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The neuronal structure of the preoptic area in the mole and the rabbit: Golgi and Nissl studies

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The present studies were carried out on the brains of the adult mole and rabbit. The preparations were made by means of the Golgi technique and the Nissl method. Two types of neurons were distinguished in the preoptic area (POA) of both species: bipolar and multipolar. The bipolar neurons have oval, fusiform or round perikarya and two dendritic trunks arising from the opposite poles of the cell body. The dendrites bifurcate once or twice. The dendritic branches have swellings, single spine-like and filiform processes. The multipolar neurons usually have triangular and quadrangular perikarya and from 3 to 5 dendritic trunks. The dendrites of the mole neurons branch sparsely, whereas the dendrites of the rabbit neurons display 2 or 3 divisions. On the dendritic branches varicosities and different protuberances were observed. The general morphology of the bipolar and multipolar neurons is similar in the mammals studied, although the neurons of the rabbit POA display a more complicated structure. Their dendritic branches show more divisions and possess more swellings and different processes than the dendrites of the neurons of the mole POA. Furthermore, of the multipolar neurons only the dendrites in POA of the rabbit were observed to have a rosary-like beaded appearance.

Key words: preoptic area, types of neurons, Nissl and Golgi studies, mole, rabbit

INTRODUCTION

The preoptic area (POA) is localised in the transitional zone between the telencephalon and the diencephalon, surrounding bilaterally the third ventricle. From the functional point of view POA is regarded as part of the hypothalamus and plays a major role in the control of many aspects of reproduction. Together with the hypothalamic nuclei POA takes part in the regulation of the hypophysiogonadal system as indicated by the presence of luteinising hormone-releasing factor [5, 21] and sex steroid hormone-concentrating neurons [14, 34]. Moreover, POA participates in both feminine and masculine sexual and communicative behaviour [22] and also in maternal behaviour [40]. The preoptic area neurons are also involved in the regulation of sleep [23] and in thermoregulation [6]. The preoptic area efferents which appear to have the most obvious implications for these functions are those to the arcuate nucleus and the median eminence [5, 10, 20, 43]. A wide variety of neurotransmitters is implicated in mediating POA functions. For example, the monoamines in the medial preoptic area (MPA) play a crucial role in the control of GnRH release and this area receives a significant number of monoaminergic fibres [44]. The greatest number of catecholaminergic fibres and cell bodies are found in the periventricular zone of POA [44]. Dopamine activity in the medial POA contributes to the control of sexual behaviour in the male rat [19]. Furthermore, the perikarya and fibres of POA contain

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many other biologically active substances, including substance P, vasoactive intestinal polypeptide, cholecystokinin, neuropeptide Y, somatostatin, neurotensin, GABA and opioid peptides [13, 15, 29, 44].

Despite the relative abundance of data concerning POA structure and topography [27, 32, 36, 38, 47], the organisation of projections [2, 7, 9, 12, 20, 45] and the ultrastructure [24, 33, 35, 37], little is known about the Golgi analysis of this region. Only a few works concerning the neuronal structure of POA restricted to single animals are available, while detailed comparative Golgi study of POA appears to be lacking. The neuronal structure of POA with reference to Golgi has been studied in the rat [16, 28, 31], mouse [46], hamster [18], ferret [8] and monkey [3]. In the light of the paucity of such studies in the mole and rabbit the aim of our work was to describe and compare POA neuronal structure of two species of placental mammals that represent two different orders, *Insectivora* and *Lagomorpha,* respectively. This would appear to be of relevance because no description of the Golgi analysis of POA of these mammals was found in the available literature.

MATERIAL AND METHODS

The studies were performed on the brains of two adult mammalian species: the mole (*Talpa europaea — Insectivora*) and the rabbit (*Oryctolagus cuniculus — Lagomorpha*). The material studied consisted of 12 brains (6 brains of males and 6 of females) of one-year-old adult moles and seven-month-old rabbits. The brains were fixed in 4% neutralised formalin for not less than three months. Preparations were made according to the Golgi technique or stained by means of the Nissl method. The paraffin sections stained with cresyl violet were 50 μm-thick. Three brains of each sex for the Golgi impregnation were placed first in a solution of 2.5% potassium dichromate for 3–6 days and then in a 3% silver nitrate for 2–5 days. After the impregnation the paraffin blocks were cut into 90 μm scraps. The microscopic images of chosen impregnated cells were digitally recorded by means of a camera coupled with a microscope and an image processing system (MultiScan 8.2, Computer Scanning System, Poland). Between 20 and 60 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. At first the neurons were not clarified to show the real microscopic images and then the neuropil was removed to clarify the pictures.

RESULTS

The preoptic area of the mammals studied appears in front of the anterior commissure and extends backward to the anterior part of the paraventricular nucleus of the hypothalamus. On the basis of the cell arrangement in the Nissl scraps we distinguished two areas within POA, namely MPA and the lateral preoptic area (LPA), and two nuclei, the periventricular preoptic nucleus (PPN) and the median preoptic nucleus (MPN) (Fig. 1). MPA and LPA contain loosely distributed perikarya and these regions are indistinctly circumscribed from the surroundings; we thus termed them areas and not nuclei. MPN and PPN are built from tightly packed neurons and their borders are quite clear-cut. PPN is situated on both sides of the third ventricle, medially to MPA, whereas dorsally it is bordered by MPN. LPA occupies the lateral part of POA, bordering medially with MPA.

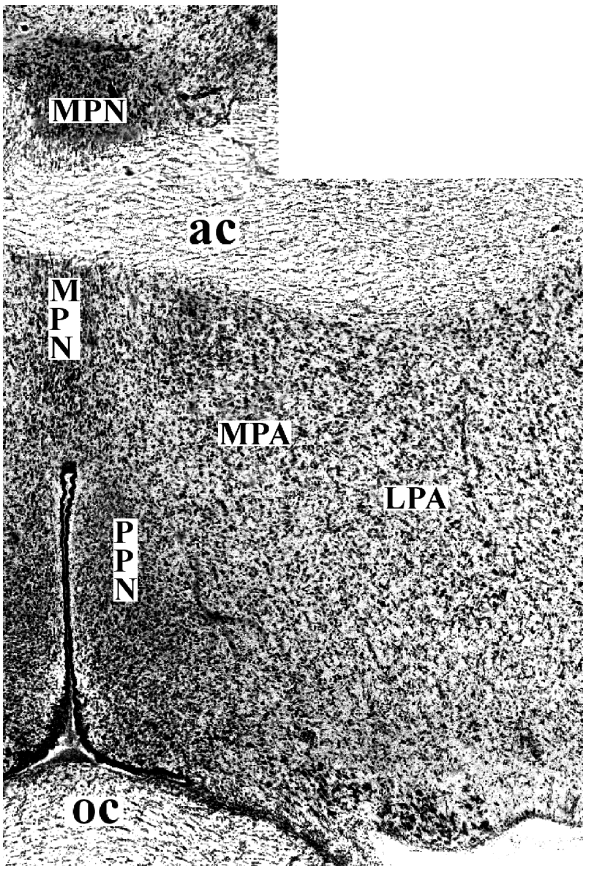

Figure 1. Microphotograph of the cross-section of the preoptic area of the rabbit. Scale bar 1 mm. MPA — medial preoptic area; LPA — lateral preoptic area; MPN — median preoptic nucleus; PPN — periventricular preoptic nucleus; ac — anterior commissure; oc — optic chiasm.

On the basis of the Golgi material analysis two types of neurons were distinguished in POA of the mammals studied, the bipolar and the multipolar.

The neuronal structure of POA of the mole

Bipolar neurons (Fig. 2). These neurons have oval, round or fusiform perikarya that measure from 12 to 28 μm. The smaller bipolar neurons were observed in MPN, whereas the larger ones were in LPA. Two dendritic trunks arise from the opposite poles of the cell body. Some neurons have elongate perikarya and thick dendritic trunks, so that it is difficult to determine the border between the soma and the dendritic trunk. The dendrites are poorly ramified and only some of them divide, usually once dichotomically but seldom twice. The first bifurcation is placed in the vicinity of the soma, about 10 μm or more from the cell body, while the second bifurcation usually occurs 20–30 μm or more from the first. Sparse swellings and processes are irregularly distributed on the dendritic branches. Generally the long axes of POA neurons lie down parallel to the fibres of the anterior commissure, whereas the long axes of MPN neurons are arranged perpendicular to these fibres. The dendritic field is not extensive and has a stream-like form (the longest dendrites reach about 120 μm). An axon emerges from the initial portion of the dendritic trunk, rarely from the perikaryon, and impregnates for a short distance (up to 25 μm).

In Nissl preparations a soma of the bipolar neurons contains a round or elongate pale stained nucleus with a darkly stained nucleolus. The tigroid substance, in the form of tiny and medium-size granules, is located on the periphery of the cell body and only in the fusiform perikarya does it penetrate the initial portions of the dendrites. The bipolar neurons were commonly observed in all POA parts, but in LPA they occurred less frequently than the multipolar ones.

Multipolar neurons (Fig. 3). Their perikarya are triangular, quadrangular or, rarely, round and measure from 14 to 30 μm. Smaller multipolar neurons occur more often in PPN and MPN, while there are larger ones in MPA and LPA. From the triangular perikarya three dendritic trunks arise conically. The remaining multipolar neurons usually have four or, sporadically, five dendrites that spread in all directions. The dendrites branch sparsely and only some of them bifurcate once, occasionally twice. The first division takes place near the cell body (after 10–15 μm), whilst the second bifurcation is located after about 40 μm. The dendritic branches follow straight course, some with a length of about 150 μm, and they are covered with delicate varicosities. An axon arises directly from the soma or from the dendritic trunk and was observed over a distance of about 35 μm.

In Nissl pictures the cells have round or oval centrally located nucleus. The tigroid substance, in the form of medium-sized granules, surrounds the nucleus and moderately penetrates the initial portions of the dendrites in the triangular and quadrangular perikarya. In the round perikarya a thin rim of cytoplasm with tiny granules of the tigroid substance is located in the cell circumference. The multipolar neurons were most frequent in LPA, whereas in the remaining parts of the mole POA they were observed in smaller numbers than the bipolar ones.

The neuronal structure of POA of the rabbit

Bipolar neurons (Fig. 4). The perikarya of these neurons are similar to those described in the bipolar neurons of the mole but they are larger and measure up to 35 μm. The place of the first division and the dendritic tree in general resemble those of the mole neurons but some differences were also observed. In the neurons with round perikarya the first division takes place at a distance of over 40–50 μm from the cell body. The second division in most bipolar neurons is located 30–40 μm from the first. A third bifurcation was observed sporadically, either in the vicinity of the previous one or in the distal portion of the dendrites (even after 100–200 μm). The dendritic branches are more varicose than the mole dendrites and possess bead-like protuberances. On the distal portions of these branches single spine-like processes were also observed. The dendritic field is elongate and vast, some dendritic branches reaching a length of about 450 μm. An axon emerges from the initial portion of the dendritic trunk and is usually impregnated at a distance of 30 μm or sometimes up to 70 μm.

Bipolar neurons preponderate in the rabbit POA. Bipolar cells with round and oval perikarya predominate in MPN and PPN, whereas neurons with fusiform perikarya preponderate in LPA and MPA. The inner structure of the perikarya is similar to that described in the mole.

Multipolar neurons (Fig. 5). The shape of the soma of the neurons is similar to the multipolar neurons of the mole but their perikarya are larger (up to 35 μm). The neurons have from three to five dendritic trunks, which show more divisions and only the single ones remain undivided. The first division

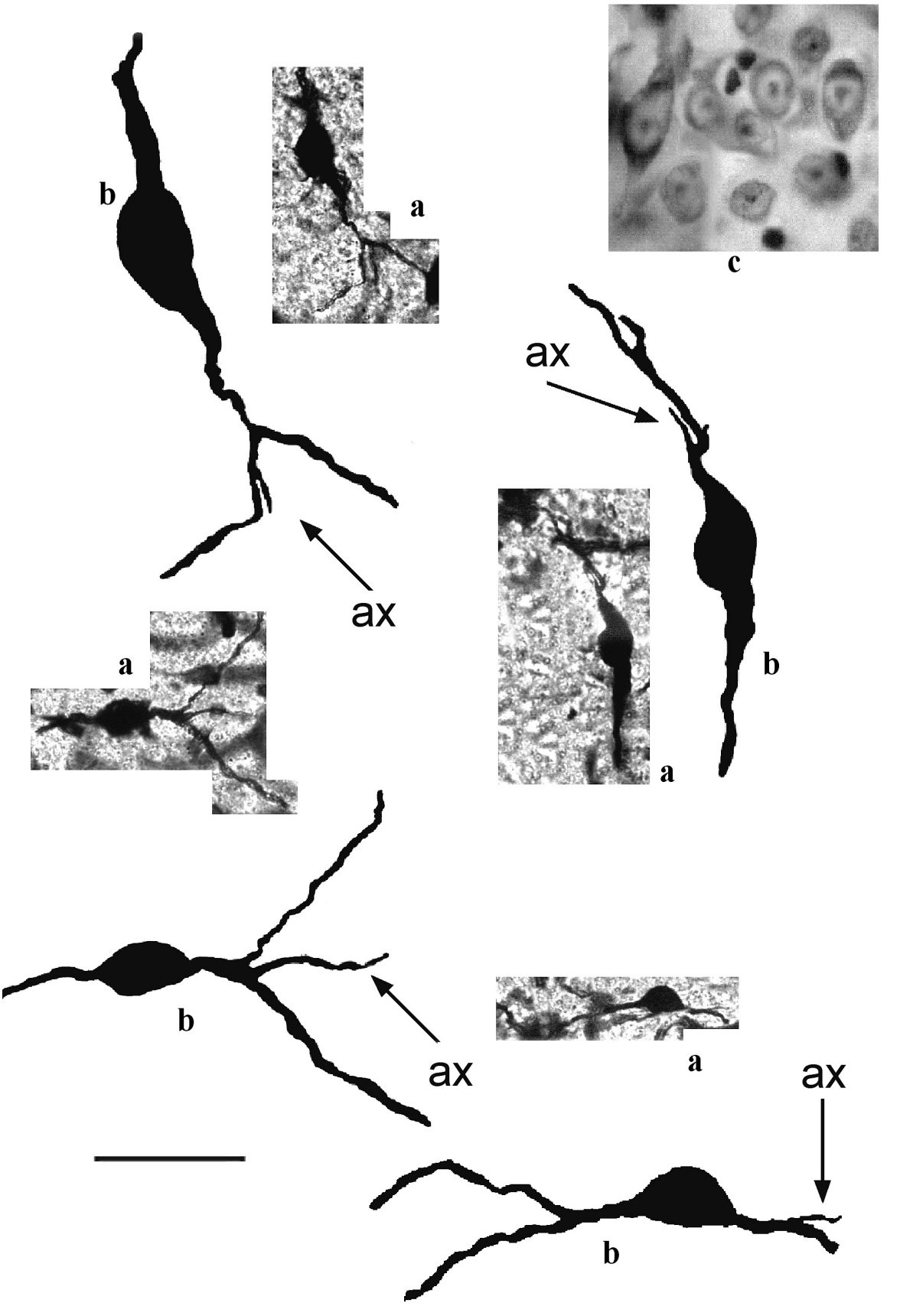

Figure 2. Bipolar neurons of POA of the mole. Scale bar 25 μm; **a.** Non-clarified Golgi impregnation; **b.** Clarified Golgi impregnation, ax — axon; **c.** Nissl stained perikarya.

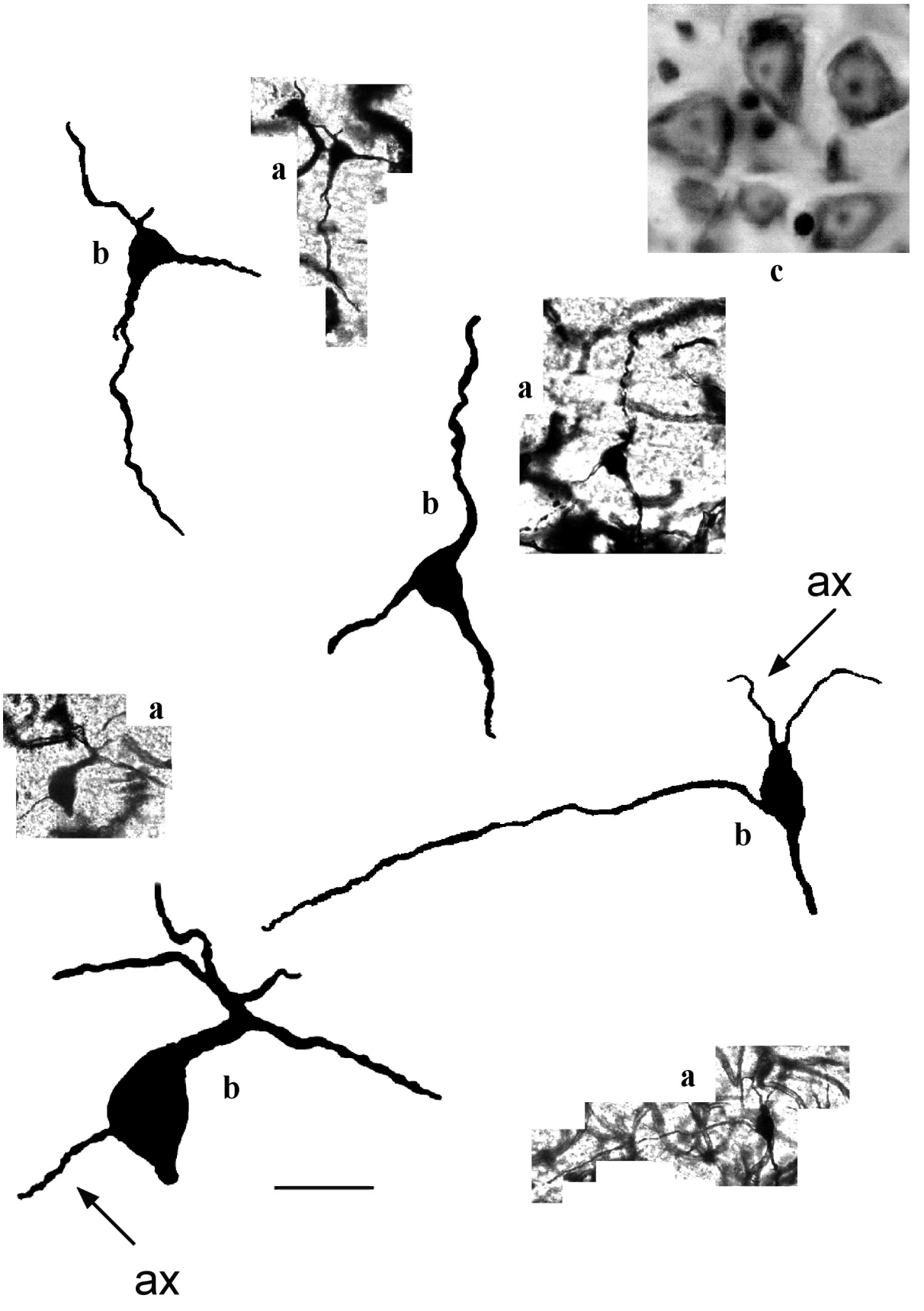

Figure 3. Multipolar neurons of POA of the mole. Scale bar 25 μm; **a.** Non-clarified Golgi impregnation; **b.** Clarified Golgi impregnation, ax — axon; **c.** Nissl stained perikarya.

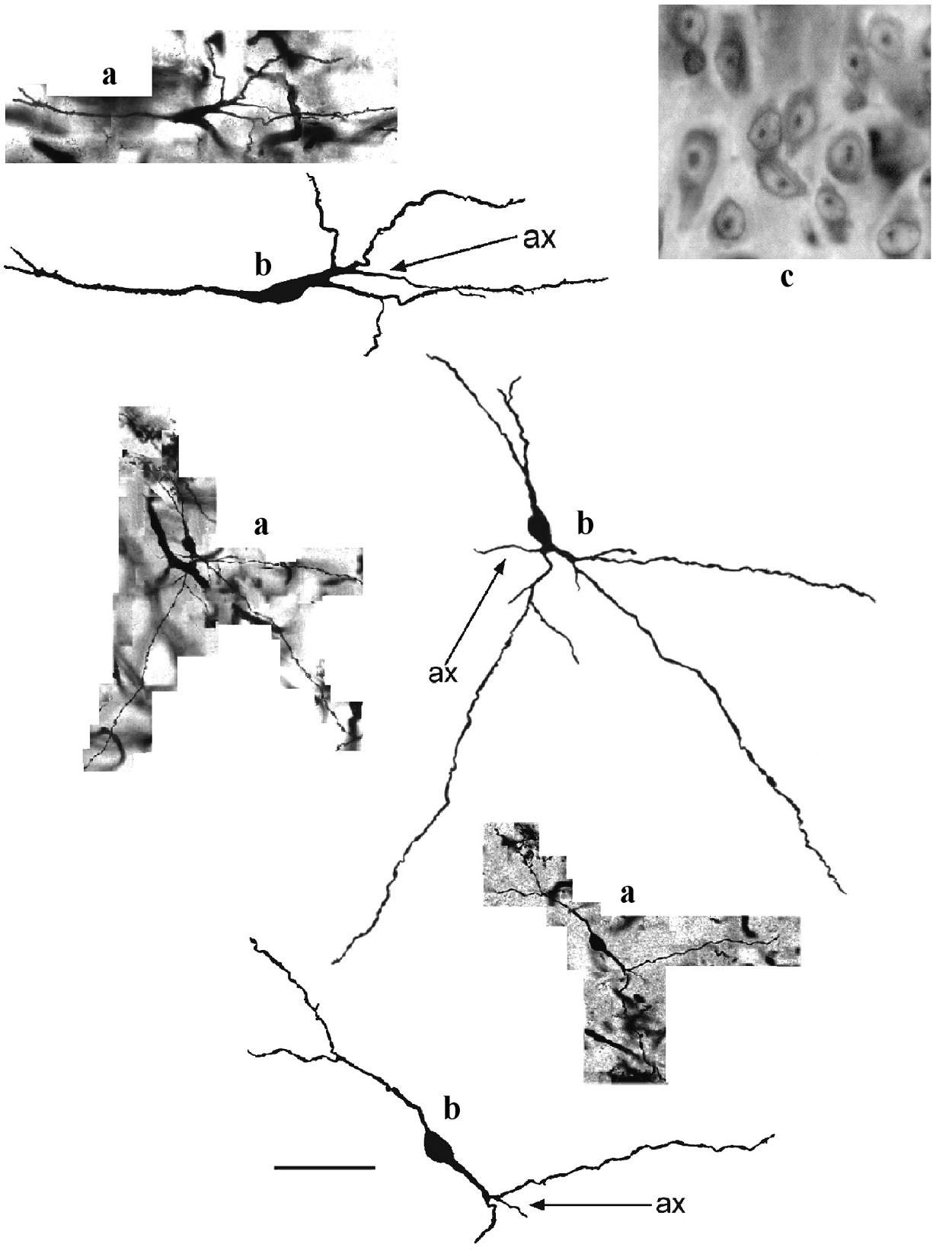

Figure 4. Bipolar neurons of POA of the rabbit. Scale bar 50 μm; **a.** Non-clarified Golgi impregnation; **b.** Clarified Golgi impregnation, ax — axon; **c.** Nissl stained perikarya.

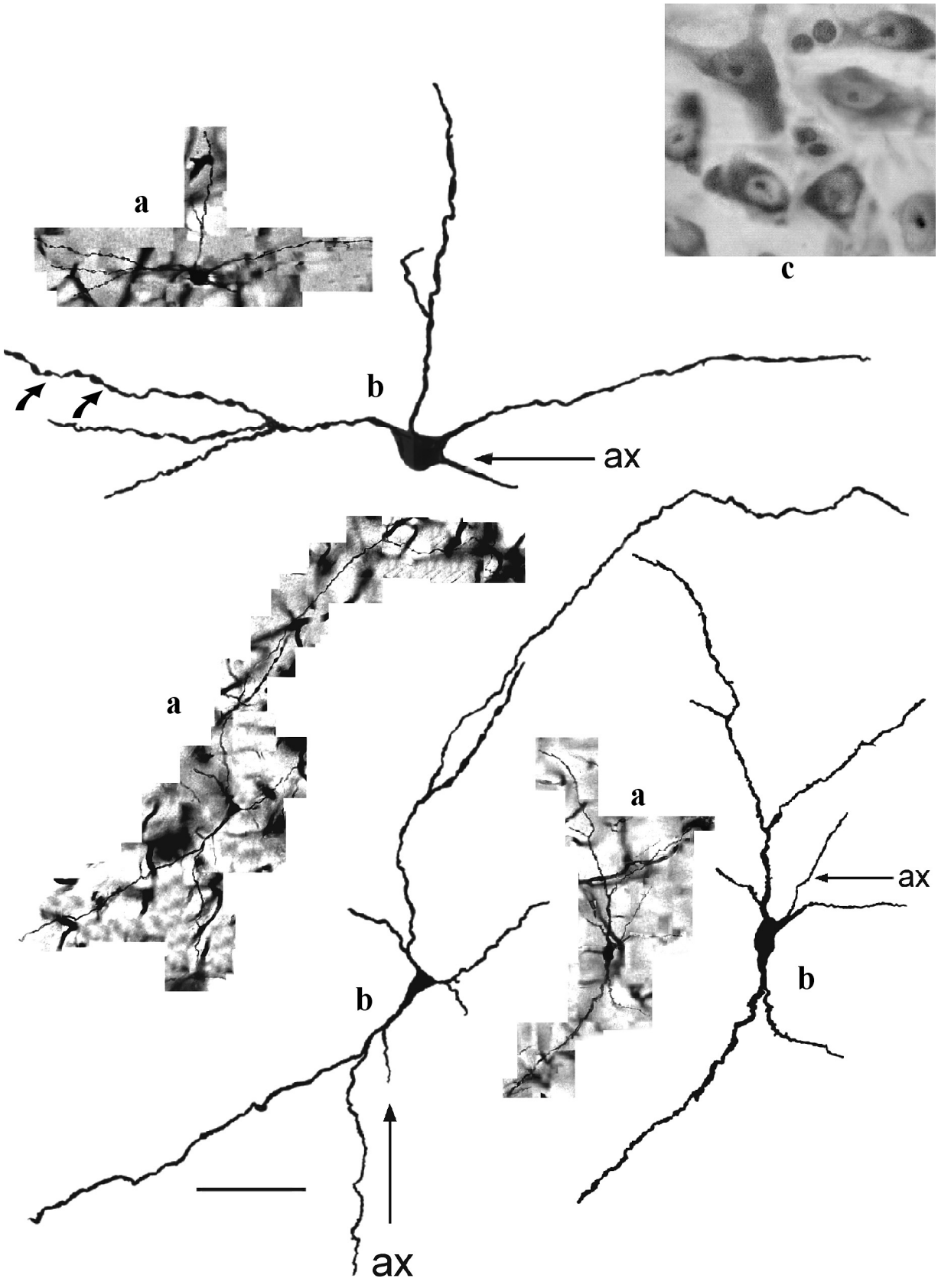

Figure 5. Multipolar neurons of POA of the rabbit. Scale bar 50 μm; **a.** Non-clarified Golgi impregnation; **b.** Clarified Golgi impregnation, arrows — "rosary-like dendrites", ax — axon; **c.** Nissl stained perikarya.

of the dendrites takes place after about 15 μm or after 50 μm. The location of the second division varies between 50–100 μm, from the previous one. Sporadically in the distal portions of the dendrites a third division was observed. Most dendritic branches are covered with varicosities and filiform or knob-like protrusions that are irregularly distributed along the dendrites. However, within the multipolar neurons there is a group of neurons with bead-like swellings that are similar in size and fairly evenly distributed on the dendritic branches. Such swellings cause the dendrites to take on a rosary-like appearance. The neurons with "rosary-like dendrites" (Fig. 5B — arrows) were observed in MPA and LPA. The dendritic field is vast and has an oval or spherical form. The longest dendrites reach to about 600 μm. An axon was observed to arise more often from the dendritic trunk than from the soma and was observed at a distance of about 40 μm, rarely at 70 μm. The multipolar neurons were observed in all parts of POA but they were less numerous than the bipolar ones.

DISCUSSION

The preoptic area of the species examined consists of two types of neurons: bipolar and multipolar. It has been reported that POA of the rat is composed of similar two types of neurons [16, 31, 37]. These neurons have from two to five sparsely branched dendrites similar to those of the mole and rabbit. The monkey POA neurons are predominantly bipolar with a relatively simple dendritic field [3]. In POA of the mole and rabbit the bipolar neurons are also prevalent besides LPA of the mole, where the multipolar ones preponderate. Only in the ferret did Cherry et al. [8] distinguish, besides the bipolar and multipolar neurons, unipolar ones. The majority of the ferret multipolar neurons have three dendrites [8], while most multipolar neurons of the mole have three or four, and in the rabbit neurons with three, four or five dendrites occur in a similar number. An *in vitro* study has revealed that the number of dendritic turns may depend on gonadal steroids [46].

In the POA of the human [21] and the rat [11] bipolar neurons contain luteinising-hormone-releasing factor (LHRH). These neurons project to the arcuate nucleus and the portal capillary in the median eminence and influence the anterior pituitary [5, 10, 20, 43]. LHRH neurons sending descending projections also make *en passant* synapses, so that LHRH acts not only as a neurohormone released from the terminals in the median eminence but also as a neurotransmitter within POA of the guinea pig [42]. Preoptic LHRH-containing neurons are modulated by the cells producing opioid peptides and NPY-immunoreactive cells of the arcuate nucleus [26, 30]. In POA of the mole and rabbit (present paper) as well as in that of the rat [16] the dendrites of neurons of the medial and lateral areas are horizontally oriented. These dendrites are the main target for the fibres of the *stria terminalis* [16, 35], which is a crucial pathway from the amygdala to POA [7]. POA innervation of the medial amygdala can regulate dopamine activity in MPA [13]. The fibres of the *stria terminalis* make synaptic contacts in POA with dendritic shafts and dendritic spines [35]. In both the species examined the dendritic branches possess varicosities and delicate swellings but bead-like and spine-like processes and "rosary-like dendrites" were characteristic only for the rabbit neurons. The dendritic morphology of the rabbit neurons resembles that of the ferret [8]. Cherry et al. [8] reported that most preoptic neurons had relatively smooth dendrites and that dendritic spines were scarce, but that swellings were commonly observed, particularly on their distal segments. Raisman and Field [35] described two types of dendrites in the rat: one with numerous spines and the other with varicosities, while McMullen and Almli [31] distinguished spiny or aspiny dendrites. The dendritic protrusions observed in our material were unevenly distributed on the branches or concentrated on their distal portions but the soma was devoid of them. Gerocs et al. [16] postulated that during maturation there are changes in the distribution of the protrusions along the dendritic tree of the preoptic neurons. In rats of 4 and 20 days of age the majority of protrusions are close to the cell body, but in 90-day old animals the vicinity of the perikaryon becomes fairly free of them [16] and axosomatic synapses form only about 2–3% of the total synapses [35].

In our qualitative study the bipolar and multipolar neurons were observed in both the male and the female. In both sexes of salmon [41] the same types of neurons were distinguished in Golgi study and no sex differences were reported. However, quantitative data on POA and the anterior hypothalamic region provide evidence that sex differences do exist in the morphology of neurons in the monkey and ferret [3, 8]. The total dendritic length, number of branches and percentage of neurons with either third or fourth order branches were significantly greater in the male than in female ferret [8]. In the juvenile monkey there were approximately 20% more dendritic branches per neuron, with a higher number of

spines in males than in females [3]. Larriva-Sahd and Gorski [24] noted no sex differences in the ultrastructural characterization of medical preoptic nucleus in the rat [24], although Raisman and Field [35] noted that the neuropil of the rat POA is sex differentiated. In the male the synapses of non-amygdaloidal origin almost all make contact directly with the dendritic shafts, while in the female contact is with the dendritic spines [35]. In the dorsomedial POA of the hamster sexual dimorphism related to the pattern of the dendritic field suggests that this region may receive differential afferent inputs in males and females [18].

Experimental studies indicate that dendritic morphology is under hormonal regulation [1, 8, 46]. For example, the dendritic length, branching and thickness increase to some degree following injections of testosterone [8]. In the mouse POA and hypothalamus neurons of gonadal steroid-treated cultures had significantly more dendrites arising from the cell body in comparison with untreated cultures [46]. Preoptic cells contain receptors for oestrogens and androgens [4] as well as aromatase, the enzyme essential for converting the testosterone to oestradiol [39]. Although some brain areas are most sensitive to gonadal hormones during perinatal development [17], such sensitivity is not limited to this period, and even in adulthood gonadal steroids can alter neuronal structure [25].

In summary, it should be pointed out that the neuron types of POA of the mole and rabbit are similar, although the dendritic morphology of the rabbit neurons is more complicated. The rabbit neurons show more dendritic divisions (up to the fourth order) and possess more dendritic swellings and different protrusions, including the "rosary-like dendrites". In contrast to the mole, the rabbit shows variation in the location of the divisions (the first division of the multipolar neurons occurs after 15 μm or 50 μm and the second at a distance of 50 to 100 μm). The dendritic field of the rabbit neurons is larger and the dendrites are three or four times longer than those of the mole. In most neurons axons were observed at a short distance (up to 40 μm) and only in some neurons of the rabbit were longer axons (up to 70 μm) seen. This may suggest that the rabbit POA neurons occur at different stages of myelinisation. The morphological differences between the mole and rabbit neurons may apply to differences that are species dependent. On the other hand, it should be borne in mind that the POA plays an essential role in a variety of reproductive functions and the dendritic morphology of preoptic neurons may, even in adulthood, be altered by the influence of hormones. In view of this, the morphological differences of neurons in the mammals studied may be related gonadal steroid levels during their reproductive activity. It should be noticed that in the mole procreation occurs once a year, whereas in the rabbit it occurs several times a year.

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