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Morphological changes in the mink area postrema during growth and under different stages of sexual activity

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Summary. The histological features of the area postrema (AP) of mink brains of both sexes were investigated at different ages and physiological conditions with light and electron microscopy. The mink AP was a twin-winged structure located at the dorsal surface of the medulla oblongata and consisted of neurons, glial cells, and both continuous and fenestrated capillaries enmeshed in a rich neuropil. The ventricular surface of the mink AP was covered by a single layer of tanycytes except at its most caudal part that was covered by a basal membrane derived from the pia mater. Supraependymal cells and intraventricular axons were also a common finding over the apical poles of tanycytes. However, our study demonstrates that the mink AP acquires the above general features at an advanced postnatal time and that, once fully developed, it undergoes morphological changes that can be directly linked to the aging process and sexual activity of the animals.

Key words: Circumventricular organs, Structure, Aging, Sexual cycle

Introduction

The area postrema (AP) is the most caudal of the circumventricular organs of the mammalian central nervous system, being located at the apex of the calamus scriptorius on the dorso-medial surface of the medulla oblongata. The AP has been examined by electron microscopy in different species of mammals, such as rats (Spacek and Parízek, 1968; Dempsey, 1973; Gotow and Hashimoto, 1980), mice (Rohrsneider et al., 1972), guinea pigs (Manni et al., 1982), cats (Klara and Brizzee, 1977; Leslie et al., 1978), monkeys (Klara and Brizzee, 1975; Ling and Wong, 1987), rabbits (Shimizu and Ishii, 1964; Leonhardt et al., 1975), sheep (Lucchi et al., 1989), and cattle (Lindberg et al., 1991). From these studies it was evidenced that the morphological features of the AP are quite consistent among different mammalian species, consisting of neurons, glial cells, a specialized ependymal covering and an unusual vascular arrangement.

Since most of the circumventricular organs lack a functional blood-brain barrier, circulating hormones gain access to the nervous tissue through these structures to trigger changes in brain function. On that score, it has been demonstrated that the AP, despite its other known receptors, contains high-affinity binding sites for steroid hormones (Stumpf et al., 1992) which, in turn, regulate gonadotrophin-releasing hormone and luteinising hormone release (Cates and O'Byrne, 2000; Ganong, 2000). However, it has not been demonstrated to date whether the structure of the AP may be influenced by endocrine factors such as sexual hormones that fluctuate during various phases of the sexual cycle and, therefore, activate and/or inhibit some functions of the organ. Thus, the present study was performed to test the hypothesis that the AP undergoes morphological modifications according to the stage of sexual activity of the animal. Moreover, this approach easily allows one to investigate whether the fine structure of the AP changes during growth, a matter that has received minor attention in the literature regarding this circumventricular organ (Gotow and Hashimoto, 1980; Lucchi et al., 1989). In this study, the mink (*Mustela vison*) was preferred because the AP has not been described in this species and because its sexual cycle is highly regulated by photoperiod. The mink is a seasonal breeder with ovulation induced by copulation and delayed implantation. Both males and females are capable of breeding at 10 months of age and mating takes place under a long-day photoperiod (from February to April, peaking in March). The young are born in spring, 28 to 30 days after implantation, and by fall, at 6 months of age, they can care for themselves. The maximum life span for a mink is usually around 10 years (Martinet and

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Allain, 1985; Pilbeam et al., 1979).

Materials and methods

A total of fifteen male and female farm minks (*Mustela vison*) of the wild variety was used in this study. All animals were maintained under natural conditions of light and temperature, fed with commercial food and euthanized at various ages and different physiological conditions: 3-week-old animals killed at the first half of suckling (group 1); 2-month-old animals killed at the second half of suckling (group 2); 6-month-old prepubertal animals killed in mid-October (group 3); 8-month-old pubertal animals killed at the end of December (group 4); 10-month-old sexually active animals killed at the beginning of March (group 5); and 1 1/2-year-old sexually inactive animals killed at the end of September (group 6). After induction with ether, the minks were anaesthetized with an intraperitoneal injection of sodium pentobarbitol and then were perfused intracardially with a solution containing 2% paraformaldehyde and 0.5% glutaraldehyde in 0.1M cacodylate buffer at pH 7.3. The brain stems were removed from the skulls and stored in fresh fixative for 2 h at 4 °C.

Two animals per group were processed for transmission electron microscopy. The portions of the medulla oblongata containing the AP were dissected out and postfixed in 1% buffered aqueous osmium tetroxide for 2 h. They were dehydrated in a graded series of ethanol and embedded in Epon 812. Semithin sections were stained with toluidine blue and photographed with a digital camera (Olympus DP10) adapted to a photomicroscope (Olympus BX50). Ultrathin sections were counterstained with uranyl acetate and lead citrate, photographed with a JEOL SX100 and negatives were digitized with a scanner (Epson GT-7000 photo).

For scanning electron microscopy, three tissue blocks of groups 2, 3 and 5, respectively, were subjected to critical point with CO_2 after dehydration in graded ethanols, shadowed with gold and examined under a JEOL T220A. The original negatives were digitized as described above. The software used to correct brightness and contrast of all digitized images was Adobe Photoshop 5.0 for Windows.

Results

General features of the mink AP

In this epigraph, we describe the morphological characteristics of the mink AP common to all groups of age analyzed, and also those features found in animals fully developed but not influenced by sexual hormones (i.e., minks of groups 2 and 3).

The mink AP consisted of symmetrical elevations of tissue on either side of the caudal part of the fourth ventricle. The two areas coalesced caudally, dorsal to the entrance of the central canal, giving the organ a twin-winged shape.

Two arteriolar vessels that entered the AP dorsally at its caudo-lateral edges (Fig. 1) provided the blood supply. Upon entering the organ, the vessels broke up into an extensive capillary network. Two types of capillaries were observed in the mink AP: fenestrated capillaries with wide diameter, sinuous course and large perivascular spaces in the caudal part of the organ (Figs. 1, 8); and continuous capillaries, smaller in diameter, with reduced or absent perivascular spaces in the rostral parts of the AP (Figs. 1, 2).

The rostral parts of the mink AP were covered entirely by ependyma. The epithelial lining of the caudal part was restricted to the protrusion of the organ into the fourth ventricle, whereas a basement membrane derived from the pia mater (dragged along with arteriolar vessels) covered the rest of the structure (Figs. 1, 3). The lining epithelium of the mink AP consisted of tanycytes, a specialized type of ependymal cell characterized by contacting vessels through a long basal process (Fig. 5).

Another consistent feature was the presence of supraependymal elements in close apposition to the ventricular surface of the organ, mainly at its caudal part. Supraependymal cells were scarce and appeared either isolated (Figs. 2, 15, 17) or arranged in clusters (Fig. 16). In scanning electron microscopy we could see intraventricular axons (Fig. 6), isolated or arranged in bundles, protruding through the ependyma, running over the tanycytic apical surface, and disappearing again through the ependyma or spanning the adjacent medulla. The images of transmission electron microscopy showed that intraventricular axons lacked myelin sheaths.

The parenchyma was composed of neurons and glial cells. The neurons were arranged in clusters of 3-4 cells, their soma were surrounded by glial and ependymal processes, and their axons characteristically lacked myelin sheaths. The neuronal soma contained a large indented nucleus and numerous cytoplasmic organelles (Fig. 4). Astrocytes were the commonest cell type in the mink AP and their soma and processes were seen around neurons (Fig. 4) and contacting the basal membrane of both vessels and pia mater (Fig. 3). Microglial cells (Fig. 4) and oligodendrocytes were also identified, with the latter being more numerous at the lateral and caudal edges of the organ where myelinated axons were a consistent finding (Fig. 17).

Structural changes according to age and sexual activity

No morphological differences were observed in the mink AP between males and females of the same group.

In group 1 (3-week-old), the most striking features were the lack of myelinated axons in the whole mink AP parenchyma (Figs. 1, 8) and the different ependymal covering between the rostral and caudal parts of the organ. The layer of tanycytes located at the caudal part of the organ exhibited a rather high cellular density and ultrastructural features of immaturity (Fig. 5), such as elongated nuclei with 2 or 3 nucleoli and uncoiled

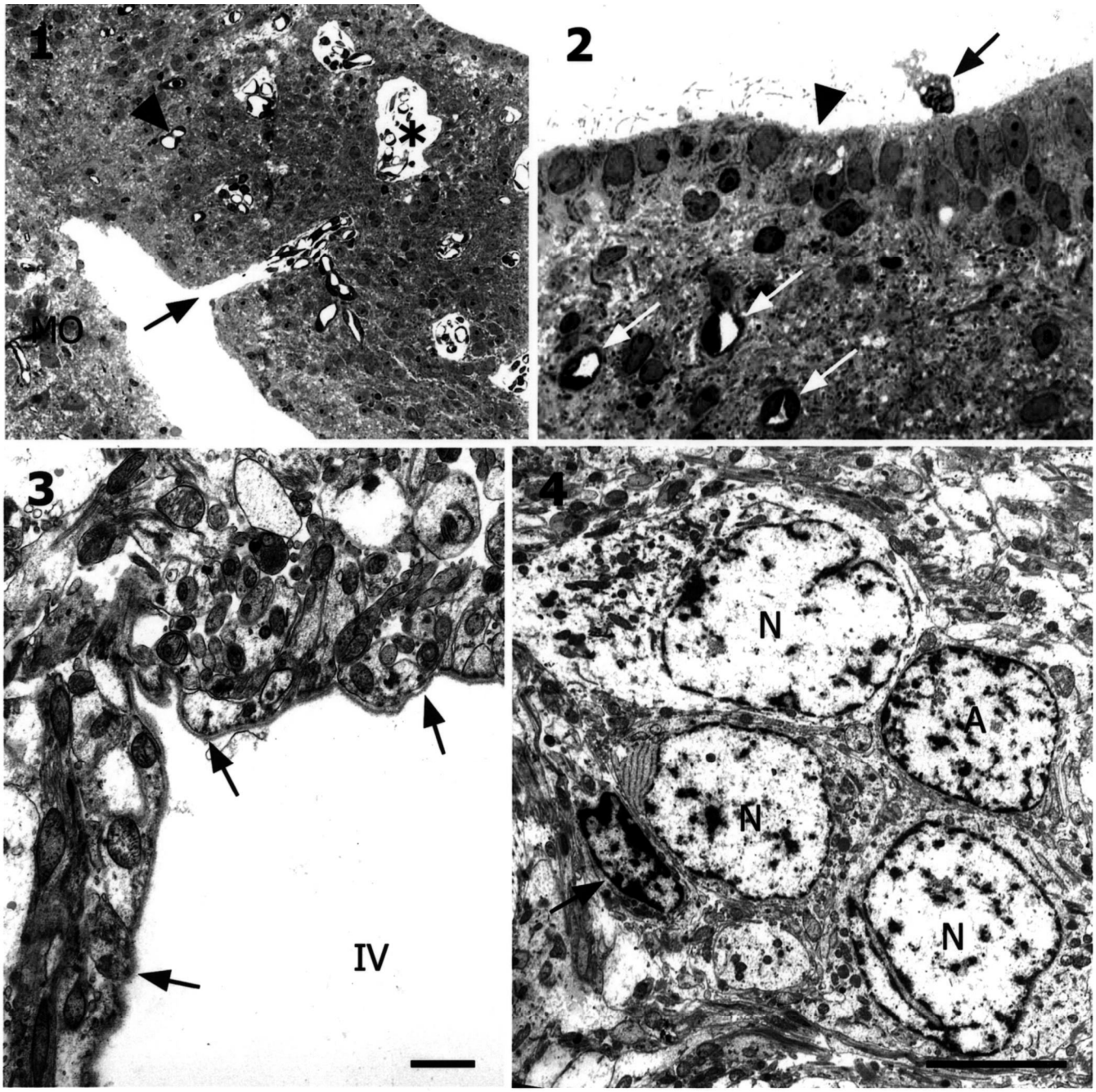


Fig. 1. Semithin section of group 1. Caudo-lateral AP and adjacent medulla oblongata (MO). Note the absence of myelinated axons and the lack of epithelial lining at the most caudal aspect of the AP where an arteriolar vessel (arrow) enters the organ. A capillary vessel with wide diameter and large perivascular space (asterisk) and a smaller one with reduced perivascular space (arrowhead). x 40

Fig. 2. Semithin section of group 1. Transition zone (arrowhead) between the ciliated and the non-ciliated ependymal covering of the mink AP. A supraependymal cell (arrow) is over the apical pole of tanycytes. White arrows point to small, continuous capillaries. x 200

Fig. 3. Thin section of group 2. Caudo-lateral edges of the mink AP lacked an ependymal lining, being covered by a basal membrane (arrows) derived from the pia mater. End feet of astrocytes terminate on the basal membrane to form the glia limitans at the surface of the AP. Bar: 1 µm.

Fig. 4. Thin section of group 3. Three AP neurons (N) surrounded by and separated from each other by the soma and processes of an astrocyte (A). The arrow points to a microglial cell. Bar: 5 µm.

chromatin, numerous ribosomes, mitochondria and rough endoplasmic reticulum cisternae, and well developed Golgi complexes. Their apical membrane in contact with the cerebrospinal fluid of the fourth ventricle exhibited numerous microvilli and bleb-like protrusions and, occasionally, a solitary cilium in the center of the cell's apical pole. In contrast to this, the rostral parts of the organ were lined by typical ciliated ependymal cells that were separated from the tanycytic covering by a sharp transition zone (Fig. 2). Mitosis figures were often seen among ependymal cells located in the transition zone between tanycytes and ciliated ependymal cells (Fig. 7) as well as in the interior of the AP (Fig. 8).

The lining ependyma of 2-month-old (group 2) and 6-month-old minks (group 3) consisted of a single layer of tanycytes both in the caudal and rostral parts of the AP. Apical portions of tanycytes were increased in size and contained round or oval nuclei with a single nucleolus and, in the cytoplasm, both filaments and mitochondria were the most prominent organelles. Their apical membrane lacked bleb-like protrusions but bore short microvilli that were specially numerous at the cell boundaries (Fig. 9). In the parenchyma, myelinated axons were apparent at the caudo-lateral edges of the organ. No mitosis figures were identified in the ependyma or in the underlying parenchyma.

The group 4 of minks (8-month-old pubertal animals) was characterized by the presence of cyst-like structures located both in the ependyma and deep in the parenchyma of the caudal AP (Fig. 10). These cysts were also a consistent finding in animals of groups 5 (10-month-old sexually active animals) (Fig. 16) and 6 (1 1.5-year-old sexually inactive animals). The cysts were spherical in shape and variable in size, and consisted of thin cytoplasmic projections which surrounded the cystic lumen (Fig. 11). The cystic lumen seemed to be confined within a single cell, as no junctional structure was found around the cytoplasm that constituted the cystic wall. Cysts were never seen in direct contact with perivascular spaces or the ventricular lumen. When they were located within the ependyma, attenuated cytoplasmic expansions from the apical surfaces of the adjacent tanycytes covered the cystic wall (Fig. 11).

The tanycytes of 10-month-old minks (group 5, sexually active animals) were less numerous but larger than those observed previously in the AP of younger animals. Another characteristic of the ependymal cells, when compared with the tanycytes of the other groups, was their rich complement of cytoplasmic filaments and mitochondria and the presence of numerous microvillous profiles and wide bleb-like protrusions on their apical membrane (Figs. 12, 13) which completely covered the cell pole in contact with the cerebrospinal fluid. Blebs contained large and irregular electron-lucent membrane-bound vesicles. Small vesicles, similar in morphology, were numerous in the supranuclear cytoplasm of tanycytes where they seemed to fuse and form the larger ones found within the apical protrusions (Fig. 13).

The loss in the number of tanycytes accompanied by hypertrophy and increased content in filaments were apparent in the mink AP of group 6 (11/2-year-old sexually inactive animals). At this age, however, the most striking feature was the presence of lipofuscin pigment in the cytoplasm of most of the neurons in the organ (Fig. 14).

With regard to the supraependymal elements, supraependymal cells appeared more frequently over the tanycytes of minks belonging to group 5 (Fig. 15 and Fig. 16), followed by animals of group 4 (Fig. 17), i.e. in sexually active and pubertal animals, respectively. No significant age or sexual differences were observed in relation to the intraventricular axons.

Discussion

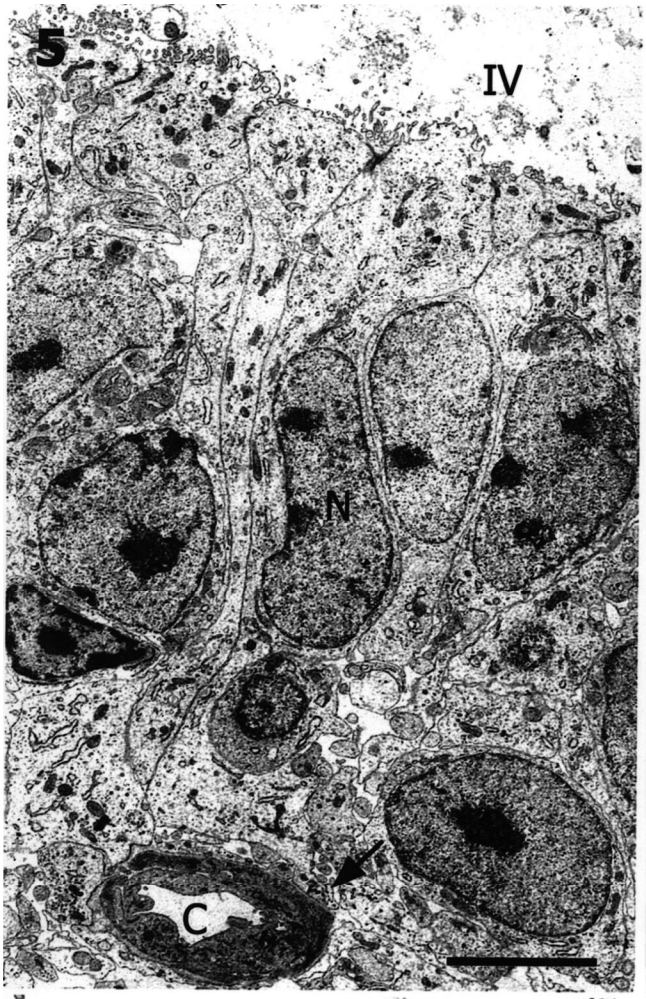
The general morphological features of the mink AP were similar to those described by several authors in different species of mammals (Shimizu and Ishii, 1964; Rohrsneider et al., 1972; Dempsey, 1973; Leonhardt et al., 1975; Klara and Brizzee, 1975, 1977; Leslie et al., 1978; Gotow and Hashimoto, 1980; Manni et al., 1982; Ling and Wong, 1987; Lindberg et al., 1991). Firstly, its shape and position on the dorsal surface of the medulla oblongata were typical of most non-rodent mammals (Borison, 1989; Olfield and McKinley, 1995). In rodents and lagomorphs, in contrast, the AP is a single midline structure embedded in the obex dorsal to the nucleus of the solitary tract (Borison, 1989; Olfield and McKinley, 1995). Secondly, the mink AP was composed of neurons, glial cells, two types of capillaries and a modified ependymal lining, the latter often associated with supraependymal cells and axons. It has to be emphasized, however, that the mink AP was not covered entirely by ependyma as was expected by its twin-

Fig. 5. Thin section of group 1. Tanycytes located at the caudal part of the organ showing features of immaturity. One of them (N, nucleus) can be seen contacting both the ventricular lumen (IV) and a capillary vessel (C) through a basal process (arrow). Bar: 5 µm.

Fig. 6. Scanning electron micrograph of group 2. Intraventricular axons (arrows) running over the tanycytic apical surfaces. Bar: 5 µm.

Fig. 7. Thin section of group 1. A cell undergoing mitosis (M) located among cells at the transition zone between the ciliated and the non-ciliated area of the mink AP. Bar: 2 µm.

Fig. 8. Semithin section of group 1. A cell undergoing mitosis (arrow) in the AP parenchyma. Note the presence of capillaries with wide perivascular spaces (arrowheads). x 200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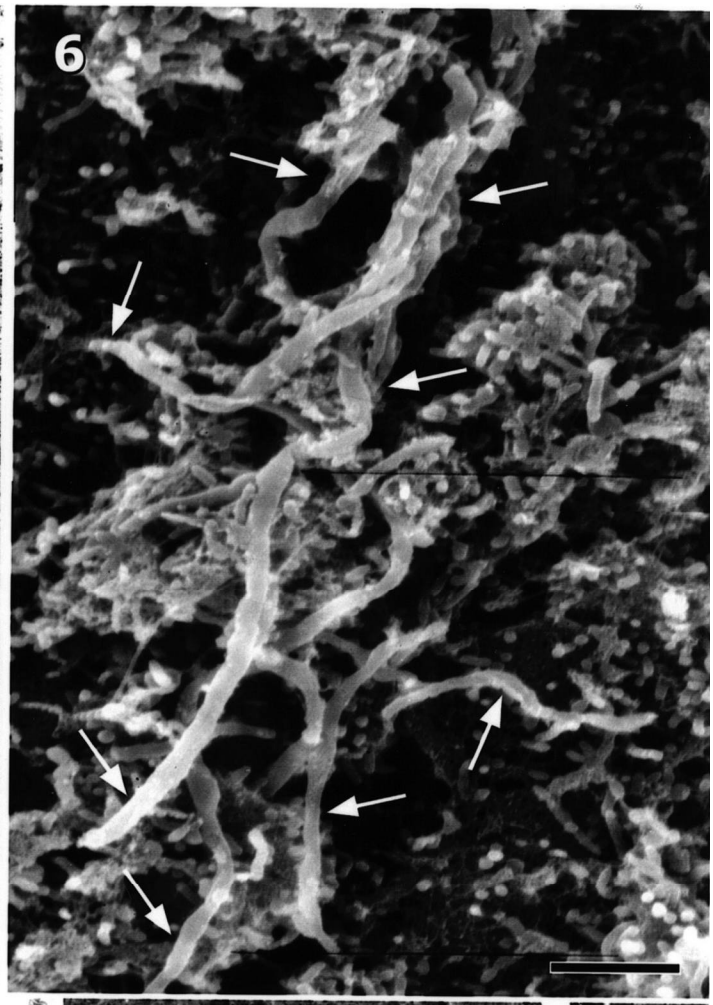
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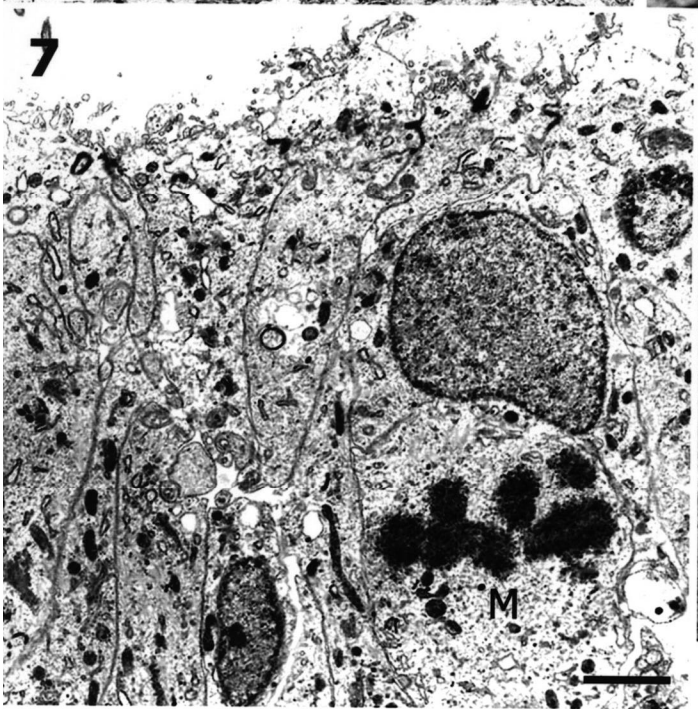
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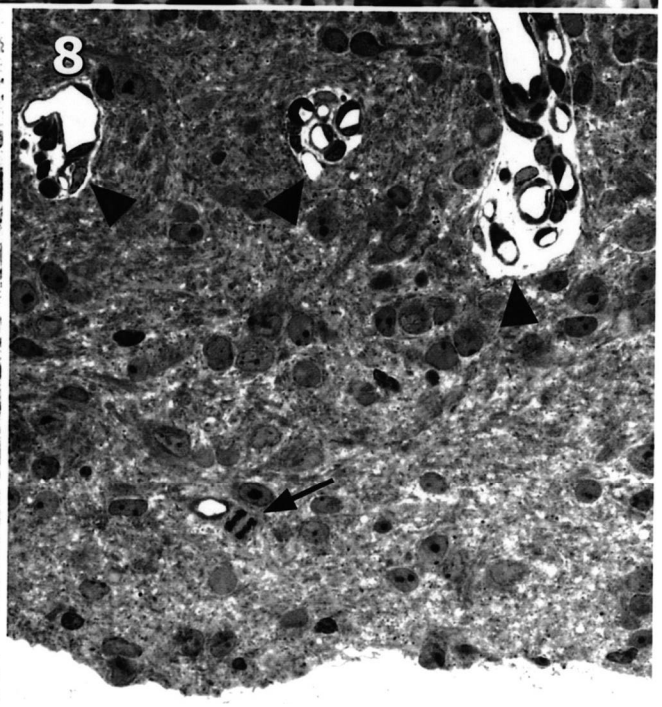
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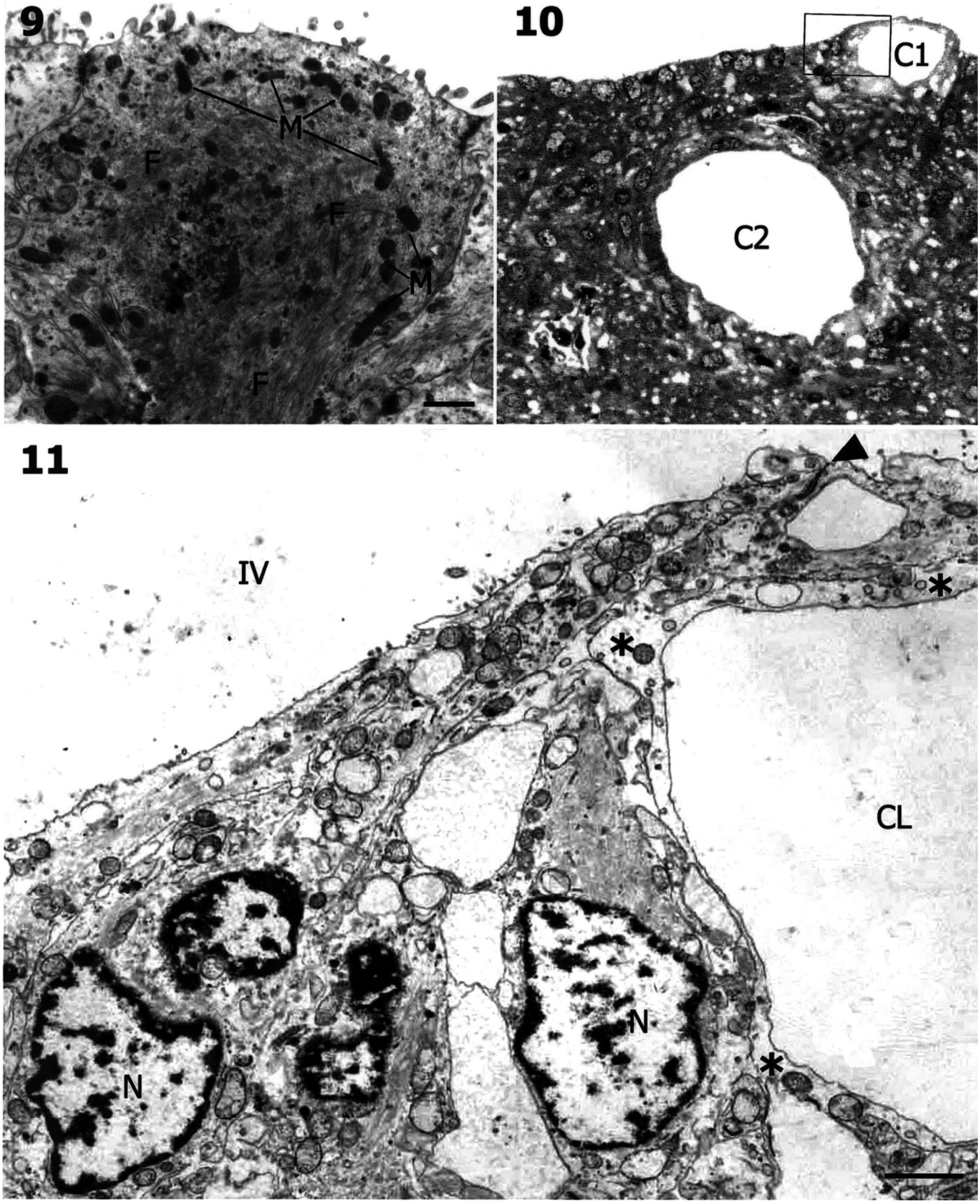


Fig. 9. Thin section of group 3. Apical portion of a tanycyte filled with bundles of filaments (F) and elongated mitochondria (M). Its apical membrane exhibits numerous short microvilli. Bar 1 µm.

Fig. 10. Semithin section of group 4. Caudal AP exhibiting both an intraepithelial cyst (C1) and a deep parenchymal cyst (C2). x 100

Fig. 11. Thin section of group 4 showing the boxed area in Fig. 8. Cystic lumen (CL), cystic wall (asterisks), adjacent tanycytes (N, nuclei), zonula occludens and adherens between two tanycytes (arrowhead), ventricular lumen (IV). Bar: 2 µm.

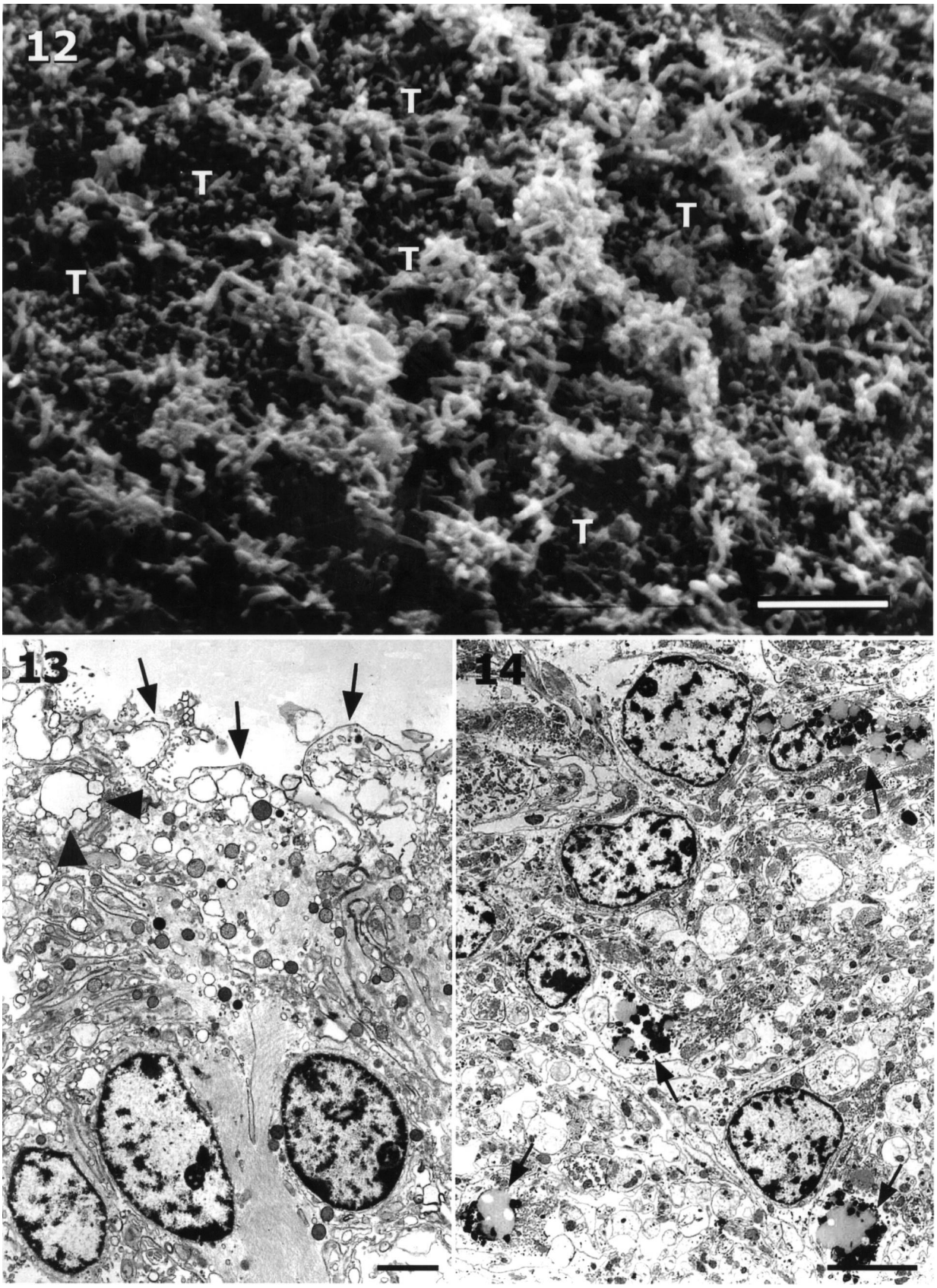


Fig. 12. Scanning electron micrograph of group 5. Membrane protrusions are concentrated at the cell boundaries, which distinctly mark the shape of individual tanycytes (T), but also are distributed on the rest of the apical pole. Bar: 10 µm.

Fig. 13. Thin section of group 5. Tanycytes showing large bleb-like protrusions (arrows) filled with irregular electron-lucent vesicles. Note the presence of numerous small vesicles of different sizes in their apical cytoplasm, some of them (arrowheads) seem to fuse. Bar: 2 µm.

Fig. 14. Thin section of group 6. Lipofuscin pigment (arrows) in neuronal processes of the mink AP. Bar: 5 µm.

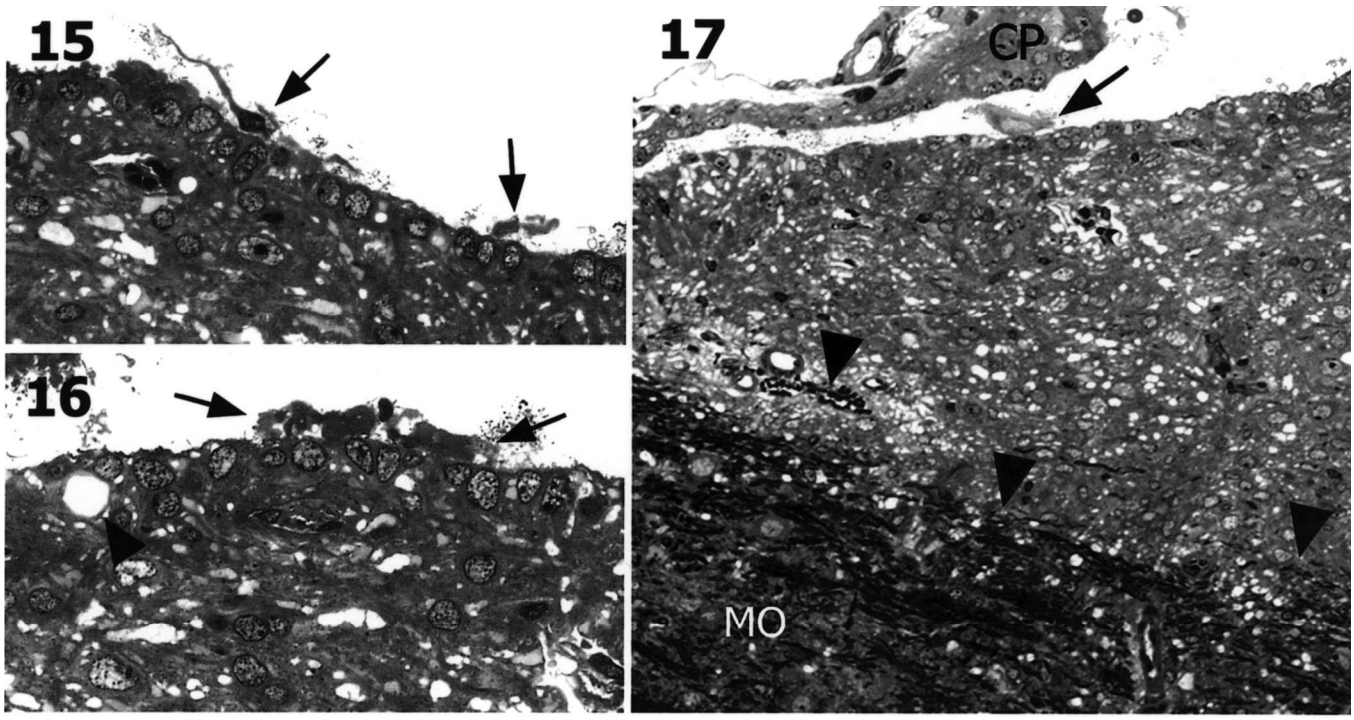
winged shape (Leslie et al., 1978; Borison, 1989; Lindberg et al., 1991), since its most caudal part was lined by a basal membrane derived from the pia mater. Thus, the AP of the mink could be considered, with regard to pial investment and according to Borison (1989), as an intermediate between typical twin-winged-AP (covered entirely by ependyma) and midline-embedded-AP species (only the rostral protrusion is endowed with ependyma).

Significantly, our study demonstrated that the histological structure of the mink AP changes during growth and under different physiological conditions.

The mink AP did not exhibit the above general morphological features until the animals were 2 months old (group 2). In the first place, minks of group 1 (3-week-old) had an AP that displayed features of immaturity, such as two types of ependymal lining separated from each other by a sharp transition zone in which cells undergoing mitosis were often found. In this way, it has been demonstrated that ependymal cells are neural stem cells that can give rise to the major classes of nervous tissue cells (Johansson et al., 1999). Therefore, a possible interpretation of our observations is that cells generated within the transition zone migrate laterally to replace the typical mural ependyma of the rostral parts of the AP by a single layer of modified ependyma. In the second place, complete myelination of nerve fibers was found in the AP of minks of group 2, whereas myelinated axons were absent in the organ of group 1. Processes of mink AP neurons lacked myelin sheaths and, therefore, myelinated axons, which could be found within the rich neuropil, represent central or peripheral afferent neural connections of the organ. Therefore, the morphological characteristics of a fully developed mink AP are acquired at an advanced postnatal time, in the period between 3 weeks and 2 months. This finding leads us to speculate that at least some of the functions of the mink AP may not be operative until the organ has fully developed.

We also observed other morphological changes in the mink AP that can be directly linked to the aging process. Such changes were a marked progressive decrease in the number of tanycytes accompanied by hypertrophy and an increase in their content in filaments and, in the case of the older animals studied (1.5-year-old minks of group 6), an obvious occurrence of lipofuscin accumulation in AP neurons.

AP tanycytes in 3-week-old minks had not fully acquired adult form when compared with those present in the organ of older animals. This finding suggests that the function of this cell population may be age-dependent and, furthermore, adds support to the hypothesis that some of the proposed functions of the AP may not be operative in the early postnatal period.



Figs. 15 and 16. Semithin sections of group 5. Supraependymal cells (arrows) isolated **(Fig. 15)** and in a cluster **(Fig. 16)**. The arrowhead points to a ependymal cyst in Fig. 16. x 200

Fig. 17. Semithin section of group 4. An isolated supraependymal cell (arrow) between the choroid plexus (CP) and the AP. Note the presence of numerous myelinated axons at the caudo-lateral AP (arrowheads) and also at the adjacent medulla oblongata (MO). x 100

Similarly, cytological modifications between developing and adult tanycytes have been previously described in different locations of the ventricular system (Walsh et al., 1978; Bruni et al., 1983). In addition, it has also been reported that tanycytes undergo morphological modifications with age similar to those observed in the mink AP throughout the studied period of time, such as their number, size and cytological features (Brawer and Walsh, 1982; Zoli et al., 1995).

Regarding lipofuscin accumulation in neurons, it is widely accepted that this occurs under normal conditions during aging and, notably, it has been reported that in the rat this accumulation occurs earlier in the AP than in other regions of the brain (Glees and Hasan, 1976).

The presence of cysts within the structure of the AP has been described previously in monkeys, cats and rats by Cammermeyer (1973), but they have only been studied in detail in rats by Gotow and Hashimoto (1980). The latter authors found them in adult rats but not in new born rats, a fact that led them to conclude that the frequency of cysts seemed to be related to the age of the animals. However, they did not investigate the precise moment of their appearance in the rat AP. In this study we have found the occurrence of cysts in the AP of 8-month-old minks (group 4) and that they were a common finding in older animals. Their presence does not seem to be related to the physiological state of the animals, since their number and size did not show evident modifications among groups, but rather to the age of the minks in agreement with the theory put forward by Gotow and Hashimoto (1980).

The present study also revealed that the mink AP undergoes morphological changes according to the stage of sexual activity. These modifications were restricted to the apical surface of the tanycytes which exhibited an increased number of microvillous profiles and bleb-like protrusions during the sexually active period (10-month-old animals of group 5) when compared with the corresponding profiles in suckling, prepubertal, pubertal, or sexually inactive minks. Moreover, supraependymal cells were also more numerous in the sexually active animals of group 5, followed by pubertal animals of group 4, than in prepubertal or sexually inactive minks. Variations in the number of tanycytic apical profiles and supraependymal cells, in both cases reaching a maximum in sexually active animals, have been registered in other species of mammals (Brawer et al., 1974; Mestres, 1976, 1981). According to Mestres (1981), modifications in the number of surface profiles may represent both absorptive and/or secretory activities of tanycytes and cell membrane turnover depending on endocrine factors. The transmission electron microscopic observations presented in this study support the idea that AP tanycytes increase at least their secretory activity in sexually active minks. In relation to supraependymal cells, the precise reason for the observed changes in their number, dependent upon the physiological state of the animal, is still unknown. It has been suggested that they may be involved in a signalling pathway between the ventricular cerebrospinal fluid and the nervous tissue (Singh et al., 1989; Ojeda and Piedra, 1998). Therefore, their increased occurrence on the surface of the mink AP in sexually active animals may play a role in transducing hormonal signals to the underlying parenchyma.

In summary, the mink AP is endowed with the morphological features that characterize this circumventricular organ in other mammalian species. In the mink, however, such features are acquired at an advanced postnatal time and, once fully developed, the organ undergoes structural modifications mainly linked to the process of aging, but also to the stage of sexual activity of the animal.

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