The neuronal structure of the ventromedial and infundibular nuclei in the guinea pig: Nissl and Golgi study

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The studies were carried out on the mesencephalons of adult guinea pigs. On the basis of the Golgi technique, as well as the Nissl and Klüver-Barrera methods, four types of neurons were distinguished in the ventromedial nucleus (VMH) and infundibular nucleus (Ni): 1. Rounded neurons (perikarya 12–18 µm) with 3–4 dendritic trunks, which divide once, twice or not at all. The dendritic branches possess varicosities and knob-like spines. These neurons predominate in VMH. 2. Fusiform neurons (perikarya 15–28 µm) with 2 dendritic trunks, which arise from the opposite poles of the cell body. Bead-like protuberances and knob-like processes are observed on the dendrites. These neurons are the most numerous in Ni. 3. Triangular neurons (perikarya 15–22 µm) possess three thick, conical dendrites, which bifurcate dichotomically. Bead-like appendages and knob-like processes were seen on the dendritic surface. 4. Multipolar neurons (perikarya 18–22 µm) with 4–5 dendritic trunks, which are poorly ramified. The dendritic branches are smooth, but varicosities can be observed on their surface. In all types of neurons an axon was observed to arise either from the dendritic trunk or from the soma.

key words: ventromedial, infundibular nuclei, types of neurons, Golgi and Nissl pictures, guinea pig

INTRODUCTION

The ventromedial and infundibular nuclei are the main centres in the tuberal region of the hypothalamus. These nuclei play a critical role in a variety of essential functions which are associated with feeding [2, 46, 47], sexual behaviour [1, 23, 28, 32], hormonal activity [2, 28, 41] and they also regulate functions necessary for survival: adaptation to stress, threat and attack or body energy balance [2, 20]. Neurophysiological and neuroanatomical studies have established that the nuclei of the tuber cinereum create projections to the posterior hypothalamic area, locus coeruleus, mammillary complex, ventral tegmental area [40] and also to the preoptic area, bed nucleus of stria terminalis and to the diagonal band of Broca [33]. VMH neurons projecting to the mesencephalic central grey [5, 28, 39, 40] may be involved in the mediation of reproductive behaviour [5]. Moreover, there are fibres passing through or arising from the infundibular nucleus projecting to the supraoptic nucleus, which are mediated by GABA [21]. On the other hand Ni and VMH receive serotonin innervation from the dorsal and medial raphe nucleus [11, 49], and from the amygdala [30, 36].
Afferent fibres from the fornix [18], subfornical organ, vasculosum of the lamina terminalis [11] and from the suprachiasmatic nucleus [48] reach the hypothalamic nuclei. According to some authors [22, 33], infundibular neurons receive fibres from the preoptic region and these neurons project in turn to the median eminence [28, 37]. The cytoarchitecture and ultrastructure of VMH and Ni were elaborated in many mammals, for example: rat [14, 16, 24, 31], insectivorous species [17], bison [38], cat [13], domestic animals [35, 42, 45], and primates [44], whereas the neuronal structure was investigated only in a few mammals: in rat [10, 25, 26] and ruminants [34]. The aim of our study was to describe the neuronal structure of VMH and Ni in the guinea pig in order to complete those data.

MATERIAL AND METHODS
The studies were performed on the mesencephalons of six adult guinea pigs. The brains were cut into frontal and sagittal planes. Preparations were made according to the Bagiński and Golgi-Kopsch techniques and stained by means of Nissl and Klüver-Barrera methods. The brains were cut into 60 µm and 10 µm sections for the Golgi and Nissl methods, respectively. The microscopic images of chosen, impregnated cells were digitally recorded by means of a camera that was coupled with a microscope and an image processing system (VIST-Wikom, Warsaw). From 50 to 100 such digital microphotographs were taken at different focus layers of the section for each neuron. The computerised reconstructions of microscopic images were made on the basis of these series. First, the neurons were not clarified to show the real microscopic images and then the neuropil was removed to clarify the picture.

RESULTS
On the basis of various criteria (shape and size of perikarya, distribution of the tigroid substance, number and arborisation of dendrites and location of axon) the following types of neurons were distinguished in the ventromedial and infundibular nuclei:

1. Rounded neurons (Fig. 1). The perikarya of the rounded neurons measure from 12 to 18 µm. These cells have 3–4 (sometimes 2) occasionally conical dendritic trunks, which spread out in all directions. The dendritic trunks usually divide into secondary dendrites, after 15–50 µm of their route, and sporadically once again at various distances from the cell body. Some of the primary dendrites may remain undivided. These undivided dendrites are more often observed in the Ni neurons. The dendritic branches have a slightly wavy course and are longer than their parent dendrites. The dendritic trunks are smooth but the undivided primary dendrites may possess bead-like protuberances and have a few spine-like processes. The secondary and tertiary dendrites have varicosities and knob-like spines, which are unevenly distributed on the surface of the dendrites and usually concentrate on their distal portions. The dendritic tree is oval or round in shape. An axon arises either from the soma close to one of the primary dendrites or from the proximal part of the dendritic trunks. The axon of VMH neurons usually directs ventrally, whereas in the Ni neurons ventrally or rarely caudally. A soma contains a large, round nucleus, which is surrounded by medium-size granules of the tigroid substance. The tigroid substance does not penetrate into the initial portions of the dendritic trunks. The rounded cells are observed both in VMH and in Ni but they predominate in the ventromedial nucleus.

2. Fusiform neurons (Fig. 2). Their cell bodies measure from 15 to 28 µm along the long axis. These neurons possess two dendritic trunks which arise from the opposite poles of the cell body. Sometimes
three dendrites are observed; in this case two dendritic trunks arise from one pole and the third dendrite arises from the opposite pole of the cell body.

These dendrites divide once, twice or not at all. The first bifurcation is placed near the soma (usually after 15–35 µm of the dendrites route) and the second division is located at a long distance from the cell body. The undivided dendrites and the dendritic branches are slightly wavy and they have varicosities on their surface. On the secondary and tertiary dendrites there are observed bead-like protuberances and knob-like processes. The fusiform neurons are evenly distributed throughout the infundibular nucleus, whereas in the ventromedial nucleus they mostly concentrate on the periphery of the nucleus. Most dendrites of the fusiform neurons run in a ventro-dorsal direction. Some of them may be followed at a distance of 400–500 µm (sagittal plane) and these dendrites were observed to go beyond the territory of the studied nuclei, especially the territory of VMH. The dendritic field has a stream-like form. An axon originates from the initial portion of the dendritic trunk or seldom from the perikaryon and takes a ventral or rostral course. The fusiform cells have a centrally located, large, round nucleus with dark stained nucleolus. Coarse and medium-size granules of the tigroid substance are concentrated at the poles of the cell body and deeply penetrate into the initial parts of the dendritic trunks. The fusiform cells are seen in both the studied nuclei and they are the most often observed neurons in the infundibular nucleus.

3. Triangular neurons (Fig. 3). Their cell bodies measure from 15 to 22 µm. They have 3 thick primary dendrites, which conically arise from the cell body. Most of the primary dendrites divide dichotomically near the soma (after 15–25 µm) into secondary dendrites, which may bifurcate once again after 10–25 µm from the first division. The dendritic trunks are smooth and devoid of protuberances but the secondary and tertiary dendrites possess unevenly distributed bead-like appendages and knob-like processes, which concentrate on their distal portions. The dendrites run in rostral, caudal and ventral directions and the dendritic tree is oval or round in shape. An axon arises from a soma (close to one of the dendrites), or rarely from the dendritic trunk and usually takes a rostral or caudo-dorsal course. These cells have a spherical, large, darkly-stained nucleus. The tigroid substance in the form of medium-size gran-
ules is located on the periphery of the cell body and delicately enters the cones of the dendrites. The triangular neurons are the least numerous neurons, both in VMH and Ni, and only single triangular neurons are observed throughout these nuclei.

4. Multipolar neurons (Fig. 4). The perikarya of the multipolar neurons measure from 18 to 22 µm. From an oval or quadrangular cell body there originate 4–5 usually conical dendritic trunks, which spread out in all directions. Generally, these dendrites are poorly ramified and only some of them divide once or twice, whereas the others remain undivided. The first bifurcation is located either near the soma, after 15–25 µm, or at a long distance (70–80 µm) from it. The dendrites are smooth but delicate varicosities may be occasionally observed on their surfaces. These dendrites have a slightly wavy route and some of them may be followed at a distance of about 200 µm. The dendritic tree is round in shape. An axon emerges directly from a soma or occasionally from the dendritic trunks and usually directs upwards. A large, rounded and darkly stained nucleus is centrally located and is surrounded by medium-size and small granules of the tigroid substance, which moderately enters the cones of the dendrites. The multipolar neurons are rarely observed in both the studied nuclei.

DISCUSSION

In the ventromedial and infundibular nuclei of the guinea pig there have been distinguished four types of neurons: rounded, fusiform, triangular and multipolar. The rounded cells are the dominant type of neurons in VMH, whereas in Ni the fusiform neurons predominate. In both the studied nuclei, there are observed less frequently the multipolar neurons and the least numerous are the triangular nerve cells. On the basis of the cytoarchitectonic data in VMH and Ni, the fusiform, rounded and multipolar cells were described in different mammals [12, 17, 35, 38, 45], but the triangular cells which were occasionally observed in our material have been reported only in sheep [35]. According to various methods and criteria used by investigators, different numbers of neurons were reported in VMH and Ni. Morphometric characterisation of the rat Ni [10] revealed six types of neurons: non-ramified unipolar, ramified unipolar, non-ramified bipolar, ramified bipolar, small multipolar and large multipolar. Kiss [16] distinguished four categories of neurons in the rat VMH mainly on the basis on the ultrastructural appearance of the endoplasmic reticulum. Kelly et al. [15] in the arcuate-ventromedial hypothalamus (ARC-VM) of the rat, according to electrical stimulation of the median eminence, stria terminals and after procin yellow injection, delineated four, five or two morphologically distinct cell types, respectively. Three types of Ni neurons: spherically-formed, elongated and flattened, were distinguished in the rat [31] and also in the cat [13]. In the cat, Ibata et al. [13] classified neurons into clear, intermediate and dark types, according to their fine structure. The moderately large cells with 2–3 long dendrites, large and irregularly shaped, as well as round-bodied and triangular neurons, were reported in the ruminants [34]. Our results revealed morphological features similar to those described above, particularly with regard to the shape of the cell bodies, number and arborisation of dendrites, as well as the dendritic morphology [34]. However, the rounded neurons, the predominant nerve cells of the guinea pig, were only sporadically seen in VMH ruminants [34]. By the Golgi technique, in the rodents, Millhouse identified category I and II VMH neurons [24] as well as type I and type II VMH [25] neurons, whereas the bipolar and unipolar neurons were described in Ni [25]. Neurons of type I (the majority of VMH neurons) possess spherical or spindle-shaped somata with 2–3 principal dendrites, whereas the neurons of type II have several principal dendrites and they are estimated.
to constitute no more than 1–2% of the neuronal population. The rounded and fusiform neurons of the guinea pig correspond most probably to the type I and bipolar neurons [25], whereas the multipolar and triangular neurons seem to be similar to the type II reported in the rat [25]. Some dendrites of the guinea pig neurons run for a long distance and they go beyond the territory of the studied nuclei. Dendrites of the VMH neurons extend into the neuropil surrounding VMH [25], so that VMH is not isolated from the remainder of the hypothalamus but, on the contrary, is intimately associated with the hypothalamus [25].

The secondary and tertiary dendrites of the rounded, fusiform and triangular nerve cells have bead-like protuberances and knob-like or spine-like processes, which are unevenly distributed on the surface of the dendritic branches, or may concentrate on their distal portions. The dendrites of the multipolar neurons are smooth and only delicate varicosities were observed on their surface. In sheep [34] VMH dendrites possess spine-like processes (especially their collaterals) and in some places display thickening, or carry several nodules [34]. Millhouse [25] in the rat found spines or spiny protrusions and varicosities along the shaft of the dendrites, whereas Kelly et al. [15] commonly observed dendritic swellings, but spine-like appendages were seldom seen. Gonzales-Burgos et al. [10] in the rat neurons reported both somatic and dendritic spines, as did Segarra and McEwen [43]. Dendritic and soma spine density is significantly higher in juvenile than peripubertal rats [43] and Leal et al. [19] suggest that aging induces regressive changes in the spine dendrites and also in the dendritic arborisation. The physiological studies indicate that the dendritic morphology is under hormonal regulation [6] and the spine density is plastic, which correlates with reproductive behaviour, and gonadal steroid has been found to influence the dendritic spine density [4, 7–9, 27, 43]. The density of the dendritic spines fluctuates during the estrous cycle, with an increased density occurring on proestrus [8]. Mathews and Edwards [23] reported that the ventromedial hypothalamic area is known to contain the greatest densities of estradiol concentrating cells in the brain [23] and VMH is the most progesterone-sensitive site [32]. Electron microscopic study [29] has shown that multipolar neurons of the infundibular nucleus contain somatostatin and there are some neurons which contain cystain C [3].

In the guinea pig, axons arise either from the proximal part of the dendritic trunks or directly from a soma and could be traced only a short distance. Similar observations were reported in the rats and ruminants [10, 15, 34] but Millhouse [25] described axon collaterals that are sent into the surrounding hypothalamus, which were not observed in our material. However, there are some differences as well as similarities in the neuronal structure of the tuberal nuclei in the guinea pig and other mammals. We conclude that these centres have the most properties in common with the analogous centres in other rodents.

**REFERENCES**


