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The neuronal structure of the mamillary nuclei in guinea pig: Nissl, Klüver-Barrera and Golgi studies

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The neurons of the mamillary body of adult guinea pigs were classified into four types: Type 1 — unidendritic cells with rounded perikarya (7–16 µm) and one thick primary dendrite, mostly dividing into tortuous secondary branches; Type 2 — bipolar cells: curly or simple ones with fusiform perikarya (13–22 µm); the curly-bipolar neurons possess 2 primary dendrites which may divide, even into tertiary dendrites, but each of them runs in screw-like or bending patterns; the simple-bipolar neurons have slender dendrites following a more straight route; Type 3 -multipolar cells with cap-like perikarya (10–20 µm) and 2–3 dendritic trunks originating from the base of the perikaryon and running in a wavy pattern; sometimes their dendrites possess spiny-like protrusions; Type 4 — multipolar cells with triangular or quadrangular perikarya (13–28 µm) and 3–4 dendritic trunks, poorly ramified, having a rather rectilinear course. In all types of neurons, dendritic spines are absent or rare. The majority of these neurons have a short impregnated axon originating from the perikaryon or primary dendrite.

key words: medial mamillary nucleus, lateral mamillary nucleus, types of neurons, Nissl and Golgi studies, guinea pig

INTRODUCTION

The nuclei of the mamillary body are a nodal link within the Papez circuit [23,27] i.e. a closed circuit of interconnected cortical and subcortical areas, which have to receive information from the environment by connections with the sensory system [25]. The classical connections of the mamillary body form the most prominent pathways: mamillary peduncule, fornix and principal mamillary fascicle containing mamillothalamic tract and mamillotegmental tract [3,4,9,11,23]. Some studies suggest that other significant or more discrete connections exist [1,16,19,20,25] varying in their chemical nature; they are serotonin [20,26], [Leu]enkephalin [10] related to mamillothalamic tract, and histamin [6] connected with magnocellular mamillary nuclei [13] and probably related to the mamillary peduncle. On the other hand in the mamillary nuclei, there are no CCK [28] and some monoaminergic cell groups [8,26] although they appear in the remaining regions of the hypothalamus. The mamillary body neurons contain enkephalins [10] and KGDHC, which may be colocalized with ChAT [5], however the latter was absent in the hypothalamus [12]. Basing on data of other investigators, Sikes and Vogt [25] suggest that the mamillary body and some other limbic structures do not contain projection neurons that synthesize ACh, serotonin, norepinephrine, substance P, and neurotensin [more see 8,19]. Thus, the mamillary body has a special position within the hypothalamus and differs from other hypothalamic nuclei in many aspects: in blood supplying [2], projections and biochemical content [5,8,10,14,17–19,27–29] and acting that still remain obscure. Many cytoarchitectonic data based on Nissl and Klüver-Barrera studies about the mamillary region in various mammals exist [3,4,9,11,23,24]. The aim of the present paper was to describe types of neurons in the mamillary body of guinea pig, using two modifications of the Golgi impregnation technique.

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MATERIAL AND METHODS

The present study was carried out on the brains of 6 adult female guinea pigs, Dunkin-Hartley strain, from The Research Institute of the Polish Mother's Health Centre in Łódź. The transversal and sagittal blocks were either cut into 10-μm-thick sections and stained by the Nissl as well as Klüver-Barrera methods or were impregnated according to the Bagiński and Golgi-Kopsch techniques and cut into 60-μm-thick sections. The specimens were afterwards analysed in a light microscope. The microscopic images of chosen, impregnated cells were digitally recorded by means of camera that was coupled with a microscope and image processing system (VIST — Wikom, Warsaw). From 40 to 80 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

Nissl study

In Cavia porcellus the mamillary body (MBs) contains two nuclei: the medial mamillary nucleus (Mm) and the lateral mamillary nucleus (Ml), and is surrounded by the supramamillary nucleus (Sm) and the posterior part of the tuberomamillary nucleus (Tmp). The last one lies close to the Ml, and their cells are mixed together, thus their cells are clearly distinguishable only on the Nissl slides but not on the Golgi preparations. The posterior part of the medial mamillary nucleus is formed by cells which are smaller and more homogenous in shape than those at the anterior one. However, the cells of the whole medial mamillary nucleus are smaller than in the lateral mamillary nucleus. Moreover, the long axes of neurons at the posterior sector of Mm are oriented horizontally, while at the anterior, usually in the direction of fibres leaving the mamillary body (principal mamillary fascicle). The glia cells at the posterior part of Mm are more numerous than at the anterior part. On the Nissl scraps the population of Mm neurons of guinea pig consists of very heterogenous perikarya. There are rounded and oval, pear-shaped and fusiform, triangular and polygonal including cap-like perikarya, generally measuring from 6 to 24 μm. Most Mm cells stain relatively dark and contain a large nucleus often irregular in shape and stain as intensively as cytoplasm. Usually perikarya contain a relatively large amount of the cytoplasm and medium-sized or large granules of the tigroid substance, which penetrate into dendritic trunks. Rather small perikarya with a light nucleus were occasionally found and they were almost devoid of cytoplasm. The cell nucleus contain intensively stained 1 rare 2 nucleoli. The cells of the lateral mamillary nucleus are bigger, up to 34 μm, and have regular form and always a lot of cytoplasm. The large-sized granules penetrate deeply even into the secondary branches of their dendrites. In Ml there is a preponderance of cells with a dark but well outlined nucleus. Within the medial mamillary nucleus were present neurosecretory-like perikarya that were placed around vessels or along the principal mamillary fascicle. Somewhat, their large sizes and appearance (the irregular shape of these cells, large tigroidal granules and light nucleus and sometimes appearing vacuoles) resemble the perikarya of the posterior part of the tuberomamillary nucleus. The cells of Tmp appear from the posterior pole of Mm along its ventral and lateral surfaces forwards and sometimes near the emanating fascicle by the dorsal edge of this nucleus. Thus, these cells may be included in the examined neuronal structure of the mamillary body in guinea pig.

Golgi study

The following criteria were taken into account in the classification of neuron types: the shape and size of perikarya, the number and arborization of dendritic trunks, the appearance of dendritic spines and also the axon, as well as inner structure of Nissl-stained cells. On the basis of these criteria, four types of neurons were distinguished in the mamillary body:

Type 1 (Fig. 1) — unidendritic cells with rounded or oval perikarya, from 7 to 20 μm. They have 1 dendritic trunk, which may divide near or at some distance (12–50 μm) from the cell body. In most cases this dendrite is thick and has a straight route and divides most often on secondary dendrites, which bend and follow a loop-like route. They are wavy and have various thread-like protrusions but typical dendritic spines are either absent or rare. Occasionally, the primary dendrite is thin, possesses varicosites and a course like the other secondary dendrites. The dendritic field has a stream-like or ball-shaped form. Usually the short axon leaves the soma tapering straight or in a spiral way. This type constitutes about 26% of the neuronal population of the medial mamillary nucleus and only 7% in the lateral mamillary nucleus.

Type 2 (Fig. 2) — bipolar cells emanating dendritic trunks from two opposite poles, with fusiform or rounded perikarya, from 10 to 36 μm. In this type, two kinds of neurons: curly and simple ones (as regards the route of their dendrites) were distin-

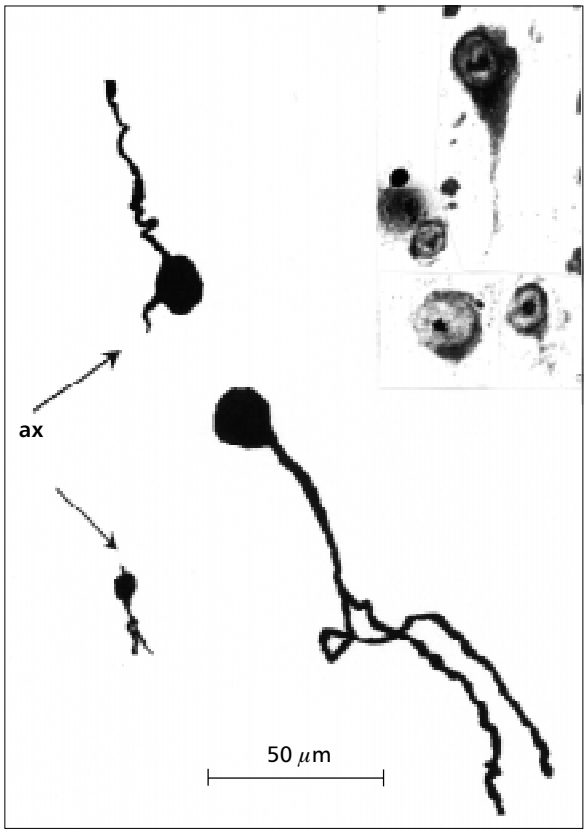

Figure 1. Computerized reconstruction of Golgi impregnated neurons of type 1; inserted microphotograph of Nissl stained perikarya.

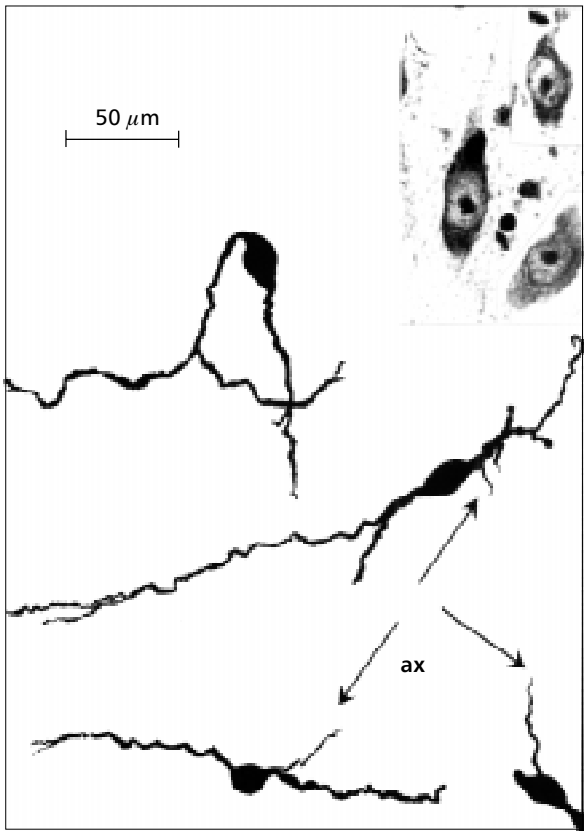

Figure 2. Computerized reconstruction of Golgi impregnated neurons of type 2; inserted microphotograph of Nissl stained perikarya.

guished. The curly-bipolar neurons possess 2 thick primary dendrites which may divide once (close to the soma) or twice, or not at all running at the distance over 100 μm, but they run in wavy, screw-like or bending patterns. These dendrites have varicosites and sometimes spine-like protrusions. Simple-bipolar neurons with thinner, wavy dendrites following a straight route were met rarely. Some of these cells reaching the smallest sizes have slender tapering dendrites with axon-like appearance and were observed near the principal mamillary fascicle and fornix. The dendritic field has a stream-like or oval shape. The axon arises from the soma alone but more often together with the dendritic trunk. These cells make both Mm and Ml in equal proportions, about 40%, but curly-bipolar ones were especially observed at the medial mamillary nucleus.

Type 3 (Fig. 3) — multipolar cells with cap-like perikarya from 10 to 25 μm possessing 2–3 dendritic trunks. Two primary dendrites conically emanated in opposite directions from the base of the soma but then they run in similar way to the dendrites of curly-bipolar cells and also possess different varicosites and spine-like protrusions. The third primary dendrite arises together with one of those described above or from the tip of the perikaryon but run in the same direction as the remaining ones. These dendrites usually at the distance 6–30 μm from the soma divide once and may give off thinner collaterals. The dendritic field is oval or ball-shaped. The axon may originate from the base or tip of the soma alone or together with the dendritic trunks emanating from the base. These axons have their own cone and go away spirally or like a stairway. In two cases axons emanate from the dendritic trunk at a distance about 6 μm from the perikaryon. There are cells which may be regarded as a transitional form between this type and type 2 (they have 2 primary dendrites at the base and axon at the tip of the cap-like perikaryon). The neurons of type 3 constitute about 30% of cells in Mm and 8% in Ml.

Type 4 (Fig. 4) typical multipolar cells with triangular or polygonal perikarya from 10 to 34 μm. They have usually 3–4 (occasionally 5) thick primary dendrites,

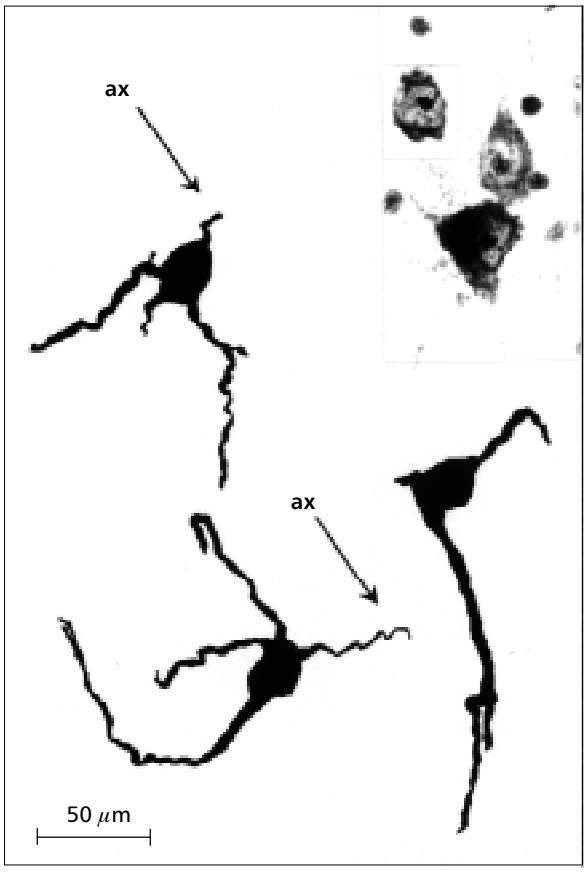

Figure 3. Computerized reconstruction of Golgi impregnated neurons of type 3; inserted microphotograph of Nissl stained perikarya.

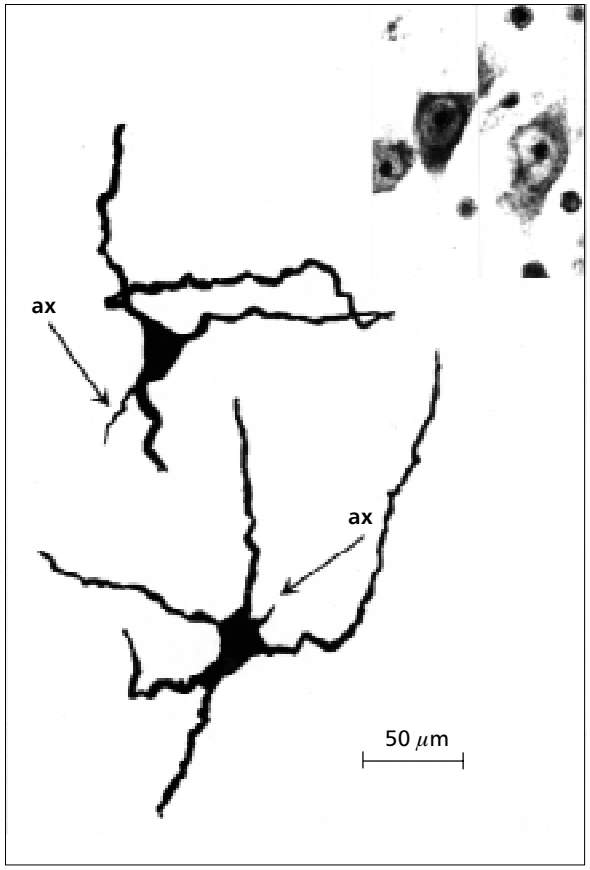

Figure 4. Computerized reconstruction of Golgi impregnated neurons of type 4; inserted microphotograph of Nissl stained perikarya.

which radiate from the polar ends of the soma in all directions. They may bifurcate closely to the cell body and then after 18 microns once again or give off collaterals. Some of them are poorly or do not divide at all and show a more rectilinear course than the ones described above. These dendrites rarely display dendritic spines although they have a varicose course and different tuberous processes. The dendritic field is oval or ball-shaped. The axon originates directly from the soma alone or together with the dendritic trunk. These neurons constitute about 4% of cells in Mm and 45% in Ml.

DISCUSSION

In mammals, within the mamillary complex there are medial mamillary nucleus and the lateral mamillary nucleus that are encapsulated by acid phosphatase positive magnocellular neurons [13] or the paramamillary nuclei [for details see 3,4,9,11,23,24]. Within both the medial and lateral mamillary nuclei there are small and multipolar cells immunostaining for the α-ketoglutarate dehydrogenase complex [5] and cells for [Leu]enkephalin [10]. Exclusively in Ml and caudal magnocellular nucleus, there are histamine-immunoreactive neurons [18] projecting to the thalamic nuclei, whereas the ventral part of the whole medial mamillary nucleus exhibits very high serotonin-like innervation although no 5-HT positive perikarya [26]. Descriptions of mamillary neuronal types, using the Golgi method are scanty due to the difficulty of staining this region; even in immature bison impregnation failed and only the paramamillary area was successfully impregnated [24]. The next investigations of the mamillary region were undertaken in guinea pig because no data in the literature have been found. Although the rodent hypothalamus was elaborated using Golgi impregnation methods, the mamillary body was not considered [15], and only brief mentions about these centres were found among other morphological classification of neuron [22]. In the present studies, although neurons of the mamillary nuclei in guinea pig show considerable variations in their morphology on Golgi scraps, they were segregated into four types: 1— un-

idendritic, 2 — bipolar (curly and simple ones), 3 — multipolar (cap-like) and 4 — multipolar (typical). Their perikarya were comparable with those seen in Nissl staining. The Mm mainly consists of types 2 and 3, whereas types 2 and 4 constitute a preponderance of Ml. The cells of type 3 have similar characteristics to type 1 neurons, the most numerous in others, rich in enkephalins hypothalamic magnocellular dorsal nucleus in guinea pig [7] and differs only in the route of their dendrites. Dendrites of cap-like neurons often bend, creating loops or rings and exclusively are specific for the medial mamillary nucleus lying mostly on its periphery. This dendritic pattern corresponds to the hodophobic tendencies of allodendritic neurons found in the analogue nucleus of cat [22], but in the guinea pig these neurons are weakly branched. Similar features were observed in unidendritic neurons of type 1. These neurons were small and scattered throughout the mamillary body; their primary dendrites either remained unbranched or branched only once. Type 4 of the neurons was observed exclusively in Ml of the guinea pig, and generally belongs to isodendritic neurons [22], which are commonly present in the hypothalamus. In the guinea pig some of these cells occasionally possess bending or crossing dendrites and differ from those multipolar cells, which were found in the paramamillary nuclei [24]. On the other hand, because ML cells are mixed with neurons of the posterior part of the tuberomamillary nucleus [11,23], some neurons of Tmp may be included in the described cell types in the guinea pig. A similar situation exists for the magnocellular praefascicular mamillary nucleus [9] and different named corresponding groups [3,4,13] located within the anterior sector of the mamillary body, squeezed between the medial mamillary nucleus and fornix. This centre is present in the guinea pig, but not in other mammals [11,23]. From similarly located magnocellular histaminergic neurons their axons enter the mamillothalamic tract, and also the mamillary peduncle as a hypothalamo-cerebellar pathway directing to the posterior cerebellar lobe, which may be involved in the coordination and integration of somatic as well as non-somatic responses [6]. On sagittal scraps within the mamillothalamic tract a few simple bipolar cells were found, which possess two main processes that should be named neurotendrils according to Ramon-Moliner [22]. They were thin, had no appendages and went away from the smallest perikarya; these cells possessed some features of the leptodendritic neurons [22]. The longest and most branched dendrites have curly bipolar cells of type 2. Their dendrites often bend and overlap one another or wrap themselves. Probably it may be a tendency to increase the possibility of contacts in dendro-dendritic communication, which may be important for local modulatory influences [8] apart from typical reception roles. Almost in all cell types of mamillary nuclei in guinea pig the dendrites were thick with various processes, which seldom had the typical appearance of dendritic spines [22] although a few were found, especially on the dendrites of the cap-like neurons in type 3. In general, various protrusions and varicosites were commonly observed but mostly on dendrites of fusiform cells in type 2. Ramon-Moliner [22] thought that dendritic spines located within a strong myelinated area may be impregnated in such a way. In the present studies also the short although generally relatively prominent initial segment of axon is impregnated in a reliable manner similar to other results in adult [21,22] and also in immature [24] material; this difficulty is discussed elsewhere. The axon emanates from the dendritic trunk most frequently in fusiform cells of type 2 in contrast to the unidendritic smaller cells of type 1. It was noticed that the points of leaving the cell by axon may be an important matter in neuronal communication [8]. In view of the above the cells in type 2 possess some attributes for dendro-dendritic communication and also to form preferentially axo-dendritic synapses. The characteristic morphological features of neurons of types 2 and 3 constituting especially the medial mamillary nucleus suggest that these cells may be the local circuit neurons. It may be serviceable to point out some anatomical relationships that are the base for functional possibilities of these centres but future studies are necessary.

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