GABA immunocytochemistry and lectin retrograde transport. Neuroscience, 29: 567–581.
11. Gronewegen HJ, Berendse HW (1990) Connections of the subthalamic nucleus with ventral striatopallidal parts of the basal ganglia in the rat. J Comp Neurol, 294: 607–622.
12. Iwahori N (1978) A Golgi study on the subthalamic nucleus of the cat. J Comp Neurol, 182: 383–398.
13. Kawana E, Watanabe K (1981) A cytparchitectonic study of zona incerta in the rat. J Hirnforsch, 22: 535–541.
14. Kitai ST, Denian JM (1981) Cortical inputs to the subthalamus: intracellural analysis. Brain Res, 214: 411–415.
15. Ma TP, Hu XJ, Anavi Y, Rafols JA (1992) Organization of the zona incerta in the macaque: A Nissl and Golgi study. J Comp Neurol, 320: 273–290.
16. Mizuno N, Veyama T, Itoh K, Satoda T, Tashiro T, Shigemoto R (1988) Direct projections from the subthalam-

ic nucleus of Luys to the spinal cord in the Japanese monkey. Neurosci Lett, 89: 13–18.
17. Nauta HJW, Cole M (1987) Efferent projections of the subthalamic nucleus: An autoradiographic study in monkey and cat. J Comp Neurol, 180: 1–17.
18. Pearson JC, Norris JR, Phelps CH (1985) Subclassification of neurons in the subthalamic nucleus of the lesser bushbaby (*Galago senegalensis*): A quantitative Golgi study using principal components analysis. J Comp Neurol, 238: 323–339.
19. Rafols JE, Fox CA (1976) The neurons in the primate subthalamic nucleus: A Golgi and electron microscopic study. J Comp Neurol, 168: 75–112.
20. Ricardo JA (1981) Efferent connections of the subthalamic region in the rat. The zona incerta. Brain Res, 214: 43–60.
21. Romansky KV, Usnoff KG, Ivanov DP, Galabov GP (1979) Corticosubthalamic projection in the cat: an electron microscopic study. Brain Res, 163: 319–322.
22. Romansky KV, Usnoff KG, Ivanov DP, Hassler R (1980) Pallidosubthalamic projection in the cat. Anat Embryol, 159: 163–180.
23. Romansky KV, Usnoff KG (1983) Electron microscopic identification of reticulo-subthalamic axon terminals in the cat. Neurosci Lett, 42: 113–117.
24. van der Kooy D, Hattori T (1980) Single subthalamic nucleus neurons project to both the globus pallidus and substantia nigra in rat. J Comp Neurol, 192: 751–768.
25. van der Kooy D, Hattori T, Shannak K, Horynkiewicz O (1981) The pallido-subthalamic projection in rat: Anatomical and biochemical studies. Brain Res, 204: 253–268.
26. Welento J (1964) Budowa i topografia jąder międzymózgowia świni. Jądra subthalamus. Ann Univ M Curie-Skłodowska. 10: 161–168.