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

Krystyna Bogus-Nowakowska, Stanisław Szteyn, Anna Robak

Department of Comparative Anatomy, University of Warmia and Mazury, Olsztyn, Poland

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].

Address for correspondence: Krystyna Bogus-Nowakowska, Department of Comparative Anatomy, University of Warmia and Mazury, ul. Żołnierska 14, 10–561 Olsztyn, Poland, tel: +89 527 60 33, tel/fax: +89 535 20 14, e-mail: odin@matman.uwm.edu.pl

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
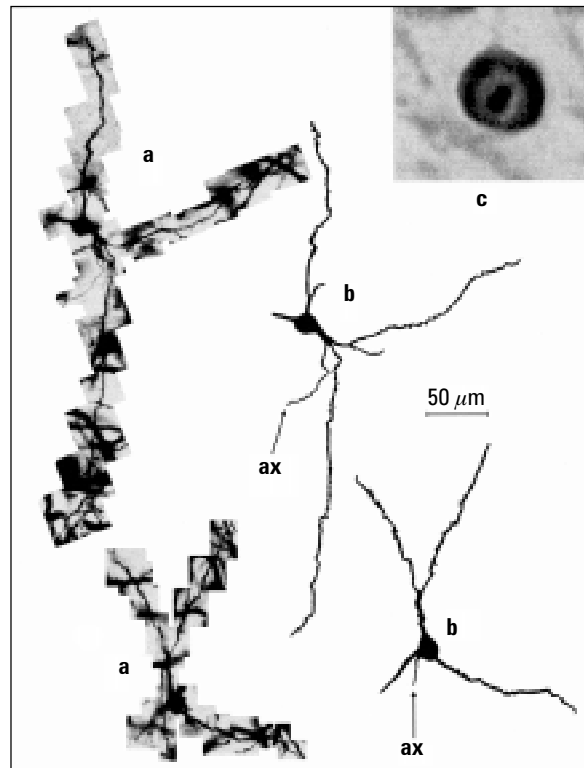
Figure 1. Rounded neurons: **a)** non-clarified Golgi impregnation; **b)** clarified Golgi impregnation, ax — axon; **c)** the Nissl stained soma.
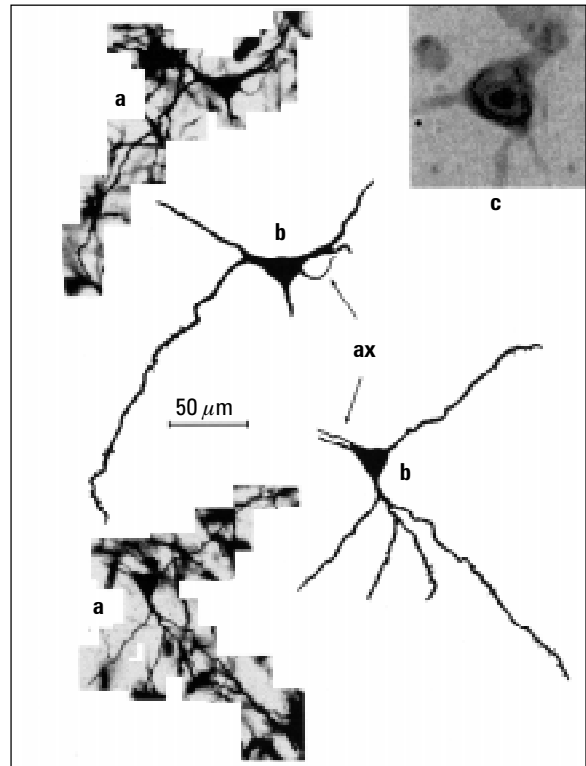
often observed in the Ni neurons. The dendritic branches have a slightly wavy course and are longer than their parent dendrites. The dendritic trunks are smooth but the undivided primary dendrites may possess bead-like protuberances and have a few spine-like processes. The secondary and tertiary dendrites 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
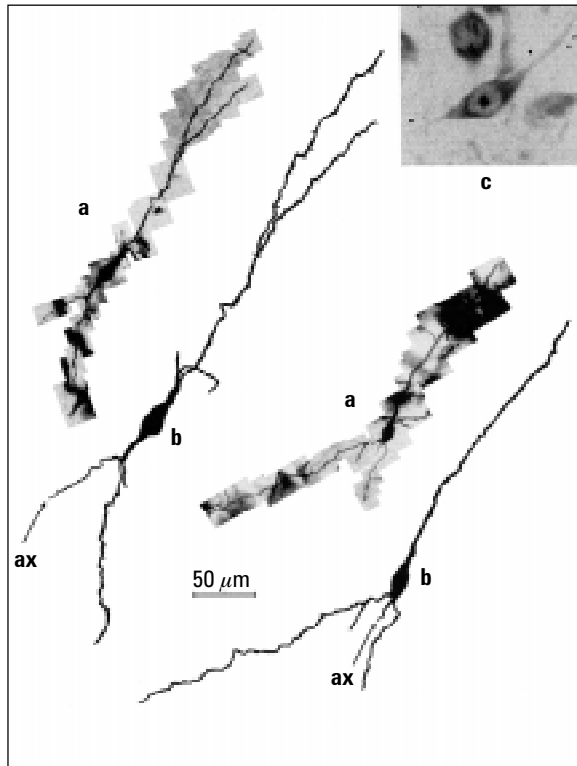
Figure 2. Fusiform neurons: **a)** non-clarified Golgi impregnation; **b)** clarified Golgi impregnation, ax — axon; **c)** the Nissl stained soma.

Figure 3. Triangular neurons: **a)** non-clarified Golgi impregnation; **b)** clarified Golgi impregnation, ax — axon; **c)** the Nissl stained soma.

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 nuceli, 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, cadal 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-

311
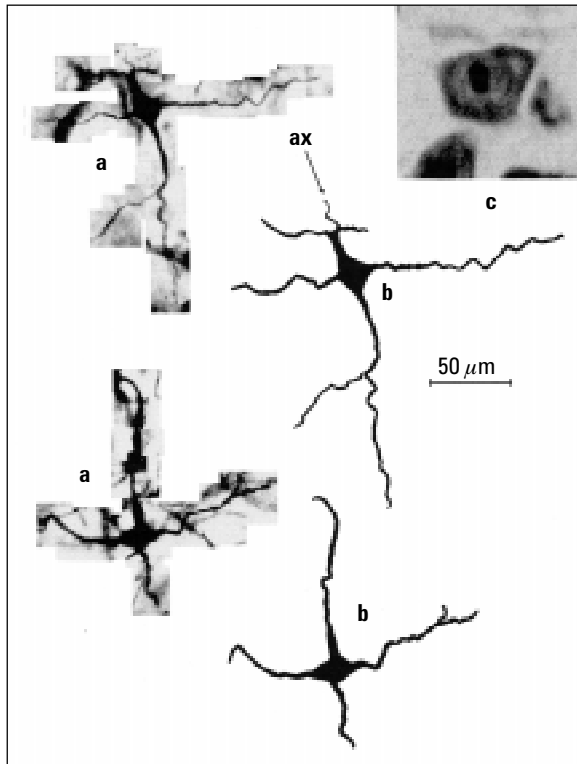
Figure 4. Multipolar neurons: **a)** non-clarified Golgi impregnation; **b)** clarified Golgi impregnation, ax — axon; **c)** the Nissl stained soma.

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 terminalis 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

1. Aou S, Oomura Y, Yoshimatsu H (1988) Neuron activity of the ventromedial hypothalamus and the medial preoptic area of the female monkey during sexual behavior. Brain Res, 455: 65–71.
2. Bell ME, Bhatnagar S, Akana SF, Choi S, Dallman MF (2000) Disruption of arcuate /paraventricular nucleus connections changes body energy balance and response to acute stress. J Neurosci, 20: 6707–6713.
3. Brenstein HG, Järvinen M, Pöllänen R, Schirpke H, Knöfel B, Rinne R (1988) Cystain C containing neurons in human postmortem hypothalamus. Neurosci Letters, 88: 131–134.
4. Calizo LH, Flanagan-Cato LM (2000) Estrogen selectively regulates spine density within the dendritic arbor of rat ventromedial hypothalamic neurons. J Neurosci, 20: 1589–1596.
5. Chung SK, Pfaff DW, Cohen RS (1990) Projections of ventromedial hypothalamic neurons to the midbrain central gray: an ultrastructural study. Neuroscience, 38: 395–407.
6. Danzer SC, McMullen NT, Rance NE (1998) Dendritic growth of arcuate neurons following orchidectomy in adult rats. J Comp Neurol, 390: 234–246.
7. Danzer SC, McMullen NT, Rance NE (2001) Testosterone modulates the dendritic architecture of the arcuate neuroendocrine neurons in adult male rats. Brain Res, 890: 78–85.
8. Frankfurt M, Gquld E, Woolley CS, McEwen BS (1990) Gonadal steroids modify dendritic spine density in the ventromedial hypothalamic neurons: a Golgi study in the adult rat. Neuroendocrinology, 51: 530–535.
9. Frankfurt M, McEwen BS (1991) 5,7-Dihydroxytryptamine and gonadal steroid manipulation alter spine density in ventromedial hypothalamic neurons. Neuroendocrinology, 54: 653–657.
10. Gonzales-Burgos I, del Angel-Meza AR, Barajas-Lopez G (1993) Morphometric characterization of the arcuate nucleus neurons of the rat. A Golgi study. Neurosci Lett, 155: 99–101.
11. Gruber K, McRae-Degueurce A, Wilkin LD, Mitchell LD, Johnson AK (1987) Forebrain and brainstem afferents to the arcuate nucleus in the rat: potential pathways for the modulation of hypophyseal secretions. Neurosci Lett, 75: 1–5.

12. Hartwing HG, Wahren W (1982) Anatomy of the hypothalamus. In: Schaltenbrand G and Walker AE (eds.). Stereotaxy of the human brain. Georg Thieme Verlag, Stuttgart.

13. Ibata Y, Nojyo Y, Matsuura T, Yoshikawa H, Sano Y (1975) Electron microscopy of the arcuate nucleus of normal and 5-hydroxydopamine treated cats. Cell Tissue Res, 160: 139–153.

14. Inagaki S, Kubota Y, Kito S, Yamano M, Tohyama M (1986) Enkephalin-like immunoreactive neurons and fibres in the ventromedial hypothalamic nucleus. Brain Res Bull, 17: 159–167.

15. Kelly MJ, Kuhnt U, Wuttke W (1979) Morphologiocal features of physiologically identified hypothalamic neurons as revealed by intracellular marking. Exp Brain Res, 34: 107–116.

16. Kiss A (1983) Effect of acute and repeated immobilization stress on the ultrastructure of neurons of the rat hypothalamic ventromedial nucleus. Z Mikrosk Anat Forsch, 97: 49–64.

17. Krawczuk-Robak A, Szteyn S, Szatkowski E (1990) Topography and cytoarchitectonics of small-cell nuclei of the tuber cinereum in Insectivota. Anat Anz, 170: 63–71.

18. Larriva-Sahd J, Rondan-Zarate A, Ramirez-Degollado M (1995) Sexually dimorphic contribution from the fornix to the ventromedial hypothalamic nucleus: a quantitative electron microscopic study. Neurosci Lett, 200: 147–150.

19. Leal S, Andrade JP, Paula-Barbosa MM, Madeira MD (1998) Arcuate nucleus of the hypothalamus: effect of age and sex. J Comp Neurol, 401: 65–88.

20. Lipp HP, Hunsperger RW (1978) Threat, attack and flight elicited by electrical stimulation of the ventromedial hypothalamus of the marmoset monkey Callithrix jacchus. Brain Behav Evol, 15: 260–293.

21. Ludwig M, Leng G (2000) GABAergic projection from the arcuate nucleus to the supraoptic nucleus in the rat. Neurosci Lett, 281: 195–197.

22. Magoul R, Dubourg O, Kah O, Tramu G (1994) Tachykinergic synaptic inputs to neurons of the medial preoptic region which project to the rat arcuate nucleus. Neurosci Lett, 169: 149–153.

23. Mathews D, Edwards DA (1977) Involvement of the ventromedial and anterior hypothalamic nuclei in the hormonal induction of receptivity in the female rat. Physiol Behav, 19: 319–326.

24. Millhouse OE (1978) Cytological observations on the ventromedial hypothalamic nucleus. Cell Tissue Res, 191: 473–491.

25. Millhouse OE (1979) A Golgi anatomy of the Rodent hypothalamus. In: Morgane and Panksepp (eds). Handbook of the hypothalamus. Vol. 1. Marcel Deker, Inc, New York, Basel.

26. Mong JA, Glaser E, McCarthy MM (1999) Gonadal steroids promote glial differentiation and alter neuronal morphology in the developing hypothalamus in a regionally specific manner. J Neurosci, 19: 1464–1472.

27. Mong JA, Roberts RC, Kelly JJ, McCarthy MM (2001) Gonadal steroids reduce the density of axospinous synapses in the developing rat arcuate nucleus: an electron microscopy analysis. J Comp Neurol, 432: 259–267.

28. Morrell JI, Rhodes CH Pfaf DW (1980) Modern neuroanatomical approaches to neuroendocrine control systems. In: Müller EE (eds.). Neuroactive Drugs in Endocrinol. North-Holland Biomedical Press, 3–18.

29. Ohtsuka M, Hisano S, Daikoku S (1983) Electronmicroscopic study of somatostatin-containing neurons in rat arcuate nucleus with special reference to neuronal regulation. Brain Res, 263: 191–199.

30. Ono T, Luiten PG, Nishijo H, Fukuda M, Nishino H (1985) Topographic organization of projections from the amygdala to the hypothalamus of the rat. Neurosci Res, 2: 221–238.

31. Perra F, Wittkowski W (1985) Size, form and orientation of the neuronal perikarya and nuclei in the nucleus infundibularis of the male rat. J Hirnforsch, 26: 91–108.

32. Pleim ET, Lipetz J, Steele TL, Barfield RJ (1993) Facilitation of sexual receptivity by ventromedial hypothalamic implants of the antiprogestin RU 486. Horm Behav, 27: 488–498.

33. Pompolo S, Rawson JA, Clarke IJ (2001) Projections from the arcuate/ventromedial region of the hypothalamus to the preoptic area and the bed nucleus of stria terminalis in the brain of the ewe; lack of direct input to gonadotropin-releasing hormone neurons. Brain Res, 904: 1–12.

34. Rajtova V, Danko J (1983) Impregnoarchitectonics of the ventromedial hypothalamic nucleus and the ventrolateral hypothalamic nucleus in Ruminants. Folia Morphol, 31:10–13.

35. Rajtova V, Horakova A (1982) The middle region of the hypothalamus in the sheep (Ovis aries L) I. The ventromedial hypothalamic nucleus. Folia Morphol, 30: 50–55.

36. Renaud LP (1976) An electrophysiological study of amygdalohypothalamic projections to the ventromedial nucleus of the rat. Brain Res, 105: 45–58.

37. Renaud LP (1979) Neurophysiology and Neuropharmacology of medial hypothalamic neurons and their extrahypothalamic connections. In: Morgane and Panksepp (eds.). Handbook of the hypothalamus. Vol. 1. Marcel Deker, Inc, New York, Basel.

38. Robak A, Bogus-Nowakowska K, Szteyn S (1998) The morphology and cell structure of some nuclei in tuberal region of Bison bonasus. Folia Morphol, 57: 309–314.

39. Sakuma Y, Tada K (1984) Evidence that two sizes of ventromedial hypothalamic neurons project to the mesencephalic central grey matter in rats. J Physiol, 349: 287–297.

40. Saper CB, Swanson LW, Cowan WM (1976) The efferent connections of the ventromedial nucleus of the hypothalamus of the rat. J Comp Neurol, 169: 409–442.

41. Sartin JL, Lamperti AA (1985) Neuron numbers in the hypothalamic nuclei of young, middle-aged and aged male rats. Experientia, 14: 109–111.

42. Seeger J (1990) Cytoarchitecture and topography of the ventromedial hypothalamic nuclei of swine (Sus scrofa domestica). J Hirnforsch, 31: 601–611.

43. Segarra AC, McEwen BS (1991) Estrogen increases spine density in ventromedial hypothalamic neurons of peripubertal rats. Neuroendocrinology, 54: 365–372.

44. Simmons RMT (1980) The morphology of the diencephalon in the Prosimii. II. The Lemuroidea and Lorisoidea part II. Epithalamus, subthalamus and hypothalamus. J Hirnforsch, 22: 493–514.

45. Szteyn S, Łakomy M, Dynowski J, Krawczuk A (1981) The stereotaxic configuration of hypothalamus nerve centres in the cow. Anat Anz, 149: 471–485.

46. Takaki A, Aou Y, Oomura E, Okada E, Hori T (1992) Feeding suppression elicited by electrical and chemical stimulation of monkey hypothalamus. Am Physiol Soc, 586–594.

47. Tarttelin MF (1976) The ventromedial nucleus of the hypothalamus of sheep (Ovis aries) and the effects on food and water intake following its electrolytic destruction. Acta Anat, 94: 248–261.

48. Watts AG, Swanson LW, Sanchez-Watts G (1987) Efferent projections of the suprachiasmatic nucleus: I. Studies using anterograde transport of Phaseolus vulgaris leucoagglutinin in the rat. J Comp Neurol, 258: 204–229.

49. Willoughby JO, Blessing WW (1987) Origin of serotonin innervation of the arcuate and ventromedial hypothalamic region. Brain Res, 418: 170–173.